Outlines of practical physiology : being a manual for the physiological laboratory, including chemical and experimental physiology, with reference to practical medicine / by William Stirling.

Contributors

Stirling, William, 1851-1932.

Publication/Creation

London : Charles Griffin, 1902 (Glasgow : Bell and Bain.)

Persistent URL

https://wellcomecollection.org/works/aheakhcq

License and attribution

This work has been identified as being free of known restrictions under copyright law, including all related and neighbouring rights and is being made available under the Creative Commons, Public Domain Mark.

You can copy, modify, distribute and perform the work, even for commercial purposes, without asking permission.

wellcome collection

Wellcome Collection
183 Euston Road
London NW1 2BE UK
T +44 (0)20 7611 8722
E library@wellcomecollection.org
https://wellcomecollection.org

GRIFFIN'S SCIENTIFIC TEXT-BOOKS


22101454216

NET BOOK.—This book is supplied to the Trade on terms which will not allow of Discount to the Public.

CHARLES GRIFFIN & CO., LTD.

OUTLINES OF
PRACTICAL PHYSIOLOGY.

Companion Volume by Prof. Stirling.

Second Edition. In Extra Cloth 8vo, with 368 Illustrations. Cloth, 12s. 6d.

OUTLINES OF PRACTICAL HISTOLOGY:

A Manual for Students.

PART I.—PRACTICAL WORK IN CUTTING, MOUNTING, AND STAINING.
PART II.—THE EXAMINATION OF ANIMAL TISSUES AND FLUIDS.

"The general plan of the work is ADMIRABLE. . . . Merits the highest praise for the ILLUSTRATIONS, which are at once clear and faithful."—*British Medical Journal.*

"We can confidently recommend this CONCISELY-WRITTEN and ADMIRABLY ILLUSTRATED work to students. A VERY USEFUL and RELIABLE GUIDE in the laboratory, or in their own room."—*Lancet.*

The Physiologist's Note Book. A Summary of the present state of Physiological Science for Students. By ALEX HILL, M.A., M.D., Master of Downing College, Cambridge. With numerous Illustrations. 12s. 6d.

WORKS BY PROFESSOR DAVIS.

An Elementary Text-Book of Biology. By J. R. AINSWORTH DAVIS, M.A., F.Z.S., Professor of Biology, University College, Aberystwith. *Second Edition* In Two Volumes. Sold separately.

Vol. I.—VEGETABLE MORPHOLOGY AND PHYSIOLOGY. With Complete Index-Glossary and 128 Illustrations. 8s. 6d.

Vol. II.—ANIMAL MORPHOLOGY AND PHYSIOLOGY. With Complete Index-Glossary and 108 Illustrations. 10s. 6d.

A Zoological Pocket-Book, or Synopsis of Animal Classification, with Definitions of Phyla, Classes, and Orders, Remarks, and Tables. By Dr. EMIL SELENKA. Authorised English Translation by Professor AINSWORTH DAVIS. Interleaved, Cloth Limp. 4s.

The Flowering Plant, with a Supplementary Chapter on FERNS and MOSSES, as Illustrating the first Principles of Botany. By Prof. AINSWORTH DAVIS. *Third Edition*, Revised and Enlarged. Large Crown 8vo, with numerous Illustrations. 3s. 6d.

Third Edition, Revised, Enlarged, and brought up-to-date. Containing all the latest Chemical Discoveries. 9s. net.

A SHORT MANUAL OF INORGANIC CHEMISTRY.

By A. DUPRÉ, Ph.D., F.R.S., and
WILSON HAKE, Ph.D., F.I.C., F.C.S., of the Westminster Hospital Medical School.

"A well written, clear, and accurate elementary manual of inorganic chemistry. WILL MAKE EXPERIMENTAL WORK TREBLY INTERESTING BECAUSE INTELLIGIBLE."—*Saturday Review.*

Outlines of Quantitative Analysis. By Prof. HUMBOLDT SEXTON. With Illustrations. Crown 8vo, Cloth. *Fourth Edition.* 3s.

Outlines of Qualitative Analysis. By Prof. HUMBOLDT SEXTON. With Illustrations. Crown 8vo, Cloth. *Third Edition.* 3s. 6d.

Practical Pharmacy and Dispensing (Elements of). By W. ELBORNE, B.A., F.L.S., F.C.S., Demonstrator of Materia Medica and Teacher of Pharmacy at University College, London; Pharmacist to University College Hospital. 8s. 6d.

GRIFFIN'S "POCKET-BOOK" SERIES.

Elegantly Bound in Leather Limp, with Rounded Corners and Gilt Edges.

A Surgical Handbook. By F. M. CAIRD, M.B., F.R.C.S., and C. W. CATHCART, M.B., F.R.C.S. *Eleventh Edition*, Revised. 8s. 6d.

A Medical Handbook. By R. S. AITCHISON, M.D.(Edin)., F.R.C.P., *Third Edition*, Revised. 8s. 6d.

Outlines of Bacteriology. By THOINOT and MASSELIN. Translated by W. ST. CLAIR SYMMERS, M.B. With Illustrations (some in Colours). 10s. 6d.

Hygiene (A Handbook of). By Lt.-Col. A. M. DAVIES, D.P.H.(Camb.) *Second Edition*, Thoroughly Revised, Enlarged, and partly Rewritten. 8s. 6d. *net.*

The Surgeon's Pocket-Book. By Major J. H. PORTER. Revised by Brigade-Surgeon C. H. Y. GODWIN. 8s. 6d.

The Diseases of Children. By GEORGE ELDER, M.D., F.R.C.P.(Edin.), and J. S. FOWLER, M.B., F.R.C.P.(Edin.). Illustrated. 10s. 6d.

LONDON: CHARLES GRIFFIN & CO., LTD., EXETER STREET, STRAND.

OUTLINES

OF

PRACTICAL PHYSIOLOGY:

BEING A

Manual for the Physiological Laboratory,

INCLUDING

CHEMICAL AND EXPERIMENTAL PHYSIOLOGY, WITH REFERENCE TO PRACTICAL MEDICINE.

BY

WILLIAM STIRLING, M.D., Sc.D.,

BRACKENBURY PROFESSOR OF PHYSIOLOGY AND HISTOLOGY IN THE OWENS COLLEGE, AND PROFESSOR IN VICTORIA UNIVERSITY, MANCHESTER.

FOURTH EDITION, REVISED AND ENLARGED.

With 465 Illustrations.



LONDON:

CHARLES GRIFFIN AND COMPANY, LIMITED

EXETER STREET, STRAND.

1902.

[*All Rights Reserved.*]

In Memoriam

CARL LUDWIG,

MY REVERED AND BELOVED MASTER.

Born at Witzenhausen, 29th December, 1816.

Died at Leipzig, 24th April, 1895.

WELLCOME INSTITUTE LIBRARY
Coll. welMOmec
Call No. QT4 1902 S860

THE FOURTH EDITION OF THIS WORK IS

Dedicated

TO

CHARLES RICHET,

PROFESSOR OF PHYSIOLOGY IN THE MEDICAL FACULTY OF THE UNIVERSITY OF PARIS,

WHO, BY HIS RESEARCHES IN PHYSIOLOGY AND ALLIED SUBJECTS HAS CONTRIBUTED IN NO SMALL DEGREE TO OUR KNOWLEDGE OF THE MEANS WHEREBY THE FELL EFFECTS OF DISEASE MAY BE SUCCESSFULLY COMBATED, AND WHO, BY HIS GENEROUS, BROAD, ENLIGHTENED, AND GENIAL SYMPATHIES, HAS ENDEARED HIMSELF TO ALL HIS PHYSIOLOGICAL COLLEAGUES.

PREFACE TO THE FOURTH EDITION.

It is with a certain amount of reluctance that I offer a Fourth Edition of this work, which is intended primarily to help my own students.

Many additions and alterations have been made, chiefly in the experimental part. I have availed myself of all the works on practical physiology published since the issue of the last edition—including those of Brodie, Birch, R. Dubois, W. S. Hall, Halliburton, Hermann, Hofmeister, Langley, Novy, Porter, Schulz, and Waller.

To many friends and colleagues—including Professors Sherrington, Lafayette B. Mendel, Dr. J. Mackenzie of Burnley, Mons. C. Verdin, and many others—I am indebted for much valuable assistance.

An account of some demonstrations of fundamental importance and of some recent methods—*e.g.*, Cryoscopy—has been added.

The Lessons on the Senses have been considerably augmented. In compiling the latter I am greatly indebted to the works of Sanford and Titchener. The student is too apt to concentrate his attention on nerve-muscle physiology to the exclusion of other phenomena which can be readily studied on himself or with the aid of a friend. It is for this reason that I have added many of the more recent experiments on the sense-

organs—a branch of physiology of great and growing importance to the practitioner of medicine, and one which can often be studied by comparatively simple means.

I had originally intended to add separate Lessons on "Physiological Psychology," a subject which has attracted much attention in America. I have, however, practically obtained my object by extending the physiology of the senses.

The number of illustrations has been nearly doubled. To all those who have lent clichés, I tender my sincere thanks.

Some References to original papers have been added—more especially to those in the *Journal of Physiology*.

I have done myself the honour of adding the name of my friend Professor Charles Richet to the dedication page, as a small token of gratitude for the many privileges I have so frequently enjoyed in his Laboratory.

WM. STIRLING.

PHYSIOLOGICAL LABORATORY,
OWENS COLLEGE, MANCHESTER,
November, 1901.

PREFACE TO THE THIRD EDITION.

IN the light of extended experience in teaching Practical Physiology, I venture to submit a Third Edition of this little work. The essential features remain unchanged; but there has been some re-arrangement of the subject-matter, and many additions have been made, including a short Appendix on Recording Apparatus.

In preparing the Chemical Part, I have made use of the Text-books of Gamgee, Halliburton, Neumeister, and Salkowski; while, for the Experimental Part, I found numerous valuable suggestions in the practical works and syllabuses of my friends Professors Gotch, Halliburton, Fredericq, and Dr. Schenck. I have to express my thanks to Professor Fick of Würzburg for several improvements in the Lessons on Muscle.

A large number of new woodcuts have been added (chiefly in the Experimental Part); and for communications and several original drawings — some of the latter illustrating new methods described by their authors—I am indebted to my friends and colleagues, Professors Birch, Gotch, Rutherford, and Schäfer, Dr. Bayliss, Dr. Gregor Brodie, and C. Herbert Hurst, Ph.D., and to the other sources acknowledged elsewhere.

I have also to thank my pupils, Messrs. Moore, Halstead, and J. H. Sheldon, for some of the drawings, and my Senior Demonstrator, Dr. J. A. Menzies, for reading the proof sheets, and for other kind assistance and suggestions.

WILLIAM STIRLING.

PHYSIOLOGICAL LABORATORY,
OWENS COLLEGE, MANCHESTER,
August, 1895.

CONTENTS.

PART I.—CHEMICAL PHYSIOLOGY.

LESSON		PAGES
I.	THE PROTEIDS,	1-13
II.	THE ALBUMINOIDS AND SOME NITROGENOUS DERIVATIVES OF PROTEIDS,	13-16
III.	THE CARBOHYDRATES,	17-31
IV.	FATS, BONE, AND EXERCISES,	32-36
V.	THE BLOOD—COAGULATION—ITS PROTEIDS,	37-47
VI.	HÆMOGLOBIN: ITS COMPOUNDS, DERIVATIVES, AND THEIR SPECTRA,	48-59
VII.	RED BLOOD-CORPUSCLES — HÆMOCYTOMETERS — HÆMOGLOBINOMETERS—WAVE LENGTH,	60-72
VIII.	SALIVARY DIGESTION,	73-77
IX.	GASTRIC DIGESTION,	77-89
X.	PANCREATIC AND INTESTINAL DIGESTION,	89-98
XI.	THE BILE,	98-105
XII.	GLYCOGEN IN THE LIVER,	105-109
XIII.	MILK, FLOUR, AND BREAD,	109-116
XIV.	MUSCLE, SKELETAL AND SMOOTH,	116-120
XV.	SOME ORGANIC SUBSTANCES — NERVE TISSUE — THYROID GLAND,	121-125
XVI.	THE URINE,	125-131
XVII.	THE INORGANIC CONSTITUENTS OF THE URINE,	131-139
XVIII.	ORGANIC BODIES IN URINE—UREA—KJELDAHL,	140-146
XIX.	VOLUMETRIC ANALYSIS FOR UREA,	147-152
XX.	URIC ACID — URATES — HIPPURIC ACID — KREATININ — PIGMENTS,	153-164
XXI.	ABNORMAL CONSTITUENTS OF URINE—ALBUMIN,	164-168
XXII.	BLOOD, BILE, AND SUGAR IN URINE,	168-173
XXIII.	QUANTITATIVE ESTIMATION OF SUGAR,	174-179
XXIV.	URINARY DEPOSITS — CALCULI — EXAMINATION OF THE URINE,	179-193

PART II.—EXPERIMENTAL PHYSIOLOGY.

LESSON		PAGES
XXV.	GALVANIC BATTERIES AND GALVANOSCOPE,	194-199
XXVI.	KEYS—COMMUTATOR—RHEOCHORD,	199-208
XXVII.	INDUCTION COIL—ELECTRODES,	208-212
XXVIII.	SINGLE INDUCTION SHOCKS—INTERRUPTED CURRENT—BREAK EXTRA-CURRENT—HELMHOLTZ'S MODIFICATION,	212-218
XXIX.	NERVE-MUSCLE PREPARATION—NORMAL SALINE,	219-222
XXX.	NERVE-MUSCLE PREPARATION—MECHANICAL, CHEMICAL, AND THERMAL STIMULI,	222-226
XXXI.	SINGLE AND INTERRUPTED INDUCTION SHOCKS—TETANUS—CONSTANT CURRENT,	226-233
XXXII.	RHEONOME — DIRECT AND INDIRECT STIMULATION — MUSCLE SOUND—DYNAMOMETERS,	233-236
XXXIII.	INDEPENDENT DIRECT MUSCULAR EXCITABILITY—CURARE,	236-243
XXXIV.	GRAPHIC METHOD—MOIST CHAMBER—SIMPLE TWITCH,	243-251
XXXV.	CHRONOGRAPHS—CRANK MYOGRAPH—AUTOMATIC BREAK—MUSCLE CURVE—HYOGLOSSUS,	252-266
XXXVI.	ISOTONIC AND ISOMETRIC CONTRACTIONS—WORK DONE—HEAT-RIGOR,	266-271
XXXVII.	PENDULUM-MYOGRAPH—SPRING-MYOGRAPH,	271-278
XXXVIII.	INFLUENCE OF TEMPERATURE, LOAD, AND VERATRIN ON MUSCULAR CONTRACTION,	278-284
XXXIX.	ELASTICITY AND EXTENSIBILITY OF MUSCLE — BLIX'S MYOGRAPH,	284-289
XL.	TWO SUCCESSIVE SHOCKS—TETANUS—COLD—METRONOME,	289-296
XLI.	FATIGUE AND RECOVERY OF EXCISED MUSCLE,	296-298
XLII.	INDEFATIGABILITY OF NERVE—SEAT OF EXHAUSTION,	298-300
XLIII.	MYOGRAPHIC EXPERIMENTS ON MAN — ERGOGRAPH — DYNAMOGRAPH,	300-305
XLIV.	THICKENING OF A MUSCLE—MUSCLE WAVE,	306-311
XLV.	GALVANOMETER—NON-POLARISABLE ELECTRODES—SHUNT—CURRENTS IN MUSCLE, NERVE, HEART,	311-319
XLVI.	CAPILLARY ELECTROMETER—MUSCLE AND HEART CURRENT—MEASUREMENT OF E.M.F.,	319-322
XLVII.	GALVANI'S EXPERIMENTS—SECONDARY CONTRACTION AND TETANUS—PARADOXICAL CONTRACTION—KÜHNE'S EXPERIMENTS,	322-328
XLVIII.	LAW OF CONTRACTION—POLAR STIMULATION OF MUSCLE,	328-335
XLIX.	ELECTROTONUS—ELECTROTONIC VARIATION OF EXCITABILITY,	335-341

LESSON		PAGES
L.	VELOCITY OF NERVE IMPULSE—CONDUCTIVITY AND EXCITABILITY OF NERVE,	341-346
LI.	CONDITIONS AFFECTING EXCITABILITY OF NERVE,	346-351
LII.	SMOOTH MUSCLE—CILIARY MOTION—CHEMOTAXIS—GALVANOTAXIS,	351-359
LIII.	FROG'S HEART—HEAT AND COLD—SECTION OF HEART—GRAPHIC RECORD OF BEAT,	360-368
LIV.	SUSPENSION METHODS FOR HEART—GASKELL'S HEART-LEVER AND CLAMP,	369-376
LV.	STANNIUS' EXPERIMENT—ALL CONTRACTIONS MAXIMAL,	376-380
LVI.	LATENT PERIOD OF HEART — REFRACTORY PERIOD—HEAT, COLD, AND CONSTANT CURRENT,	380-385
LVII.	INNERVATION OF FROG'S HEART—INTRACARDIAC NERVES—VAGUS—AUGMENTOR NERVES,	385-393
LVIII.	ENDO-CARDIAL PRESSURE—FROG'S MANOMETER—TONOMETER,	394-401
LIX.	ACTION OF SALTS, ALKALOIDS, DRUGS, AND EXTRACTS ON FROG'S HEART,	401-408
LX.	MAMMALIAN HEART AND ITS NUTRITION — PERFUSION THROUGH BLOOD-VESSELS,	408-414
LXI.	CARDIAC IMPULSE—SOUNDS—CARDIOGRAPHS—ILLUMINATED HEART,	414-422
LXII.	PULSE—SPHYGMOGRAPHS—SPHYGMOSCOPE,	422-430
LXIII.	INSTRUMENTS FOR MEASURING AND RECORDING BLOOD-VESSELS IN MAN,	430-436
LXIV.	PRINCIPLES OF CIRCULATION—SCHEMATA—PULSE DELAY—STROMUHR,	437-443
LXV.	CAPILLARY PRESSURE — LYMPH HEARTS AND SACS — PLETHYSMOGRAPHS,	443-449
LXVI.	BLOOD-PRESSURE IN ANIMALS—MERCURY KYMOGRAPH—MEMBRANE MANOMETERS—VAGUS—DEPRESSOR,	449-465
LXVII.	MOVEMENTS OF CHEST WALL—ELASTICITY OF LUNGS—HYDROSTATIC TEST—STETHOGRAPH—APNŒA,	466-472
LXVIII.	VITAL CAPACITY—EXPIRED AIR—GASES OF BLOOD AND AIR,	473-481
LXIX.	LARYNGOSCOPE—VOWELS,	481-484
LXX.	CRYOSCOPY—SALIVARY SECRETION—INJECTION OF PEPTONES—KIDNEY SECRETION—BILE FISTULA—ABSORPTION—EXCRETION,	484-491
LXXI.	REFLEX ACTION—ACTION OF POISONS—STRYCHNIN—KNEE-JERK,	492-497
LXXII.	SPINAL NERVE ROOTS—OVERLAP OF SENSORY NERVES—BRONDGEEST'S TONUS,	498-499

LESSON		PAGES
LXXIII.	REACTION TIME—CEREBRAL HEMISPHERES—HYPNOSIS, .	500-508
LXXIV.	FORMATION OF AN IMAGE—DIFFUSION—ABERRATION—ACCOMMODATION — SCHEINER'S EXPERIMENT — NEAR AND FAR POINTS—PURKINJE'S IMAGES—PHAKOSCOPE —ASTIGMATISM—PUPIL, ...	509-519
LXXV.	BLIND SPOT—FOVEA CENTRALIS—DIRECT VISION—CLERK-MAXWELL'S EXPERIMENT — PHOSPHENES — RETINAL SHADOWS—ADAPTATION—AFTER-IMAGES, . . .	520-530
LXXVI.	PERIMETRY—IRRADIATION—GEOMETRICAL ILLUSIONS—IMPERFECT VISUAL JUDGMENTS, ...	530-540
LXXVII.	COMPLEMENTARY COLOURS—MIXING COLOUR-SENSATIONS —COLOUR BLINDNESS—CONTRAST—FLICKER, . .	541-556
LXXVIII.	STEREOSCOPE—HAPLOSCOPE—OPHTHALMOSCOPE—INTRA-OCULAR PRESSURE—OPHTHALMOTONOMETER—KÜHNE'S ARTIFICIAL EYE, ...	557-565
LXXIX.	CUTANEOUS SENSATIONS—COLD, HOT, PRESSURE, AND PAIN SPOTS—MUSCULAR SENSE—RESISTANCE, . . .	565-580
LXXX.	TASTE—SMELL—HEARING—COMPENSATORY MOVEMENTS —STEREOGNOSTIC SENSE, ...	580-587
	APPENDIX, ...	588
	INDEX, ...	609

LIST OF ILLUSTRATIONS.

FIG.		PAGE
1.	Apparatus for coagulation temperature (*Gamgee*),	12
2.	Dextrose (*Hill*),	19
3.	Phenyl-glucosazone (*Stirling*),	21
4.	Cane sugar (*Hill*),	22
5.	Maltose (*Hill*),	23
6.	Phenyl-maltosazone (*Stirling*),	23
7.	Lactose (*Hill*),	25
8.	Phenyl-lactosazone (*Stirling*),	25
9.	Potato starch,	25
10.	Potato starch viewed with crossed Nicols (*Stirling*),	26
11.	Laurent's polarimeter (*Laurent*),	30
12.	Gad's experiment (*Stirling, after Gad*),	33
13.	Desiccator (*Gscheidlen*),	41
14.	Bernard's apparatus for estimating sugar (*Stirling*),	46
15.	Incineration of a deposit (*Gscheidlen*),	46
16.	Rat's hæmoglobin crystals (*Stirling*),	48
17.	Spectroscope (*Browning*),	49
18.	*a.* Platinum wire support. *b.* Spectroscope stand,	49
19.	Spectra of hæmoglobin (*Landois and Stirling*),	50
20.	Absortion by oxy-hæmoglobin (*Rollett*),	51
21.	Absorption by reduced hæmoglobin (*Rollett*),	51
22.	Spectrum of methæmoglobin (*v. Jaksch*),	54
23.	Spectra of derivatives of hæmoglobin (*Landois and Stirling*),	55
24.	Hæmin crystals (*v. Jaksch*),	57
25.	Hermann's hæmatoscope (*Rollett*),	57
26.	Hæmochromogen apparatus (*Fredericq*),	58
27.	Gower's hæmocytometer,	60
28.	Thoma-Zeiss counting chamber,	62
29.	Oliver's hæmocytometer,	63
30.	Hæmoglobinometer of Gowers,	64

FIG. PAGE
31. Oliver's hæmoglobinometer (*Tintometer Co., Ltd.*), . . . 67
32. Fleischl's hæmometer, ... 69
33. Spectroscope for wave-lengths (*Landois and Stirling*), . . 70
34. Wave-lengths of hæmoglobin and its compounds (*Preyer and Gamgee*), 71
35. Spectra of derivatives of hæmoglobin (*Preyer and Gamgee*), . 72
36. Saliva and buccal secretion (*v. Jaksch*), ... 73
37. Digestion bath (*Stirling*), ... 80
38. Kühne's dialyser (*Stirling*), ... 86
39. Crystals of tyrosin (*Stirling*), ... 97
40. Crystals of cholesterin (*Stirling*), ... 101
41. Double-walled funnel (*Gscheidlen*), ... 103
42. Hot air-oven (*Gscheidlen*), ... 106
43. Milk and colostrum (*Stirling*), ... 109
44. Porous cell for filtering milk (*Stirling*), ... 114
45. Lactoscope, ... 115
46. Kreatin (*Brunton*), ... 118
47. Urinometer (*Landois and Stirling*), ... 126
48. Deposit in acid urine (*Landois and Stirling*), ... 129
49. Deposit in alkaline urine (*Landois and Stirling*), . . . 130
50. Stellar phosphate (*v. Jaksch*), ... 135
51. Triple phosphate, ... 135
52. Triple phosphate, feathery forms (*v. Jaksch*), ... 136
53. Allen's burette and stand. Made by *Baird & Tatlock*, . . 136
54. Burette meniscus, and Schellbach's burette (*Lautenschläger*), . 138
55. Erdmann's float, ... 138
56. Oxalate of lime, ... 139
57. Urea and urea nitrate (*Landois and Stirling*), ... 141
58. Urea oxalate, ... 142
59. Kjeldahl's N-apparatus. Made by *Baird & Tatlock* and *Reynolds & Branson*, ... 145
60. Dupré's urea apparatus, ... 148
61. Steell's apparatus for urea, ... 149
62. Ureameter of Doremus (*Southall*), ... 149
63. Hüfner's urea apparatus (*v. Jaksch*), ... 151
64. Gerard's urea apparatus (*Gibbs, Cuxson & Co.*), ... 152
65. Uric acid, ... 154
66. Uric acid, ... 154
67. Hippuric acid (*Landois and Stirling*), ... 159
68. Kreatinin zinc-chloride (*Landois and Stirling*), ... 160
69. Esbach's tube (*v. Jaksch*), ... 168
70. Phenylglucosazone from urine (*v. Jaksch*), ... 172
71. Bulb test tube (*Neumann*), ... 172

FIG.		PAGE
72.	Johnson's picro-saccharimeter,	175
73.	Einhorn's fermentation saccharimeter (*Stirling*),	176
74.	Sacchar-ureameter (*Gibbs, Cuxson & Co.*),	177
75.	Apparatus for distilling acetone (*Sahli*),	178
76.	Hand centrifuge (*Lautenschläger*),	180
77.	High-speed centrifuge,	180
78.	Hæmatocrit attachment and milk bottle for centrifuge,	181
79.	Centrifuge for water pressure (*Lautenschläger*),	182
80.	Oxalate of lime,	184
81.	Acid urate of ammonium (*v. Jaksch*),	184
82.	Cystin,	184
83.	Leucin and tyrosin,	184
84.	Daniell's cell (*Stirling*),	195
85.	Leclanché cell,	195
86.	Grove's cell,	196
87.	Bichromate cell,	196
88.	Detector (*Elliott*),	197
89.	Hand electrodes (*Stirling*),	198
90.	Du Bois-Reymond's key—old form,	200
91.	Newer form of Du Bois-Reymond's key (*Cambridge Scientific Inst. Co.*),	200
92.	Scheme of fig. 90 (*Stirling*),	200
93.	Scheme of fig. 90 (*Stirling*),	200
94.	Mercury key (*Cambridge Scientific Inst. Co.*),	202
95.	Morse key (*Stewart & Gee*),	202
96.	Spring key (*Elliott*),	202
97.	Contact key (*Zimmermann*),	202
98.	Ostwald's key (*Petzold*),	203
99.	Pohl's commutator,	204
100.	Pohl's commutator, with crossbars and leads,	204
101.	Simple rheochord, with detector (*Stirling*),	205
102.	Simple rheochord (*Stirling*),	206
103.	Rheochord, Oxford pattern (*Stirling*),	207
104.	Du Bois-Reymond induction coil (*Elliott*),	209
105.	Vertical inductorium,	210
106.	Ewald's sledge coil (*Hurst*),	211
107.	Du Bois electrodes,	212
108.	Induction coil for single shocks (*Stirling*),	213
109.	Du Bois coil,	214
110.	Break extra-current (*Stirling*),	215
111.	Helmholtz's modification,	216
112.	Equalised make and break shocks (*Stirling*),	217

FIG. PAGE
113. Brodie's rotating key, ... 217
114. Frog's leg-muscles (*Ecker*), ... 220
115. Frog's sciatic nerve (*Ecker*), ... 220
116. Nerve-muscle preparation, ... 223
117. Straw flag (*Stirling*), ... 224
118. Scheme for single induction shocks (*Stirling*), ... 227
119. Scheme of constant current (*Stirling*), ... 229
120. Frog's sartorius and thigh muscles (*Ecker*), ... 230
121. Rheonome (*Stirling*), ... 233
122. Dynamometers—Colin's and Chicago form, ... 236
123. Curare experiment, ... 238
124. Scheme of curare experiment, ... 242
125. Revolving cylinder of Ludwig. Made by *Petzold*, . . . 244
126. Scheme of moist chamber (*Stirling*), ... 247
127. Moist chamber (*Cambridge Sc. Inst. Co.*), ... 247
128. Universal stand (*Zimmermann*), ... 248
129. Record of make and break contractions (*Stirling*), . . . 250
130. Muscle-curves—make and break (*Stirling*), ... 251
131. Lever and abscissa recorder (*Zimmermann*), ... 251
132. Desprez signal or chronograph (*Cambridge Sc. Inst. Co.*), . . 252
133. Electric signal (*Stirling*), ... 253
134. Chronograms (*Stirling*), ... 253
135. Crank-myograph on stand, ... 254
136. Crank-myograph (*Stirling*), ... 254
137. Arrangement for automatic break (*Stirling*), ... 256
138. Simple muscle-curve (*Stirling*), ... 257
139. Muscle-curve of hyoglossus, ... 258
140. Dissection of hyoglossus (*Ecker*), ... 259
141. Muscle-curve of gastrocnemius (*Stirling*), ... 260
142. Muscle-curve of hyoglossus (*Stirling*), ... 260
143. Correction for lever (*Schenck*), ... 261
144. Tigerstedt's lever and Ludwig's form, ... 261
145. Chronographs (*Castagna*), ... 262
146. Time marker (*Petzold*), ... 263
147. Pfeil's signal, ... 263
148. Recording tuning-fork (*Zimmermann*), ... 264
149. Drum and tambour (*Castagna*), Transmission chronograph, . 265
150. Signal in circuit to record (*Verdin*), ... 265
151. Drum of Pye & Co., Cambridge, ... 266
152. Scheme of Fick's tension recorder (*Schenck*), ... 267
153. Isotonic and isometric curves (*Stirling*), ... 268
154. Isotonic and isometric curves (*Gad*), ... 268

FIG. PAGE
155. Myotonometer of B. Sanderson (*Cambridge Sc. Inst. Co.*), . 269
156. Curve obtained with fig. 155 (*Stirling*), ... 269
157. Ludwig's apparatus for heat-rigor (*Petzold*), ... 271
158. Pendulum-myograph, ... 272
159. Pendulum-myograph curve (*Stirling*), ... 273
160. Scheme of pendulum-myograph curve (*Stirling*), . . . 273
161. Spring-myograph of du Bois-Reymond, ... 274
162. Revolving drum for time relations of muscle-curve, . . . 275
163. Marey's simple myograph. Made by *Verdin*, ... 276
164. Marey's transmission myograph. Made by *Verdin*, . . 276
165. Spring-myograph (*Fredericq*), ... 279
166. Effect of temperature on muscle-curve (*Stirling*), . . . 279
167. Effect of heat and cold on muscle (*Stirling*), ... 279
168. Effect of heat and cold on hyoglossus (*Stirling*), . . . 280
169. Apparatus for heating nerve and muscle (*Bernard*), . . . 280
170. Muscle-curve with load-pendulum (*Stirling*), ... 281
171. Muscle-curve with load-drum (*Stirling*), ... 281
172. Veratrin curve (*Stirling*), ... 283
173. Veratrin and a normal curve (*Stirling*), ... 283
174. Weights to load muscle (*Baird & Tatlock*), ... 285
175. Curves of elasticity of frog's muscle and india-rubber, . . 285
176. Blix's myograph (*Fick*), ... 287
177. Apparatus for elasticity of Marey (*Verdin*) and Nicolaides (*Petzold*), ... 287
178. Curve of two superposed contractions (*Stirling*), . . . 289
179. Drum (Sherrington) with "striker," 290
180. Scheme for tetanus (*Stirling*), ... 291
181. Genesis of tetanus curves (*Stirling*), ... 292
182. Tetanus interrupter (*Stirling*), ... 293
183. Tracing of simple and tetanic contraction (*Stirling*), . . 293
184. Tetanus curve of hyoglossus (*Stirling*), ... 294
185. Metronome (*Petzold*), ... 295
186. Magnetic interrupter (*Cambridge Scientific Instrument Co.*), . 295
187. Fatigue curve—form of curve (*Stirling*), ... 297
188. Fatigue curve, slow drum—height of lift (*Stirling*), . . . 297
189. Mosso's ergograph, ... 301
190. Ergographic curves, ... 303
191. Fick's tension myograph (*Schenck*), ... 305
192. Apparatus to record thickening of muscle (*Langendorff*), . . 306
193. Apparatus for recording thickening of muscle, ... 307
194. Marey's registering tambour and pince myographique (*Marey*), 307
195. Muscle wave apparatus, ... 309

FIG. PAGE
196. Wild's apparatus (*Stirling*). 310
197. Thomson's galvanometer (*Elliott*), ... 312
198. Lamp and scale for fig. 197, ... 312
199. Non-polarisable electrodes, ... 313
200. Shunt (*Elliott*), ... 314
201. Scheme for galvanometer (*Stirling*), ... 314
202. Non-polarisable nerve electrodes, ... 317
203. Brush electrodes (*v. Fleischl*), ... 317
204. D'Arsonval's electrodes (*Verdin*), ... 318
205. Capillary electrometer (*v. Frey*), ... 319
206. Scheme for measuring E.M.F. of muscle, ... 321
207. Galvani's experiment with metals (*Stirling*), ... 322
208. Galvani's experiment without metals (*W. Rutherford*), . . 323
209. Scheme for demarcation current of muscle (*Schenck*), . . 324
210. Secondary contraction, ... 324
211. Scheme of secondary contraction (*Stirling*), ... 325
212. Scheme of paradoxical contraction (*Stirling*), ... 325
213. Kühne's experiment on nerve current (*Stirling*), . . . 327
214. Scheme of Pflüger's law of contraction (*Stirling*), . . . 329
215. Hering's double myograph. Made by *Rothe*, ... 330
216. Apparatus for polar stimulation of muscle (*Stirling*), . . 331
217. Polar stimulation of sartorius (*W. Rutherford*), . . . 332
218. Results obtained with fig. 217 (*W. Rutherford*), . . . 333
219. Polarisation of electrodes, ... 334
220. Scheme of electrotonic excitability (*Stirling*), ... 335
221. Scheme of electrotonus (*Stirling*), ... 336
222. Curve of electrotonus (*Stirling*), ... 337
223. Scheme of electrotonus (*Landois and Stirling*), ... 338
224. Scheme for kathodic stimulation, ... 339
225. Du Bois-Reymond's rheochord, ... 340
226. Scheme of velocity of nerve-impulse (*Stirling*), ... 342
227. Scheme for Grünhagen's experiment on conductivity, . . 344
228. Nerve fibres in frog's sartorius (*Mays*), ... 345
229. Kühne's gracilis experiment (*Stirling*), ... 346
230. Scheme for unequal excitability of a nerve (*Stirling*), . . 347
231. Effect of temperature on galvanic stimulation (*Gotch and Macdonald*), ... 349
232. Effect of ether on nerve (*Gotch*), ... 350
233. Gas chamber for ciliary motion, ... 354
234. Ranvier's apparatus for ciliary motion, ... 356
235. Slide for study of paramæcium (*Jennings*), ... 357
236. Paramæcia round drop of acid (*Jennings*), ... 357

FIG. PAGE
237. Paramæcia round drop of alkali (*Jennings*), ... 357
238. Galvanotaxis of paramæcium (*Verworn*), ... 358
239. Electrodes and chamber for galvanotaxis (*Verworn*), . . 358
240. Frog's heart from the front (*Ecker*), ... 361
241. Frog's heart from behind (*Ecker*), ... 361
242. Simple lever for frog's heart, ... 365
243. Lever for frog's heart. Made by *Verdin*, ... 366
244. Arrangement for excised frog's heart (*Gotch*), ... 367
245. François-Frank's lever for heart of tortoise (*Verdin*), . . 367
246. Cowl's frog holder. Made by *Lautenschläger*, ... 368
247. Gaskell's lever (*Stirling*), ... 369
248. Tracing of frog's heart taken with fig. 247 (*Stirling*), . . 370
249. Frog's heart lever, ... 370
250. Heart-tracing, varying speed of drum (*Stirling*), . . . 371
251. Heart-tracing, effect of heat and cold (*Stirling*), . . . 371
252. Frog cardiograph, ... 372
253. Tracing showing effect of heat and cold on heart (*Stirling*), . 372
254. Tracing of frog's heart (*Stirling*), ... 373
255. Tracing of frog's heart. Effect of snip of heart, . . . 373
256. Gaskell's heart clamp (*Cambridge Scientific Inst. Co.*), . . 374
257. Modified Gaskell clamp (*Stirling*), ... 375
258. Tracing of auricles and ventricle (*Stirling*), ... 375
259. Effect of first Stannius ligature (*Stirling*), ... 378
260. Effect of second Stannius ligature (*Stirling*), ... 378
261. Staircase of heart (*Stirling*), ... 379
262. Latent period of heart muscle (*Stirling*), ... 381
263. Single beat of frog's heart on quick drum (*Stirling*), . . 381
264. Single contraction of frog's ventricle (*Stirling*), . . . 382
265. Refractory period of heart (*Dastre*), ... 383
266. Compensatory pause of heart-beat (*Fans*), ... 384
267. Support for excised frog's heart (*Stirling*), ... 384
268. Staircase character of heart-beat (*Stirling*), ... 384
269. Heart and cardiac nerves exposed (*Lyon*), ... 386
270. Tracing showing arrest of heart-beat (*Stirling*), . . . 387
271. Arrest of frog's heart by "crescent" stimulation (*Stirling*), . 387
272. Metronome to record seconds (*Verdin*), ... 388
273. Scheme of frog's vagus (*Stirling*), ... 389
274. Vagus curve of frog's heart (*Stirling*), ... 391
275. Scheme of frog's sympathetic (*Gaskell*), ... 392
276. Scheme of Kronecker's frog manometer (*Stirling*), . . . 394
277. Complete frog-heart manometer of Kronecker (*Wertheimer*), . 395
278. Heart-tracing during perfusion (*Stirling*), ... 395

FIG. PAGE
279. Kronecker's frog-heart cannula, ... 396
280. Heart-plethysmograph (*Schäfer*), ... 397
281. Scheme of Roy's tonometer (*Stirling*), ... 398
282. Tonometer (*Cambridge Scientific Instrument Co.*), . . . 398
283. Simple frog-heart manometer (*Fredericq*), ... 399
284. William's heart apparatus (*Langendorff*), ... 400
285. Valve for fig. 284 (*Langendorff*), ... 401
286. Effect of muscarin and atropin on frog's heart (*Stirling*), . . 403
287. Effect of nicotin on frog's heart (*Stirling*), ... 405
288. Supra-renal extract on frog's heart containing blood (*Stirling*), 406
289. Supra-renal extract on heart (*Stirling*), ... 407
290. Supra-renal extract on bloodless heart (*Stirling*), . . . 407
291. Cannula tied in mammalian heart (*Pratt*), ... 411
292. Perfusion apparatus for heart of mammal (*Pratt*), . . . 411
293. Glass cannula, ... 414
294. Marey's cardiograph, ... 415
295. Receiving and recording tambour (*Petzold*), ... 416
296. Human cardiogram (*Langendorff*), ... 417
297. Effect of swallowing on heart-beat (*Wertheimer*), . . . 417
298. Polygraph of Rothe, ... 418
299. Cardiac impulse tracing (*Knoll*), ... 419
300. Rothe's receiving tambour, ... 419
301. Radial pulse and respirations (*Knoll*), ... 420
302. Radial pulse and cardiac impulse (*Knoll*), ... 420
303. Illuminated ox-heart (*Fredericq* after *Gad*), ... 421
304. Phonendoscope, ... 422
305. Marey's sphygmograph (*Bramwell*), ... 424
306. Sphygmograms (*Marey*), ... 424
307. Dudgeon's sphygmograph, ... 425
308. Sphygmogram (*Dudgeon*), ... 425
309. Tracing with Richardson's modification of fig. 307, . . . 425
310. Ludwig's sphygmograph, ... 426
311. Arm support for fig. 310, ... 426
312. Pulse tracing taken with Jacquet's sphygmograph, . . . 427
313. Marey's transmission sphygmograph (*Verdin*), ... 428
314. Hürthle's receiving tambour, ... 428
315. Waller's digital sphygmograph, ... 429
316. Gas sphygmoscope, ... 429
317. Hæmo-dynamometer of Oliver, ... 431
318. Mode of applying fig. 317, ... 433
319. Sphygmometer (*Hill & Barnard*), ... 433
320. Pressure-gauge (*Hill & Barnard*), ... 433

FIG. PAGE
321. Sphygmomanometer of von Basch (*Castagna*), ... 434
322. Sphygmomanometer of Mosso, ... 434
323. Scheme of fig. 322 (*Morat*), ... 435
324. Clinical polygraph of Dr. J. Mackenzie of Burnley, . . . 436
325. Marey's scheme of rigid and elastic tubes, ... 440
326. Rheometer or Stromuhr of Ludwig, ... 441
327. Scheme of fig. 326, ... 441
328. Scheme of circulation (*Fredericq*), ... 442
329. Apparatus for estimating capillary pressure (*v. Kries*), . . 444
330. Holmgren's apparatus for circulation in lung (*Ranvier*), . . 444
331. Apparatus for circulation in mesentery (*Ranvier*), . . . 445
332. Posterior lymph-hearts of frog (*Ecker*), ... 446
333. Mosso's plethysmograph. Made by *Verdin*, ... 447
334. Ludwig's Hg-kymograph. Made by *Zimmermann*, . . . 450
335. Hürthle's kymograph. Made by *Albrecht*, ... 451
336. Blood-pressure tracing with Hering's kymograph (*Langendorff*), 451
337. Ludwig's Hg-manometer. Made by *Zimmermann*, . . . 452
338. Dissection of nerves in neck of rabbit (*Cyon*), ... 452
339. Kymograph, simple form. Made by *Verdin*, ... 453
340. Modified rabbit-holder of Czermak (*Cambridge Sc. Inst. Co.*), . 454
341. Cowl's animal holder. Made by *Lautenschläger*, . . . 454
342. Malassez's rabbit-holder. Made by *Lautenschläger*, . . 454
343. Shielded electrodes. Made by *Verdin*, ... 455
344. Arterial cannula of François-Franck, ... 455
345. Normal B.P. tracing of rabbit (*Langendorff*), ... 456
346. B.P. and plethysmograph tracing (*Bayliss*), ... 457
347. Dog-holder, mouth part. Made by *Lautenschläger*, . . . 458
348. Dissection nerves of neck of dog (*Schmiedeberg*), . . . 459
349. B.P. tracing of dog, ... 460
350. B.P. tracing of dog—stimulation of vagus (*Stirling*), . . 461
351. B.P. tracing of dog—vagus stimulation (*Laulanie*), . . . 461
352. B.P. tracing of dog—section of vagi (*Laulanie*), . . . 462
353. B.P. tracing of dog—stimulation of vagus (*Laulanie*), . . 462
354. B.P. tracing of dog—stimulation of central end of vagus, (*Laulanie*), ... 463
355. Fick's spring kymograph, ... 463
356. Hürthle's spring manometer. Made by *Albrecht*, . . . 464
357. Hürthle's membrane manometer. Made by *Albrecht*, . . 464
358. Tracing taken with fig. 357, ... 465
359. Marey's respiration double tambour, ... 466
360. Stethographic tracing (*Stirling*), ... 467
361. Marey's stethograph (*Verdin*), ... 467

FIG. PAGE
362. Stethographic tracing (*Stirling*), ... 467
363. Apparatus for recording respiratory pressures (*Langendorff*), . 469
364. Relation of B.P. to respiratory movements (*Laulanie*), . . 470
365. Hering's apparatus for effects of diaphragmatic action (*Rothe*), 471
366. Bellows for artificial respiration (*Verdin*), ... 472
367. Tracheal respiration cannula, ... 472
368. Müller's valves (*Stirling*), ... 473
369. Faraday's experiment (*Stirling*), ... 474
370. L. Hill's gas pump. Made by *Baird & Tatlock*, . . . 475
371. Muscle chamber for fig. 370, ... 476
372. Gases collected over mercury (*Gscheidlen*), ... 476
373. Haldane's apparatus for estimation of O, ... 477
374. Hempel's apparatus for expired air, ... 478
375. Hempel's absorption pipette, ... 478
376. Zuntz's apparatus for CO_2, modified by Waller (*Baird & Tatlock*), 479
377. Fredericq's apparatus for CO_2 (*Verdin*), ... 480
378. View of larynx during inspiration, ... 483
379. Larynx during vocalisation, ... 483
380. König's flame apparatus, ... 483
381. Beckmann's apparatus for freezing point (*Reynolds & Branson*), 486
382. Dissection of submaxillary region of dog (*Bernard*), . . . 487
383. Hammer for knee-jerk (*Verdin*), ... 497
384. Reaction-time, pendulum method (*Rutherford*), . . . 501
385. Result obtained with fig. 384 (*Rutherford*), ... 501
386. Reaction-time for touch, sight, hearing (*Rutherford*), . . 502
387. Reaction-time (*Stirling*), ... 503
388. Neuramœbimeter of Exner, ... 504
389. Experimentum mirabile of Kircher, ... 505
390. Frog's brain (*Landois and Stirling*), ... 505
391. Frog in attitude of general reflex tone (*Verworn*), . . . 507
392. Frog like fig. 391, but placed on its back (*Verworn*), . . 507
393. Ludwig's apparatus for involuntary hand movements (*Petzold*), 507
394. Method of recording muscular contraction (*Verdin*), . . 508
395. Diagram for v. Bezold's experiment, ... 511
396. Scheiner's experiment, ... 513
397. Diffusion (*Helmholtz*), ... 514
398. Phakoscope (*Helmholtz*), ... 516
399. Model of plates of ophthalmometer (*Auber*), ... 516
400. Apparatus for vision of a point (*Ludwig*), ... 519
401. Mariotte's experiment, ... 520
402. Mariotte's experiment (another way), ... 520
403. Blind spot (*Helmholtz*), ... 521

FIG. PAGE
404. Stuart's model for Mariotte's experiment (*Camb. Sc. Inst. Co.*), 521
405. Volkmann's experiment on the blind spot, ... 521
406. Bergmann's experiment (*Helmholtz*), ... 523
407. Anderson Stuart's model for identical points (*Camb. Sc. nst Co.*), 524
408. Disc for Talbot's law (*Helmholtz*), ... 526
409. Charpentier's disc for "black band," 527
410. Charpentier's disc for coloured field, ... 527
411. Priestley Smith's perimeter and Asher's perimeter (*Jung*), . 531
412. Scheme for wheel movements of eye (*Hering*), ... 532
413. Positive irradiation (*Helmholtz*), ... 533
414. Irradiation (*Helmholtz*), ... 534
415. Irradiation (*Helmholtz*), ... 534
416. Folded card illusion (*Mach*), ... 535
417. Illusion of fluctuating blocks (*Scripture*), ... 535
418. Arrowhead and feather illusion (*Müller-Lyer*), ... 536
419. Zöllner lines, ... 536
420. Helmholtz's chess-board (*Helmholtz*), ... 537
421. Hering's experiment with falling bodies (*Jung*), . . . 539
422. Spiral disc for radial movement (*Helmholtz*), ... 540
423. Bidwell's illusion of motion (*Bidwell*), ... 540
424. Rothe's rotation or colour-mixing apparatus, ... 543
425. Perimetric colour chart (*Meyer*), ... 544
426. Apparatus to mix coloured light (*Hering*), ... 545
427. Scheme of fig. 426 (*Hering*), ... 545
428. Disc for contrast (*Helmholtz*), ... 548
429. Disc for simultaneous contrast (*Helmholtz*), ... 549
430. Ragona Scina's experiment (*Rood*), ... 550
431. Hering's apparatus for fig. 430 (*Hering*), ... 550
432. Apparatus for simultaneous contrast (*Hering*), ... 551
433. Simultaneous contrast apparatus (*Hering*), ... 551
434. Disc for successive contrast (*Sherrington*), ... 553
435. Disc for successive contrast (*Sherrington*), ... 553
436. Modified Benham's spectrum top (*Hurst*), ... 555
437. Tropostereoscope of Ludwig (*Petzold*), ... 559
438. Bird and cage experiment, ... 559
439. Ophthalmoscope of Walker Hall (*Armstrong & Brother*), . . 560
440. Michel's carriage for rabbit (*Stirling*), ... 561
441. Priestley Smith's demonstrating ophthalmoscope, . . . 562
442. Kühne's artificial eye. Made by *Jung*, ... 564
443. Æsthesiometer of Jastrow (*Chicago Supply and Scale Co.*), . 566
444. Æsthesiometer with guarded points (*Chicago Supply and Scale Co.*), ... 567

FIG. PAGE
445. Aristotle's experiment, ... 568
446. Von Frey's hair æsthesiometers, ... 574
447. Algometer (*Chicago Supply and Scale Co.*), ... 577
448. Aly's algesometer, ... 577
449. Simple olfactometer. Made by *Zimmermann*, ... 582
450. Double olfactometer. Made by *Petzold*, ... 582
451. Exner's model of middle ear. Made by *Castagna*, . . . 583
452. Galton's whistle, ... 584
453. Exner's model of semicircular canals (*Castagna*), . . . 585
454. Exner's model of otoliths, ... 585
455. Comparison spectroscope (*Schmidt & Haensch*), . . . 599
456. Scheme of straight vision spectroscope (*Schmidt & Haensch*), . 599
457. Frog pistol of du Bois-Reymond (*Verdin*), ... 599
458. Muscle telegraph of du Bois-Reymond (*Verdin*), . . . 600
459. Recording drum, American pattern (*Chicago Supply Co.*), . 601
460. Modified crank myograph, used in Owens College, . . . 601
461. Jaquet's graphic chronograph (*Jaquet*), ... 602
462. Scheme of Birch's recording arrangements (*de Burgh Birch*), . 603
463. Heart chamber of Sherrington for application of gases or vapour, ... 604
464. Railway myograph, Waller's pattern. Made by *Hoyer*, . . 604
465. Moist chamber, Waller's pattern. Made by *Hoyer*, . . 605

ERRATA.

Page 270, line 8, for "Arbeitssammler" *read* "Arbeitsammler."

,, ,, ,, 12, after "twitch" *add* "(A. Fick, 1869)."

PRACTICAL PHYSIOLOGY.

PART I.—CHEMICAL PHYSIOLOGY.

LESSON I.

PROTEIDS.

Students are required to bring the following:—One dozen ¾-inch test tubes, with stand and brush; one 4-oz. and one 6-oz. flask; two beakers, No. 2 size; two porcelain capsules, No. 3 size; two funnels; glass rods; pipette graduated from 50 to 5 c.c.; platinum wire in holder; Bunsen burner; wire gauze; tripod; india-rubber tubing; and duster.

As a type of this group take white of egg, egg-white, or egg-albumin. In nature proteids occur only as constituents or products of living organisms, and their presence is indispensable for the occurrence of the phenomena of life. In animals they form the principal organic solids of the muscular, nervous, and glandular tissues, and also of blood-plasma and lymph. Normally they are absent—or practically so—from bile, urine, tears, and sweat. They possess certain chemical reactions in common, and are closely related to one another. Their elementary composition varies within the following limits:—

	C.	H.	N.	O.	S.	
From	50	6·8	15·0	22·8	0·4	per cent.
To	55	7·3	18·2	24·1	5·0	,,

For the most part they are amorphous and colloid, but many representatives have now been crystallised. The question of *solubility* is an important one. All are insoluble in alcohol or ether; some are soluble in water, others insoluble. Some (globulins) are soluble only in dilute saline solutions. Many are

precipitated by more or less complete saturation with neutral salts. They all rotate the ray of polarised light to the left, and are thus lævorotatory. In strong acids and alkalies they are dissolved, but they undergo decomposition in the process. When decomposed they yield a large number of products, so that their constitution is exceedingly complex. In the body, after undergoing a series of metabolic changes, they are excreted chiefly in the form of urea, and a number of more or less closely related nitrogenous bodies. Besides the general characters stated below, most yield aromatic bodies—*e.g.*, tyrosin and phenol.

1. Solution of egg-albumin—*Soluble in water.*—Place the unboiled white of an egg in a porcelain capsule (taking care that none of the yolk escapes), and cut it freely with scissors to liberate the albumin from the membranes. Add 10 volumes of distilled water, and mix thoroughly in a flask. The mixture will be slightly opalescent. The opalescence is partly due to the precipitation of a small quantity of ovo-mucin. Strain through several folds of wet linen. The reaction is alkaline. Egg-white contains about 11 to 12 p.c. egg-albumin, with small quantities of globulins, ovo-mucin, grape-sugar, and salts (1 p.c.).

N.B.—Unless otherwise stated, take a fresh sample of the material for each reaction.

General Reactions—(A) Colour reactions.

(*a*) Xanthoproteic reaction.—Add nitric acid = a white precipitate, which on being boiled turns yellow. After cooling (water tap) add ammonia = the yellow colour or precipitate becomes orange.

(*b*) Millon's test = a whitish precipitate which becomes dull brick-red on boiling. A red colour of the fluid is obtained if only a trace of proteid be present. This reaction is due to the presence of an aromatic nucleus. It is given by tyrosin, phenol, &c.

(*c*) Piotrowski's reaction.—Add excess of strong solution of caustic soda (or KHO), and then a drop or two of *very dilute* solution of cupric sulphate (1 p.c.) = a transparent *violet* colour. The reaction occurs more quickly if heat is applied, and the colour deepens.

[Perform a control test with water, noting that the hydrated oxide of copper formed on adding the alkali is not dissolved.]

A violet colour is obtained with albumins, globulins, albuminates, and gelatin. The peptones and albumoses give a *rose-pink*

colour, instead of a violet, if only a trace of copper sulphate is used (biuret reaction).

(B) Precipitation by neutral salts, $MgSO_4$ $(NH_4)_2SO_4$ (conveniently written Am_2SO_4).

(*a*) Saturate by adding crystals of $MgSO_4$ and shake vigorously = slight white precipitate of egg-globulin. Filter. The filtrate contains egg-albumin. If this filtrate be saturated with Na_2SO_4, so that the solution is saturated with both $MgSO_4$ and Na_2SO_4, = bulky precipitate of egg-albumin.

(*b*) Add an equal volume of saturated solution of Am_2SO_4 (*i.e.*, "half-saturation") = precipitation of globulin; the albumin remains in solution and is not precipitated.

(*c*) Add crystals of Am_2SO_4 and shake until the solution is completely saturated (difficult to do this) = precipitate of *both* the globulin and albumin, which collect on the surface of the fluid. Filter. The filtrate is (or may be) devoid of proteid.

[N.B.—*Complete saturation with* Am_2SO_4 *precipitates all proteids except peptones.*]

(C) Effects of acids, metallic salts, &c.

(*a*) Add a drop or two of strong HNO_3 = white precipitate. Or the HNO_3 may be gently poured down the side of the tube = white cloud at junction.

(*b*) Acidulate strongly with acetic acid ($C_2H_4O_2$) and add strong potassium ferrocyanide (K_4FeCy_6) = whitish-yellow precipitate. (Peptone and some albumoses do not give this reaction.)

(*c*) Add a salt of a heavy metal—*e.g.*, copper sulphate, mercuric chloride, or lead acetate = precipitate.

(*d*) Add one drop of $HgCl_2$ = heavy white cloud or precipitate. Divide into two portions. (i.) Add 10 p.c. NaCl, the precipitate is dissolved. (ii.) Add 2 volumes of undiluted egg-white, the precipitate dissolves.

(*e*) Picric acid in cold = yellowish-white precipitate.

(D) Other reagents.

(*a*) Alcohol (5 vols.) = precipitate or opalescence in an acid medium, due to partial precipitation of proteid. The precipitation is favoured by the addition of 10 p.c. NaCl. (Peptone is precipitated with difficulty.)

(*b*) Ether (equal vol.) = cloudiness at junction in a neutral or acid fluid.

(*c*) Tannic acid = white precipitate or opalescence.

(*d*) Acidulate strongly with HCl, then add phosphotungstic acid (or phosphomolybdic acid) = white precipitate.

(*e*) Ethyl or methyl alcohol (50 p.c.) or acetone, 10 c.c. to 1 c.c. of egg white give a distinct jelly (*G. Mann*).

(E) Coagulation by heat.

(*a*) Boil, no coagulum of albumin is formed. Test the solution with litmus paper—it is alkaline—and then acidify by adding, drop by drop, dilute acetic acid (2 p.c.) until a flaky coagulum of coagulated insoluble albumin separates (about 70° C.).

(*b*) Boil and add HNO_3 = a white or yellowish coagulum.

(*c*) Acidify strongly with acetic acid, add an equal volume of a saturated solution of Na_2SO_4, and boil = coagulation.

(*d*) *Temperature of coagulation.* — Neutralise or slightly acidulate the egg-white with 0·2 p.c. acetic acid. [To neutralise a fluid add 0·1 p.c. acetic acid or HCl (or 0·1 p.c. NaHO) until it does not change the colour of neutral litmus paper.] Use an enamelled half-pint jug (or beaker) as a water bath. Over its mouth is stretched wire netting to hold the test tubes. Place the test tube in the water bath, and heat the water fairly rapidly to about 70° C. Place a thermometer in the test tube, and observe the temperature at which the solution becomes opalescent, or when particles of insoluble albumin separate out. If the reaction of the fluid is slightly acid complete coagulation occurs, and the fluid becomes clear. The amount of the coagulum varies with the amount of proteid present and other conditions.

(*e*) *Dilution of egg-albumin with water makes it non-coagulable by heat.*—Label four test tubes, A B C D, and place egg-white in them.

Egg-white.		*Result.*
A. Undiluted.	Boil in water bath 10 minutes.	Coagulated, solid.
B. Diluted, 1 : 5		May be more or less opalescent, but not coagulated.
C. ,, 1 : 10		
D. ,, 1 : 50		

Dilution of egg-white therefore renders it non-coagulable by heat.

The addition of a trace of acetic acid or NaCl greatly favours coagulation.

Make a similar test with blood-serum, and observe the difference.

CLASSIFICATION OF PROTEIDS.

I. Native Albumins are *soluble in water*, in dilute saline solutions, saturated solutions of NaCl, and $MgSO_4$, and are

not precipitated by alkaline carbonates, NaCl, or very dilute acids. They are precipitated by completely saturating their solutions with Am_2SO_4. These solutions are coagulated by heat at 70° to 73° C., although the temperature varies considerably according to the conditions. When dried at 40° C. they yield a clear yellow mass, "**soluble albumin**," which is soluble in water.

(1) **Egg-albumin.**—Its properties have already been considered.

(2) **Serum-albumin.**—Blood-serum contains serum-albumin and serum-globulin (see "Blood"). Dilute blood-serum with ten times its volume of water; the slight opalescence is due to precipitation of some serum-globulin. Neutralise the solution with very dilute acid until a faint haziness is obtained.

(*a*) Repeat the tests for egg-albumin.

(*b*) Add crystals of $MgSO_4$ to saturation, shaking the flask vigorously to do so = a white precipitate of serum-globulin. Filter. The filtrate contains serum-albumin, which gives the same general reactions as egg-albumin, but there are some points of difference, for which see "Blood." Some of the differences are indicated below.

(*c*) Saturate serum with $(NH_4)_2SO_4$ = white precipitate of both serum-albumin and serum-globulin. Filter. The filtrate contains no proteids.

Egg-albumin.	*Serum-albumin.*
(i.) Readily precipitated by HCl, but the precipitate is not readily soluble in excess.	(i.) It also is precipitated by HCl; the precipitate is soluble in excess.
(ii.) A non-alkaline solution is gradually coagulated by ether.	(ii.) It is not coagulated by ether.
(iii.) The precipitate with HNO_3 is soluble with difficulty in excess of the acid.	(iii.) The corresponding precipitate is much more soluble in excess of acid ($\frac{1}{2}$ vol. HNO_3).
(iv.) The nitric acid precipitate does not disappear on adding an equal volume of absolute alcohol.	(iv.) The corresponding precipitate largely disappears.
(v.) The precipitate obtained by boiling is but slightly soluble in boiling HNO_3.	(v.) The corresponding precipitate is soluble in strong HNO_3.
[(vi.) When injected under the skin, or introduced in large quantities into the stomach or rectum, it is given off by the urine.]	[(vi.) When injected under the skin, it does not appear in the urine.]

(3) **Lact-albumin**, see "Milk."

II. **Globulins** are insoluble in pure water, *soluble in dilute*

saline solutions—*e.g.*, NaCl, $MgSO_4$, $(NH_4)_2SO_4$—but insoluble in concentrated or saturated solutions of these neutral salts. Their solutions are coagulated by heat, though the T° of heat coagulation varies considerably. They are soluble in dilute acids and alkalies, yielding acid- and alkali-albumin respectively. Most of them are precipitated from their saline solution by saturation with NaCl, $MgSO_4$, Am_2SO_4, and some other neutral salts.

(1) **Serum-globulin** forms about half the total proteids of blood-serum. It is insoluble in water, readily soluble in dilute saline solutions (NaCl, $MgSO_4$). Its solutions give the general reactions for proteids. Its NaCl solution coagulates at about 75° C.

Repeat all the general reactions for proteids.

(*a*) Neutralise 5 c.c. blood-serum with dilute acetic or sulphuric acid (0·1 p.c.), then add 75 c.c. distilled water, and allow the precipitate to settle. Pour off the fluid and divide the precipitate into two portions, noting that it is insoluble in water, but soluble in excess of acid.

(*b*) Boil a portion of the neutralised fluid = coagulation.

(*c*) Saturate blood-serum with crystals of $MgSO_4$, shaking briskly for some time. Serum-globulin separates out and floats on the surface. Filter, and test the filtrate for serum-albumin.

(*d*) Pour upon some blood-serum in a tube a saturated solution of $MgSO_4$. Where the two fluids meet there is a white zone of serum-globulin.

(*e*) Saturate blood-serum with crystals of NaCl or neutral Am_2SO_4 = separation of serum-globulin.

(*f*) Precipitate the serum-globulin with $MgSO_4$, and filter. To the filtrate add Na_2SO_4 in excess, which gives a further precipitate. The filtrate may still give the reactions for proteids.

(*g*) Let a few drops of serum fall into distilled water = faint opalescence due to serum-globulin, or precipitate after standing.

(2) **Fibrinogen**, see "Blood."

(3) **Myosin**, see "Muscle."

(4) **Crystallin** is obtained from the crystalline lens.

(5) **Globin** the proteid constituent of hæmoglobin.

III. Derived Albumins (Albuminates) are compounds of proteids with mineral substances. Those produced by the action of acids or alkalies on albumins or globulins, yield respectively acid-albumin and alkali-albumin. They are insoluble in pure water and in solutions of NaCl, but readily soluble in dilute

HCl and dilute alkalies. The solutions are not coagulated by heat.

(1) **Alkali-albumin** or **Alkali-albuminate.**

(*a*) To dilute egg-albumin add a few drops of 0·1 p.c. NaHO, and keep it at 40° C. for 5 to 10 minutes = **alkali-albumin.** Boil; it does not coagulate, and is alkaline.

(*b*) Colour some with litmus, and neutralise carefully with 0·1 p.c. HCl or H_2SO_4 = a precipitate on neutralisation, which is soluble in excess of acid or alkali.

(*c*) Repeat (*b*); but, before neutralising, add sodium phosphate solution (10 p.c.). The alkaline phosphates prevent precipitation on neutralisation, until at least sufficient acid is added to convert the basic phosphate into acid phosphate. The solution must be decidedly acid before a precipitate falls.

(*d*) Precipitate by saturating with crystals of NaCl or $MgSO_4$.

(*e*) Lieberkühn's jelly is a strong solution of alkali-albumin. To undiluted egg-white add strong KHO. The whole mass becomes a jelly, so that the tube can be inverted without the mass falling out.

(*f*) Liberation of sulphur. Boil a strong solution of alkali-albumin with lead acetate solution = black precipitate of lead sulphide.

(2) **Acid-albumin** [or **syntonin**]. **Preparation.**

(A) To dilute egg-albumin, add 0·1 p.c. H_2SO_4, and warm gently for several minutes = **acid-albumin.**

(B) To finely-minced well washed muscle, *e.g.*, of frog or rabbit, add ten volumes of dilute HCl (4 c.c. of acid in a litre of water), allow it to stand for several hours and stir it frequently; filter, the filtrate is a solution of a globulin combined with an acid—**syntonin.**

(C) Allow concentrated HCl to act on fibrin for a time, and filter.

(D) Dissolve myosin in excess of 1 p.c. HCl, and after a time neutralise the solution with sodium carbonate.

(E) To undiluted egg-white, add acetic acid = a jelly of acid-albumin.

Use the clear filtrate from (A) or (B) for testing acid reaction.

(*a*) Boil; it does not coagulate.

(*b*) Add litmus solution, and neutralise with very dilute NaHO = a precipitate soluble in excess of the alkali or acid.

(*c*) Repeat (*b*), but add sodium phosphate before neutralising; acid-albumin is precipitated on neutralising. Thus sodium phosphate does not interfere with its precipitation.

(*d*) Add strong HNO_3 = a precipitate which dissolves on heating, producing an intense yellow colour.

(*e*) It is precipitated by saturation with neutral salts, *e.g.*, NaCl, $MgSO_4$, $(NH_4)_2SO_4$, like globulins.

(*f*) Boil with lime-water = partial coagulation.
(*g*) It gives no sulphur reaction (unlike alkali-albumin).

IV. Proteoses or Albumoses are formed as intermediate products in the peptic and tryptic digestion of proteids. In peptic digestion of albumin, acid-albumin is first formed, and finally peptone. Between the two is the group of proteoses or albumoses—primary and secondary. These proteoses have been subdivided into albumoses, globuloses, caseoses, &c., according as they are derived from albumin, globulin, or casein. (See "Digestion.") Witte's peptones (*i.e.*, "**Commercial peptones**") usually contain a small amount of peptone and much albumose. Make a strong solution by dissolving some in warm water, or 10 p.c. NaCl. This gives a solution of albumoses.

(*a*) They are soluble in water; not coagulated by heat; and are precipitated by complete saturation with neutral ammonium sulphate. The precipitate with $(NH_4)_2SO_4$ partly disappears on heating, and reappears on cooling.

(*b*) Add nitric acid = a white precipitate which *dissolves with heat* (yellow fluid) and *reappears on cooling*. Run tap water on the tube, the precipitate reappears. This is a characteristic reaction, and occurs best in the presence of NaCl. [Salicyl-sulphonic acid gives the same result.]

(*c*) Acetic acid and ferrocyanide of potassium = precipitate which *disappears on heating, and reappears on cooling.*

(*d*) It is precipitated by acetic acid and saturation with NaCl. The precipitate disappears on heating, and reappears on cooling.

(*e*) It, like peptone, gives a *rosy-pink* with Piotrowski's test, *i.e.*, $CuSO_4 + NaHO$.

(*f*) They are precipitated but not coagulated by alcohol.

(*g*) To 5 c.c. + 5 c.c. saturated solution of Am_2SO_4 and 4 grms. of the powdered solid. Saturate the mixture in water bath at 35° C. = sticky deposit of albumoses. Filter, and test filtrate for peptones.

(*h*) Add 1 c.c. dil. H_2SO_4 (1 : 4) + $ZnSO_4$ = precipitate.

V. Peptones are hydrated proteids, and in the body are produced by the action of proteolytic ferments on proteids. They are exceedingly soluble in water, but they are not precipitated by NaCl, acids, or alkalies, nor are they coagulated by heat. They are precipitated by tannic acid, and with difficulty by a large excess of absolute alcohol. Not precipitated by $(NH_4)_2SO_4$ (unlike all other proteids.)

Use a solution of Darby's fluid meat or commercial peptones in warm water. Commercial peptones contain only a small amount of peptone, and much albumose.

(*a*) Boil ; it is not coagulated.

(*b*) *Xanthoproteic reaction.*—Add HNO_3; boil = a yellowish colour, no precipitate ; cool, and add ammonia = orange colour.

(*c*) Acidify strongly with acetic acid, and add ferrocyanide of potassium = no precipitate.

(*d*) Test separate portions with tannic acid; potassio-mercuric iodide; mercuric chloride; picric acid (saturated solution); and lead acetate. Each gives a precipitate. The picric acid precipitate disappears on heating, and reappears on cooling.

(*e*) *Biuret reaction.*—Add excess of NaHO, and then a few drops of *very dilute* solution of $CuSO_4$ = a *rose-pink* colour; on adding more $CuSO_4$, it changes to a violet.

(*f*) Add two drops of Fehling's solution = a *rose-pink* colour; add more Fehling's solution, the colour changes to violet.

(*g*) Neutralise another portion = no precipitate.

(*h*) Add excess of absolute alcohol = a precipitate of peptone, but not in a coagulated form.

(*i*) It is not precipitated by saturation with NaCl or $MgSO_4$, nor by boiling with Na_2SO_4 and acetic acid.

(*j*) Peptone is not precipitated by saturation with neutral Am_2SO_4. *N.B.*—The other proteids are. Hence this salt is used to precipitate other proteids in the presence of peptones, the latter remaining in solution.

(*k*) It also gives Millon's test. No precipitate, but red colour.

(*l*) Saturate the solution of commercial peptones with $(NH_4)_2SO_4$ = a precipitate of albumoses or proteoses. Filter. The filtrate contains the pure peptone.

(*m*) *Diffusibility.*—Place a solution of peptones in a dialyser covered with an *animal* membrane, and test the diffusate after some time for peptones. Peptones do not diffuse through a parchment tube.

VI. Coagulated proteids are insoluble in water, weak acids, and alkalies, but are dissolved when digested at 40° C. in gastric juice (acid medium), or pancreatic juice (alkaline medium), forming first proteoses and finally peptones. There are two subdivisions :—

(A) *Proteids coagulated by heat.*

Preparation.—Use chopped-up, hard-boiled white of egg.

(*a*) Test its insolubility in water, weak acids, and alkalies.

(*b*) It is partially soluble in acids and alkalies, when boiled.
(*c*) Bruise some in water, and test with Millon's reagent.

(B) *Proteids coagulated by ferment action.*

(i.) **Fibrin** is insoluble in water and weak solutions of NaCl. It is a white, fibrous, soft, and very elastic substance, which exhibits fibrillation under a high magnifying power (see "Blood").

(*a*) Place well-washed, chopped-up fibrin in a test-tube, add 0·2 p.c. HCl. It swells up and becomes transparent in the cold, but does not dissolve. On neutralising with dilute Na_2CO_3 it regains its original size.

(*b*) Repeat (*a*), but keep on a water-bath at 60° C. for several hours; filter, and test the filtrate for acid-albumin by neutralisation with very dilute KHO or Na_2CO_3.

(*c*) To a very dilute solution of $CuSO_4$ add fibrin. The latter becomes greenish, while the fluid is decolourised. Add NaHO, the flock becomes violet.

(*d*) To chopped-up fibrin suspended in water apply the xanthoproteic and Millon's tests for proteids.

(*e*) For the effect of a dilute acid with pepsin (see "Digestion"). These digest fibrin, and convert it into proteose, and ultimately into peptone.

(*f*) It decomposes hydric peroxide, and turns freshly-prepared tincture of guaiacum blue (see "Blood").

(*g*) Digest fibrin in 10 p.c. NaCl for two days. A small part is dissolved; boil the fluid = coagulation.

(ii.) Myosin (see "Muscle").
(iii.) Casein (see "Milk").
(iv.) Gluten (see "Bread").

[**VII. Combined or compound proteids** consist of proteids combined with non-proteid bodies—*e.g.*, hæmatin, carbohydrates, or nucleic acid.

(*a*) *Chromo-proteids*, . { Hæmoglobin ("Blood"). Histohæmatin. Hæmocyanin.
(*b*) *Gluco-proteids*, . . { Mucins. Mucoids.
(*c*) *Nucleo-proteids*, . . { Caseinogen (see "Milk"). Vitellin. Nucleo-histon.]

[**VIII. Protamines**—regarded by Kossel as the simplest pro-

teids—exist in combination with nuclein in fish sperm. They yield bases containing 6 atoms of carbon, and these bases as a group have been called *hexones—e.g.*, lysin, arginin, histidin.]

Additional Exercises.

1. **Reaction of Adamkiewicz.**—To white of egg add glacial acetic acid, and heat to get it in solution; gradually add concentrated H_2SO_4 = a violet colour with slight fluorescence (not given by gelatine). (Impurity in $\overline{A}$ acid—Hopkins, *Proc. R. S.*, 1901.)

2. **Liebermann's reaction.**—Wash finely powdered albumin with alcohol and then with cold ether, and heat the washed residue with concentrated HCl acid = a deep violet-blue colour. This is best done in a white porcelain capsule, or on a filter-paper in a funnel; in the latter case the boiling acid is poured gently down the side of the filter-paper; or to 3 c.c. HCl + 2 drops undiluted egg-white. Boil = pink or violet colour. Avoid excess of water.

Colour reactions with cobalt (see Pickering, *Journ. of Phys.*, xiv., 1893).

The proteid molecule contains several aromatic groups:—(1) Oxyphenol group, represented by phenol and tyrosin. The xanthoproteic and Millon's reactions are due to the formation of nitro-products from this group. (2) Phenyl group, by phenyl-acetic acid. (3) The indol group, by indol and skatol. The Adamkiewicz reaction is due to this group (*Novy*). Liebermann's reaction does not appear to be due to the aromatic group.

3. **Indiffusibility of egg-albumin** (and most proteids).—Place egg-white solution in a dialyser or parchment-paper sausage-tube. Suspend in a tall glass jar (Lesson IX.) of distilled water. The salts (crystalloids) diffuse readily (test for chlorides by $AgNO_3$ and HNO_3). Test the diffusate for proteids = result negative. (Peptones diffuse through animal membranes.)

4. **Nitrogen and sulphur in albumin.**—(*a*) Place powdered dried albumin in a reduction tube, and into the mouth of the tube insert (1) a red litmus paper, and (2) a lead acetate paper. On heating the tube, the former becomes blue from the escape of ammonia, which can also be smelt (odour of burned feathers), and the latter black from the formation of lead sulphide

(*b*) Heat dry proteid with excess of soda-lime in a hard dry tube or spoon. It is charred, and ammonia vapour is evolved. The charring shows the presence of C and the ammonia the presence of N.

(*c*) Heat in a dry hard tube—at first slowly, and then strongly—dry proteid with a small piece of metallic sodium, cool, add 3 c.c. water to the NaCy residue, filter, and to the filtrate add a few drops of ferric chloride and ferrous sulphate, and then excess of HCl. If nitrogen be present, there is a precipitate of Berlin blue, sometimes only seen after standing for a time.

(*d*) To a solution of albumin add an equal volume of solution of caustic potash and a few drops of lead acetate and boil for some time = slowly a brownish colouration, due to lead sulphide.

5. **Temperature of coagulation** (fig. 1).—The reaction of the fluid must be neutral or feebly acid. "A glass beaker containing water is placed within a second larger beaker also containing water, the two being separated by a ring of cork. Into the water contained in the inner beaker there is immersed a test-tube, in which is fixed an accurately graduated

thermometer, provided with a long narrow bulb. The solution of the proteid, of which the temperature of coagulation is to be determined, is placed in the test-tube, the quantity being just sufficient to cover the thermometer bulb. The whole apparatus is then gradually heated, and the experimenter notes the temperature at which the liquid first shows signs of opalescence" (*Gamgee*).

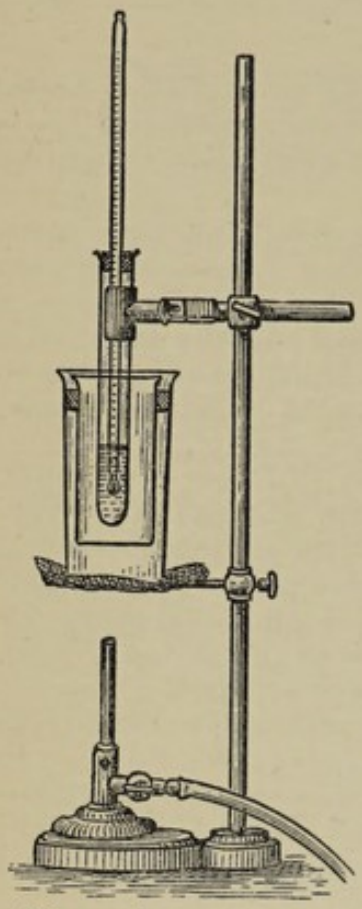


Fig. 1.—Apparatus for determining the Coagulation Temperature of Proteids.

6. **Circumstances modifying coagulating temperature.**—Place 5 c.c. of solution of albumin in each of three test-tubes, colour with neutral solution of litmus, and label A, B, C. To A add a drop of very dilute acetic acid (0·1 p.c. diluted five or six times); to B a very dilute solution of NaHO (0·1 p.c. similarly diluted); C is neutral for comparison. Place all three tubes in a beaker with water and heat gradually. Coagulation occurs first in A, next in C, and not at all in B, the alkaline solution. Use thermometer.

7. **Influence of salts on coagulation.**—To blood-serum diluted one-fifth add an equal volume of saturated solution of NaCl. Divide into two portions. Boil one portion = coagulation; acidify the other with acetic acid; it may become slightlv turbid, and on heating it coagulates. The more salts present in an albuminous solution the less is the coagulation temperature dependent on the reaction of the fluid. The less the amount of salts the nearer neutral—or even slightly acid—must the reaction be to ensure coagulation.

8. **Crystallisation of proteids**—*e.g.*, *Egg-albumin.*—Various methods are employed, but the most convenient is that of Hopkins and Pinkus,* which is a modification of the original method by Hofmeister [1891].

Fresh egg-white is thoroughly mixed with an equal bulk of saturated neutral filtered solution of Am_2SO_4, the latter being gradually added—10 c.c. to 15 c.c. at a time—and the mixture stirred with an egg-whisk after each addition. The mixture is strongly alkaline and smells of ammonia. Let the well-whisked mixture stand overnight, filter, and to the clear filtrate add from a burette very cautiously more Am_2SO_4 until a permanent precipitate is obtained. Add a few drops of distilled water until the solution is again clear. Drop in from a burette 10 p.c. acetic acid until a distinct and bulky precipitate is obtained. Cork the bottle, and let it stand for 24 hours, when abundance of small rosettes or fan-shaped aggregations of microscopic needle-shaped crystals are obtained when a preparation is examined with $\frac{1}{6}$-inch objective. On adding the Am_2SO_4 to the proteid, ammonia is evolved, and this free alkali interferes with the separation of crystals, hence the addition of acetic acid. Pure egg-albumen, crystals of serum-albumin (*Gürber*), and allied bodies have thus been obtained in large quantities.† If a dye be added during crystallisation the crystals are correspondingly coloured.

**Journ. of Phys.*, xxiii., 132, 1898. †*Journ. of Phys.*, xxv., 306, 1900.

9. **Halogen proteid derivatives**—*i.e.*, artificial compounds of proteids with iodine and other halogens. These have a certain importance in view of the iodine compounds obtainable from the thyroid gland (*F. Blum*).

Egg-albumin + 15 water is slightly acidulated and filtered. To the clear filtrate Br, I, or Cl is added, the mixture being well-shaken, when the corresponding halogen proteids are obtained * (*Blum. Hopkins* and *Brook*). They do not give Millon's reaction.

10. **Removal of proteids.**—The following, amongst other methods, are used for removing proteids from liquids containing them. In this way other substances present may be more easily detected.

Wenz's Method.—Saturate with $(NH_4)_2SO_4$. This precipitates all proteids except peptones.

Boiling.—Acidulate faintly with acetic acid and boil. This removes globulins and albumins.

Brücke's Method.—Acidulate with HCl, and then add potassio-mercuric iodide (see "Liver" and "Glycogen").

Alcohol.—Acidify feebly with acetic acid, add several volumes of absolute alcohol. After 24 hours all proteid is precipitated.

Girgensohn's Method.—Add half its volume of a saturated solution of NaCl, and then tannic acid in slight excess. This precipitates all proteids.

11. **Lardacein, or amyloid substance,** occurs in organs—*e.g.*, liver and kidney—undergoing amyloid, waxy or wax like, degeneration. It is insoluble in dilute acids or alkalies, and is not acted on by gastric juice.

(*a*) A solution of iodine in iodide of potassium gives a deep brown or mahogany stain when poured on a section of a fresh waxy organ.

(*b*) With iodine and sulphuric acid a blue reaction is occasionally obtained.

(*c*) Methyl-violet and gentian-violet give a rose-pink with the waxy parts, while the healthy parts are coloured blue or purple.

LESSON II.

ALBUMINOIDS—NUCLEO-PROTEIDS.

THE group of albuminoids includes a number of bodies which in their general characters and elementary composition resemble proteids, but differ from them in many respects. They are amorphous; some contain sulphur, and others do not. The decomposition-products resemble those of proteids.

I. **Collagen** is the name given to the albuminoid basis of connective tissues—such as tendon, ligaments, and bone. **Gelatin** is obtained from them by prolonged boiling.

Preparation of a solution.—Use best French commercial gelatin.

* *Journ. of Phys.*, xxii., 1898.

Cut it into small pieces, add cold water. The gelatin swells up by imbibition of water, but does not dissolve. Heat, the gelatin dissolves. Allow it to cool; it gelatinises if the solution is strong enough.

(A) General proteid tests for gelatin.

(*a*) Xanthoproteic test.—Add HNO_3 and boil = a *light* yellow colour with no previous precipitate; the fluid becomes lemon-coloured on adding ammonia. This reaction is feeble, owing to the absence of the phenol group, C_6H_5OH.

(*b*) Millon's reagent = no pinkish-red precipitate on boiling, due to the absence of the tyrosin nucleus. This reaction may be obtained with commercial gelatin, but not with pure gelatin.

(*c*) Piotrowski's test = a blue-violet, rather than a violet colour, with NaHO and $CuSO_4$ (resembling somewhat proteids).

(*d*) Not precipitated by acetic acid and potassium ferrocyanide (unlike albumin, but like peptone).

(*e*) Not coagulated by heat (unlike albumin).

(*f*) Not coagulated by boiling with Na_2SO_4 and acetic acid (unlike albumin).

(*g*) Precipitated by saturation with $MgSO_4$ or $(NH_4)_2SO_4$.

(B) Special reactions for gelatin.

(*h*) It is not precipitated by acetic acid (unlike mucin) or alkalies.

(*i*) Mercuric chloride = no precipitate (unlike albumose and peptone).

(*j*) Lead acetate = no precipitate (unlike proteids). The heavy metals do not precipitate it (unlike proteids).

(*k*) Tannic acid = white precipitate, insoluble in excess.

(*l*) Picric acid (sat. sol.) = yellowish-white precipitate, which disappears on heating and reappears on cooling.

(*m*) It is precipitated by alcohol, and also by platinic chloride.

II. Chondrin is obtained by the prolonged boiling of hyaline cartilage, which largely consists of the substance "Chondrigen."

Preparation.—Boil costal cartilages (perichondrium removed) several hours in water = opalescent fluid, which gelatinises on cooling.

(*a*) Acetic acid = white precipitate, soluble in great excess.

(*b*) Dilute mineral acids = white precipitate, readily soluble in excess.

(*c*) Not precipitated by acetic acid and potassium ferrocyanide.

III. Mucin, see "Saliva." It is also found in the ground substance of connective tissue and tendon. There are probably

several mucins. On heating with dilute H_2SO_4 they yield a reducing sugar, and they are regarded as glucosides, compounds of a proteid (globulin?) with animal gum. It has been called a "glucoproteid."

Preparation.—(A) Place small pieces of tendon for 3 days in lime-water, which dissolves the mucin. Add acetic acid = precipitate of mucin.

(B) Mince the submaxillary gland of an ox, rub it up with sand in a mortar, and add half a litre of 0·1 p.c. KHO solution. Stir from time to time, and after an hour or two strain through wet linen and filter through coarse filter paper.

(*a*) Mucin makes fluids viscid and slimy. It is not coagulated on boiling. Add acetic acid = stringy precipitate insoluble in excess of acid.

(*b*) It gives the xanthoproteic test and Millon's reaction.

(*c*) Add NaHO and $CuSO_4$ = violet colour.

(*d*) It is not precipitated by tannic acid (unlike gelatin) or by potassium ferrocyanide (unlike proteids, except peptone).

(*e*) Add lead acetate = precipitate (unlike gelatin).

(*f*) Boil with a few drops of H_2SO_4 (25 p.c.). It is decomposed and yields a reducing sugar. Apply Trommer's test.

IV. Elastin is the chemical basis of elastic tissue, ligamentum nuchæ, ligamenta subflava, &c.

Preparation.—Boil fresh ligamentum nuchæ of an ox successively in alcohol, ether (to remove fats), water (to remove gelatin), and finally in acids and alkalies.

(*a*) It is insoluble in water, but is soluble in strong caustic soda.

(*b*) It gives the xanthoproteic test.

(*c*) It is precipitated from a solution by tannic acid.

V. Keratin is the chemical basis of some epithelial structures—*e.g.*, surface layers of the epidermis, hairs, horn, hoof, and nails. It contains a large percentage of sulphur, part of which is loosely combined. It is very insoluble (though soluble in strong alkalies), and resists putrefaction for a long time. A closely-allied body, **Neuro-Keratin**, is found in nerve fibres.

(*a*) Burn a paring of horn, and note the characteristic smell.

(*b*) Heat a paring of nail or horn with strong NaHO and lead acetate = black or brown colouration, due to lead sulphide.

(*c*) Test for sulphur. (Lesson I., Add. Exs.).

VI. Nucleo-proteids are compounds of proteids with the complex body nuclein, which contains phosphorus, nuclein being so-called because it is obtained chiefly from the nuclei of cells, though it also occurs in protoplasm. In some animals the so-called mucin of bile is a nucleo-proteid. When digested with gastric juice they yield from the proteid partner—peptone—and an insoluble nuclein which contains phosphorus; thus differing

from true mucins, which yield no nuclein. They react somewhat like globulins and mucin.

Preparation.—As it is most abundant in nuclei it is best prepared from cellular organs—*e.g.*, lymphatic glands, thymus.

A. Sodium chloride method.—Grind finely-divided thymus gland in a mortar with an equal volume of NaCl and a little water. Pour the viscous mass into distilled water, when stringy masses rise to the surface. Collect and dissolve these in 1 p c. sodium carbonate solution.

B. Grind up thymus or lymphatic glands of ox with sand, and extract with 10 to 20 vols. of water (1 to 2 hours, better 24 hours). Strain, and, if possible, centrifugalise. Add acetic acid, dropwise, until acid, when the nucleo-proteid "tissue fibrinogen" of Wooldridge is precipitated.

(*a*) Dissolve the precipitate in 1 p.c. Na_2CO_3, or dilute alkali = opalescent solution.

(*b*) Boil (*a*) = no coagulation. The original solution in an acid medium is coagulated by heat.

(*c*) Add acetic acid = precipitate of nucleo-proteid.

(*d*) Saturate with $MgSO_4$ or Am_2SO_4 = precipitate of nucleo-proteid.

(*e*) It yields the ordinary proteid reactions.

(*f*) Digested in gastric juice it yields an insoluble residue of nuclein, which when collected and boiled with HNO_3 and ammonium molybdate yields a yellow colour indicating phosphorus.

(*g*) A few c.c. of the solution injected into the blood-vessels of a rabbit produce extensive intra-vascular clotting, especially in the veins.

Record the results obtained with the following substances and tests:—

	Egg-Albumin	Serum-Albumin.	Serum-Globulin.	Albumose.	Peptone.	Gelatin.
Xanthoproteic, . . .						
Millon, ...						
$CuSO_4$ + NaHO, . .						
Nitric acid, . . .						
Boiling and cooling, . .						
$C_2H_4O_2$ + K_4FeCy_6, . .						
Tannic acid, . . .						
$HgCl_2$, ...						
Alcohol, ...						
Sat. with $MgSO_4$, . .						
,, Am_2SO_4, . .						

[*Chemie der Eiweisskörper*, by Otto Cohnheim, Braunschweig, 1900. *The chemistry of proteid bodies*, translated by Gustav Mann, M.D. *Die Proteïne*, by G. Ruppel, Marburg, 1900.]

LESSON III.

THE CARBOHYDRATES.

THE **carbohydrates**—represented in our foods by starch and sugar—occur especially in plants. They also occur in animals, to a smaller extent, chiefly in the form of glycogen, dextrose, and lactose.

They consist of **C**, **H**, and **O**, with the H and O in the same proportion as in water (*i.e.*, 2H : 1O). As this proportion obtains in other substances which are not carbohydrates—*e.g.*, acetic acid ($C_2H_4O_2$), lactic acid ($C_3H_6O_3$), the definition must be extended. The chief groups include substances that do not contain less than 6 atoms of carbon, although many carbohydrates contain multiples of this. To every 6 atoms of C there are at least 5 atoms of O, so that on the one hand acetic acid is excluded, and pyrogallic acid ($C_6H_6O_3$) on the other. Recently, however, bodies—having the constitution of an aldehyde-alcohol and called *aldoses*, or of a ketone-alcohol—*ketoses*, and containing 3 to 9 atoms of carbon, have been included in this group. The number of C atoms always equals those of O, and according to the number of C atoms they are called trioses, tetroses, pentoses, hexoses, &c. The pentoses have been found abnormally though rarely in urine.

General characters.—They are indifferent bodies, with a neutral reaction which form loose combinations, specially with bases. Other general characters some possess directly, *e.g.*, dextrose, or they can be converted into bodies which have the following features in common. One or other character may fail, but, as a group, they have the following reactions :—

(*a*) They *reduce alkaline metallic solutions*, and are coloured yellow by alkalies.

(*b*) *Rotate the plane of polarised light.*

(*c*) With yeast they yield alcohol and CO_2—*i.e.*, undergo *fermentation.* (Some do not).

(*d*) Boiled with HCl or H_2SO_4 they are decomposed with the formation of *lävulinic acid*, humin substance, and formic acid.

(*e*) Give a deposit of yellow needles with **phenyl-hydrazine.**

(*f*) Various *colour reactions* with acids and aromatic alcohols.

(*g*) Some, *e.g.*, cellulose and starch, are *insoluble* in water ; others are very *soluble.* Those which are insoluble in water can usually be dissolved by heating with an acid—*i.e.*, by hydrolysis.

In absolute alcohol (and ether) almost all are soluble with difficulty, or insoluble.

(*h*) When strongly heated they char, decompose, and yield a variety of products.

Classification of some Carbohydrates.

I. Glucoses or Monosaccharides ($C_6H_{12}O_6$). (Hexoses.)	II. Saccharoses or Disaccharides ($C_{12}H_{22}O_{11}$).	III. Amyloses or Polysaccharides ($n(C_6H_{10}O_5)$).
+ *Dextrose.*	+ Cane-sugar.	+ Starch.
− Lævulose.	+ *Lactose.*	+ Soluble starch.
+ Galactose.	+ Maltose.	+ Dextrins.
	+ Iso-maltose.	+ *Glycogen.*
		Cellulose.
		Animal gum.

The + and − signs indicate that, as regards polarised light, the substances are dextro- and lævorotatory respectively. Those in *italics* occur in the organs or secretions.

The amyloses are anhydrides of the glucoses [$n(C_6H_{12}O_6) - nH_2O = (C_6H_{10}O_5)_n$], while the saccharoses are condensed glucoses ($C_6H_{12}O_6 + C_6H_{12}O_6 - H_2O = C_{12}H_{22}O_{11}$)]. The saccharoses are converted into glucoses on boiling with dilute sulphuric acid.

$$O \begin{cases} C_6H_{11}O_5 \\ C_6H_{11}O_5 \end{cases} + H_2O = 2C_6H_{12}O_6.$$

Emil Fischer has shown that the monosaccharides are aldehydes or ketones of a hexatomic alcohol, $C_6H_8(OH)_6$. Just as aldehyde, C_2H_4O, is formed by oxidising ethylic alcohol, C_2H_6O, so from mannitic alcohol the simplest carbohydrate, $C_6H_{12}O_6$, is formed. When two molecules of such monosaccharides polymerise with the loss of water, they form the disaccharides, which may split up again and yield monosaccharides. When there is further polymerisation with loss of water we get bodies with molecules of greater molecular weight—the simpler members being dextrins, the more complex starch and glycogen, forming the group of polysaccharides. These in turn may break down and yield monosaccharide or disaccharide molecules. Thus the transformation undergone by carbohydrates in the organism, their conversion from one form to another, are rendered more easy of comprehension.

I. MONOSACCHARIDES.

1. Glucose, Dextrose, or Grape-sugar ($C_6H_{12}O_6$).—In commerce it occurs in warty uncrystallised masses of a yellowish or yellowish-brown colour. It exists in fruits, honey, and in small

quantities in the blood (0·1 to 0·2 p.c.) and other fluids and organs. It is the form of sugar found in diabetic urine. After ingestion of large quantities of sugar, a reducing sugar may appear in the urine ("Alimentary glycosuria"). It is very soluble in water and in alcohol. Dissolve a small quantity in hot water (1 p.c.).

(*a*) It is not so sweet as cane-sugar.

(*b*) Add iodine solution = no reaction.

(*c*) Heat with H_2SO_4; darkens slowly. (Distinction from cane-sugar.)

(*d*) Dissolve some in boiling absolute alcohol. It crystallises in transparent prisms when the alcohol cools (fig. 2).

(*e*) Heat gently some dry glucose = melts—then yellow and brown = (caramel) with odour of burnt-sugar.

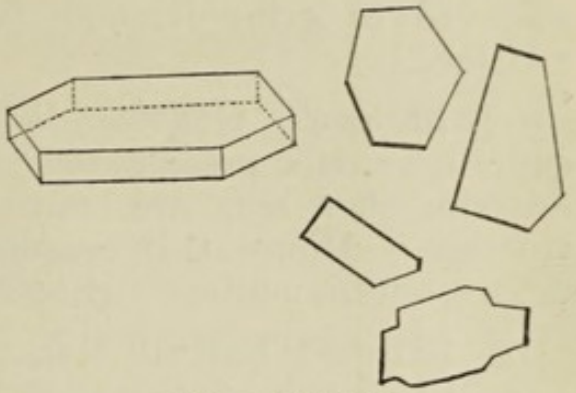


Fig. 2.—Dextrose.

A. Some important tests depend on its power of reducing various metallic oxides, especially those of copper. Glucose, like all aldehydes (*e.g.*, formaldehyde, $HC\overset{O}{\underset{H}{<}}$) is readily oxidised. Substances, therefore, that readily give up O are reduced.

(*a*) **Trommer's test.**—Add a drop or two of $CuSO_4$ solution (10 p.c.), and afterwards NaHO (or KHO) in excess, *i.e.*, until the precipitate—hydrated oxide of copper—first formed is re-dissolved, and a clear blue fluid is obtained. The hydrated oxide of copper is held in solution by dextrose (and by all the glucoses). Heat the upper stratum slowly, turning the tube in the flame. A little below the boiling point, if grape-sugar be present the blue colour disappears, and a yellow (cuprous hydrate) or red (cuprous oxide) precipitate is obtained which contrasts sharply with the deep blue below. The precipitate is first yellow, then yellowish-red, and finally red. The colour is best seen in reflected light. If excess of copper be added, the copper hydrate precipitate masks a small amount of the cuprous precipitate. If no sugar be present, only a black colour may be obtained. [Make a control test with water to show this.]

[Ammonia or ammoniacal salts retard the reaction, as the Cu_2O is not precipitated at once. Test this by adding some NH_4Cl, then perform Trommer's test.]

(*b*) **Add Fehling's solution**; boil = a yellow or yellowish-red precipitate of cuprous oxide or hydrate. It is better to proceed thus:—Boil Fehling's solution; if it retains its dark blue clear appearance it is in good condition. Add the sugar solution and boil again = yellow or yellowish-red precipitate of cuprous oxide or hydrate due to reduction, as in Trommer's test. [For the method of making Fehling's solution, the precautions to be observed in using it, and for other tests for glucose, see "Urine."]

(*c*) **Barfoed's reagent.**—When heated with dextrose red cuprous oxide is precipitated. Lactose, cane-sugar, maltose, and dextrin, when they are boiled with it for a short time, give no reaction. Hence this reagent has been used to distinguish dextrose from maltose. (See Appendix for its preparation).

(*d*) **Böttger's bismuth test.**—Heat the fluid with NaHO and a small quantity of dry basic bismuth nitrate = a grey or black reduction product of bismuth oxide. For Nylander's modification, see "Urine." In all reactions depending on reduction, one must recollect that some substances which are in no way related to the glucoses—*e.g.*, uric acid, kreatinin, phenylhydrazine—may cause reduction, and thus lead to error.

$$Bi(OH_2) + NaOH = Bi(OH)_3 + NaHO.$$
$$2Bi(OH)_3 - 3O = Bi_2 + 3H_2O.$$

B. Other Tests.

(*e*) **Mulder's test.**—Add freshly prepared sulphindigotate of soda solution and Na_2CO_3, and boil; the blue changes to violet, red, yellow, and finally becomes colourless—*i.e.*, the indigo is reduced to indigo-white. Cool the tube under the water tube, and shake; the colours reappear in the inverse order to blue.

(*f*) **Picric acid.**—Add an equal volume of saturated solution of picric acid, and some NaHO; boil = deep red solution (see "Urine").

(*g*) **Molisch's test.**—(i.) Add a drop or two of a 15 p.c. alcoholic solution of α-naphthol, and slowly 1 to 2 vols. of concentrated H_2SO_4. The colour which first appears is violet; water causes a bluish-violet deposit. (ii.) If, instead of the naphthol, an alcoholic solution of thymol be used, a red colour is obtained. Seegen, however, points out that this reaction can be obtained with other substances, *e.g.*, albumin, which, however, is denied by Molisch. It is not a reliable test. It is given by all carbohydrates, and is a general reaction due to the formation of furfurol ($C_5H_4O_2$).

(*h*) **Nitro-propiol** (*Gebhardt*).—Dilute 10 to 15 drops of test fluid (or diabetic urine) with 10 c.c. distilled water. Add a nitro-propiol tablet. Boil. If glucose be present the fluid becomes greenish, and if heating be continued it becomes blue-black, due to indigo blue, which is deposited on the tube cooling. The colour may be extracted by chloroform. (Tablets made by Teusch.)

(*i*) Benzoylchloride and excess of NaHO = precipitate.

[The furfurol reaction is often used not only for testing for the presence of carbohydrates, but also for that of carbohydrate groups in proteid bodies. Besides the Molisch-Udransky test above described, resorcin with HCl (*Seliwanoff*), phloroglucin with HCl (*Tollens*), and orcein (*Tollens*) have been used. On heating a sugar solution containing 18 p.c. HCl with orcein, the solution becomes first red, then violet, and lastly bluish-green, and then blue-green flakes are deposited. This is used as a test for pentoses. (Neuberg, *Zeits. f. phys. Chem.*, xxxi., 564).]

C. The **Osazone** reactions are of prime importance.

Phenyl-hydrazine test.—(*a*) Two parts of phenyl-hydrazine hydrochloride and three of acetate of soda are mixed in a test-tube with 6 to 10 c.c. of dextrose solution. Boil in a water-bath for 30 minutes, and then place the tube in cold water or dextrose. A yellow crystalline deposit is formed, which, microscopically, consists of bright sulphur-colour yellow needles either detached or arranged in rosettes or feathery groups (fig. 3). The substance formed is **phenyl-glucosazone** ($C_{18}H_{22}N_4O_4$), with a melting-point of 204° C. The arrangement of the acicular crystals I find frequently varies. Sometimes they are in rosettes (see "Urine"), and at other times more feathery. They are soluble in alcohol, and may be recrystallised from it.

The phenyl-hydrazine test with sugars is a most important and reliable reaction for their identification. It forms with most sugars—when sufficiently heated—**osazones**, the several sugars yielding corresponding yellow osazones, which differ in solubility, crystalline form, and melting point (see "Appendix"). It is usually stated that the best proportions are 1 dextrose, 2 hydrochloride of phenyl-hydrazine, 3 sodium acetate, and 20 water. The substance formed is but slightly soluble in water. According to E. Fischer, the following is the reaction which takes place :—

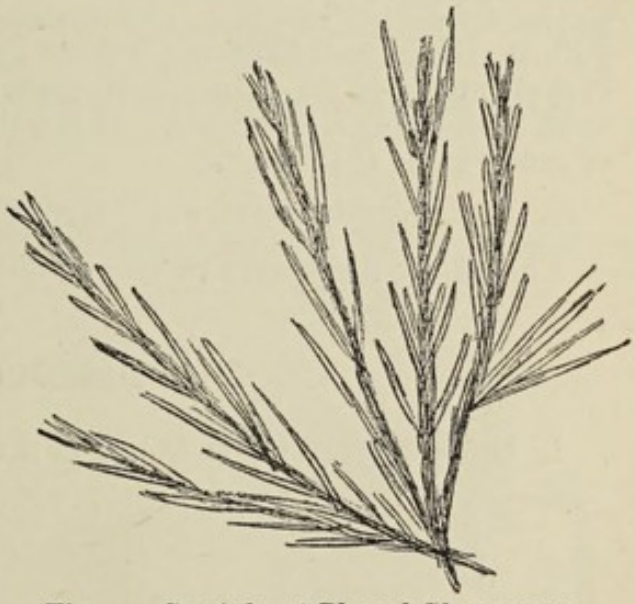


Fig. 3.—Crystals of Phenyl-Glucosazone, × 120.

$$C_6H_{12}O_6 + 2C_6H_5N_2H_3 = C_{18}H_{22}N_4O_4 + 2H_2O + 2H.$$

Phenyl-glucosazone.

The full equation is—

$$C_6H_{12}O_6 + H_2N.NH.C_6H_5 = C_6H_{12}O_5 : N.NH.C_6H_5 + H_2O.$$

$$C_6H_{12}O_5 : N.NH.C_6H_5 + 2C_6H_5NH.NH_2 =$$

$$C_6H_{10}O_4(:N.NH.C_6H_5)_2 + C_6H_5NH_2 + NH_3 + H_2O.$$

Glucosazone. Anilin.

(*b*) A *simple method* is to place in a test-tube half an inch of hydrochloride of phenyl-hydrazine (powder), then another half inch of sodium acetate (crystals), and then half fill the test-tube with the sugar solution. Boil for 2 minutes. Let the tube stand for half an hour or longer (*Williamson*). See "Urine" for other points of *clinical* importance, and for an important modification by Neumann.

(*c*) Or add to the dextrose solution half its volume of acetic acid and 2 or 3 drops of phenyl-hydrazine (fluid). Boil as above with a similar result.

(*d*) *Riegler's modification.*—Dissolve 0·1 grm. phenyl-hydrazine and 0·5 grm. sodium acetate in a little water. Boil for several seconds with 1 c.c. of a glucose solution (or diabetic urine), then add 1 c.c. of NaHO (10 p.c.). Within a minute or two a deep red-violet colour appears. It is applicable for the lactose in milk.

D. Moore's test—Yellow colouration with caustic soda or potash.—Heat the solution with half its volume of NaHO = a yellow or brown colour due to the formation of glucic and melassic acids. The non-appearance of a yellow colour indicates the absence of dextrose, but the following substances also give a yellow colour with NaHO :—All the glucoses, together with milk-sugar and lactose. It is only used as a rough test. Estimation of glucose, see "Urine."

E. Fermentation test, see "Urine."

The other members of the glucose group are purposely omitted.

II. DISACCHARIDES.

I. Cane-sugar ($C_{12}H_{22}O_{11}$) is a *non-reducing* sugar.

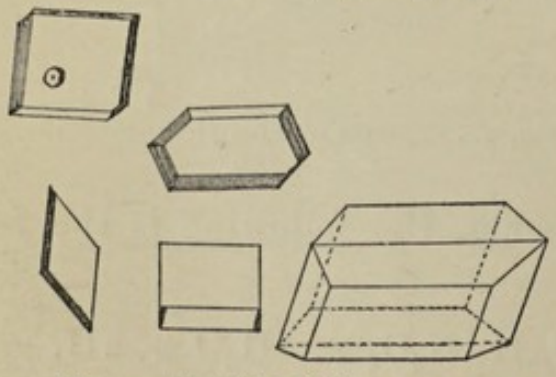


Fig. 4.—Crystals of Cane-sugar.

(*a*) Note its crystalline form (fig. 4) and sweet taste.

(*b*) Its pure solutions do not reduce Fehling's solution.

(*c*) Trommer's test = a clear blue fluid. Heat = no reduction.

(*d*) Barfoed's reagent is *not* reduced.

(*e*) It does not form a corresponding

osazone with phenyl-hydrazine, but after inversion it forms phenyl-glucosazone.

(*f*) Pour strong H_2SO_4 on cane-sugar in a beaker, add a few drops of water ; the whole mass is quickly charred.

(*g*) Heat cane-sugar with caustic soda = it darkens slowly.

(*h*) Practically insoluble in absolute alcohol, but its solubility greatly increases with the dilution of the alcohol.

(*i*) **Inversion of cane-sugar.**—Boil for 5 minutes a solution of cane-sugar with a few drops of HCl (or 25 p.c. of H_2SO_4). The cane-sugar is "inverted." Cool, neutralise with NaHO. The solution contains dextrose and lævulose—both reducing sugars. Test its reducing power with Fehling's solution and apply the other tests.

$$\underset{\text{Cane-sugar.}}{C_{12}H_{22}O_{11}} + H_2O = \underset{\text{Glucose.}}{C_6H_{12}O_6} + \underset{\text{Lævulose.}}{C_6H_{12}O_6}.$$

(*j*) **Estimation of cane-sugar.**—To 10 c.c. of cane-sugar solution, add 1 c.c. of a 25 p.c. solution of H_2SO_4. Boil for half an hour, and then make up bulk of fluid to its original volume. The cane-sugar is converted into a reducing sugar—dextrose. Place the fluid in a burette, and estimate its reducing power on Fehling's solution (see "Urine"); 95 parts of glucose corresponds to 100 parts of cane-sugar.

II. Maltose ($C_{12}H_{22}O_{11}$) forms a fine, white, warty mass of needles, and is the chief sugar formed by the action of diastatic

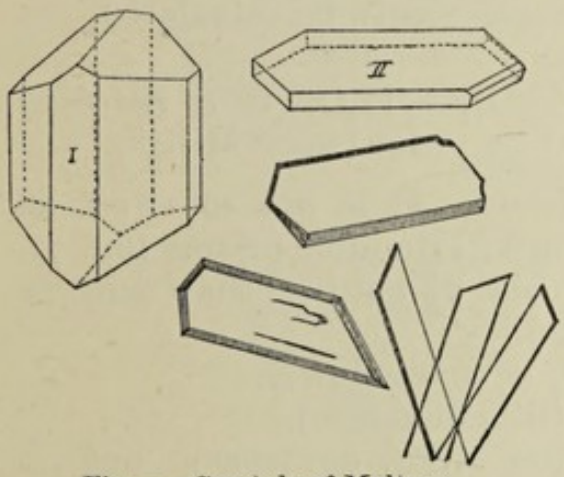


Fig. 5.—Crystals of Maltose.

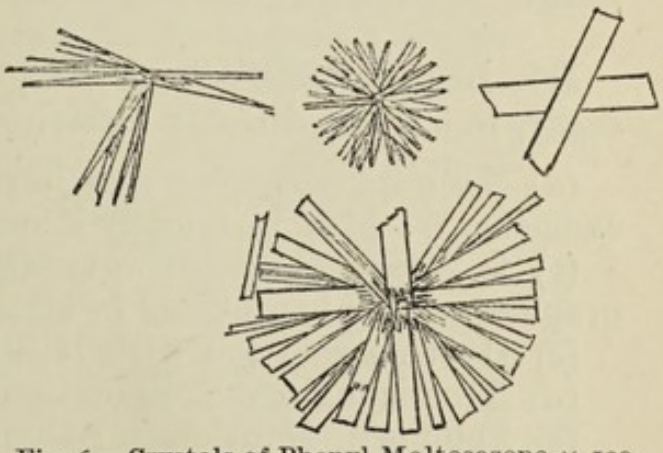


Fig. 6.—Crystals of Phenyl-Maltosazone × 120.

ferments on starch—*e.g.*, diastase on sprouting barley or starch. It is a *reducing sugar*. It reduces Fehling's solution more feebly than does dextrose ; 10 c.c. Fehling = ·07 grm. maltose, Barfoed's reagent helps to distinguish it (negative result) from dextrose.

(*a*) Use 1 p.c. solution of maltose or mix 1 grm. ground malt with 10 volumes water. Keep at 60° C. for half an hour. Boil and filter; the filtrate contains maltose and dextrin.

(*b*) Test for a reducing sugar with Fehling's solution or other suitable test. (See also "Salivary digestion.")

(*c*) Apply phenyl-hydrazine test (boil $1\frac{1}{2}$ hours) = **phenyl-maltosazone** ($C_{24}H_{32}N_4O_9$), which crystallises in yellow needles (fig. 6).

(*d*) Barfoed's reagent. Boil = no reduction. In this respect, and in some others, it differs from dextrose.

(*e*) It is soluble in water and alcohol. Examine its crystals (fig. 5). Its specific rotatory power is + 150°—*i.e.*, it is greater than that of dextrose, but its reducing power (on Fehling's solution) is only two-thirds of that of dextrose.

Estimation of maltose.—(i.) Determine its reducing power on 10 c.c. of Fehling's solution (see "Urine").

(ii.) Convert it into dextrose by boiling ($\frac{1}{2}$ hour) 50 c.c. of the solution with 1 c.c. H_2SO_4. Cool and bring the solution to the original volume (50 c.c.) by adding water. Again determine its reducing power by Fehling's solution. If x = c.c. of maltose solution necessary to reduce 10 c.c. of Fehling's solution, then as the respective reducing powers of glucose and maltose are as $2 : 3$ $\frac{2x}{3}$ = c.c. of dextrose solution necessary for the same purpose. As 10 c.c. of Fehling correspond to 0·05 grm. dextrose, the strength of the maltose solution can easily be calculated.

III. Lactose (Milk-Sugar), $C_{12}H_{22}O_{11} + H_2O$, is a *reducing sugar*, but is not directly fermented by yeast (see "Milk").

(*a*) Note its whiteness and hardness. It is not so sweet as cane-sugar. Microscopically it occurs in rhombic prisms (fig. 7).

(*b*) It is less soluble in water (6 cold, $2\frac{1}{2}$ boiling) than cane- or grape-sugar, and insoluble in alcohol.

(*c*) Heat its solution with H_2SO_4 = chars slowly.

(*d*) **Trommer's** test = reduction (like dextrose).

(*e*) **Fehling's** solution = reduction (like dextrose), but it requires 10 parts of lactose to reduce the amount of Fehling's solution that is reduced by 7 of dextrose—*i.e.*, only about two-thirds as strong as dextrose.

(*f*) Barfoed's reagent is *not* reduced.

(*g*) It is precipitated from its saturated watery solution by absolute alcohol.

(*h*) Phenyl-hydrazine test (fig. 8) yields **phenyl-lactosazone** ($C_{24}H_{32}N_4O_9$), but the solution must be boiled for a long time.

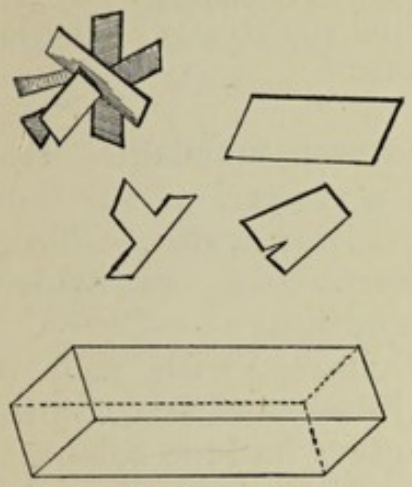


Fig. 7.—Crystals of Lactose.

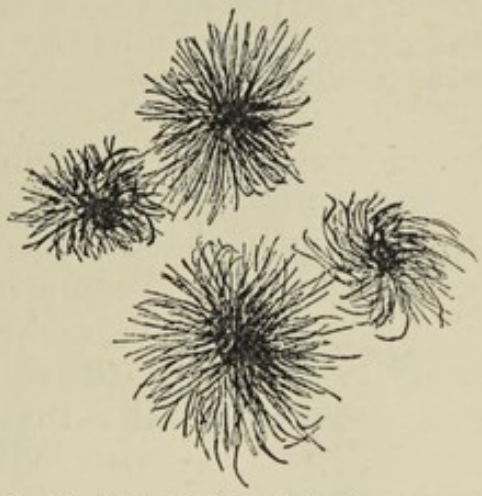


Fig. 8.—Crystals of Phenyl-lactosazone, × 120.

III. POLYSACCHARIDES.

I. Starch $(C_6H_{10}O_5)_n$.—The n is not less than 4, and may be 10 or 20; indeed, some place the formula at 200 ($C_{12}H_{20}O_{10}$). Starch is one of the most widely distributed substances in plants, where it occurs either (*a*) as a direct or indirect product of the assimilation of CO_2 in the leaves of the plant, or (*b*) as reserve material in the roots, seeds, or shoots.

1. (*a*) Examine (with microscope) potato starch; note that each starch-granule shows an eccentric hilum with concentric markings (oyster-shell) (fig. 9). Add a dilute solution of iodine. Each granule becomes blue, while the concentric markings become more distinct. (Iodide of starch.)



Fig. 9.—Potato Starch.

(*b*) Compare the microscopical characters of other starches—*e.g.*, rice, arrowroot, &c.

(*c*) Examine starch granules with a **polarisation** microscope. With

crossed Nicol's, when the field is dark, each granule shows a dark cross on a white refractive ground (fig. 10). They are doubly refractive. If a plate of mica be placed on the stage of the microscope under the starch grains, the latter, with polarised light, exhibit interference colours.

(*d*) Squeeze dry starch powder between the thumb and forefinger, and note the peculiar crepitation sound and feeling.

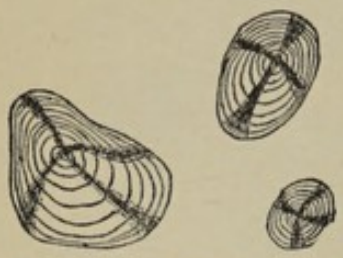


Fig. 10.—Potato Starch Granules.

View in polarised light with crossed Nicol's, × 300.

2. Solution of starch mucilage.—Rub up 1 grm. starch in a mortar. Pour the mixture into 50 c.c. boiling water. Boil, cool = solution of starch—*i.e.*, an opalescent, but not perfect, solution.

(*a*) Shake dry starch with cold water. It is insoluble. Filter, and test the filtrate with iodine. It gives no blue colour.

(*b*) Boil starch with water = opalescent solution, which if strong sets on cooling = starch paste or mucilage.

(*c*) Add iodine solution = a blue colour, which disappears on heating (iodide of starch is dissociated by heat) and reappears on cooling—provided it has not been boiled too long. Cool the test-tube with cold tap water.

(*d*) Make the starch solution alkaline (+ NaHO solution). Add iodine = no blue colour.

(*e*) Acidify with a dilute acid, then add iodine = blue colour.

(*f*) Starch does not give Trommer's or Fehling's test.

(*g*) Tannic acid = precipitate, which dissolves on heating.

(*h*) Starch or its iodide is precipitated by some neutral salts —*e.g.*, Am_2SO_4. (See Lesson III.)

(*i*) Place strong starch solution in a dialyser or parchment tube in distilled water. Suspend either for some time, and test the diffusate for starch. Result negative. Starch is a *colloid*.

3. Conversion of starch into glucose.—Boil starch solution with a few drops of 20 p.c. H_2SO_4 until the fluid is clear. Neutralise with Na_2CO_3; test for glucose. A large number of intermediate products are formed (see "Saliva"). They are—

Starch, ...	Blue with iodine.
Soluble starch (amidulin or amylodextrin),	
Varieties of Dextrin { Erythrodextrin, . . .	Iodine gives violet and red.
{ Achroodextrin, . . .	No reaction with iodine.
Maltose, ...	Fehling's solution reduced. Barfoed's not.
Dextrose, ...	Both are reduced.

4. Hydration of starch.—Successive stages in the hydration of starch by the action of any of the following processes :—Acids, heating water under pressure, enzymes or ferments (after Novy).

Substance.	Iodine.	Fehling.	Barfoed.	Taste.
Starch, . . .	blue.	0	0	Tasteless.
Soluble starch, .	,,	0	0	,,
Erythrodextrin, .	red.	+ very slight.	0	,,
Achroodextrin, .	0	+ slight.	0	,,
Maltodextrin, . .	0	0	0	Sweetish.
Isomaltose, . .	0	+	0	Sweet.
Maltose, . . .	0	+	0	,,
Dextrose, . . .	0	+	+	,,

II. Dextrin (British Gum) ($C_6H_{10}O_5$) is an intermediate product in the hydration of starch. There are several varieties—*e.g.*, **erythrodextrin**, which gives a red colour with iodine; and **achroodextrin**, which gives no colour with iodine solution (see "Saliva"). Commercial dextrin is made by heating dry starch to 200° C.

Note its fawn-colour. It is gummy and amorphous. Smell it. Dissolve some in boiling water; the solution is not opalescent.

(*a*) Add iodine solution = reddish-brown colour, which disappears on heating and returns on cooling. Make a control test with water.

(*b*) Make it alkaline by NaHO. No red-brown colour with iodine. Acidify and the reddish-brown colour appears.

(*c*) Alcohol = precipitate.

(*d*) Tannic acid = no precipitate (distinction from starch, gelatin, albumin).

(*e*) Not precipitated by basic lead acetate alone (unlike glycogen).

(*f*) Precipitation occurs on adding ammonia and basic lead acetate. The ammonia gives a white precipitate with lead acetate, which carries down dextrin.

(*g*) Saturate the solution or the iodide with Am_2SO_4 = precipitate.

III. Glycogen or Animal Starch $n(C_6H_{10}O_5)$.—Prepare a solution (see "Liver").

(*a*) The solution is *opalescent* (unlike dextrin); add iodine

solution = red-brown or port-wine red colour. As in the dextrin test, use a control test. Take two test-tubes, one with water and the other with glycogen; add I., and compare the difference in colour. The colour disappears on heating and reappears on cooling. It also disappears on the addition of alkalies, which break up the feeble compound.

(*b*) Trommer's test = a blue solution, boil = no reduction.

(*c*) Add basic lead acetate (boil plumbic acetate with litharge for 10 minutes, and filter) = a precipitate (unlike dextrin).

(*d*) Add ammonia and basic lead acetate = a precipitate.

(*e*) Boil with dilute HCl = a reducing sugar. Neutralise with dilute NaHO, and test with Fehling's solution for dextrose.

(*f*) The solution is precipitated by alcohol (2 parts absolute alcohol to 1 part of the solution).

(*g*) Saturate the solution or iodide with Am_2SO_4 = flocculent precipitate.

(*h*) Heated with potash or acetic acid the opalescence diminishes, and the solution becomes clear.

(*i*) It does not reduce Barfoed's reagent.

Precipitation of carbohydrates by neutral salts.—The ordinary crystalline carbohydrates are not precipitated by these salts, but the colloid varieties are precipitated quite as easily as proteids.*

(*a*) Starch paste, soluble starch, and glycogen are precipitated by saturation with Am_2SO_4 or $MgSO_4$, but not by NaCl.

(*b*) The corresponding soluble iodide compounds are, as a rule, more readily precipitated.

As saturation of a glycogen solution with Am_2SO_4 or $MgSO_4$ precipitates all the glycogen, it can thus be distinguished from erythrodextrin, which is not so precipitated.

Additional Exercises.

1. **Preparation of maltose.**—Make potato-starch paste (1 : 10 water); digest with a filtered extract of low-dried malt (200 grms. to 1 litre of water) for an hour at 57° to 60° C., filter, evaporate, precipitate the dextrin with alcohol, concentrate the filtrate to a syrup, and allow the maltose to crystallise.

2. **Preparation of lactose** ($C_{12}H_{22}O_{11} + H_2O$).—Acidulate milk with acetic acid = precipitate of caseinogen and fat; filter; boil filtrate to precipitate albumin, and filter again; evaporate the filtrate to small bulk; set aside to crystallise.

* Young, *Journ. of Phys.*, xxii., 401, 1898.

Milk-sugar is soluble in 6 parts of cold and 2½ parts of hot water, but not in alcohol.

3. **Dextrin from starch.**—Make 10 grms. of starch into a paste with 20 c.c. of water, add 30 c.c. of a 20 p.c. solution of H_2SO_4. Mix and heat in a water-bath at 90° C. Cool and precipitate the dextrin by alcohol. Collect the white deposit, wash with alcohol, and dry it.

4. **Invert-sugar**—a mixture of grape-sugar and fruit-sugar—is widely distributed throughout the vegetable kingdom, and is so called because it rotates the plane of polarised light to the left, the specific rotatory power of the lævulose being greater than that of dextrose at ordinary temperatures.

5. **Cellulose**, $(C_6H_{10}O_5)n$, is a polysaccharide, and occurs in the tissues of the higher plants, where it forms the walls of cells, and the great mass of the hard parts of wood. Cotton-wool may be used to test its reactions.

(*a*) Insoluble in water and all the feebler solvents.
(*b*) Soluble in Schweitzer's reagent, or a solution of ammonio-cupric oxide. Dissolve slips of copper in ammonia in an open flask, or dissolve precipitated hydrated oxide of copper in 20 p.c. ammonia. The former is prepared by precipitating a solution of sulphate of copper by soda in the presence of ammonium chloride.
(*c*) Soluble in concentrated acids, and a gelatinous precipitate—called amyloid—falls on the addition of water. The substance precipitated gives a blue colour with iodine. It is also soluble in zinc chloride.
(*d*) Gives a blue colour with H_2SO_4 and iodine, but not with the latter alone.

Polarimeters—Circumpolarisation.—Certain substances when dissolved possess the power of rotating the plane of polarised light—*e.g.*, the proteids, sugars, &c. The extent of the rotation depends on the amount of the active substance in solution. The direction of rotation—*i.e.*, to the right or the left—is constant for each active substance. Light of the same wave-length must be used. The light obtained from the volatilisation of common salt or borax is used.

The term "specific rotatory power," or "specific rotation" of a substance, is used to indicate the amount of rotation expressed in degrees of the plane of polarised light which is produced by 1 grm. of the substance dissolved in 1 c.c. of liquid, when examined in a layer 1 decimetre thick.

Those substances which cause specific rotation are spoken of as "*optically active;*" those which do not, as "*inactive.*"

If a = the observed rotation;
p = the weight in grms. of the active substance contained in 1 c.c. of liquid;
l = the length of the tube in decimetres;
$(a)_D$ = the specific rotation for light corresponding to the light of a sodium flame;
then

$$(a)_D = \pm\frac{a}{pl}$$

The sign + or − indicates that the substance is dextro- or lævo-rotatory. Various instruments are employed. Use

Laurent's Polarimeter, which is a so-called "half-shadow polarimeter." It is used in a dark room (fig. 11).

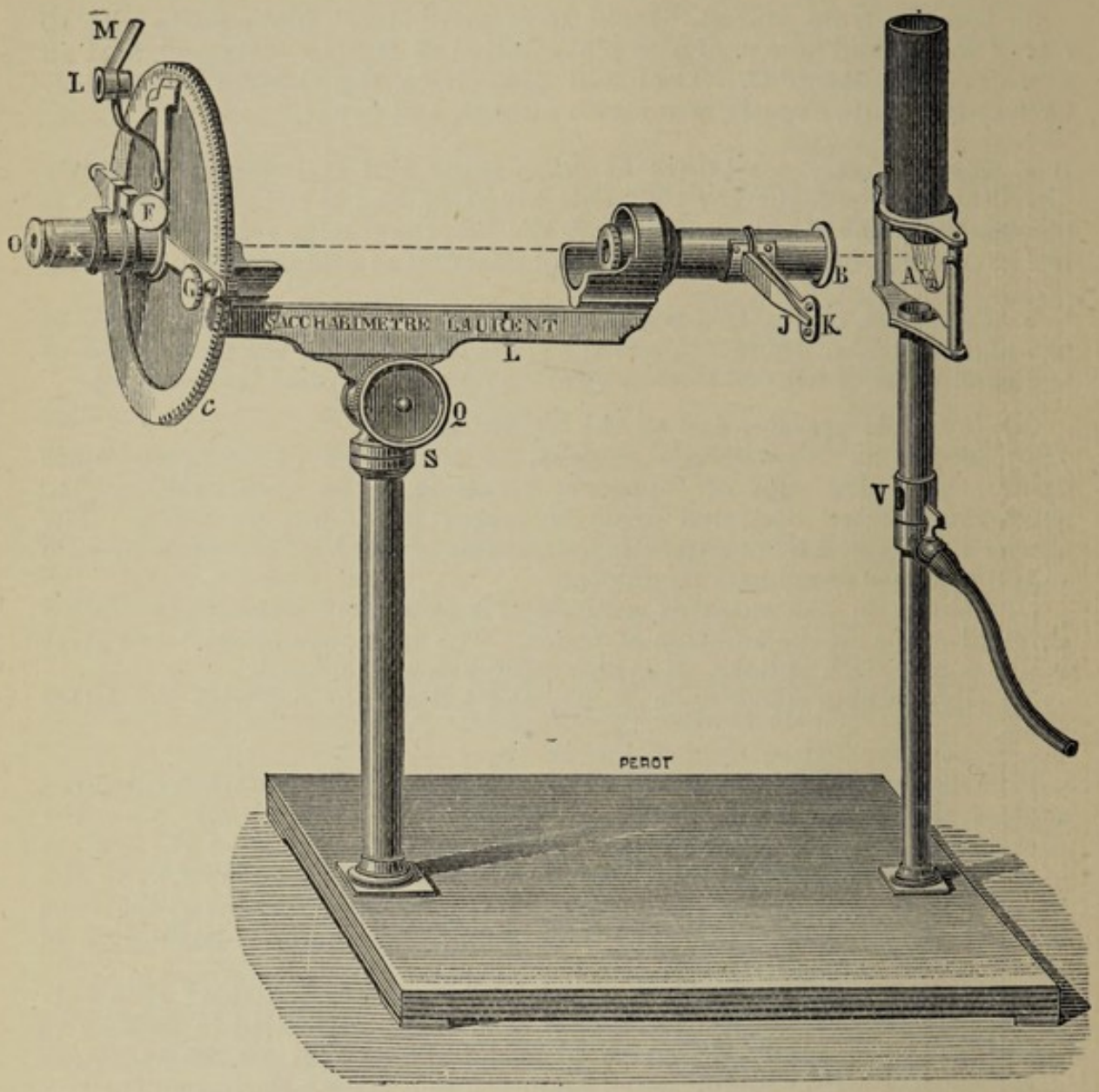


Fig. 11.—Laurent's Half-Shadow Polarimeter.

Specific Rotatory Power of Dextrose.

(*a*) Fill one of the decimetre tubes with distilled water, taking care that no air-bubbles get in. Slip on the glass disc horizontally, and screw the brass cap on the tube, taking care not to do so too tightly. Place the tube in the instrument, so that it lies in the course of the rays of polarised light.

(*b*) Place common salt (or fused common salt and sodium carbonate) in the platinum spoon (A), and light the Bunsen's lamp, so that the soda is volatilised. If a platinum spoon is not available, tie several platinum wires together, dip them into slightly moistened common salt. In the newer form of the instrument there are two Bunsen burners, placed the one behind the other, which give more light.

(*c*) Bring the zero of the vernier to coincide with that of the scale. On

looking through the eye-piece (O), and focussing the vertical line dividing the field vertically into two halves, the two halves of the field should have the same intensity when the scale reads zero. If this is not the case, then adjust the prisms until it is so by means of the milled head placed for that purpose behind the index dial and above the telescope tube. It is well to work with the field not too brightly illuminated.

(*d*) Remove the water-tube, and substitute for it a similar tube containing the solution of the substance to be examined—*e.g.*, a *perfectly clear solution* of pure dextrose. Place the tube in position, and proceed as before. The two halves of the field are now of unequal intensity. Rotate the eye-piece until equality is obtained.

(*e*) Repeat the process several times, and take the mean of the readings. The difference between this reading and the first at (*c*), when the tube was filled with distilled water—*i.e.*, zero = is the rotation due to the dextrose = *a*.

(*f*) Place 10 c.c. of the dextrose solution in a weighed capsule, evaporate to dryness over a water-bath, let the capsule cool in a desiccator, and weigh again. The increase in weight gives the amount of dextrose in 10 c.c. ; so that the amount in 1 c.c. is got at once = *p*.

(*g*) Calculate the specific rotatory power by the above formula (about + 53°).

For practice, begin with a solution of dextrose containing 11 grms. per 100 c.c. of water. Make several readings of the amount of rotation, and take the mean.

Example.—In this case, the mean of the readings was 11·6°.

$$(a)_D = \frac{11{\cdot}6^\circ}{{\cdot}11 \times 2} = 53^\circ.$$

Repeat the process with a 4 and 2 p.c. solution. It is necessary to be able to read to 2 minutes, but considerable practice is required to enable one to detect when the two halves of the field have exactly the same intensity.

Test the rotatory power of corresponding solutions of cane-sugar, and any other sugar you please.

Test also the rotatory power of a proteid solution.

The following indicate the S.R. for yellow light :—

Proteids.—Egg-albumin – 35·5° ; serum-albumin – 56° ; syntonin – 72° ; alkali-albumin prepared from serum-albumin – 86°, when prepared from egg-albumin – 47°.

Carbohydrates.—Glucose + 56° ; maltose + 150° ; lactose + 52·5°.

N.B.—A complication may arise in connection with carbohydrates, as the S R. is sometimes much altered by the temperature ; thus the S.R. of lævulose, when heated from 20° to 90° C., falls in the proportion of 3 : 2. It is best, therefore, to work at a constant temperature, say 20° C. Again, some solutions have not the same S.R. when they are first dissolved that they have 24 hours afterwards. This is called birotation, and it is therefore well to use the solution 24 hours after it is made.

LESSON IV.

FATS—BONE—EXERCISES ON THE FOREGOING.

NEUTRAL FATS.

Fat occurs in adipose tissue; also in considerable quantity in the yellow marrow of bones, and in milk, and in small quantity in many animal cells. The fats of adipose tissue consist of a mixture of the neutral fats **stearin, palmitin,** and **olein,** the former two being solid at ordinary temperatures, while olein is fluid. It keeps the other two in solution at the temperature of the body (37° C.). The fats differ amongst themselves in constitution, solubility, and melting point; stearin melting at 53° to 66°, palmitin at 45°, and olein at −5° C.

Neutral fats are derivatives of the triatomic alcohol glycerin,

$$\left.\begin{matrix} C_3H_5 \\ H_3 \end{matrix}\right\} O_3,$$

and are glycerides or compound ethers of palmitin, stearin, and olein, in which three of the hydrogen atoms of the glycerin are replaced by as many equivalents of the acid radical.

I. Reactions.—(*a*) Lighter than water; S.G., ·91 to ·94.

(*b*) Almond or olive oil or lard are *soluble* in ether, chloroform, and hot alcohol, but *insoluble* in water.

(*c*) Dissolve fat in ether. Let a drop of the solution fall on paper, *e.g.*, cigarette (not filter) paper = a greasy stain, which does not disappear with strong heat.

(*d*) To olive oil or suet add KHO, and boil. Stearin is present in the suet and is glycerin-stearate, while olein in olive oil is glycerin-oleate. When stearin is boiled with a caustic alkali—*e.g.*, potash—a potassic stearate or soap is formed, and glycerin is set free. This is the process of **saponification.**

$$\underset{\text{Tri-stearin.}}{\left.\begin{matrix} 3C_{18}H_{35}O \\ C_3H_5 \end{matrix}\right\} O_3} + \underset{\text{Potash.}}{3\left.\begin{matrix} K \\ H \end{matrix}\right\} O} = \underset{\text{Potassic stearate (Soap).}}{3\left.\begin{matrix} C_{18}H_{35}O \\ K \end{matrix}\right\} O_3} + \underset{\text{Glycerin.}}{\left.\begin{matrix} C_3H_5 \\ H_3 \end{matrix}\right\} O_3}.$$

(*e*) Heat lard and NaHO solution in a capsule to form a soap ; decompose the latter by heating it with dilute H_2SO_4 ; the liberated fatty acids float on the top.

(*f*) Proceed as in (*d*), and add to the soap solution crystals of sodium chloride until the soaps separate.

(*g*) Dissolve by gentle heat in a water-bath a piece of fat—*e.g.*, suet or lard—size of a pea, in 5 c.c. of a mixture of equal parts of ether and alcohol. Cool, and examine microscopically the crystals which separate out. Sketch them. Note in which the crystals separate more rapidly.

(*h*) Dissolve suet in 5 c.c. alcohol. Test the reaction with blue litmus paper, or, better, add a drop of alcoholic solution of rosolic acid = yellow colour, indicating an acid reaction due to a free fatty acid. Why? Fats become rancid on standing, due to liberation of free fatty acids.

(*i*) To a few c.c. alcohol add a few drops ·04 p.c. NaHO, then rosolic acid or phenolphthalein solution to give a red colour. Fresh fat dissolved in ether added gives no change in colour, so that *fresh* fat is neutral in reaction.

(*j*) Shake oil containing a fatty acid—*e.g.*, De Jongh's cod-liver oil—with a few drops of a dilute solution of sodium carbonate, The whole mass becomes white = **emulsion**. Examine it microscopically, and compare it with milk, which is a typical emulsion.

In a perfect **emulsion** of oil the particles are broken up into finer particles, which do not run together again.

(*k*) Shake up olive oil with a (i.) solution of albumin in a test-tube = an emulsion. Examine it microscopically. (ii.) 1 p.c. sodium carbonate, (iii.) alkali-albumin, (iv.) acid-albumin. Test how long, hours or days, each emulsion lasts. It will be found that alkali-albumin is the most potent in conserving the emulsion (Moore and Krumbholz, *Journ. of Phys.*, 1898).

(*l*) **Gad's emulsion experiment.**—Place in a watch-glass a solution of sodium carbonate (·25 p.c.), and on the latter a drop of rancid oil. The drop comes to rest, but soon the oil drop shows a white rim, and at the same time a white milky opacity extends over the soda solution. With the microscope, note the lively movement in the neighbourhood of the fat droplet, due to the separation of minute particles of oil. The white fluid is a fine and uniform emulsion (fig. 12). This experiment has an important bearing on the formation of an emulsion in the intestine in connection with the pancreatic digestion of fats.



Fig. 12.—Gad's Experiment.

(*m*) **Ranvier's emulsion experiment.**—To a drop of lymph taken from the peritoneal cavity of a frog, add a drop of olive oil. On examining with

a microscope where the two fluids come into contact, one sees the process of emulsification due to the formation of fine particles of oil like the molecular basis of chyle (*Comptes rendus*, 1894). Lesson X. 5 (*d*).

(*n*) Heat (one hour) in a porcelain capsule lard mixed with plumbic oxide and a little water. The fat is split up, yielding glycerin and a lead-soap.

BONE.

2. Roughly the solids of bone consist of one-third organic and two-thirds inorganic or mineral matter. The chief salts of the latter are calcium phosphate (84 p.c.) and calcium carbonate (13 p.c.), with smaller quantities of other salts.

(A) **Organic basis**—(*a*) **Decalcify bone.**—Place a small bone in dilute HCl (1 : 8) for a few days. Its mineral matter is dissolved out; it retains its original form, loses its rigidity, and becomes pliable and so soft that it can be cut with a knife. What remains is the **organic matrix** or **ossein.** Keep the solution obtained.

(*b*) Wash the decalcified bone thoroughly with water, then with solution of Na_2CO_3 and again with water. Boil in water; it yields gelatin. Neutralise with Na_2CO_3. The solution gelatinises. Test the solution for gelatin (Lesson II. 1).

(B) **Mineral matter in bone.**—(*a*) Examine the successive stages produced by incinerating a bone in a clear fire. At first the bone is black from the carbon of its organic matter, but ultimately it is a white brittle **calcined bone,** having the form of the original bone. Powder some of the white bone-ash. If incineration be carried out without free access of air, a black mass of animal charcoal or "bone-black" remains.

(*b*) Dissolve bone-ash in HCl or HNO_3; bubbles of gas (CO_2) are given off, indicating the presence of a carbonate. Dilute the solution, add excess of ammonia = a white precipitate of **phosphate of lime** and **phosphate of magnesia.**

(*c*) Filter, and to the filtrate add ammonium oxalate = a white precipitate of oxalate of lime, showing that there is lime present, but not as a phosphate.

(*d*) To the solution of mineral matters 2 (A) (*a*) add sodium acetate until free acetic acid is present (smell); add ammonium oxalate = a copious white precipitate of lime salts.

(*e*) Use solution of mineral matters obtained in 2 (A) (*a*). Add NH_4HO to alkalinity = copious precipitate; add acetic acid, which dissolves all except a small flocculent residue of phosphate of iron (perhaps in part derived from the blood of bone). Filter; use a part to test for phosphoric acid and the rest for calcium and magnesium (Filtrate A).

(i.) The undissolved flocculent precipitate is washed and dissolved in a few c.c. dilute HCl. Test for *iron oxide* by ferrocyanide of potassium (= blue), and *phosphoric acid* by molybdate of ammonium (see "Urine").

(ii.) With the filtrate A test for *phosphoric acid* by uranium acetate = yellowish-white precipitate of uranium phosphate, $(UrO_2)HPO_4$.

(iii.) *Calcium*, by ammonium oxalate = $Ca_2C_2O_4 + H_2O$. Filter, and when the filtrate is clear and gives no precipitate with ammonium oxalate, make it alkaline with NH_4HO = after a time crystalline precipitate of ammonio-magnesium phosphate, $MgNH_4PO_4 + 6H_2O$, showing presence of *magnesium*.

(*f*) *Bone-black.*—Heat a light blue solution of indigo with bone-black and filter. The filtrate will be clear, showing the great absorptive power of bone-black for colouring matter. (See "Urine.")

EXAMINATION OF A SOLUTION FOR PROTEIDS AND CARBOHYDRATES.

I. Appearance.

(*a*) Note and record **transparency, opacity,** or **viscidity.** Glycogen solution is opalescent; starch and some proteid solutions less so.

(*b*) **Taste.** Salt solution may contain globulin. A sweet taste indicates a sugar.

(*c*) **Smell.** The beef-tea odour of albumose and peptone solution, and the smell of dextrin are characteristic.

(*d*) **Reaction** to litmus paper.

(*e*) **Other characters.** Thus a persistent froth is suggestive of an albuminous solution.

[Concentrate a portion. Let some evaporate nearly to dryness, but complete the evaporation on a water-bath.]

II. Test for proteids by xanthoproteic, Millon's, and Piotrowski's tests. Do not depend on a single test. Always use, if possible, confirmatory tests. If a yellow colouration instead of a precipitate is obtained, suspect gelatin or pure peptone. Confirm by other tests. If proteids are present:

1. Test **reaction** to litmus paper. If acid or alkaline, test for **acid-** or **alkali-albumin,** and if either is present, **neutralise** with weak Na_2CO_3 or 1 p.c. acetic acid and filter off precipitate. Test filtrate for proteoses and peptones as in 4 and 5.

2. If original solution is neutral, acidulate faintly and **boil.** A coagulum may consist of native albumin or globulin, or both. Filter; and test filtrate for proteoses and peptones as in 4 and 5.

3. Distinguish between albumin and globulin by (*a*) dropping solution into water, precipitate indicates **globulin,** (*b*) saturating

solution with $MgSO_4$, precipitate = **globulin**, but may also contain proto- and hetero-albumose. If precipitate obtained by (*b*), filter and boil filtrate, coagulum = **native albumin.** Distinguish between egg- and serum-albumin by ether test.

4. Add excess of NaHO, then very dilute $CuSO_4$, pink colour indicates proteoses or peptones, or both.

5. Separate proteoses from peptones by saturating solution with Am_2SO_4. Precipitate = **proteoses.** Filter; and to filtrate add *large excess* of solution of NaHO, then dilute $CuSO_4$. Pink colour indicates **peptones.**

[6. **Gelatin** (albuminoid) gives **xanthoproteic** (modified) and Millon's reactions, gives a *violet* colour with NaHO and $CuSO_4$, is not coagulated by boiling, and is not precipitated by acetic acid and potassium ferrocyanide.]

III. Test for Carbohydrates. First remove derived albumins by neutralising and filtering, and native albumin and globulin by boiling and filtering.

1. Acidulate if necessary and add iodine.

(*a*) *Blue* colour, disappearing on heating and returning on cooling, indicates **starch.**

(*b*) *Mahogany-brown* colour, disappearing on heating and returning on cooling, indicates **glycogen** or **dextrin.** Add basic lead acetate, precipitate (if proteids are absent) = **glycogen.**

2. Test for **reducing sugar** by Trommer's test. If present, distinguish glucose, maltose, and lactose by the phenyl-hydrazine test, and use Barfoed's reagent.

3. If no starch, dextrin, glycogen, or reducing sugar, examine for cane-sugar by inversion test.

Record your results as follows :—

Experiment.	*Observation.*	*Result and Inference.*

LESSON V.

BLOOD—COAGULATION—ITS PROTEIDS.

BLOOD contains about 77 p.c. of water and 23 of solids. Of the latter hæmoglobin is about 14 p.c., proteids 7 to 8, salts 1·5, and extractives—*e.g.*, sugar 0·1, and urea 0·2 p.c.

1. Reaction.—Constrict the base of the middle finger by means of a handkerchief. When the finger is congested, with a clean sewing needle or triangular saddler's needle prick the skin at the root of the nail. Or prick the lobe of the ear. Touch the blood with a strip of dry, smooth, *glazed*, neutral litmus paper. After 15 seconds wash off the blood with distilled water, when a blue spot upon a red or violet ground will be seen, indicating an *alkaline* reaction, due chiefly to Na_2HPO_4 and sodium carbonate.

2. Colour.—Arterial is scarlet, and venous dark crimson or purplish.

3. Opacity.—(*a*) Try to read print through a thin layer of defibrinated blood on a glass slide.

(*b*) **Transparent or laky blood.**—Place 5 c.c. defibrinated blood in each of three test-tubes, A, B, and C (A for comparison). To B add 5 vols. water, and warm slightly. Note the change of colour by reflected and transmitted light. By reflected light it is darker, but by transmitted light it is transparent. Test this by looking at printed matter. To C add solution of sodium taurocholate or bile. Test the transparency.

4. Specific gravity of blood.—(*a*) A number of solutions of sulphate of soda, varying in S.G. from 1·035 to 1·070 are ready. At least twenty separate solutions are required, each with a definite S.G., or use a series of stock standard solutions (S.S.) of glycerin and water with S.G. varying from 1035 to 1070 by difference of 5 degrees (*Roy, Lloyd Jones*). The average in health is about 1055 to 1060. Pour a small quantity of each of the solutions into glass thimbles. A thin glass tube is drawn out in a gas-flame to form a capillary tube, which is bent at *a right angle*, and closed above with a small caoutchouc cap. A drop of blood is obtained from a finger, and by pressing lightly on the caoutchouc cap a quantity of the freshly-shed blood is drawn up into the capillary part of the tube. The tip of the fine capillary tube is at once immersed in one of the solutions of sodium sulphate, and a drop of the blood expressed into the saline solution, and it is noted whether it sinks or floats. The operation is repeated with other solutions until one is found in which the blood neither sinks nor floats. The S.G. of blood varies from 1045 to 1075, the average S.G. being 1056 to 1059 (*Journ. of Phys.*, v., 1884).

(*b*) Other methods.—Hammerschlag (1892) uses a mixture of chloroform and benzol, and Haycraft a mixture of toluol (S.G. 800) and benzyl chloride (S.G. 1100) to obtain a fluid with S.G. of 1070. Label this A. Make another with the S.G. 1025. Label this B.

Method.—With a pipette place a measured quantity of A in a warm cylindrical glass. Add a drop of the blood. It will float; now add B until the blood neither floats nor sinks. Suppose 1·5 c.c. of B has been added to 1 c.c. of A, then

1 c.c. of A (1070) =	1070
1·5 c.c. of B (1025) =	1537
2·5 c.c.	2607

Divide this by the total volume 2·5 c.c. = 1043, the S.G. of the blood.

5. Action of a saline solution.—To 2 c.c. blood in a test-tube (D) add 5 vols. of 10 p.c. NaCl solution. It changes to a very bright brick-red colour. Compare colour with A, B, C. It is opaque.

6. Hæmoglobin does not dialyse.—Place a watery solution of blood in a dialyser (bulb form or parchment tube), and suspend it in distilled water. Test the dialyser beforehand to see that there are no holes in it.

(*a*) After several hours no Hb has passed into the water.

(*b*) Test diffusate for chlorides (HNO_3 + $AgNO_3$).

7. Defibrinated blood and fibrin.—At a slaughter-house allow blood to run into a vessel; with a bundle of twigs beat or whip the blood steadily for some time. White fibres of **fibrin** collect on the twigs, while the blood remains fluid. This is **defibrinated blood**, which does not coagulate spontaneously. Wash away all the colouring-matter from the fibrin in water.

(*a*) Fibrin is white, fibrous, and elastic. Stretch some fibres (extensibility); on freeing them they regain their shape (elasticity).

(*b*) Add 0·2 p.c. HCl *in the cold.* It swells up, becomes transparent, but does not dissolve. Place the test-tube in a water-bath at 60° C.; part of the fibrin is dissolved, forming acid-albumin. Test for the latter (Lesson I. 7).

(*c*) Pour hydric peroxide over fibrin in a watch-glass; bubbles of oxygen are given off. Boiled fibrin does not do so. Is result due to presence of leucocytes, and not to the fibrin itself? Place a flock in freshly-prepared alcoholic tincture of guaiacum (5 p.c. resin), and then in hydric peroxide, when a blue colour is developed, due to the ozone liberated by the fibrin striking a

blue with the resin. If the fibrin contains much water, it is preferable to place it first of all for a short time in rectified spirit to remove the water. [Other substances give a blue colour under similar conditions.]

(*d*) Place some fibres in alcohol to rob them of water. They lose their elasticity.

(*e*) Fibrin gives the xanthoproteic reaction and Millon's test (Lesson I.).

(*f*) Fibrin is partly dissolved by the prolonged action of 5 p.c. NaCl, 2 p.c. sodium fluoride, and similar neutral salts. Test the fluid for proteids after 24 hours.

8. Clotting or coagulation of blood.—(*a*) Decapitate a rat, and allow the blood to flow into a watch-glass or thimble. Within a few minutes the blood **congeals**, and when the vessel is tilted the blood no longer moves as a fluid, but as a **solid**. It then **coagulates** completely. Allow it to stand, and after an hour or so, pale-yellow coloured drops of fluid—**blood-serum**—are seen on the surface, being squeezed out of the red mass, the latter being the **clot**, which consists of fibrin and the corpuscles.

(*b*) Examine coagulated blood from the slaughter-house—*i.e.*, the blood of a sheep or ox has been collected in a perfectly dry cylindrical vessel, and allowed to coagulate. Place it on ice for a day. Observe the **serum** and the **clot**. Pipette off the pale, straw-coloured serum, and note the red clot, which has the shape of the vessel, although it is smaller than the latter. The clot consists of the new formed body, fibrin, entangling the blood-corpuscles.

(*c*) If the blood of a horse can be obtained, study it, noting that the upper layer of the clot is paler in colour; this is the **buffy coat**.

9. Blood-serum is blood plasma minus the substances that go to form fibrin. It contains two proteid bodies—**serum-albumin** and **serum-globulin** (or para-globulin)—and an allied substance, the **fibrin ferment**. The proteids are approximately 7 p.c.—*i.e.*, 4 p.c. serum-albumin and 3 p.c. serum-globulin.

Pipette off the serum from coagulated blood. If a centrifugal apparatus is available, any suspended blood-corpuscles can easily be separated by it (fig. 77). Note its colour and musky odour. Ox-serum is yellowish-red, human is yellow. Its reaction *alkaline*. S.G. = 1028 to 1034.

(*a*) **General proteid reactions.**—Dilute 10 c.c. serum to 70 c.c. with water. Usually a slight cloudiness occurs due to precipitation of a small amount of serum-globulin.

(*b*) Test separate portions by neutralisation and heat = coagulation; xanthoproteic reaction—*i.e.*, HNO_3 and the sub-

sequent addition of ammonia; acetic acid and ferrocyanide of potassium; Millon's reagent; and the NaHO and $CuSO_4$ reaction (Lesson I.) Alcohol causes precipitation.

(*c*) **Coagulation temperature.**—Heat diluted serum in a water bath (Lesson I.) to 80° C. (thermometer in tube). The proteids are not all coagulated (fluid is alkaline). The fluid only becomes opalescent. Acidulate and heat again; the coagulation begins at 73° or 75° and is complete about 80°, a clear fluid being obtained.

10. Proteids of Serum.—A. Serum-globulin (or para-globulin).

(*a*) *Hammarsten's method.*—Saturate serum with crystals of $MgSO_4$. Serum-globulin is precipitated. The undissolved crystals fall to the bottom, and on their surface is precipitated a dense white flocculent mass of serum-globulin. Filter. Wash the precipitate on the filter with a saturated solution of $MgSO_4$; add a little distilled water to the precipitate; it is dissolved, as it is a globulin. The solution does not coagulate spontaneously. It gives all the reactions for proteids with the special reactions of a globulin. Determine the coagulation temperature of the dilute $MgSO_4$ solution (75° C.).

(*b*) *Kauder's method.*—Add to serum half its volume of a saturated solution of Am_2SO_4 (*i.e.*, half saturate it) = serum-globulin. [Filter. Saturate the filtrate with crystals of Am_2SO_4 = precipitate of serum-albumin. Filter. Test filtrate for proteids. Negative result.]

This method enables one rapidly to separate globulin and then the albumin by the use of one salt.

(*c*) Let a few drops of serum fall into a large quantity of distilled water. Observe the milky cloud = precipitated serum-globulin (black surface behind).

(*d*) Saturate with Am_2SO_4. This precipitates all the proteids, globulin and albumin. Filter; the filtrate is proteid-free. Test. It contains no peptones.

B. Serum-albumin.—From **10 A.**, (*a*) or (*b*), filter off the precipitate, and test the filtrate for the usual proteid reactions. The filtrate still contains a proteid—serum albumin (Lesson I.). To the filtrate add Na_2SO_4, when serum-albumin is precipitated. Na_2SO_4 alone, however, gives no precipitate with pure serum.

C. Fibrin-ferment.—(*a*) Add to blood-serum a large excess of alcohol, collect the copious precipitate—consisting of the proteids and fibrin-ferment. Cover it with absolute alcohol, and allow it to stand at least a

month, when the proteids are rendered insoluble. Dry the precipitate at 35° C., and afterwards over sulphuric acid (fig. 13). Keep it as a dry powder in a well-stoppered bottle. When a solution is required extract some of the dry powder with 100 volumes of water; filter. The filtrate contains the ferment.

(*b*) Or soak washed fibrin in 5 p.c. NaCl solution for some hours in a water-bath at 40° C. Strain; the filtrate contains fibrin-ferment.

11. Precipitation of serum proteids by other salts.

(*a*) Precipitate serum with potassium phosphate. All the proteids are thrown down after prolonged shaking.

(*b*) Precipitate serum with magnesium sulphate and sodium sulphate, or the double salt sodium magnesium sulphate. All proteids are thrown down. The filtrate should give no proteid reactions. No peptone occurs in blood.

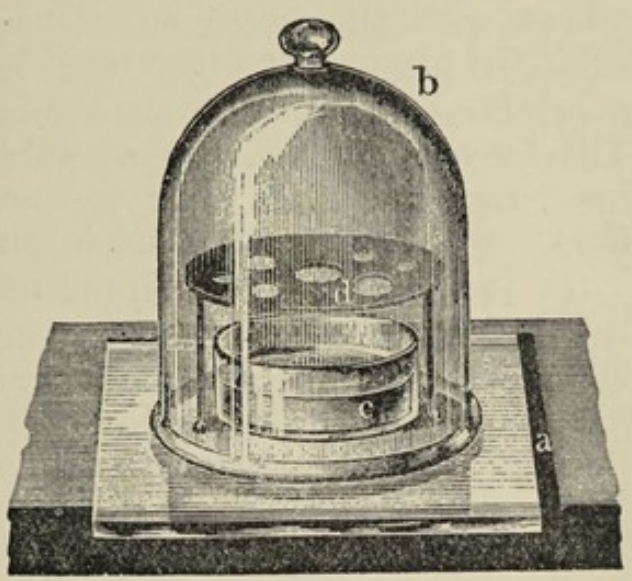


Fig. 13.—Desiccator for drying a precipitate over H_2SO_4.

12. Coagulation temperature of serum-proteids. — Saturate serum with $MgSO_4$. Filter, keep the filtrate, label it B. Wash the precipitate—*i.e.*, the **serum-globulin**—with saturated solution of $MgSO_4$ until the washings give no reaction for albumin. This takes a long time, and had better be done previously by the demonstrator. Dissolve the precipitate in distilled water = an opalescent solution. Label it A. Acidify it slightly with a drop of 2 p.c. acetic acid, and determine the temperature at which it coagulates (75° C.). The liquid in the test-tube should just cover the bulb of the thermometer.

The filtrate B contains **serum-albumin.** Dilute with an equal volume of water, faintly acidify and heat, as above. A precipitate falls about 77° to 79° C., and on filtering this off, and again acidifying, another precipitate is obtained on heating to 84° to 86° C. ("Fractional heat-coagulation").

Blood plasma and its proteids.—It contains **serum-albumin, serum-globulin** (like blood-serum), but, in addition, a small amount of **fibrinogen.** It coagulates spontaneously, so that means must be taken to keep it fluid. The following methods are important:—

13. Salted plasma—Neutral salts.—At the slaughter-house allow blood to run into an equal volume of saturated solution of Na_2SO_4 (or one-quarter of its volume of a saturated solution of

$MgSO_4$); mix. The blood does not clot, but remains fluid. Place the vessel on ice, and note that the corpuscles subside, leaving a narrow clear yellowish layer on the surface—plasma mixed with the saline solution—"**salted plasma.**" To obtain sufficient plasma the blood must be "centrifugalised" (fig. 77).

(*a*) Heat undiluted "salted plasma" in a water-bath to 60° C. The fibrinogen coagulates at 56° C. Filter. The filtrate does not coagulate, even after the addition of fibrin-ferment and $CaCl_2$, as there is no fibrinogen present.

(*b*) Place 15 c.c. of salted plasma in a tall, narrow, cylindrical, stoppered glass vessel. Add crystals of NaCl, and shake vigorously; a white flocculent precipitate is formed. Allow the precipitate to subside. Decant the supernatant fluid. Filter through a filter moistened with a *saturated* solution of NaCl. Wash the precipitate on the filter with a saturated solution of NaCl. This is the **plasmine** of Denis. With a spatula scrape the washed precipitate off the filter.

Dissolve the plasmine in a small quantity of distilled water, and filter quickly. The filtrate, if set aside, will ultimately clot. Do the several operations rapidly.

(*c*) **Fibrinogen.**—Half saturate plasma with NaCl—*i.e.*, add an equal volume of saturated solution of the salt. Fibrinogen is precipitated. Filter. To the precipitate add a little water; this forms a dilute saline solution which dissolves the fibrinogen. Put some of the fibrinogen solution in a warm bath at 40° C. It may clot.

(*d*) To diluted salted plasma add fibrin-ferment. Place in a warm bath. Clotting takes place more quickly than without fibrin-ferment.

14. Oxalated plasma or Decalcified plasma.—Potassium oxalate prevents blood from coagulating when present to the extent of 0·2 p.c. Dissolve 1 grm. in 10 to 20 c.c. normal saline, place it in a vessel capable of holding 500 c.c., and allow blood from a slaughtered animal to run in to fill the vessel; or allow blood —pigs' blood is very good—to run into one-fifth of its volume of a 1 p.c. solution of potassium oxalate (*Arthus* and *Pagès*). Mix the two fluids. The blood usually does not coagulate, but remains fluid. I have sometimes found it coagulated after standing for some time. Centrifugalise it to obtain oxalated clear plasma without corpuscles, which may be siphoned off. The oxalate precipitates—as oxalate of lime—the calcium which is necessary for coagulation.

(*a*) To oxalated plasma add a few drops of 2 p.c. $CaCl_2$ solution = coagulation ; more quickly at 40° C.

(*b*) Prepare **fibrinogen**, as above, by half-saturation with NaCl. Dissolve the precipitated fibrinogen and divide the solution into two portions: (i.) Add a drop of $CaCl_2$; (ii.) do not add anything. Keep both at 40° C. The one to which $CaCl_2$ has been added clots ; the other does not. *Lime is necessary for clotting.*

15. Coagulation experiments.—(*a*) Place salted plasma into each of three tubes and dilute with about 10 volumes of liquid.

A. With distilled water.
B. With water, but add fibrin ferment.
C. With water, but add fibrin ferment and drop $CaCl_2$.

Keep at 40° C. in water-bath. B and C coagulate rapidly. A very slowly or not at all

(*b*) Add fibrin ferment to a fibrinogen solution prepared as in 13 (*c*) and observe effect (water-bath).

(*c*) Heat fibrin-ferment solution to 65° C. Its ferment is at once destroyed.

(*d*) To decalcified plasma add a few drops of $CaCl_2$ (at 40° C.) = coagulation.

(*e*) *Andrew Buchanan's experiment* (1836-45).—Mix 5 c.c. fresh **serum** (preferably horse's) with 5 c.c. **hydrocele fluid**, and keep at 35° C. ; coagulation occurs, a pellucid clot of fibrin being obtained.

(*f*) Add fibrin-ferment to 5 c.c. of hydrocele fluid, and keep in a water-bath (40° C.) ; coagulation takes place.

(*g*) To 2 c.c. salted plasma (which is known to clot slowly on the addition of water), add 10 volumes—*i.e*, 20 c.c. watery solution of fibrin-ferment = coagulation.

(*h*) Add to oxalate plasma a few drops of a 2 p.c. $CaCl_2$ solution. It coagulates ; more quickly at 40° C.

16. Hydrocele fluid does not coagulate spontaneously. Prepare **fibrinogen.**

(*a*) Half saturate with NaCl solution—*i.e.*, add to it an equal volume of saturated solution of NaCl. Fibrinogen is precipitated in small amount. Filter, and keep the filtrate.

(*b*) Dilute 10 c.c. with 150 to 200 c.c. water, and pass through it a stream of CO_2, when there is precipitated a small quantity of a somewhat slimy white body, fibrinogen. (Schmidt's method, 1861.)

(*c*) Dissolve the fibrinogen and add to it fibrin-ferment (water-bath 40° C.) = clot.

(*d*) To the filtrate (*b*) add $MgSO_4$ = precipitate of serum-globulin. Hydrocele fluid contains fibrinogen and serum-globulin. It does not coagulate, because the fibrin-ferment is absent.

17. Salts, sugar, and urea of serum.—The usual salts may be tested for directly with serum diluted with water (after removal of proteids) as follows:—Faintly acidulate with acetic acid, boil, filter off the coagulated proteids (traces remain in the filtrate); or the following:—

Dilute blood and boil it; filter.

Colourless filtrate, which can be tested for salts and sugar.	Coagulum coloured brown by hæmatin.

To ascertain the presence of substances dissolved in blood the proteids must be removed—*e.g.*, by boiling and faint acidulation with dilute acetic or sulphuric acid, pouring blood into 5 vols. absolute alcohol, or by adding an equal vol. of trichloracetic acid. Filter, and concentrate the filtrate by evaporation. Test for sugar. Urea requires special precautions. Peptones are absent.

The blood is heated with 6 to 8 times its volume of water, and slightly acidulated. The filtrate is evaporated to a small bulk. When a drop of the concentrated filtrate is placed on a slide, cubes of common salt separate out.

To the colourless filtrate of **17**—

(*a*) Add silver nitrate = white curdy precipitate soluble in ammonia, but insoluble in nitric acid = *chlorides*.

(*b*) Add barium chloride = white, heavy precipitate insoluble in nitric acid = *sulphates*.

(*c*) Add nitric acid and molybdate of ammonium and heat = yellow precipitate = *phosphates*.

(*d*) Test with Fehling's solution or $CuSO_4$ and NaHO and boil = red cuprous oxide = *reducing sugar*, which is glucose.

Additional Exercises.

18. Red corpuscles.—(*a*) To defibrinated ox blood (or, better, dog's blood), add 20 vols. dilute solution NaCl (·5 to 2 p.c.). The red corpuscles subside, and the supernatant fluid can be poured off. Wash the corpuscles several times in this way. They will be required for the preparation of hæmoglobin (Lesson VI.).

(*b*) A solution containing 0·6 to ·75 p.c. of NaCl in tap water is called "normal saline." It alters the tissues (frog) but slightly, and is said to be *isotonic* with them. For mammalian tissues a 0·9 p.c. solution is required.

(*c*) Put 2 drops of defibrinated blood (sheep) in each of four test tubes, and add to

(*a*) 5 c.c. normal saline (0·9 p.c.)
(*b*) ,, distilled water.
(*c*) ,, 2 p.c Na_2CO_3.
(*d*) ,, normal saline + 20 drops ether.

Shake and let them stand for 2 hours. Note if the fluid is coloured and the depth of the tint. With *isotonic* solutions the colour (Hb) is not discharged from the corpuscles (Hamburger's method).

19. Frog's blood—Coagulation of plasma.—Place 5 c.c. of normal saline (0·75 p.c. salt solution) in a glass thimble surrounded with ice. Expose the heart of a pithed frog, and open the ventricle, allowing the blood as it escapes to flow into the normal saline. Mix; the corpuscles (owing to their greater specific gravity) after a time subside. After they have subsided, remove the supernatant fluid—the plasma mixed with normal saline—by means of a pipette. Place it in a watch-glass, and observe that it coagulates.

20. Clot and serum.—Draw out a glass tube into a fine capillary pipette at both ends, leaving a bulb in the middle, and suck some uncoagulated blood, either from one's finger, or from the heart of a frog, into it, seal up the ends of the tube, allow the blood to coagulate, and examine the tube under a microscope. Observe the small red shrunken clot, and the serum squeezed out of the latter.

21. Circumstances influencing coagulation—Cold.—(*a*) Place a small platinum capsule—a brass or glass thimble will serve—on a freezing mixture of ice and salt. Decapitate a frog or rat, and allow the blood to flow directly into the cooled vessel. At once it becomes solid or congeals, but it is not coagulated. As soon as the blood becomes solid, remove the thimble and thaw the body by placing it on the palm of the hand, when the blood becomes fluid, so that it can be poured into a watch-glass; if the vessel be once more placed on the freezing mixture, the blood again congeals and solidifies, and on its being removed becomes fluid. Observe at the same time that the colour and transparency of the blood are altered. The blood is darker in colour and transparent. This is the *laky* condition due to the discharge of hæmoglobin from the corpuscles. Place the vessel with the fluid blood on the table, and it clots.

(*b*) **Coagulation-time.**—For the "Determination of the coagulation-time of blood," see Brodie and Russell, *Journ. of Phys.*, xxi., 403, 1897; and Wright, *Lancet*, 1390, 1893.

22. Precipitation of serum-globulin.—(*a*) *A. Schmidt's method.*—To 10 c.c. serum add 200 c.c. ice-cold water, and pass a stream of CO_2 through it = a white precipitate of serum-globulin. The precipitation is not complete. The serum must be diluted.

(*b*) *Panum's method.*—Dilute 1 c.c. serum with 15 c.c. water; add 5 drops of a 2 p.c. solution of acetic acid = a white precipitate of serum-globulin. All the serum-globulin is not precipitated.

23. **Grape-sugar in blood—Estimation.**—This is difficult because the proteids must be got rid of as they interfere with reduction and keep part of the oxide in solution. It should be done at once as the sugar disappears if blood be kept.

(*a*) Place 20 grms. of crystallised Na_2SO_4 in each of three porcelain capsules, and to each add exactly 20 grms. of the blood to be investigated. Mix the blood and salt together. Boil them until the froth above the clot becomes white, and the clot itself does not present any red specks. Weigh again, adding water to make up the loss by evaporation. The whole is then placed in a small press, and the fluid part expressed, collected in a capsule, and afterwards filtered. The filtrate is placed in a burette.

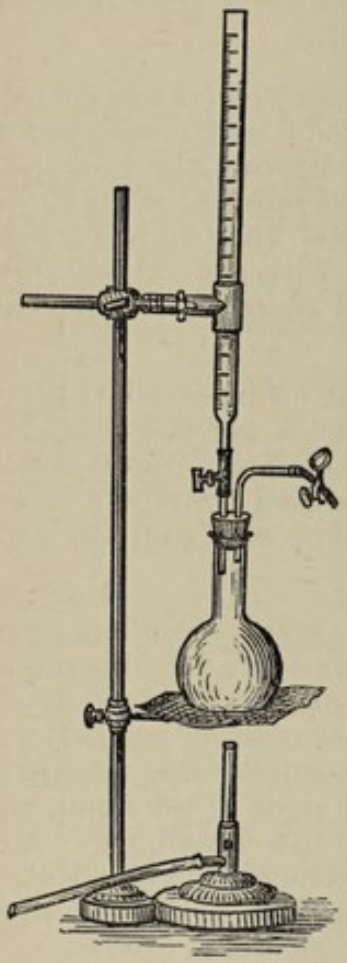


Fig. 14.—Bernard's Apparatus for Estimating the Sugar in Blood.

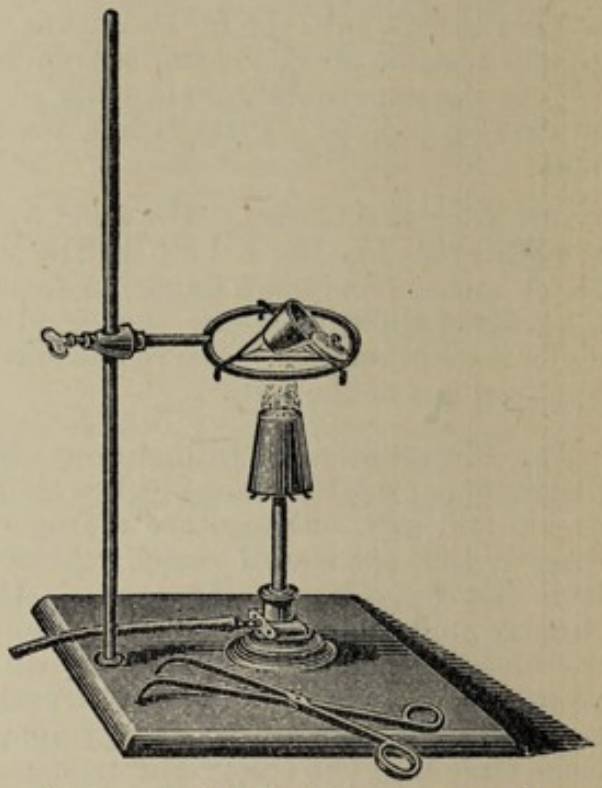


Fig. 15.—Method of Incinerating the Deposit to obtain the Ash.

In a flask place 1 c.c. Fehling's solution, and to it add a few small pieces of KHO and 20 c.c. distilled water. Boil, and from the burette allow the clear filtrate of the blood to drop into the boiling dilute Fehling's solution until the latter loses every trace of its blue colour (fig. 14). As in all sugar estimations, the process must be repeated several times to get accurate results. Hence the reason why several capsules are prepared.

Read off, on the burette, the number of c.c. of the filtrate used, *e.g.* = n c.c. Bernard's formula gives

$$S = \frac{8}{n} =$$

in grms. the weight of sugar per kilogrm. of blood.

(*b*) Add dextrose to blood to give a 1 p.c. solution. Estimate the amount after 24 hours. It will be much less. Is this loss due to the assumed glycolytic action of blood?

(*c*) *Diabetic* v. *non-diabetic blood*—Methylene blue test.—Place 40 c.mm. water in a narrow tube. Get 20 c.mm. of blood from a diabetic person's finger as directed for the hæmoglobinometer (Lesson VII.). Blow the blood into the water, add 1 c.c. of a 1 in 6000 watery solution of methylene blue, and then 40 c.mm. of liquor potassæ. Make a control with normal blood. Boil both in a beaker for 4 minutes. The diabetic blood becomes dirty pale yellow, the other remains blue (*R. T. Williamson*).

24. **Ash of hæmoglobin.**—Incinerate a small quantity of oxy-hæmoglobin in a platinum capsule, shown in fig. 15, where the capsule is placed obliquely, and its contents heated in a Bunsen-flame until only the ash remains. The ash is red, and contains oxide of iron. Dissolve some in HCl; add potassium sulphocyanide = a red precipitate; or ferrocyanide of potassium = a blue precipitate.

25. **Iron.**—Dissolve dried blood in HNO_3. Heat. Filter; test filtrate with potassium ferrocyanide = blue colour.

26. **The Centrifuge** precipitates very minute particles suspended in a fluid—*e.g.*, blood-corpuscles in serum may be readily separated by this apparatus.

The liquid is placed in strong glass tubes, and these are in turn placed in metallic cases, which can move on a horizontal axis, the cases themselves being placed in a horizontal disc which is driven at the rate of 1000 revolutions per minute; this causes the tubes to take a horizontal position, and after 30 to 40 to 60 minutes rotation the precipitate or other suspended particles are found at the outer end of the tube. Serum can thus be obtained perfectly free from corpuscles.

There are various forms of this apparatus. Some can be driven by the hand for small quantities of fluid, such as those sold by Muencke of Berlin (fig. 76), or that made by Watson & Laidlaw of Glasgow. When large quantities of fluid are required, that made by Fr. Runne of Heidelberg is one of the best. It requires a water or gas-motor to drive it (see *Appendix*).

27. **The Hæmatocrit** (Hedin's or Daland's) is a modified centrifugal machine into which is placed a graduated capillary tube filled with blood. When "centrifuged" the corpuscles are packed at the outer end of the tube, the percentage of corpuscles being read off directly on the graduated tube (1 large division = 100,000 corpuscles per c.c.). It is useful, but inexact. (Figs. 77 and 78.)

28. **Cryoscope**, applied to serum (see Lesson LXX.).

LESSON VI.

HÆMOGLOBIN: ITS COMPOUNDS, DERIVATIVES, AND THEIR SPECTRA.

1. **Hæmoglobin crystals** ($C_{600}H_{960}N_{154}O_{179}SFe$). — (*a*) *Rat's blood.*—Place a drop of defibrinated rat's blood on a slide, add three drops of water, mix, and cover with a cover-glass. After a few minutes examine with a microscope, when yellowish-red crystals of oxy-hæmoglobin begin to form, especially at the edges of the preparation, and gradually grow larger, and in the form of thin rhombic plates arranged singly or in groups (fig. 16). Some may be colourless.

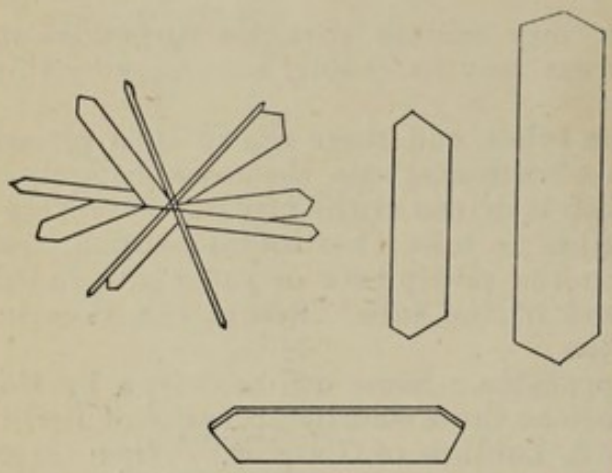


Fig. 16.—Hæmoglobin crystals from rat's blood.

(*b*) *Guinea-pig's blood.*—Treat as directed for rat's blood, or mount defibrinated blood in pure Canada balsam or stir it in clove oil. Tetrahedral crimson crystals are obtained.

(*c*) Mix defibrinated blood of rat, guinea-pig, horse, cat, or dog with an equal solution of 2 p.c. sodium fluoride, and leave for 8 or 10 days. No putrefaction occurs, as this is prevented by 1 p.c. NaFl. Crystals are readily obtained (*Arthus*).

2. **Ozone test for Hb.**—Mix freshly-prepared alcoholic solution of guaiacum with ozonic ether or old oil of turpentine; the mixture becomes turbid. On adding a dilute solution of hæmoglobin, a blue colour results, due to oxidation of the resin by the ozone liberated from the ozonic ether by the hæmoglobin.

The blue colour is given by Hb-CO, Met-Hb, neutral and alkaline, alkali-hæmatin, acid-hæmatin, but not by hæmochromogen. The blue colour is obtained when only 0·5 p.c. of blood is present.

3. **Spectroscopic examination of blood.**—Use a small Browning's straight-vision spectroscope (fig. 17), or that of Zeiss or Schmidt and Haensch (see *Appendix*).

Preliminary.— Observe the **solar spectrum** by placing the

spectroscope before the eye, adjusting the slit, and directing it to bright daylight. Note the spectrum from the red to the violet end, with the intermediate colours, and focus particularly the dark Fraunhofer's lines, known as D in the yellow, E, the



Fig. 17.—Browning's straight-vision spectroscope.

next well-marked line in the green, *b* and F, their position and relation to the colours. Make a diagram of the colours, and the dark lines, indicating the latter by their appropriate letters.

(*a*) Fix the spectroscope in a suitable holder, and direct it to a gas-flame, the edge of the flame being towards the slit in the spectroscope, noting that the spectrum shows no dark Fraunhofer lines (fig. 18*b*).

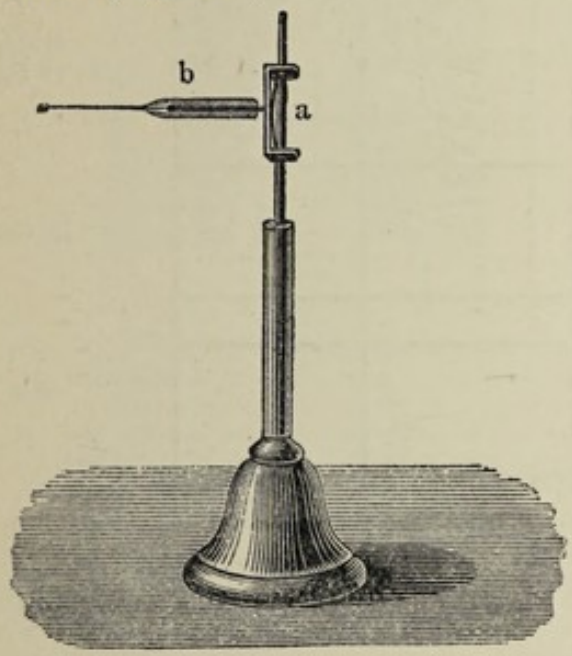


Fig. 18*a*.—Stand for platinum wire.

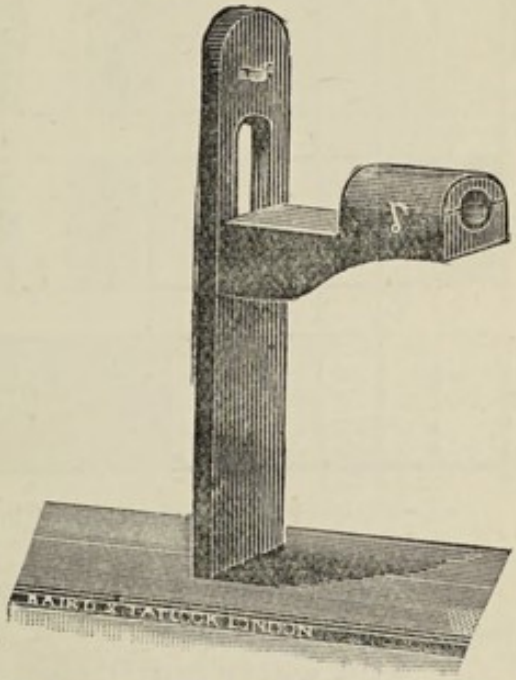


Fig. 18*b*. Stand for hand spectroscope.

(*b*) Dip a loop of platinum wire in water and then in common salt or biborate of soda, and burn the salt in the gas-flame, having previously directed the spectroscope towards the flame, and so arranged the latter that it is seen edge-on. Note the position of the bright yellow sodium line in the position of the D line (fig. 18*a*).

A. SPECTRUM OF HÆMOGLOBIN AND ITS COMPOUNDS WITH GASES.

I. Oxy-hæmoglobin—HbO_2.—(*a*) Make a solution of defibrinated blood (ox, sheep) in water, and place some in a test-tube.

(*b*) Light a fan-tailed gas-burner, fix the spectroscope in a suitable holder, and between the light and the spectroscope place the test-tube (or a wedge-shaped bottle) with the blood solution. Focus the *long image* of the gas-flame on the slit of the spectroscope. The focal point can be readily ascertained by holding a sheet of white paper behind the test-tube.

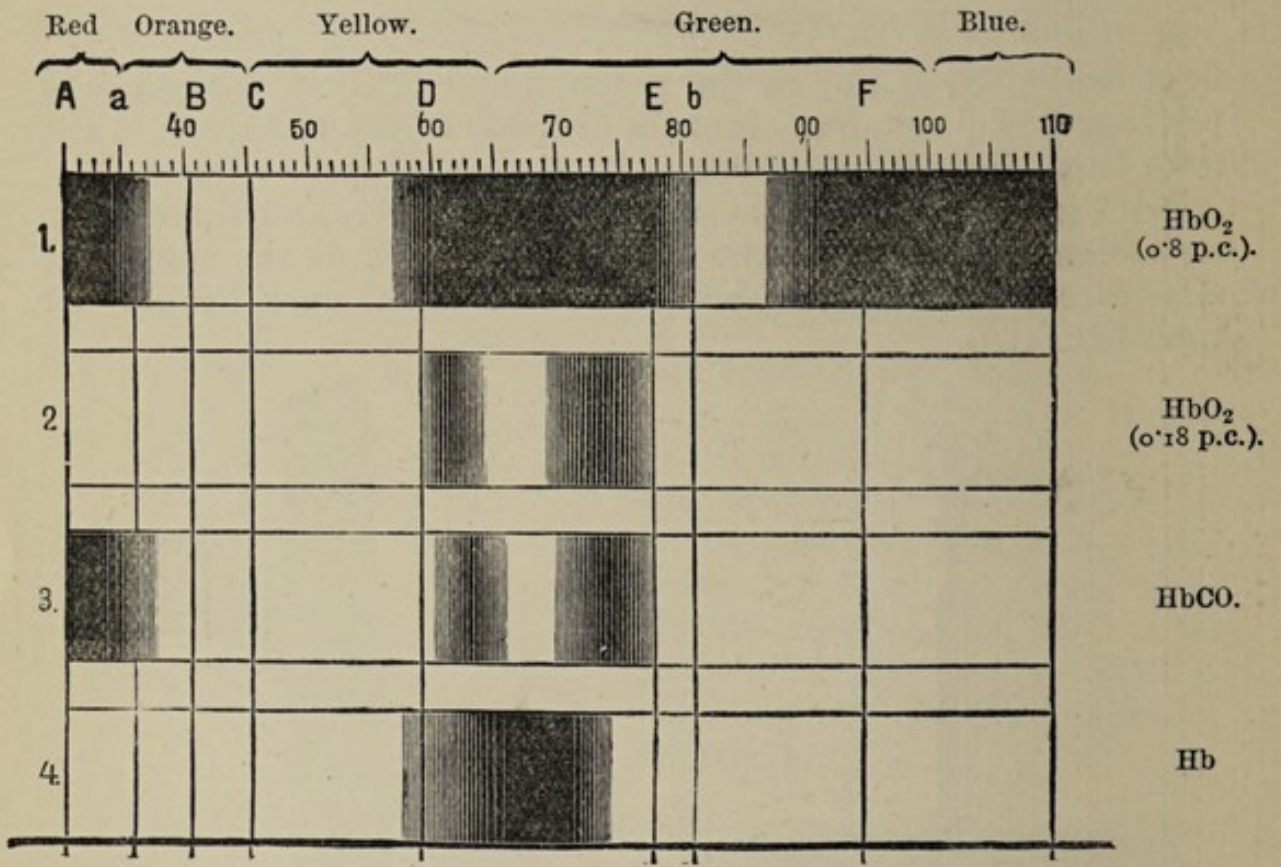


Fig. 19.—Spectra of hæmoglobin, and its compounds.

(*c*) Add 10 to 15 vols. water; only the red part of the spectrum is visible.

(*d*) Add water until the green appears, and observe a single dark absorption-band between the red and green (fig. 19, 1). Dilute until this single broad band is resolved into two by the transmission of yellow-green light. Burn a bead of NaCl in the flame. Note the D line, and observe that of the two absorption-bands the one nearer D, conveniently designated by the letter α, is darker, sharply defined, and narrow; the other band, β, nearer the violet, is broader and fainter. At the violet end the

spectrum is shortened by absorption (fig. 19, 2). On further dilution β disappears first, about 0·01 p.c. If α has disappeared, the p.c. is less than 0·1 of Hb. [The mid line of α corresponds to W.L. or λ 578, that of β about 540 (fig. 34).]

Using coloured chalks or pencils, sketch the appearances seen with each dilution, and indicate opposite each the degree of the latter.

[Prof. Engelmann has recently published coloured spectral charts which are very serviceable.]

(*e*) Make a solution of blood, showing one undivided band; add water, so that it mixes but slightly with the upper strata of the blood. The same result is obtained by using a wedge-shaped bottle for the blood solution. Move the tube so as to examine first the deeper strata until the surface is reached. At first a single band; but as the solution is more dilute above, two

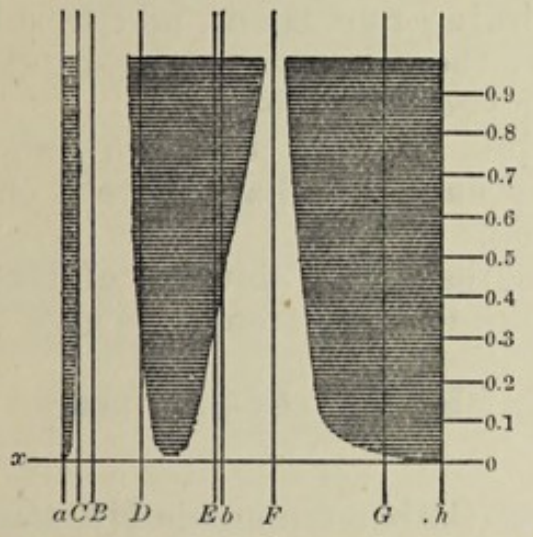


Fig. 20.—Graphic representation of the absorption of light in a spectrum by solutions of oxy-hæmoglobin, of different strengths. The shading indicates the amount of absorption of the spectrum, and the numbers at the side the strength of the solution.

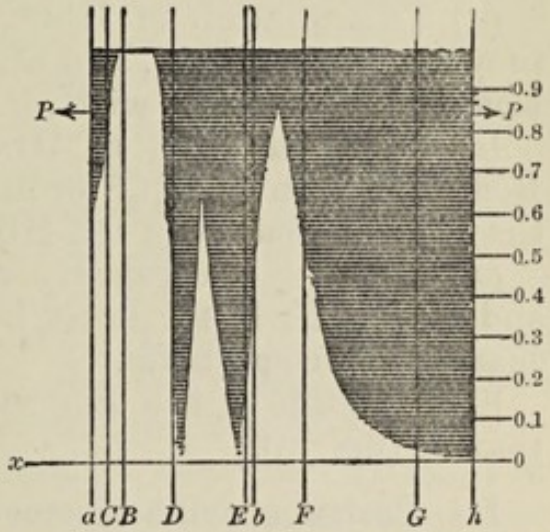


Fig. 21.—Graphic representation of the absorption of light in a spectrum by solutions of reduced Hb, of different strengths. The shading indicates the amount of absorption of the spectrum, and the numbers at the side the strength of the solution.

bands begin to appear, and as the solution is weaker above, β-band disappears, until, finally, with a very weak solution, such as is obtained in the upper strata only, the α-band alone is visible. Fig. 20 shows the amount of light absorbed by solutions of HbO_2 (1 cm. in thickness) and of various strengths.

(*f*) *Gradually increase the strength of the solution*, and observe how more and more of the spectrum is cut off, both at the red and blue ends, but especially at the latter. The two bands run together to form one continuous broad band—light now only passes through red and green. If made stronger the green is cut out, and only red passes.

(*g*) Hold up the hand before a strong light with the fingers erect and

pressed together. Along the line of contact of two fingers a single absorption-band due to the thick layer of HbO_2 is seen.

II. Hæmoglobin (reduced Hb, gas-free Hb).—(*a*) To a solution of HbO_2 showing two well-defined absorption-bands, with the inter-linear green well marked, add a few drops of 0·6 p.c. ammonium sulphide, and warm *gently*. The solution becomes *purplish* or *claret-coloured*.

(*b*) Note that the two bands of HbO_2 are replaced by **one absorption-band** between D and E, not so deeply shaded, and with its edges less defined (fig. 19, 4). Shake the solution vigorously with air, and examine at once, the two bands re-appear for a short time. Observe the absorption at the red and violet ends according to the strength of the fluid.

(*c*) Dilute the solution, and observe that the single band is not resolved into two bands, but gradually fades and disappears.

(*d*) To a solution of HbO_2, showing two bands, add freshly-prepared Stokes' fluid, and observe the single absorption-band of hæmoglobin. Shake with air, the two bands re-appear.

(*e*) Use a solution of HbO_2 just showing the two bands. Reduce it with $(NH_4)_2S$ or Stokes' fluid. Perhaps there is only the faintest shadow of the Hb band.

(*f*) Compare the relative strengths of the solution of HbO_2 and Hb. The latter must be considerably stronger to give its characteristic spectrum.

[The middle of the Hb band is about λ, 565—*i.e.*, nearer D than E (fig. 34).]

III. Carbonic oxide hæmoglobin (Carbon-monoxide Hb, Carb. oxy-Hb, Hb-CO).—Through a diluted solution of blood pass a stream of carbonic oxide—or coal gas (4 p.c. CO)—20 mins. Note the cherry-red colour with a bluish tinge.

(*a*) Dilute the solution and observe its spectrum ; **two absorption-bands** nearly in the same position as those of HbO_2, but slightly nearer the violet end (fig. 19, 3). A stronger solution is required than with HbO_2, to show the absorption-bands. [Midline position of *α* about λ 572, *β* about λ 535 (fig. 34).]

(*b*) The bands are *not* affected by the addition of reducing agents, *e.g.*, $(NH_4)_2S$ or Stokes' fluid—*i.e.*, HbCO *is not* reduced by $(NH_4)_2S$, while HbO_2 *is*. HbCO that has been kept for a long time may be ultimately—days—reduced by Am_2S.

(*c*) To a solution of HbCO add 10 p.c. solution of NaHO and boil = cinnabar-red colour. Compare with a solution of HbCO similarly treated. The latter gives a brownish-red mass.

(*d*) Dilute 1 c.c. blood with 20 c.c. water + 20 c.c. NaHO (S. G. 1·34).

If the blood contains CO, the fluid first becomes white and cloudy, and then red. When allowed to stand, flakes form and settle on the surface. Normal blood gives a dirty brown colouration.

(*e*) The HbCO compound is very stable and resists putrefaction. It gives the HbCO spectrum after being kept for many years.

[Nitric oxide hæmoglobin is omitted.]

N.B.—The band, known as the band of Soret, seen in the ultra-violet part of the spectrum in the photographic spectrum of HbO_2 and other Hb compounds, is purposely omitted. It requires a fluorescent screen to be seen ("Photographic spectrum").

IV. Neutral Methæmoglobin (Met-Hb).—This substance is formed on adding oxidising reagents to a solution of blood or HbO_2. It sometimes occurs in the urine, and is therefore of practical importance. It is a compound of Hb with O_2, and is related to oxy-hæmoglobin; but the relations between the Hb and O_2 are different in the two cases, as Met-Hb does not yield its O_2 to a vacuum, while HbO_2 does. Like HbO_2, however, it crystallises, and with it reducing agents yield hæmoglobin. It is often present in the blood of hæmatemesis.

Ferricyanide of sodium or potassium not only changes diluted HbO_2 into Met-Hb, but it also liberates the linked oxygen. If laked blood be treated with potassium ferricyanide and a little carbonate of soda added to absorb the CO_2, the O_2 liberated can be collected and measured. As all the O_2 is set free, this method can be used to estimate the amount of O_2 present. Perhaps the O_2 of the HbO_2 is detached, then the Hb takes an equal quantity of O_2 from the reagent added, according to the following provisional equation (*Haldane*) [see "Respiration"]:—

$$\underset{\text{Oxy-Hb.}}{HbO_2} + 4Na_3Cy_6Fe + 4NaHCO_3 = \underset{\text{Met-Hb.}}{HbO_2} + 4NaCy_6Fe + 4CO_2 + 2H_2O + O_2.$$

(*a*) To a medium solution of oxy-hæmoglobin add a few drops of a freshly-prepared 10 p.c. solution of ferricyanide of potassium, heat gently, observe the change of colour—to brownish-red—and examine spectroscopically. If the two bands of HbO_2 are still present, allow it to stand and examine again. If they are still visible, add ferricyanide until the two bands disappear. Note **one absorption-band** in the red near C, nearly in the same position, but nearer D than the band of acid hæmatin; the violet end is much shaded (fig. 22). Three other bands are described—two in the green, and one in the blue, especially in dilute solutions; but the student may find it difficult to recog-

nise a dim band in the green. Add ammonia to render the solution alkaline, the band in the red disappears, and is replaced by a faint band near D. Filter if necessary.

(*b*) To an alkaline solution of methæmoglobin add $(NH_4)S_2$. Observe (i.) the spectrum, first of oxy-hæmoglobin and then (ii.) that of hæmoglobin; (iii.) on shaking with air, oxy-hæmoglobin is formed.

(*c*) *Nitrites.*—To undiluted defibrinated blood add a few drops of an alcoholic solution of amyl nitrite. The blood assumes a chocolate colour (*Gamgee*).

(*d*) To diluted blood add solution of potassium or sodium nitrite = dark port wine colour rather than chocolate colour.

(*e*) To portions of (*c*) and (*d*) add ammonia; the chocolate gives place to a red colour.

(*f*) Observe the spectrum of (*c*) and (*d*). The band in the red is distinct, and is best seen when the solution is of such strength that only the red rays are transmitted. On dilution, other bands are seen in the green. Add ammonia, and with the change of colour described in (*e*) the spectrum changes as described in (*a*). Add $(NH_4)_2S$, or Stokes' fluid, the spectrum of reduced hæmoglobin appears, and on shaking up with air the bands of oxy-hæmoglobin appear.

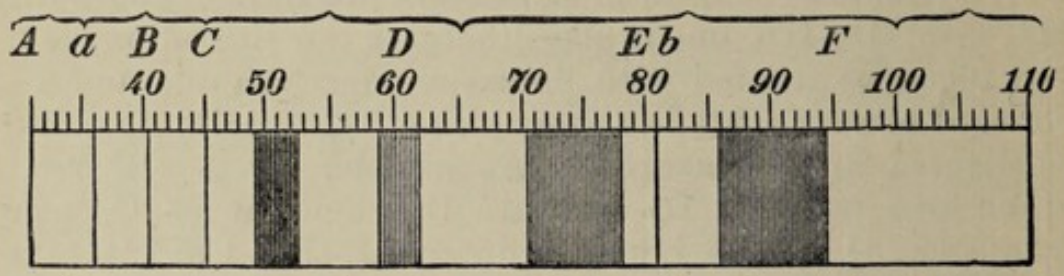


Fig. 22.—Spectrum of methæmoglobin in acid and neutral solutions.

(*g*) *Crystals.*—To a drop of blood partly dry on a slate add pyridine. Cover and examine.

(*h*) *Liberation of O from Met-Hb.*—Add a saturated solution of potassium ferricyanide and a little sodium bicarbonate to blood diluted—*i.e.*, laked with water (1 : 2); not only is methæmoglobin formed, but bubbles of gas—nearly pure oxygen—are given off. HbCO by the same means yields CO. Thus the affinity of the Hb molecule for O or CO is abolished by the reagent. The ferricyanide is reduced to ferrocyanide (*Haldane*).* Methæmoglobin, though it does not yield its O to a vacuum, does so in other ways even more readily than HbO_2.

[To methæmoglobin solution add a few drops of $(NH_4)_2S$ = spectrum of HbO_2, which lasts some time before the HbO_2 is finally reduced to Hb.]

* *Journ. of Phys.*, xxii., 298, 1898.

(*i*) *Alkaline methæmoglobin.*—To Met-Hb prepared with potassium ferricyanide add 2 or 3 drops of strong ammonia = ruby-red colour. The spectrum shows a band on the red side of D and another in the green.

B. SPECTRA OF DECOMPOSITION PRODUCTS OF Hb.

I. Acid-hæmatin.—(*a*) To diluted blood add a few drops of acetic acid, heat *gently;* the mixture becomes brownish, owing to the formation of acid hæmatin. [Dilute defibrinated sheep's blood with an equal volume of water. Take 150 c.c., heat to 35° C., and add 5 c.c. glacial acetic acid. Filter.]

(*b*) Spectrum shows **one absorption-band** to the red side of D near C (fig. 23, 5) with considerable absorption of the blue.

(*c*) The band is not affected by $(NH_4)_2S$ or Stokes' fluid. Sulphur is precipitated if $(NH_4)_2S$ is used. If the fluid is made alkaline hæmochromogen is formed.

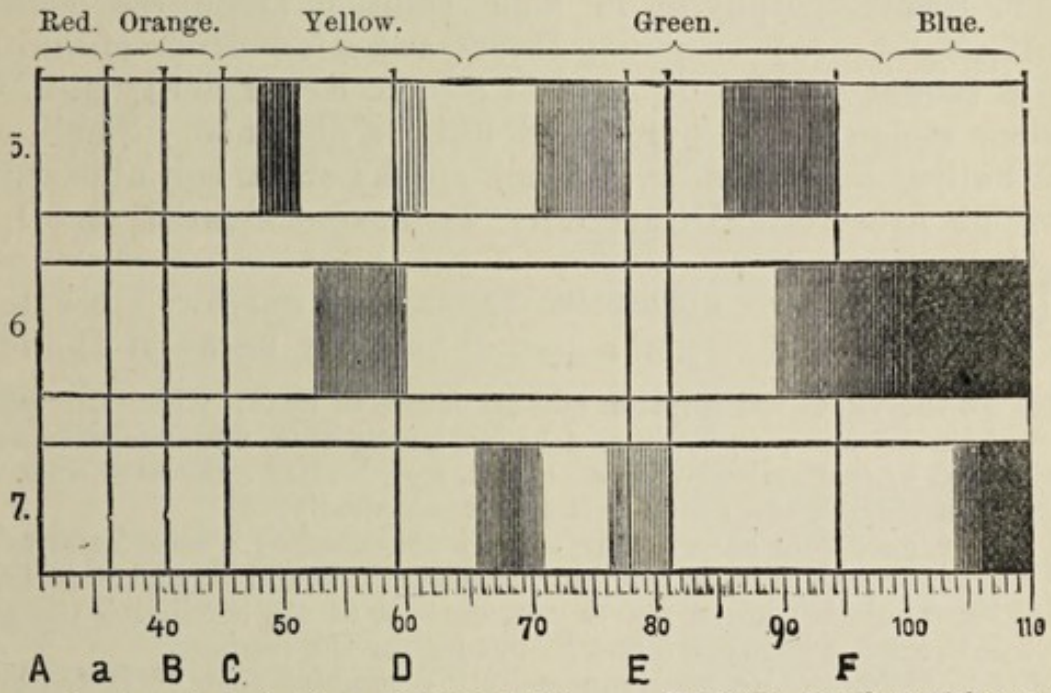


Fig. 23.—Spectra of derivatives of hæmoglobin. 5. Hæmatin in ether with sulphuric acid. 6. Hæmatin in an alkaline solution. 7. Reduced hæmatin.

(*d*) *Acid-hæmatin in ethereal solution.*—To undiluted blood add glacial acetic acid, which makes the mixture brown. Extract with ether, shake, and a dark brown ethereal solution of hæmatin is obtained. Pour it off and observe its *four* absorption bands—one in the red between C and D, corresponding to the watery acid-hæmatin, and on diluting further with ether a narrow faint one near D, one between D and E, and a fourth between *b* and F (fig. 23, 5). The last three bands are seen only in ethereal solutions, and require to be looked for with care.

II. Alkali-hæmatin.—(*a*) To diluted blood add solution of KHO, and heat gently. The colour changes to a brownish-green, and is dichroic.

(*b*) Shake (*a*) **oxy-alkali-hæmatin** with air. Its spectrum shows **one absorption-band** just to the *red* side of D, but nearer D than that of acid hæmatin (fig. 23). Much of the blue end of the spectrum is cut off.

III. Reduced alkali-hæmatin or **hæmochromogen.**—(*a*) Add to alkali-hæmatin a few drops of $(NH_4)_2S$, and heat gently. Note the change of colour = **reduced alkali-hæmatin, Stokes' reduced hæmatin** or **hæmochromogen.** The spectrum shows **two absorption-bands** in the green between D and E (HbO_2 and HbCO), but they are nearer the violet. The first band to the violet side of D is well defined; the second, still nearer the violet (in fact, it nearly coincides with the E line), is less defined. Both bands disappear after shaking with air, and re-appear on standing, provided sufficient $(NH_4)_2S$ be added.

IV. Hæmatoporphyrin in acid solution (Iron-free hæmatin, $C_{16}H_{18}N_2O$).—(*a*) To strong H_2SO_4 add a few drops of undiluted blood (about 5 drops of blood to 8 to 10 c.c. H_2SO_4); mix, when a clear violet-red or purple-red fluid is obtained. Smell, odour of "halitus sanguinis." If black specks of carbon appear, filter through asbestos. Dilute only with concentrated H_2SO_4, not H_2O.

(*b*) Observe **two absorption-bands**, one narrow, close to and on the red side of D, and a second half-way between D and E.

(*c*) To the violet-red solution add an excess of water, which throws down part of the hæmatoporphyrin as a brown precipitate, which is more copious if the acid be neutralised with an alkali, *e.g.*, NaHO. Dissolve some of the deposit in NaHO, and examine it spectroscopically.

(*d*) The spectrum shows **four absorption-bands**; a faint band midway between C and D, another similar one between D and E, but close to D; a third band near E; and a fourth one, darkest of all, occupying the greater part of the space between *b* and F, but nearer the former.

Picro-carmine.—Its spectrum closely resembles that of HbO_2, but the two bands are much nearer the violet, one being midway between D and E, and the other to the violet side of E. The bands are unchanged on addition of $(NH_4)_2S$ or Stokes' fluid. The solution does not give proteid reactions. Try also carmine, magenta, hæmatoxylin, saffranin, eosin.

V. Detection of blood-stains—*e.g.*, rag stained with blood.

(*a*) Add glycerin to part of the stain, and after a time express the liquor, and observe it microscopically for blood-corpuscles.

(*b*) Suspend in distilled water by means of a thread a part of the rag, and leave it until the colouring-matter is extracted. Observe the coloured fluid spectroscopically.

(*c*) Boil some of the extract with HCl, and add potassium ferrocyanide; a blue colour indicates the presence of iron.

(*d*) Use the stain for the hæmin test.

VI. Hæmin Crystals.—To powdered dried blood on a slide, or blood smeared on a slide, add crystal of NaCl, and *glacial* acetic acid. Cover, and heat until bubbles of gas are given off. Examine microscopically, when—after cooling—brown or black rhombic crystals of hæmin are seen (fig. 24). To preserve them irrigate with water, dry, and mount in Canada balsam.



Fig. 24.—Hæmin crystals.

ADDITIONAL EXERCISES.

1. Hæmoglobin—*Preparation*—(*a*) *Dog's blood.*—To 15 c.c. of defibrinated dog's blood add, drop by drop, 1 c.c. of ether, shaking the tube after each addition of ether. The blood is rendered *laky*. Add no more ether. Place the tube in a freezing mixture of ice and salt; as the temperature falls, crystals of hæmoglobin may separate. If the crystals do not separate at once, keep the tube in the freezing mixture for one or two days. Examine the crystals microscopically. Dog's blood, containing 1 p.c. of sodium fluoride, after standing for several days, deposits crystals of Hb (*Arthus*).

(*b*) Run blood into an equal volume of sodium fluoride or oxalate of potash (2 p.c.), and leave it for 8 or 10 days. Crystals of Hb are deposited (*Arthus*).

2. Reduction of HbO_2 by putrefying bodies.—To a test-tube containing a dilute solution of HbO_2 or blood, add a drop of putrid meat infusion, cork the vessel tightly to make it air-tight, and allow it to stand. The HbO_2 is *reduced* to hæmoglobin; the colour changes to purple-red, and the fluid shows the spectrum of hæmoglobin. Seal up the blood in a tube. It need not be mixed with putrid matter in order to observe after a time the reduction.

3. Hæmatinometer.—For accurate observation, instead of a test-tube the blood is introduced into a vessel with parallel sides, the glass plates being exactly 1 cm. apart (fig. 33, D).

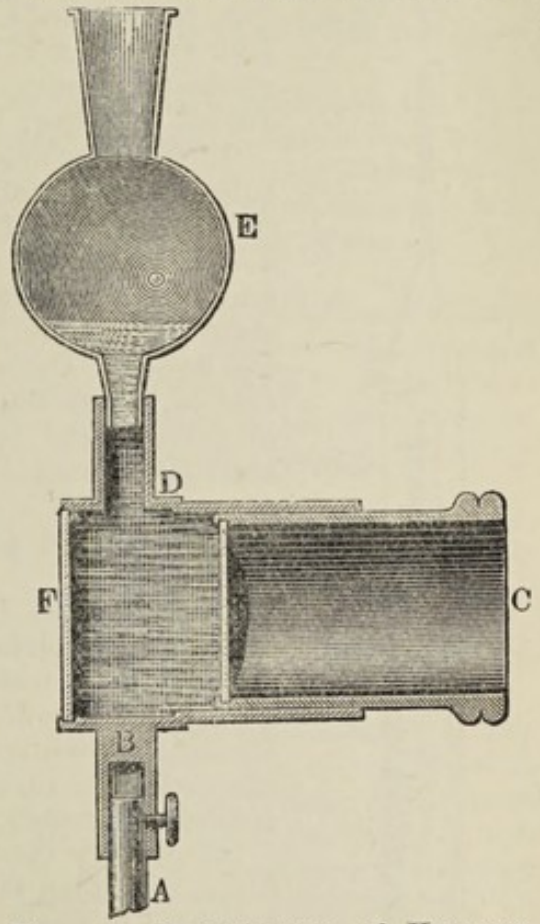


Fig. 25.—Hæmatoscope of Hermann. F. Glass plate; C. Piston-like tube, closed by a glass plate. By moving C the space B F can be increased or diminished, and the thickness of the stratum of fluid varied. E. Vessel for holding surplus fluid. A. Support.

4. Hæmatoscope (fig. 25).—By means of this instrument the depth of the stratum of fluid to be investigated can be varied, and the variation of the spectrum, with the strength of the solution, or the thickness of the stratum through which the light passes, at once determined.

To a few c.c. of defibrinated blood (rat, guinea-pig), add an equal number of drops of amyl nitrite, and shake the mixture for a minute or two = dark chocolate tint of methæmoglobin. A drop of this fluid transferred at once to a slide, and covered, yields crystals of methæmoglobin (*Halliburton*).

5. Methæmoglobin crystals.—To a litre of concentrated solution of hæmoglobin add 3-4 c.c. of a concentrated solution of ferricyanide of potassium and also a quarter of a litre of alcohol, and freeze the mixture. After two days, brown crystals of methæmoglobin separate.

6. Action of drugs—Formation of Met-Hb.—Make a solution of 3 parts of defibrinated blood in 100 parts of water. Take 15 c.c. in separate test tubes, add reagents, and observe at once change in colour and spectrum. If necessary, hasten by heating in water-bath at 40° C.

(*a*) 25 drops saturated $KClO_3$.
(*b*) 25 ,, 10 p.c. $NaNO_2$.
(*c*) 2 ,, $KMnO_4$.
(*d*) 1 drop phenyl-hydrazine.
(*e*) 25 drops 25 p.c. pyrogallol (Met-Hb spectrum and precipitate of hæmogallol).
(*f*) 5 p.c. pot. ferricyanide. When Met-Hb band is well marked, add 1 drop 2 p.c. HCN. Note band of Cyan-Met-Hb (resembles reduced). (*Sollmann.* Also, G. N. Stewart, *Journ. of Phys.*, xxvi., 470.)

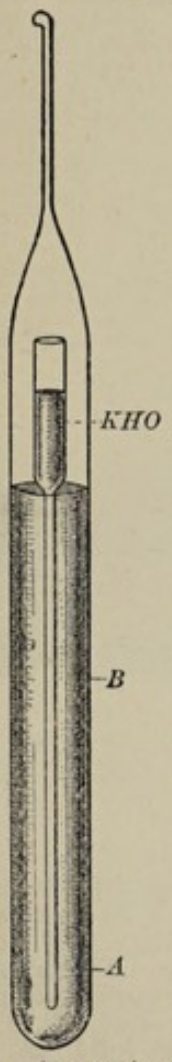


Fig. 26.—Apparatus for making hæmochromogen.

7. Hæmochromogen and hæmatin.—Seal in a glass tube a solution of HbO_2 with NaHO. Hoppe-Seyler recommends the following method, but it is unnecessary. Arrange a tube as in fig. 26. Place solution of hæmoglobin in A, and into a narrower cup-shaped glass tube (B), with a long stem place some NaHO, and place B inside A, as shown in the figure. Draw out the end of tube A in a gas flame, and seal it in the flame. Mix the two solutions. At the end of three weeks break off the narrow end of the tube, and shed the contents upon a white plate. The contents consist of red hæmochromogen, but the latter, as soon as it is exposed to the air, becomes brown, and is converted into hæmatin.

8. Hæmatin ($C_{32}H_{33}N_4O_4Fe$)—*Preparation.*—(*a*) Make defibrinated blood into a paste with potassic carbonate and dry on a water-bath. Place the paste in a flask, add 4 volumes alcohol, and boil on a water-bath. Filter; an alkaline brown solution of hæmatin is obtained. Re-extract the residue several times with boiling alcohol, and mix the alcoholic extracts. The solution is dichroic.

(*b*) Acidify the alkaline filtrate of (*a*) with dilute H_2SO_4, filter, and keep the filtrate. Observe the spectrum of acid hæmatin in the filtrate (figs. 23, 5, and 35, 5).

(*c*) Add excess of ammonia to the acid filtrate of (*b*), and filter off the precipitate, keep the filtrate, and observe that it is dichroic. Observe the spectrum of alkali hæmatin in the filtrate (fig. 23, 6).

(*d*) Evaporate the filtrate from (*c*) to dryness on a water-bath. Extract the residue with boiling water. The black residue is washed on a filter with distilled water, alcohol, and ether, and dried in a hot chamber at 120° C.

(*e*) It is convenient to keep in stock hæmatin prepared as ollows:—

Extract defibrinated blood or blood-clot (ox or sheep) with rectified spirit containing pure sulphuric acid (1 : 20). Filter; the solution gives the spectrum of acid hæmatin. Add an equal volume of water and then chloroform. The chloroform becomes brown, and there is a precipitate of proteids. Separate the chloroform extract, wash it with water to remove the acid. Separate the chloroform, and allow it to evaporate. The dark brown residue is impure hæmatin. When dissolved in alcohol and caustic soda it gives the spectrum of alkali hæmatin, and on adding ammonium sulphide that of hæmochromogen. If it is dissolved in H_2SO_4, and filtered through asbestos, the red filtrate gives the spectrum of hæmato-porphyrin (*MacMunn*).

9. Sulph-hæmoglobin is obtained when sulphuretted hydrogen is passed through diluted blood. It gives a special band in the red.

10. Spectrum of circulating blood.—(*a*) Hold up the hand vertically towards a bright light or to the sun, and direct a spectroscope towards the crimson glow seen between the fingers, a single-banded spectrum of HbO_2 is seen. The thinnest part of the ear of an albino rabbit just yields a two-banded spectrum. Constrict the base of one ear. In 30 seconds the double-band fades into the single on Hb—*i.e.*, in this short time the HbO_2 is reduced to Hb.

(*b*) Examine the circulation in the web of a frog's foot and then use a spectroscope, the expanded web being between the light and the spectroscope. A two-banded spectrum of HbO_2 is seen. Ligature the thigh. Even after 2 hours the HbO_2 is not reduced. Thus the reducing power of the amphibian tissues (cold-blooded) is far less than that of the mammalian (warm-blooded).

11. CO_2 on blood.—(*a*) Pass a stream of CO_2 through *undiluted* defibrinated blood and observe the purple or dark "venous" colour. Observe the spectrum of Hb in the transparent purplish bubbles before they burst. Does the CO_2 really form a CO_2 – Hb, or is it locked up in some peculiar combination in the red corpuscles themselves? The combination is extremely unstable; the CO_2 is at once displaced by O.

(*b*) Pass CO_2 for an hour through *diluted* (1 p.c.) blood. The red corpuscles are destroyed by the water, but with Hb in solution CO_2 does not cause a venous hue, and the HbO_2 is not reduced. The blood still shows the spectrum of HbO_2. To effect the change of hue the Hb must be in the red corpuscles, not in solution. Blood reduced by $(NH_4)_2S$ can be kept for a long time and still show the band of Hb.

12. Colour of dilute HbO_2 *v.* HbCO.—(*a*) Compare the colour of a very dilute solution of HbO_2. – few drops of blood in a litre of water, with a similar dilution of HbCO. Note yellowish tinge of former and pinker hue of latter. Haldane has based a simple colorimetric method on this reaction for estimating the amount of CO*—*i.e.*, its percentage estimation of Hb of the red corpuscles with CO during life. The affinity of CO for Hb is about 140 times that of oxygen (see "Respiration"). The colouring power of blood in different animals varies in exact proportion to its oxygen capacity (*Haldane* and *Lorrain Smith*).†

(*b*) Precipitate Hb with weak tannic acid, and observe change in colour of the two precipitates.

---

* "The Action of CO on Man," *Journ. of Phys.*, xviii., 430, 463, 1895.

† *Journ. of Phys.*, xxvi., 497, 1901.

LESSON VII.

RED BLOOD-CORPUSCLES—HÆMOCYTOMETERS.

HÆMOGLOBINOMETERS (W. L. of Hb).

Enumeration of corpuscles.—Several forms of instruments are in use—*e.g.*, those of Malassez, Zeiss, Bizzozero, and Gowers.

1. Gowers' hæmocytometer (fig. 27) consists of—

(1) A small pipette, which, when filled to the mark on its stem, holds 995 c.mm. (fig. 27, A). (2) A capillary tube to

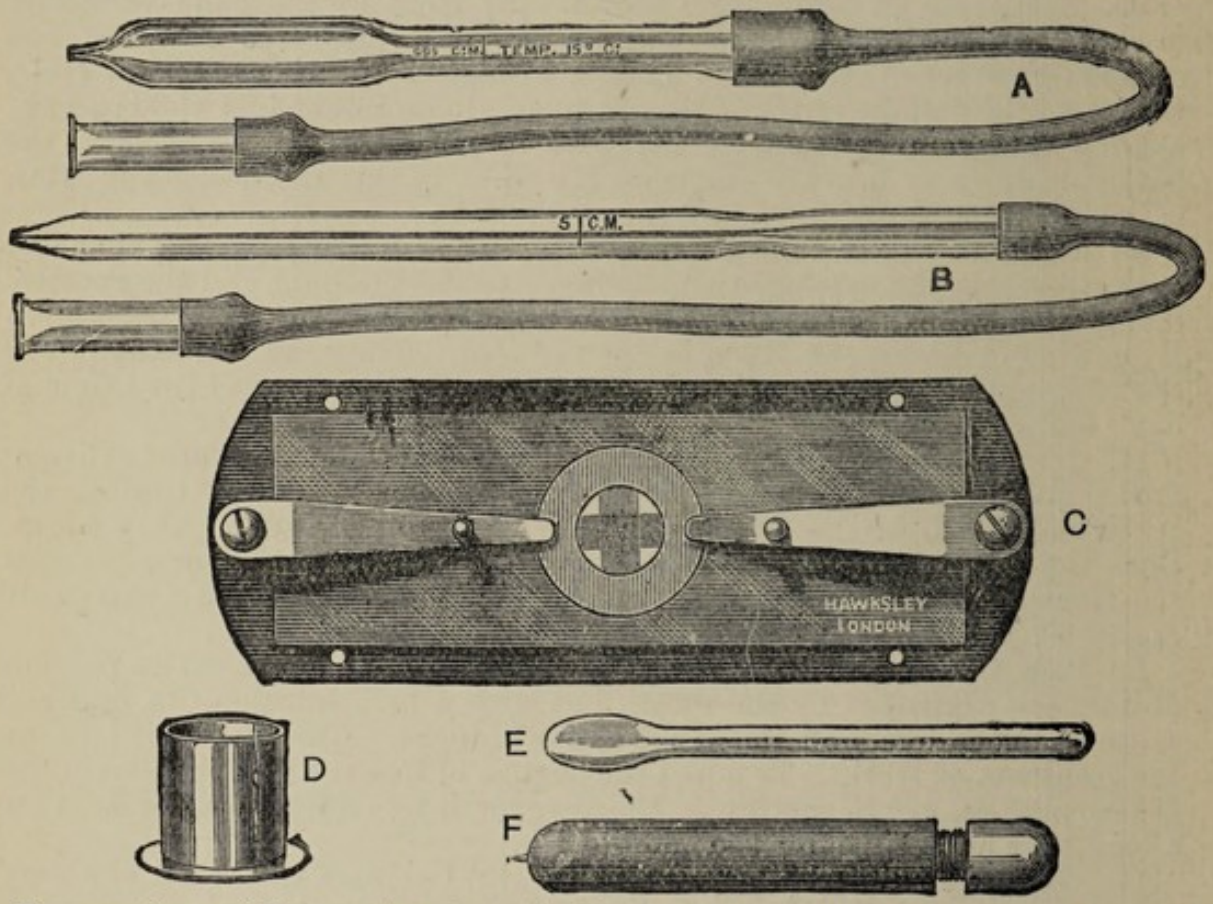


Fig. 27.—Gowers' Hæmocytometer. A. Pipette for measuring the diluting solution. B. For measuring the blood. C. Cell with divisions on the floor, mounted on a slide, to which springs are fixed to secure the cover-glass. D. Vessel in which the solution is made. E. Spud for mixing the blood and solution. F. Guarded spear-pointed needles.

hold 5 c.mm. (B). (3) A small glass jar in which the blood is diluted (D). (4) A glass stirring rod (E). (5) Fixed to a brass plate a cell $\frac{1}{5}$ of a millimetre deep, and with its floor divided into squares $\frac{1}{10}$ mm., in which the blood-corpuscles are counted. The diluting solution consists of a solution of sodium sulphate in distilled water—S.G. 1025.

Process.—(*a*) By means of the pipette (A) place 995 c.mm. of the diluting solution in the mixing jar (D).

(*b*) Puncture a finger near the root of the nail with the lancet projecting from (F), and with the pipette (B) suck up 5 c.mm. of the blood, and blow it into the diluting solution, and mix the two with the stirrer (E).

(*c*) Place a drop of the mixture on the centre of the glass cell (C), see that it exactly fills the cell, and cover it gently with the cover-glass, securing the latter with the two springs. Place the cell with its plate on the stage of a microscope, and focus for the squares ruled on its base.

(*d*) When the corpuscles have subsided, count the number in ten squares, and this, when multiplied by 10,000, gives the number in a cubic millimetre of blood.

(*e*) Wash the instrument and the cell with distilled water.

Each square has an area of $\frac{1}{100}$ of a square mm., so that 10 squares have an area of $\frac{1}{10}$ of a square mm. As the cell is $\frac{1}{5}$ mm. deep, the volume of blood in 10 squares is $\frac{1}{10} \times \frac{1}{5} = \frac{1}{50}$ c.mm. On counting the number of corpuscles in 10 squares, and multiplying by 50, this will give the number in 1 c.mm. of the *diluted* blood. On multiplying this by $\frac{1000}{5}$, we get the number in 1 c.mm. before dilution. Thus we see why we multiply the number in 10 squares by 10,000 to get the number of corpuscles in 1 c.mm. of blood.

2. **Thoma-Zeiss Hæmocytometer** (fig. 28).—This consists of (i.) a mixer or dilution pipette graduated from 0·1 to 0·5, to 1, and to 101, with a bulb containing a glass bead for mixing (*S M*). (ii.) A counting chamber, consisting of a small raised area or platform ruled into squares, each one of which encloses $\frac{1}{400}$ mm. square—*i.e.*, each has a side of $\frac{1}{20}$ mm. (fig. 28). Surrounding this is a trench or moat, and outside a rim which is $\frac{1}{10}$ mm. higher than the central plateau, which is ruled into squares. When a cover-glass rests in position over a drop of blood, the layer of blood is 0·1 mm. thick, and consequently the amount of blood over each square is $\frac{1}{4000}$ of a cubic millimetre. Groups of 16 squares are marked off by additional lines.

Process.—The pipette must be clean and dry, and the diluting fluid ready (Gowers or Hayem's). Then puncture the finger or lobe of ear freely with a sharp triangular needle. Suck blood into the pipette up to the mark 0·5 on the stem of the mixer. Wipe the point dry. Suck in the diluting fluid until the mark 101 on the pipette is reached (dilution 1 to 200). Mix the fluids thoroughly by rolling the pipette to and fro. Blow out a few drops of the liquid in the capillary part of the pipette, and then blow a drop of blood on the ruled platform. Cover with a cover-slip, taking care that no blood passes into the trench. Place the counting slide under the microscope (× 300, Zeiss D). Wait a minute until the corpuscles subside.

Newton's coloured rings should be seen between the cover-slip and the raised margin. Arrange a set of 16 squares in the field and count all the corpuscles in these, beginning at the left-upper square, the eye travelling from left to right. Count all corpuscles lying on lines bounding the left and upper sides of any square. By way of compensation omit those on the right and lower margins. Write down the number in 16 squares. Count the number in other 16 squares, and again in a third set. Suppose the numbers were respectively 91, 98, and 99 = 288 for 48 small squares—*i.e.*, 6 to each square. Each square has a

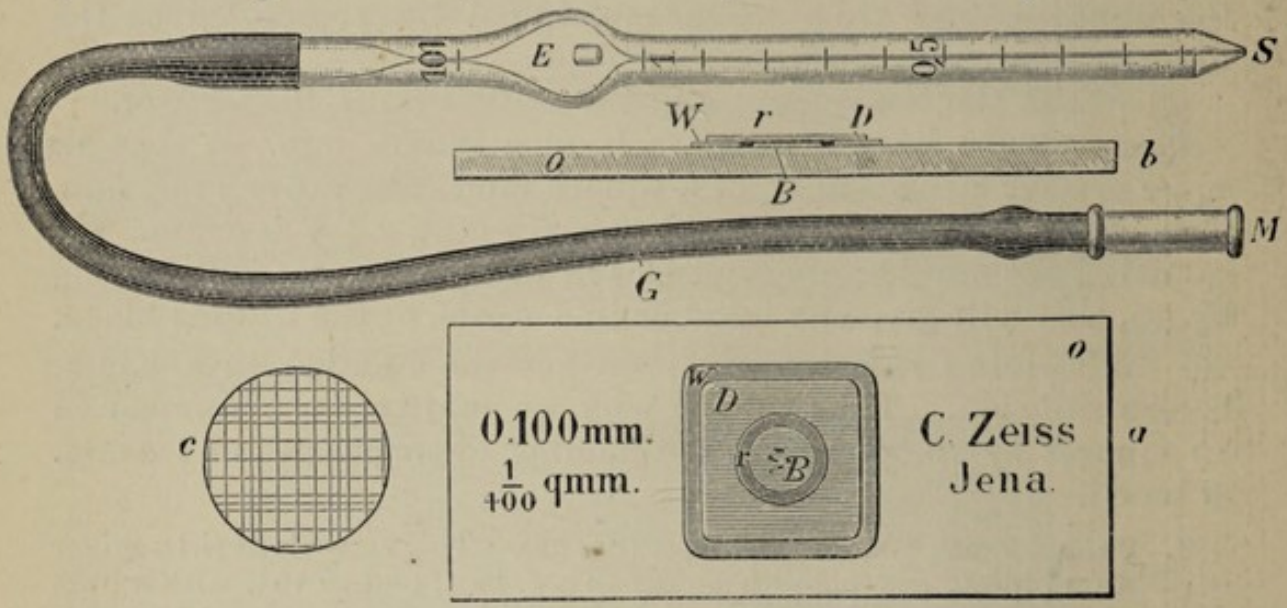


Fig. 28.—Thoma-Zeiss Hæmocytometer (two-thirds full size).—*a* and *b*, Counting chamber. *c*, Ruled bottom of cell magnified. *S M*, Mixing pipette.

capacity of $\frac{1}{4000}$ c.mm., and dilution was 1 to 200. The total number of red corpuscles in a millimetre will be

$$6 \times 4000 \times 200 = 4{,}800{,}000.$$

The normal number in the adult male is taken as 5,000,000 per cubic mm.

3. Counting leucocytes.—Collect the blood in the same way, but use the large bore pipette, so that the dilution is 1 to 20 or 1 to 10, using 0·3 p.c. glacial acetic acid as the diluting fluid; tinged it may be with methyl-green or methyl-violet 5 B; slightly stains the leucocytes. The acid diluting fluid makes the red corpuscles disappear. Aspirate blood to 0·5, and dilute to 1 to 20—*i.e.*, to line marked 11. Count the white corpuscles in the whole set of 16 large squares = 256 small squares. Suppose the number was 30, we get—

$$\frac{30}{256} \times 4000 \times 20 = 9037.$$

The leucocytes usually number about 7000 per c.mm., or 1 to

700 red corpuscles. However valuable the number may be, this process should be combined with the study of film preparations of blood, to enable one to determine which variety of leucocyte is really altered in number or character.

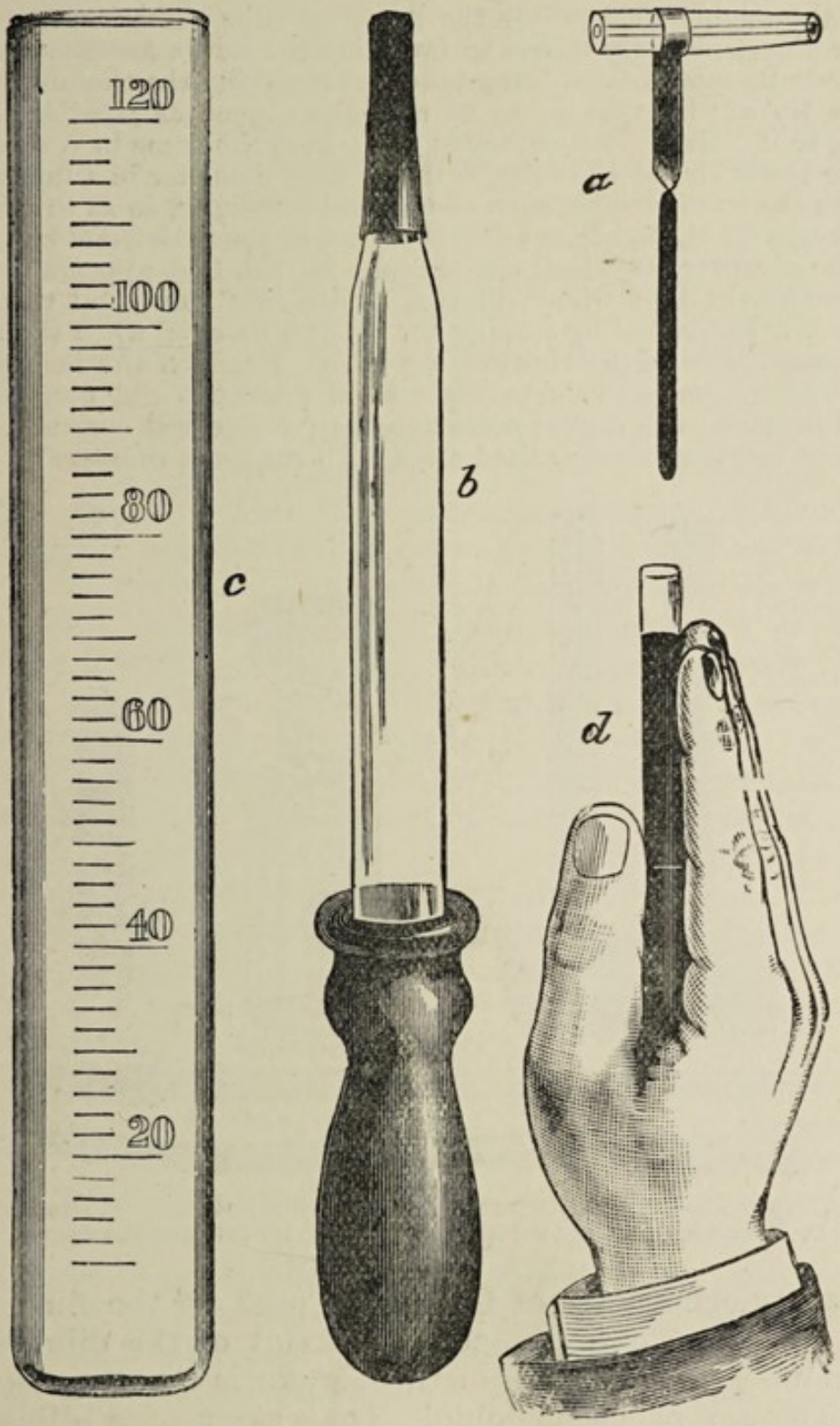


Fig. 29.—Oliver's hæmocytometer (Tintometer Co., Ltd., Salisbury).

4. **Oliver's hæmocytometer** (fig. 29, use in dark room).—*The apparatus*

consists of (1) a graduated flattened tube; (2) an automatic blood-measurer; (3) a mixing pipette; (4) a candle; and (5) a bottle of Hayem's solution.

Process.—The pipette, previously dried by drawing a thread through it, having been accurately filled with blood, the rubber nozzle of the mixer filled with Hayem's solution is then applied over its bevelled end, and the blood is thoroughly washed into the flattened tube. The contents of the tube are then uniformly mixed by inverting the tube a few times with the thumb over its mouth, care being taken on removing the thumb to draw it over the lip of the tube so as to restore as much as possible the fluid adhering to it. The observer stands 10 ft. from the flame in a dark room. Place the lower end of the tube, with its long diameter in a line with the candle, in the concavity between thumb and forefinger, so as to make as it were a frame for it, and then bring this part of the tube *quite close* to the eye. The observer must look out sharply for the first appearance of the transverse line of light which dilution brings into view, and the earliest indications are obtained by turning the tube on its axis, when the line will first become visible at the sides of the tube. Read off the height of the fluid. As 100° corresponds to the normal 5,000,000 red corpuscles per cubic millimetre, each degree corresponds to 50,000 red corpuscles. The instrument has been standardised with a Thoma-Zeiss counter.

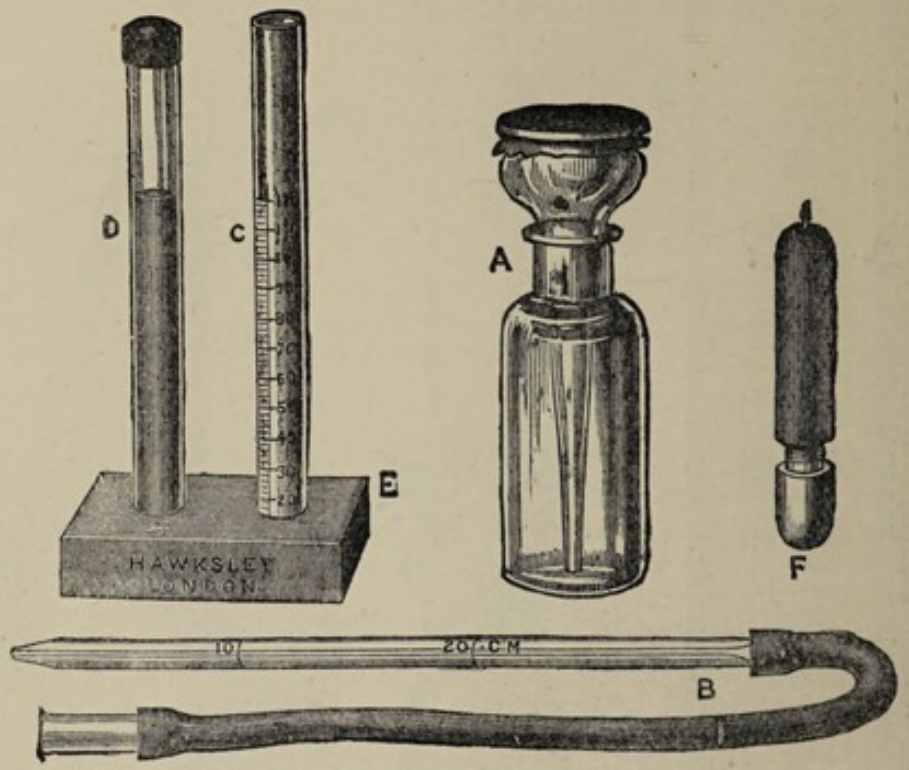


Fig. 30.—A. Pipette bottle for distilled water. B. Capillary pipette. C. Graduated tube. D. Tube with standard dilution. F. Lancet for pricking the finger.

5. Hæmoglobinometer of Gowers is used for the clinical estimation of hæmoglobin (fig. 30). The tint of the dilution of a given volume of blood with distilled water is taken as the index of the amount of hæmoglobin. The colour of a dilution of average normal blood (100 times) is taken as the standard. The quantity of hæmoglobin is indicated by the amount of distilled

water needed to obtain the tint with the same volume of blood under examination as was taken of the standard. Owing to the instability of a standard dilution of blood, tinted glycerin jelly is employed. The apparatus consists of two glass tubes of exactly the same size. One contains (D) a standard of the tint of a dilution of 20 c.mm. of blood, in 2 c.c. of water (1 in 100); the second tube (C) is graduated, 100° = 2 c.c. (100 times 20 c.mm.).

(*a*) Place a few drops of distilled water in the tube (C).

(*b*) Puncture the skin at the root of the nail with the shielded lancet (F), and with the pipette (B) suck up 20 c.mm. of the blood, and eject it into the distilled water, and rapidly mix them.

(*c*) Add distilled water drop by drop from the pipette stopper (A) until the tint of the dilution is the same as that of the standard. The amount of water which has been added (*i.e.*, the degree of dilution) indicates the amount of hæmoglobin.

"Since average normal blood yields the tint of the standard at 100° of dilution, the number of degrees of dilution necessary to obtain the same tint with a given specimen of blood is the percentage proportion of the hæmoglobin contained in it, compared to the normal. For instance, the 20 c.mm. of blood from a patient with anæmia gave the standard tint of 30° of dilution. Hence it contained only 30 p.c. of the normal quantity of hæmoglobin. By ascertaining with the hæmocytometer the corpuscular richness of the blood we are able to compare the two. A fraction of which the numerator is the percentage of hæmoglobin, and the denominator the percentage of corpuscles, gives at once the average value per corpuscle. Thus the blood mentioned above containing 30 p.c. of hæmoglobin contained 60 p.c. of corpuscles; hence the average value of each corpuscle was $\frac{30}{60}$ or half of the normal. Variations in the amount of hæmoglobin may be recorded on the same chart as that employed for the corpuscles."

The quotient of the percentage of Hb over the percentage number of corpuscles is termed the "worth" of the corpuscles.

"In using the instrument, the tint may be estimated by holding the tubes between the eye and the window, or by placing a piece of white paper behind the tubes; the former is perhaps the best. In practice it will be found that during 6° or 8° of dilution, it is difficult to distinguish a difference between the tint of the tubes. It is therefore necessary to note the degree at which the colour of the dilution ceases to be deeper than the standard, and also that at which it is distinctly paler. The degree midway between these two will represent the hæmoglobin percentage."

(*d*) **Haldane's modification.**—T. Haldane (*Journ. of Phys.*, xxvi., 497, 1901) points out the difficulty in standardising colour glasses or picrocarmine. He uses a 1 p.c. solution of HbCO, which is placed in D and

hermetically sealed. C holds 2·0 c.c., and both C and D are accurately calibrated. The oxygen capacity of Hb is taken as 18·5 p.c. The tube is graduated in percentages of 2 c.c. "The capillary tube holds 20 c.mm. (=0·02 c.c.) to the mark. Hence, if blood filling it is ejected into B and diluted with water so that, after mixing, the liquid stands at the mark 100, a 1 p.c. solution of the blood is obtained; and this solution, if the blood be exactly normal, will, after saturation with CO or coal gas, have the same tint as the solution in A. If the blood contains more, or less, than the normal proportion of hæmoglobin the dilution required to match the tint of A will be proportionally greater or less, the height of the liquid indicating the percentage of the normal.

"Before using a lancet it is sterilised in a gas flame or spirit lamp. It is held between the forefinger and thumb, leaving sufficient of the point projecting to make the puncture.

"A small tube and cap for attachment to a gas burner is provided, and serves to deliver gas for saturating the diluted blood with CO.

"*Method of Use.*—Sufficient water is first placed in the graduated tube to dilute the blood so far as safely possible. A puncture is then made in a finger or the lobe of an ear, and the capillary pipette (which must be clean and dry) at once filled to a little beyond the mark 20 from the drop of blood obtained. The point of the pipette is wiped, and dabbed on any convenient surface until the contained blood stands exactly at the mark. The blood is then gently blown out into the graduated tube, where it sinks: the pipette is rinsed with the water in the graduated tube and withdrawn. The piece of rubber tube attached to a gas burner is now introduced into the graduated tube to near the level of the water, and gas allowed to pass for a few seconds. As the tube is withdrawn (with the gas still passing) the end is closed with the forefinger, and the liquid made to pass up and down in the tube—not violently shaken—at least a dozen times, so as to saturate the hæmoglobin with CO. During this manipulation the tube is held in a handkerchief, otherwise it will become heated and liquid will spurt out when the finger is withdrawn. Water is now added drop by drop with the pipette stopper, the tube after each addition being inverted, until the point is reached at which the tints of the liquids in the two tubes are just equal. In judging of the equality the tubes should be held against the light from the sky, or, if artificial light be used, from an opal glass shade. It is also absolutely necessary to transpose the tubes repeatedly: otherwise serious errors may arise. The level is read off on the graduated tube after half a minute has elapsed since the last drop added was mixed with the rest of the liquid by inverting the tube. The observation is repeated after the addition of another drop of water, and if necessary another, until the point is reached when the tints are again unequal. The true result is the mean of the readings giving equality. The error in any single determination ought not to exceed 1 p.c.

"The result obtained is the percentage actually present of the average proportion of hæmoglobin in the blood of healthy adult men. The blood of healthy men, however, contains more hæmoglobin on an average than the blood of healthy women or children. Women give an average of 89 p.c., and children of 87 p.c. of the proportion in men. The results may be expressed in terms of oxygen capacity (the number of volumes of oxygen taken up in combination from air by 100 volumes of blood) if it be borne in mind that 100 p.c. on the scale corresponds to an oxygen capacity of 18·5." The apparatus is made by Hawksley.

6. Oliver's hæmoglobinometer (fig. 31). Use in dark room.

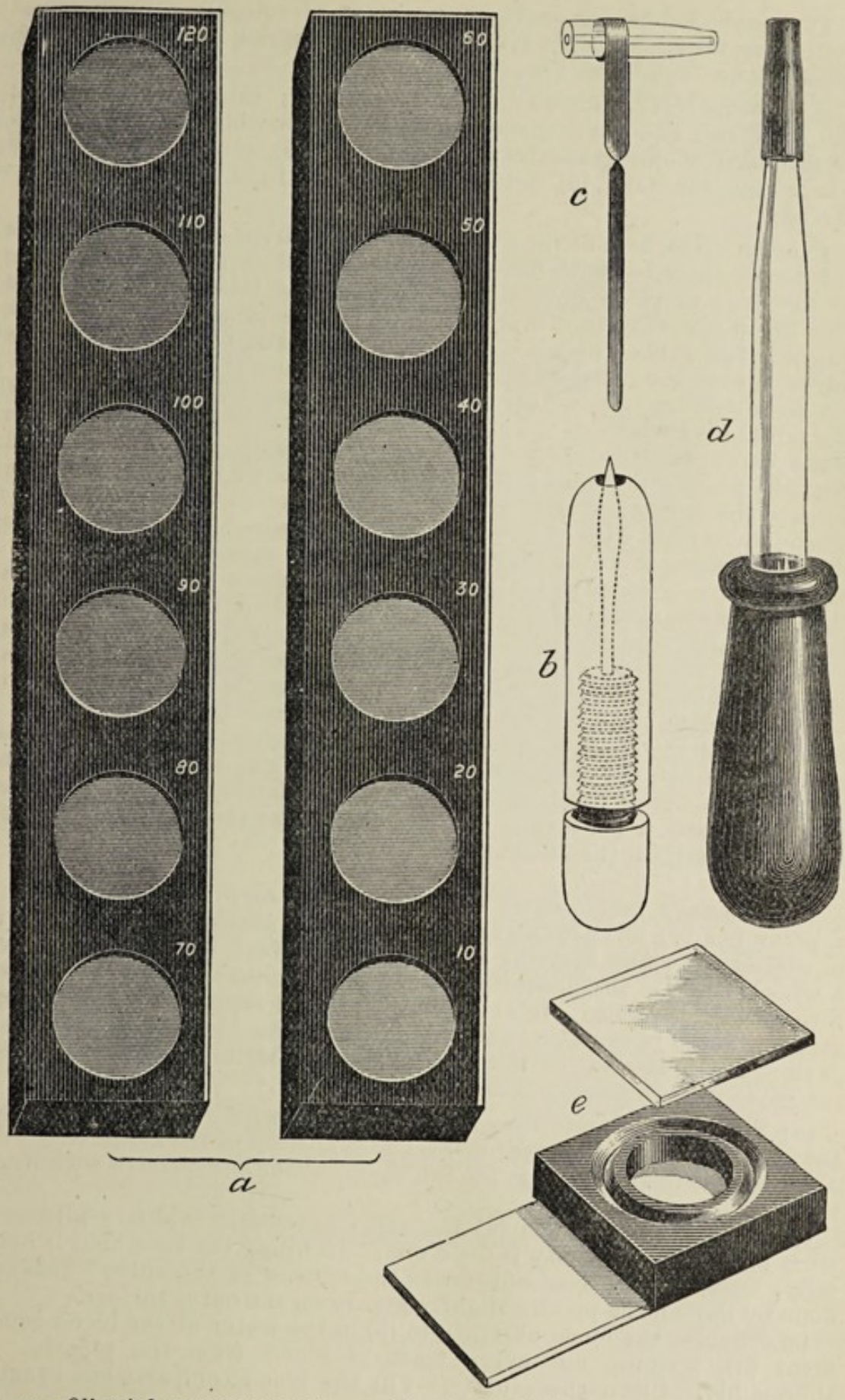


Fig. 31.—Oliver's hæmoglobinometer.—*a*, Standard gradations. *b*, Lancet. *c*, Capillary measuring pipette. *d*, Mixing pipette. *e*, Blood-cell and cover-glass. (Tintometer Co., Ltd., Salisbury.)

The method depends on the comparison of suitably diluted blood in a shallow white vessel, with standard tests prepared by Lovibond's colour-glasses (The Tintometer Co., Salisbury).

Apparatus.—(1) Automatic blood-measurer; (2) mixing pipette; (3) the blood-cell and cover-glass, the latter of faint blue glass; (4) the sets of standard colour gradations; (5) riders; (6) camera tube; (7) light, (Christmas candle); (8) bottle of antiseptic fluid, lancet, needles, and thread.

Process.—The bore of the blood-measurer is dried out by passing through it a needle threaded with darning cotton, and then the point is presented to the drop of blood obtained by pricking the lobe of the ear or finger. Any blood adhering to either end must be carefully wiped away with the finger. The rubber nozzle of the mixing pipette, charged with water, is adjusted over the polished end of the pipette, and the blood is washed into the blood-cell by pressing through the water drop by drop. The handle of the pipette is then used as a stirrer, and the further additions of water are made to impinge on it, for it serves to graduate the size of the drops required to accurately fill the cell. It is easy to do this when the observer catches the reflection of a window on the surface of the fluid. A final thorough mixing with the handle will be required, and perhaps another slight addition of water may be necessary to secure a level filling. The cover-glass is then adjusted, when a small bubble should form, showing that the cell is overfilled. The blood-cell is then placed by the side of the standard gradations. The eye quickly recognises its approximate position on the scale. Place the lighted candle about 10 cm. equidistant from the blood-cell and standard. Then the camera tube will more accurately define it. Do not look longer at a time than 10 seconds. If it is found that the blood solution is matched in depth of colour by one of the standard grades, the observation is at an end; but if it is observed to be higher than one gradation, but lower than that above it, the blood-cell is placed opposite to the former, and the riders (not shown in illustration) are added to complete the estimation.

7. Fleischl's hæmometer (fig. 32) consists of a horse-shoe stand, with a pillar bearing a reflecting surface (S) and a platform. Under the table or platform is a slot carrying a glass wedge stained red (K), and moved by a wheel (R). On the platform (M) is a small cylindrical vessel (G), divided into two compartments (a and a') by a vertical septum. In one compartment is placed pure water, and in the other the blood to be investigated. A scale (P) on the slot of the instrument enables one to read off directly the percentage of hæmoglobin.

(a) Fill with a pipette the compartment (a') with distilled water, and see that the surface of the water is level with the top of the cylinder. Fill the other compartment (a), that for the blood, about one-quarter with distilled water.

(b) Prick the finger, and fill the short automatic capillary pipette tube with blood. Its capacity is 6·5 c.mm. In filling the tube hold it horizontally. See that no blood adheres to the surface of the tube. This can be done by having the pipette slightly greasy on the outer surface.

(c) Dissolve the blood obtained in (b) in the water of the blood compartment (a), washing out every trace of blood from the pipette. Mix thoroughly. Clean the pipette. Fill the blood compartment exactly to the surface with distilled water.

(*d*) Arrange a candle in front of the reflector (S)—which is white, and with a smooth matt surface made of plaster-of-Paris—so as to throw a beam of light vertically through both compartments. Look down vertically upon both compartments, and move the wedge of glass by the milled head (T) until the colour in the two compartments is identical. Read off the scale, which is so constructed as to give the percentage of hæmoglobin.

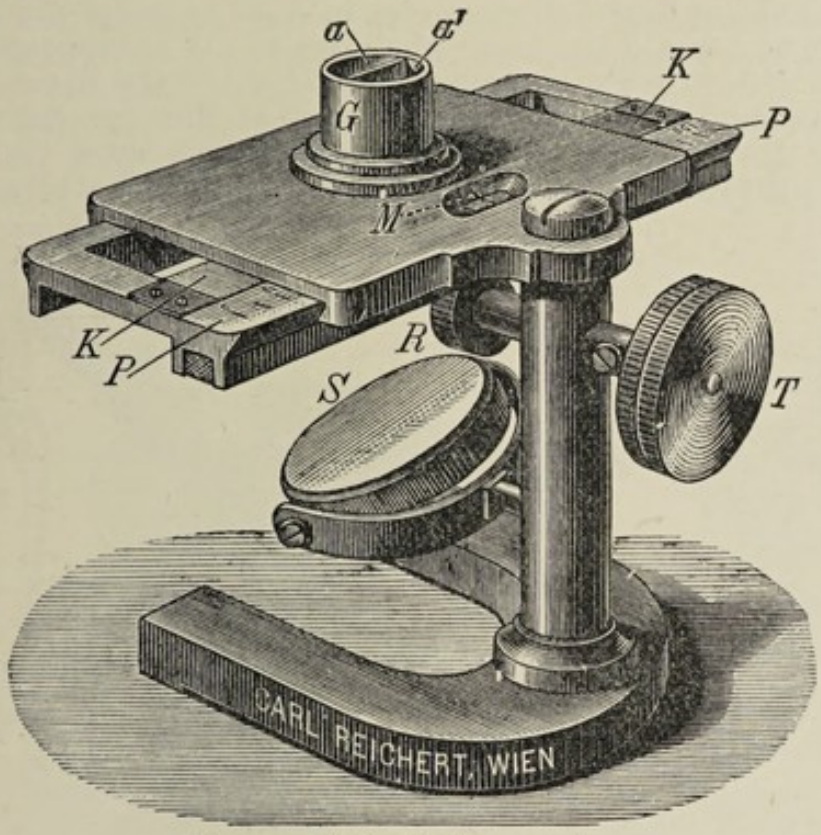


Fig. 32.—Fleischl's hæmometer.

8. Hæmoglobin scale.—T. W. Tallqvist, Helsingfors, has recently introduced a colour scale test. Special paper is used to soak up the blood, and immediately after the blood dries and has lost its humid gloss, its colour is compared, in direct daylight, with the colour scale which is supplied with the porous papers. (Lesson V., 18.)

9. Isotonic solutions.—When defibrinated blood is mixed with a watery solution of a neutral salt—*e.g.*, NaCl, according to the strength of the solution, the volume of each red corpuscle may be increased or diminished. For each animal's blood there is a certain concentration of the saline solution (usually about 0·75 p.c. NaCl) which does not affect the size of the corpuscles. Such a solution is said to be isotonic with blood serum. Hyperisotonic solutions diminish the size of the red corpuscles, and hypoisotonic increase their size. The Hb may pass into solution (Hamburger's method).

Additional Exercises.

Determination of wave-lengths.—Use Zeiss's spectroscope, which is provided with an illuminated scale for this purpose.

I. W.L. of absorption bands of oxy-hæmoglobin.—(*a*) Arrange apparatus as shown in fig. 33. A is the telescope through which the observer looks at the spectrum obtained by the light passing through B, and dispersed by the flint-glass prism in the centre of the apparatus. In C is fixed a scale photographed on glass and illuminated by a fan-tailed burner. D is the hæmatinometer containing the dilute blood.

(*b*) Place black velvet over the prism; light both lamps; look through A; adjust the slit in B, and the telescope in A, so as to get a good view of the spectrum, and over it the image of the scale. D is supposed not to be in position at first. On platinum wire, burn NaCl in the flame to get the yellow sodium line D. Adjust the scale so that this line corresponds to the figures 58·9 on the scale, and fix the spectroscope tubes (A and C) in this position; the scale is now accurately adjusted for all other parts of the spectrum.

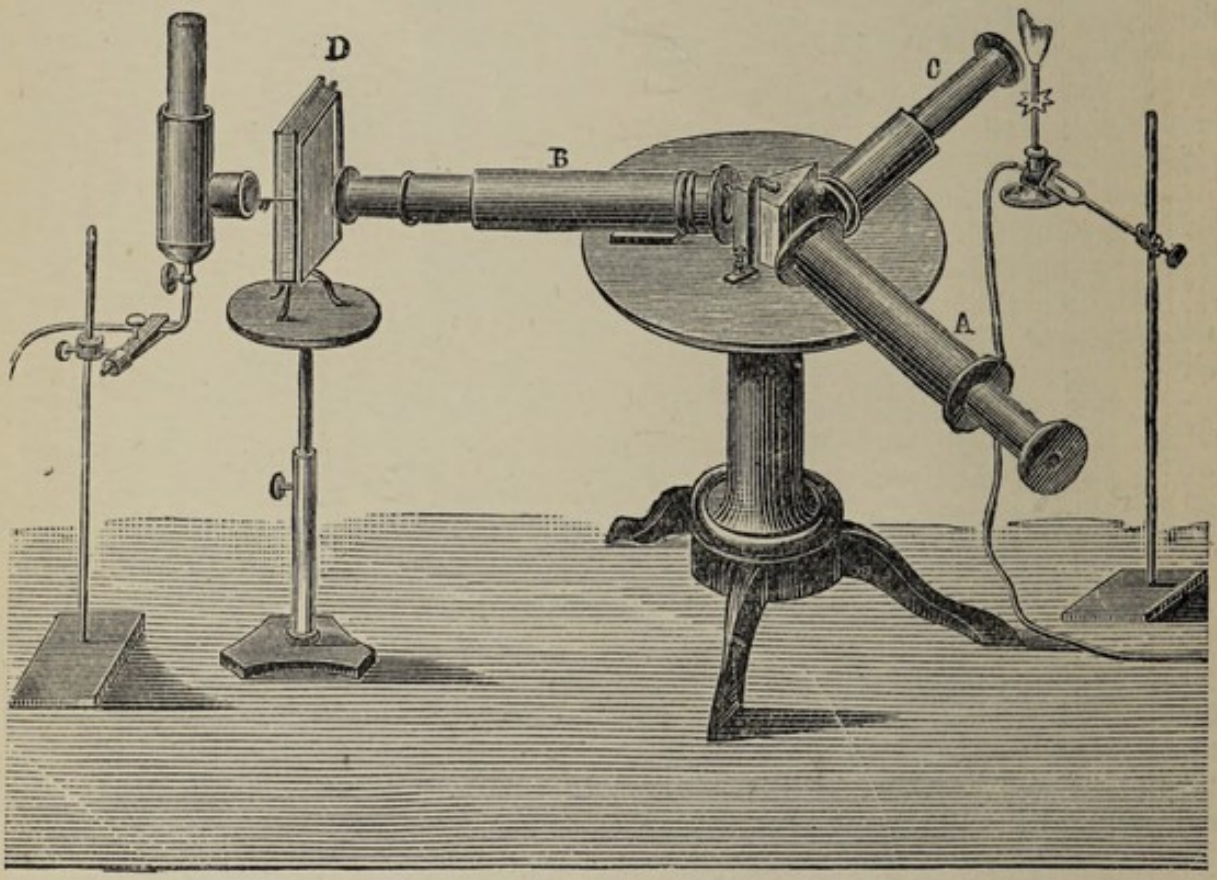


Fig. 33.—Arrangement of the Spectroscope for determining wave-lengths.—A, Telescope. B, Collimator tube. C, Scale tube. D, Hæmatinometer.

"The numbers on the scale indicate wave-lengths expressed in one hundred thousandths of a millimetre, and each division indicates a difference in wave-length equal to one hundred thousandth of a millimetre" (*Gamgee*).

Thus, Fraunhofer's line, D, which corresponds to division 58·9 of the scale, has a wave-length of 589 millionths of a millimetre. The wave-lengths of Fraunhofer's lines are:—A = 760·4, B = 687·4, C = 656·7, D = 589·4, E = 527·3, F = 486·5.

(*c*) Using a blank map supplied with Zeiss's spectroscope—the maps correspond to the scale seen in the spectroscope—fill in, in wave-lengths, the position of Fraunhofer's lines B to F.

(*d*) Pour diluted solution of blood or hæmoglobin—1 part in 1000 of water—into a hæmatinometer, D. Place the latter between the flame and the spectroscope (fig. 33). The spectrum shows the two absorption bands of oxy-hæmoglobin between D and E. The narrower, sharper, and darker band near D has its centre corresponding with the W.L. 579, and it may conveniently be expressed by the letter *α* of the oxy-hæmoglobin spectrum.

The other absorption band near E (*β*) is broader, not so dark, and has less sharply defined edges than *α*. Its centre corresponds to the W.L. 543·8. The other parts of the spectrum are visible, there being only slight cutting off of the red, and a slightly greater absorption of the violet.

(*e*) Using a stronger blood-solution, observe how the two bands become fused into one, while more and more of the red and violet of the spectrum are absorbed as the solution is made stronger, until finally only a little red light is transmitted.

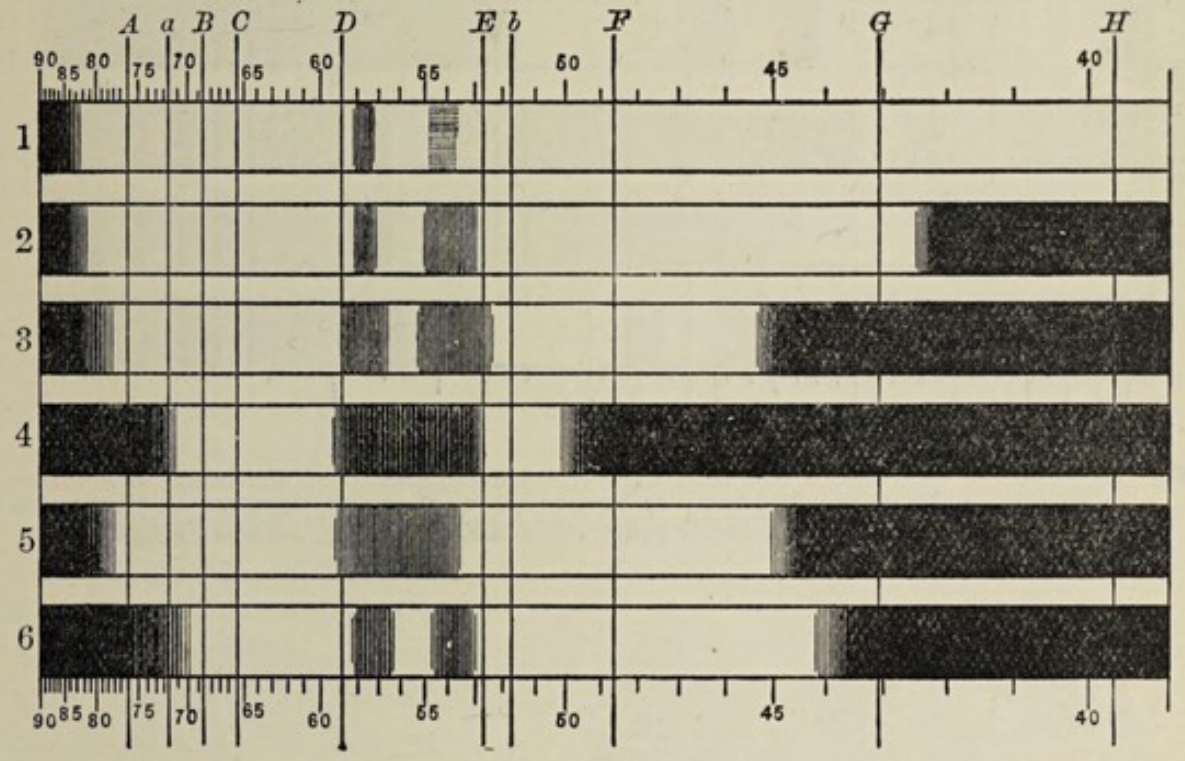


Fig. 34.—Spectra of Oxy-Hæmoglobin (1, 2, 3, 4), 1 = 0·1, 2 = 0·2, 3 = ·37, 4 = ·8 p.c. of Oxy-Hæmoglobin, Hæmoglobin, (5), and Carbonic Oxide Hæmoglobin (6). Wave-lengths added. The numbers attached to the scale indicate W.L. expressed in 100,000ths of a millimetre.

II. W.L. of absorption band of reduced Hb.—(*a*) Adjust the apparatus as before, but reduce HbO_2 solution with Stokes' fluid—noticing the change of the colour to purplish or claret—until a solution is obtained which gives the single characteristic absorption band of reduced Hb. This is usually obtained with a solution of Hb of about 0·2 p.c.

(*b*) Observe a single absorption band less deeply shaded, and with less defined edges between D and E—*i.e.*, *α* band, which extends between W.L. 595 and 538, and is not quite intermediate between D and E, is blackest opposite W.L. 550, so that it lies nearer D than E. Both ends of the spectrum are more absorbed than with a solution of HbO_2 of the same strength. On further dilution the band does not resolve itself into two, but diminishes in width and intensity (fig. 34, 5).

III. Bands of carbonic oxide hæmoglobin (W.L.).—(*a*) Use a dilute solution of HbCO with two characteristic absorption bands, *α* and *β*—like those of HbO_2—but both are *very slightly* more towards the violet. *α* extends from about W.L. 587 to 564, and *β* from 547 to 529.

(*b*) No reduction by reducing agents on adding $(NH_4)_2S$ or Stokes' fluid (fig. 34, 6).

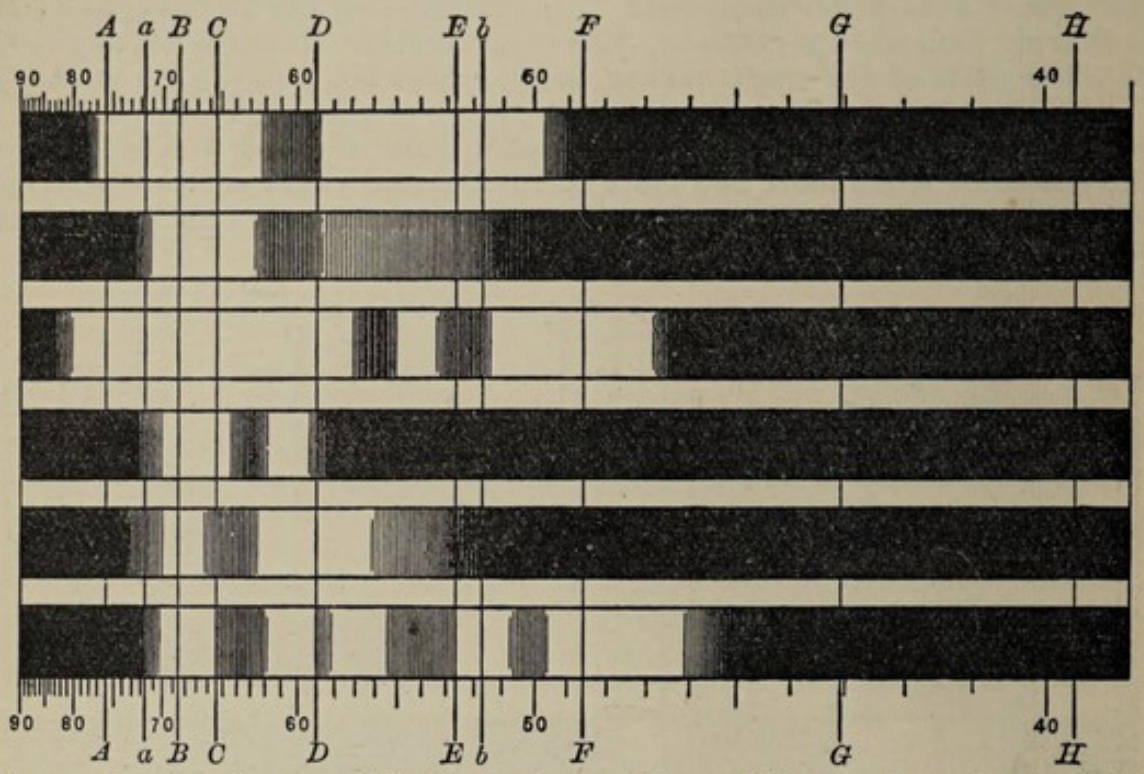


Fig. 35.—Spectra of some of the Derivatives of Hæmoglobin. 1. Hæmatin in alkaline solution. 2. The same, but more concentrated. 3. Hæmochromogen. 4. Methæmoglobin. 5. Acid hæmatin (acetic acid). 6. Acid hæmatin in ethereal solution.

LESSON VIII.

SALIVARY DIGESTION.

1. Mixed saliva.—(*a*) Rinse out the mouth with water. Inhale the vapour of ether, glacial acetic acid, or even cold air through the mouth, which causes a reflex secretion of saliva. In doing so, curve the tongue so as to place its tip behind the incisor teeth of the upper jaw. Or chew a piece of india-rubber or pure paraffin. In a test-glass collect the saliva with as few air-bubbles as possible. If it be turbid or contain much froth, pass it through a small filter perforated at its apex by a pin-hole.

(*b*) **Salivary extract.**—Instead of saliva an aqueous solution of a ptyalin-yielding gland may be used—*e.g.*, parotids of rabbit extracted for several hours and the opalescent extract filtered. Label "Salivary extract." The proteids present are apt to obscure the sugar reaction.

Neither saliva nor the salivary extract reduce $CuSO_4$. The specific ferment present is the amylolytic or diastatic ferment **ptyalin**, which ultimately converts boiled starch into a reducing sugar, maltose. The amylolytic action is greatest in the saliva collected before breakfast.

2. Microscope.—With a high power observe the presence of (1) squamous epithelium, (2) salivary corpuscles (8 to 11 μ), (3) perhaps *débris* of food, (4) possibly air-bubbles, and (5) fungi—especially various forms of bacteria (fig. 36).

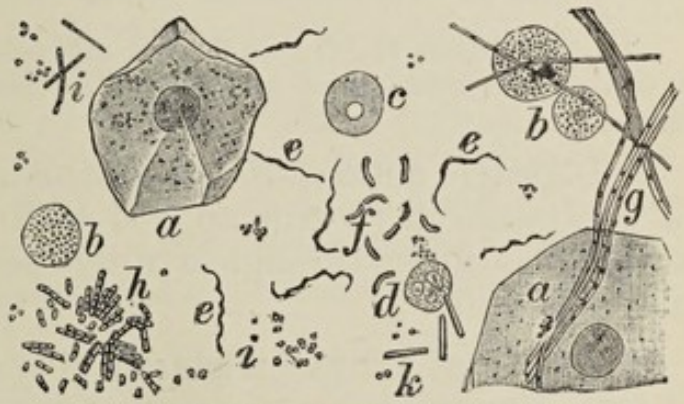


Fig. 36.—Microscopic appearances of saliva.

3. Characters (S. G. 1002 to 1006).—It is a slimy, thickish slightly opalescent or clear fluid mixed usually with air-bubbles.

(*a*) On standing, it separates into two layers; the lower one is cloudy and turbid, and contains in greatest amount the morphological constituents.

(*b*) Its **reaction** is alkaline to neutral litmus paper, to lacmoid, but it reacts acid to phenolphthalein. The alkalinity is due to hydrogen alkali phosphates. It contains no sodium carbonate.

(*c*) Add acetic acid = a precipitate of **mucin** not soluble in excess. Filter.

(*d*) With the filtrate from (*c*), test for traces of **proteids** (serum-albumin and globulin) with the xanthoproteic reaction and Millon's test, or add a drop of strong solution of ferrocyanide of potassium. The slight precipitate is due to a trace of **proteids.**

(*e*) To a few drops of saliva in a porcelain vessel add a drop of *dilute* HCl and then dilute ferric chloride = a red colouration due to **potassic sulpho-cyanate.** The colour does not disappear on heating, or on the addition of an acid, but is discharged by mercuric chloride. Meconic acid yields a similar colour, but it is not discharged by mercuric chloride. The sulpho-cyanate is present only in parotid saliva, and is generally present in mixed saliva. Pure saliva yields characteristic results with xanthoproteic, Millon, and $CuSO_4$ + NaHO, due chiefly to mucin.

(*f*) Compare very dilute potassic sulpho-cyanate with (*e*).

(*g*) *Gscheidlen's* method. Dip filter paper in weak acidulated (HCl) ferric chloride solution, and allow it to dry. Contact with a drop of saliva gives a reddish stain.

(*h*) The **salts** are tested for in the usual way (see "Urine"). Test for chlorides (HNO_3 and $AgNO_3$), carbonates (acetic acid), and sulphates (barium chloride and nitric acid).

(*i*) Nitrites are sometimes present. Add saliva to starch paste, containing a little H_2SO_4 and KI. Nitrites if present give an intense blue colour.

(*j*) Add dilute H_2SO_4, and then meta-diamido-benzol. Yellow colour indicates the presence of nitrites. This reaction does not succeed in all cases.

4. Digestive action—*Starch solution.*—Make a 0·5 p.c. solution of potato-starch (1 grm. to 200 boiling water). To get rid of air-bubbles filter through a filter perforated at its apex by a pin-hole.

5. Action of saliva on starch (Ptyalin, a diastatic enzyme).

(*a*) Dilute saliva with 5 volumes water, and filter it.

(*b*) Label three test-tubes A, B, and C. In A place starch mucilage, in B saliva, and in C 1 volume of saliva and 3 volumes of starch mucilage. Place them in a water-bath at 40° C. for 10 minutes. Test for a reducing sugar in portions of all three, by Fehling's or Trommer's test. A and B give no evidence of sugar, while C reduces the Fehling, giving a yellow or red deposit of cuprous oxide. Therefore, starch is converted into

a reducing sugar by the saliva. This is done by the ferment ptyalin contained in it.

(*c*) Test C with solution of iodine; if no blue colour is obtained all the starch has disappeared, being converted into a reducing sugar or maltose.

(*d*) Place *thick* starch mucilage in test-tubes A and B. Keep A for comparison, add saliva to B, and keep both at 40° C. A is unaffected, while B soon becomes fluid and loses its opalescence; this liquefaction is a process quite antecedent to the saccharifying process which follows.

(*e*) Mix crumbs of bread with salivary extract, and incubate at 40°. Disintegration of the bread takes place, and a marked increase in the amount of reducing sugar.

6. Stages between starch and maltose—*Course of salivary digestion.*—Mix starch and saliva as in 5 (*a*) C, and place in a water-bath at 40° C. At intervals of 2 minutes take a drop of the liquid and add it to a drop of iodine solution on a white porcelain plate.

Note the following stages:—At first there is pure blue with iodine due to the soluble starch formed giving also a blue with iodine, later a deep violet, showing the presence of erythro-dextrin, the violet resulting from a mixture of the red produced by the dextrin and the blue of the starch. Then the blue reaction entirely disappears, and a reddish-brown colour, due to erythro-dextrin alone, is obtained. After this the reaction becomes yellowish-brown, and finally there is no reaction with iodine at all, achroo-dextrin being formed—the *achromic point*—along with a reducing sugar or maltose. The latter goes on forming after iodine has ceased to react with the fluid, and its presence is easily ascertained by Fehling's solution. The soluble starch is precipitated by alcohol, while maltose is not. In this way this body may be separated. Saturation with $(NH_4)_2SO_4$ precipitates the starch and soluble starch, but not the erythro-dextrin or maltose.

7. Conditions affecting salivary digestion.—(*a*) Label three test-tubes A, B, and C. Place in A saliva, *boil* it, and add starch mucilage. In B and C place starch mucilage and saliva, to B add a few drops of hydrochloric acid (to 0·3 p.c.), and to C caustic potash. Place all three in a water-bath at 40° C., and after a time test them for sugar by Fehling's solution. No sugar is formed—in A because the *ferment was destroyed by boiling*, and in B and C, because strong acids and alkalies arrest the action of ptyalin on starch.

(*b*) If a test-tube containing starch mucilage and saliva be prepared as in 5 (*a*) C, and placed in a freezing mixture, the conversion of starch into

a reducing sugar is arrested; but the ferment is not destroyed, for on placing the test-tube in a bath at 40° C., the conversion is rapidly effected. *Hence a temperature of 0° C. only arrests, but does not destroy the action of saliva.*

(*c*) Mix raw starch with saliva and keep it at 40° C. Test it after half an hour, when little or no sugar will be found.

(*d*) To 5 c.c. of neutralised saliva add 5 c.c. 0·2 p.c. HCl (mixture = 0·1 p.c. HCl). Keep at 40° for 10 minutes. Neutralise with 4 c.c. 0·4 p.c. Na_2CO_3. Add starch solution and keep at 40°. No sugar will be formed, as the mineral acid has destroyed the ptyalin.

Additional Exercises.

8. Starch is a colloid. Sugar is a crystalloid and dialyses.

(*a*) Place in a sausage parchment tube 20 c.c. starch mucilage (A), and into another starch mucilage with saliva (B). Suspend A and B in distilled water in separate vessels. Sugar dialyses, starch does not.

(*b*) After some hours test the diffusate in the distilled water. No starch will be found in the diffusate of either A or B, but sugar will be found in that of B, proving that sugar dialyses, while starch does not. Hence the necessity of starch being converted into a readily diffusible body during digestion.

9. Action of malt-extract on starch.

(*a*) Rub up 10 grms. of starch with 30 c.c. of distilled water in a mortar, add 200 c.c. of boiling water, and make a strong starch mucilage.

(*b*) Powder 5 grms. of pale *low-dried* malt, and extract it at 50° C. for half an hour with 30 c.c. of distilled water, and filter. Keep the filtrate.

(*c*) Place the starch paste of (*a*) in a flask, and cool to 60° C., add the extract of (*b*), and place the flask in a water-bath at 60° C.

(*d*) Observe that the paste soon becomes fluid, owing to the formation of soluble starch, and if it be tested from time to time (as directed in 6), it gives successively the tests for starch and erythro-dextrin. Continue to digest it until no colour is obtained with iodine—*i.e.*, until all starch and erythro-dextrin have disappeared.

(*e*) Take a portion of (*d*) and precipitate it with alcohol = achroo-dextrin. The liquid also contains maltose (*f*).

(*f*) Boil the remainder of the fluid, cool, filter, and evaporate the filtrate to 20 c.c. Add 6 volumes of 90 p.c. spirit to precipitate the dextrin; boil, filter, and concentrate to dryness on a water-bath, and dissolve the residue in distilled water. The solution is maltose ($C_{12}H_{22}O_{11} + H_2O$). If the alcoholic solution be exposed to air, crystals of maltose are formed.

10. Compare the reducing power of maltose and dextrose.

(*a*) With Fehling's solution estimate the reducing power of the solution obtained in 9 (*f*). (See "Urine.")

(*b*) Boil in a flask for half an hour 50 c.c. solution of maltose with 5 c.c. HCl. Neutralise with NaHO, make up the volume to 50 c.c., and determine by Fehling's solution the reducing power. The acid has converted the maltose into dextrose, and the ratio of the former estimation (*a*) to the present one should be 65 to 100.

(*c*) The reducing power of pure dextrose treated as in (*b*) is not affected.

11. Drugs on salivary action (after *Sollmann*).—Test the effect of several of the following on salivary digestion of starch. Carbolic acid 1 p.c. and 5 p.c. Saturated watery solution of any of the following:—Salicylic, benzoic, boric acid. Alcohol 10 and 50 p.c. (stopper these test-tubes). $HgCl_2$, 2 : 1000. Quinine bisulphate 1 and 2 p.c. Water.

Put in each test-tube—

1 vol. boiled starch, 2 p.c.	$\frac{1}{2}$ vol. saliva.
1 ,, Na_2CO_3, 5 p.c.	$2\frac{1}{2}$ vols. of drug.

Shake and set in water-bath at 40° for an hour. Compare activity of digestion thus:—Add to each tube an equal volume of 10 p.c. NaOH, and boil for 2 minutes. The depth of colour roughly corresponds to the amount of digestion.

The action of saliva on actual articles of diet is omitted here. (See *Digestion and Diet*, by Sir William Roberts, 2nd edit., 1897.)

Note that mixed saliva, whether collected by mechanical stimulation or without effort, varies both in composition and amylolytic power. With mechanical stimulation after alcohol or ether, the saliva secreted contains more alkaline-reacting salts and more ferment. More mucus is obtained with chemical stimuli. ("Variations in power and composition of saliva," Chittenden and Richards, *Amer. Journ. of Phys.*, i., 461, 1898.)

LESSON IX.

GASTRIC DIGESTION.

Gastric juice has an acid reaction due to the presence of free HCl, and consists essentially of a watery solution of hydrochloric acid, the ferments pepsin and rennin, and certain salts. Both a free acid—preferably dilute HCl—and pepsin are necessary for the gastric digestion of proteids. There seems to be some special relation between the HCl and pepsin, sometimes written "pepsin-acid." Approximately, it contains 99·4 p.c. water and 0·6 p.c. of solids. Of the latter 0·33 are organic (chiefly pepsin) and 0·02 to 0·2 p.c. free HCl, 0·1 NaCl, and the remainder chiefly other chlorides and phosphates. S.G. 1002 to 1006.

1. Artificial gastric juice.—(*a*) Take part of the cardiac end of the pig's stomach, which has been previously opened and washed rapidly in cold water, and spread it, mucous surface

upwards, on the convex surface of an inverted capsule. Scrape this surface firmly with a scalpel, and rub up the scrapings in a mortar with fine sand. Add water, and rub up the whole vigorously for some time, and filter through linen. The filtrate is an aqueous solution of pepsin. It is better, however, not to filter but to add an equal volume of 0·2 p.c. HCl and leave it to extract for 24 hours. Strain through linen or cotton wool. Label *Acidulated aqueous extract of pepsin.* It is used as an artificial gastric juice when diluted with 3 vols. of 0·1 p.c. HCl.

(*b*) *V. Wittich's method.*—From the cardiac end of a pig's stomach detach the mucous membrane in shreds, dry them between folds of blotting-paper, place them in a bottle, and cover them with strong glycerin for several days. The glycerin dissolves the pepsin, and on filtering through fine linen a glycerin extract is obtained, which keeps indefinitely. Add, when it is to be used, 10 to 20 vols. of a 0·2 p.c. of HCl.

(*c*) *Kühne's method.*—Place 130 grms. of the cardiac mucous membrane of a pig's stomach in 5 litres of water containing 80 c.c. of 25 p.c. HCl—*i.e.*, 0·2 p.c. Heat for twelve hours at 40° C. Almost all the mucous membrane is dissolved. Strain through flannel and then filter. This is a powerfully peptic fluid, but it contains peptones. It can be kept for a long time. The test of an active preparation of gastric juice is that a thread of fibrin, when placed in the fluid and warmed, should be dissolved in a few minutes.

(*d*) Benger's liquor pepticus, or the pepsin of Burroughs, Wellcome & Co., or that of Parke, Davis & Co., when added to hydrochloric acid of the proper strength, have high digestive powers.

[(*e*) *Hydrochloric acid* (0·2 p.c.).—Add 6·5 c.c. of ordinary commercial HCl to 1 litre of distilled water.]

2. Pepsin and acid (HCl) necessary for gastric digestion.

(*a*) Take three beakers or large test tubes, label them A, B, C. Put into A water and a few drops of glycerin extract of pepsin, B 0·2 p.c. of HCl, and C 0·2 p.c. of HCl and a few drops of glycerin extract of pepsin. Put into all three a small quantity of well-washed fibrin, and place them in a water-bath at 40° C.

(*b*) Examine them from time to time. In A the fibrin is unchanged; in B the fibrin is clear and swollen up; on neutralisation it will appear unaltered; in C it has disappeared, having first become swollen up and clear, and then completely dissolved. It will ultimately be converted into peptones. Therefore, both acid and ferment are required for gastric digestion.

Tube A.	Tube B.	Tube C.
Water. Pepsin. Fibrin.	Water. HCl (0·2 p.c.). Fibrin.	Water. HCl (0·2 p.c.). Pepsin. Fibrin.
AFTER TWENTY MINUTES.		
Unchanged.	Fibrin swells up, becomes clear, and traces of acid-albumin formed.	Acid-albumin formed; traces of albumoses; fibrin dissolved.
AFTER ONE HOUR.		
Unchanged.	More acid-albumin formed.	Small amount (or no) acid-albumin; some albumoses, and peptone.

The chief digestive action of gastric juice is to convert—by a process of hydrolysis—the proteids of the food into soluble and diffusible peptones. The proteid molecule takes up water and yields certain intermediate bodies—acid-albumin and propeptones or proteoses, and finally, after prolonged action, peptones. If albumin be digested, the stages are—

(1) Acid-albumin or syntonin.

(2) Proteoses, { (*a*) Proto-albumose, } Called "primary proteoses," because they are first formed.
{ (*b*) Hetero-albumose, }
{ (*c*) Deutero-albumose, } "Secondary proteoses."

(3) Peptone.

In the stomach itself the process may be divided into three stages:—The *first stage* (15 to 40 mins.), when the total acidity rises, but *free HCl* is absent, having combined with proteids. In this stage digestion of starch by the ptyalin of the saliva may still go on in the stomach; hence it has been sometimes called the "amylolytic stage." Lactic acid may be present, either from the food or by the action of bacteria. The *second stage* (15 or 40 mins. to 2 to 4 hours), when the acidity rises and free HCl is present, but much combined with proteids; lactic acid absent or in traces. The *third stage* (till emptying of stomach), when the total acidity falls, more free HCl and less combined acid.

It is to be noted that the gastric contents generally have a higher acidity than the juice itself.

3. **Products of gastric digestion and its conditions.**—(*a*) Half fill three large test-tubes or beakers, labelled A, B, C, with HCl 0·2 p.c. Add to each 5 drops of glycerin extract of pepsin or Benger's "liquor pepticus." Boil B, and make C neutral with sodium carbonate. The alkalinity may be noted by adding previously some neutral litmus solution. Add to each an equal amount of well-washed fibrin—which has been previously steeped for some time in 0·2 p.c. HCl, so that it is swollen up and transparent. Keep in a water-bath (fig. 37) at 40° C. for an hour, and examine them at intervals of 20 minutes.

(*b*) After 10 minutes or so, the fibrin in A is dissolved, and the fluid begins to be turbid. In B and C there is no change. Even after long exposure to 40° C. there is no change in B and C. Why? In B, because pepsin loses its activity on boiling; in C the pepsin has not lost its power, because if more acid be added, and the mixture kept at 40° C., digestion will take place. *Neutralisation suspends but does not destroy the action of pepsin, while boiling does so.* After three-quarters of an hour filter A and part of B and C. Keep the filtrates.

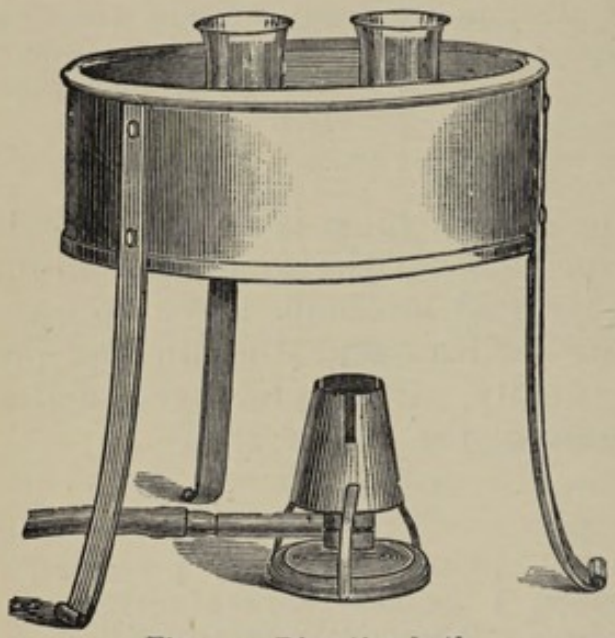


Fig. 37.—Digestion-bath.

(*c*) Carefully neutralise the filtrate of A with dilute NaHO = a precipitate of **acid-albumin.** Filter off this precipitate, dissolve it in 0·2 p.c. HCl. It gives proteid reactions (Lesson I.).

(*d*) Test the filtrate of (*c*) for **albumoses or proteoses.** Repeat all the tests for albumose (Lesson I.). **Albumose** is soluble in water, and gives all the ordinary proteid reactions. It is precipitated by HNO_3 in the cold in presence of NaCl; the precipitate disappears on heating, and reappears on cooling. This is a characteristic reaction. It is precipitated by acetic acid and ferrocyanide of potassium; by acetic acid and a saturated solution of sodic sulphate; and by metaphosphoric acid: while peptones are not. It gives the biuret reaction and is soluble in water (like peptone).

(i.) Few drops HNO_3 = a white precipitate, which *disappears* on *heating* (yellowish) and *reappears* on *cooling* (proto-proteose).

(ii.) Two drops acetic acid and ferrocyanide of potassium = a white precipitate, which *disappears* on *heating* and *returns* on *cooling* (proto- and deutero-proteose).

(iii.) Biuret reaction—*i.e.*, NaHO + $CuSO_4$ = *pink* colour (peptone gives the same). A solution giving (iii.) and not (i.) and (ii.) would indicate peptone.

(iv.) Saturate the filtrate of C with Am_2SO_4. This precipitates the proteoses. A more complete precipitation is obtained by adding dilute acetic acid and boiling with Am_2SO_4, and then neutralising the acid. The peptones are not so precipitated.

(*e*) Filter and test the filtrate for **peptones** (Lesson I.). In the biuret reaction, owing to the presence of Am_2SO_4, a great excess of soda has to be added.

(*f*) Neutralise part of the filtrates of B and C. No precipitate nor reactions for peptones. In B pepsin was destroyed by boiling, in C it cannot act in an alkaline medium.

(*g*) Add acid to the remainder of C, and keep at 40° C.; digestion takes place, so that neutralisation has not destroyed the activity of the ferment.

4. Proteoses.—For the methods used by Kühne to isolate the varieties of albumose see Add. Excs. The following can be carried out by the student in a gastric digest with the acid-albumin precipitated and filtered off, and with filtrate showing well-marked reactions for proteose:—

(*a*) *Primary proteoses* (proto- and hetero-proteose).—Saturate part of the filtrate with NaCl crystals = precipitate chiefly of these proteoses. Filter.

(*b*) *Secondary proteoses* (deutero-proteose).—To the filtrate add acetic acid = precipitate of deutero-proteose (and a little proto-proteose). [The filtrate contains peptone.]

5. Albumose tests (Lesson I.).—It is precipitated by the following substances: Nitric acid; acetic acid and NaCl; acetic acid and ferrocyanide of potassium. The precipitates are *soluble on heating and reappear on cooling.* In all these respects it differs from peptone. Like peptone, however, it gives the biuret (*rose pink*) reaction, and is not coagulated by heat.

6. Peptone tests (Lesson I.).—The following table from Halliburton shows the chief characters of the final product peptone,

and the intermediate albumoses in contrast with those of a native proteid like albumin :—

Proteid.	Heat.	Alcohol.	Nitric acid.	Am_2SO_4.	NaHO + $CuSO_4$.	Diffusibility.
Albumin.	Coagulated.	↓ Then coagulated.	↓ In cold, not readily soluble on heating.	Precipitated.	Violet colour.	Nil.
Proteoses (Albumoses).	Not coagulated.	↓ But not coagulated.	↓ In cold, soluble on heating, return on cooling.	Precipitated.	Rose-red colour (biuret reaction).	Slight.
Peptones.	Not coagulated.	↓ But not coagulated.	Not precipitated.	Not precipitated.	Rose-red (biuret reaction.	Great.

(The ↓ indicates precipitated).

7. Gastric juice is antiseptic.—Set aside a gastric digest, and observe that it does not putrefy readily.

8. Gastric juice on milk.—(*a*) Mix 5 c.c. of fresh milk in a test-tube with a few drops of *neutral* artificial gastric juice; keep at 40° C. In a short time the milk curdles, so that the tube can be inverted without the curd falling out. By-and-by whey is squeezed out of the clot. The curdling of milk by the rennet ferment present in the gastric juice is quite different from that produced by the "souring of milk," or by the precipitation of caseinogen by acids. Here the casein (carrying with it most of the fats) is precipitated in a neutral fluid.

(*b*) To milk in a test-tube add 5 c.c. of 0·4 p.c. hydrochloric acid, and keep at 40° C. for 2 hours. The pepsin in the presence of the acid digests the casein, gradually dissolving it forming a straw-yellow-coloured fluid containing peptones. The "peptonised milk" has a peculiar odour and bitter taste.

(*c*) *Peptonised milk.*—To 5 c.c. of milk in a test-tube add a few drops of Benger's liquor pepticus, and place in a water-bath. The caseinogen first clots, and is then partially dissolved to form a yellowish-coloured fluid, with a bitter taste and peculiar odour. There generally remains a very considerable clot of casein; and, in fact, the gastric digestion of milk is slow, especially if compared with its tryptic digestion (Lesson X. 8). Test the fluid for peptones with the biuret reaction, and observe the

light-pink colour obtained. The bitter taste renders milk "peptonised" by gastric juice unpalatable.

9. Rennet on milk.—(Rennin the enzyme).

(*a*) To milk in a test-tube add a drop or two of rennet—*e.g.*, Clark's commercial rennet—and keep in the warm bath at 40° C. Rennet is obtained from the fourth stomach of the calf. The milk becomes solid in a few minutes, forming a **curd**, and by-and-by the curd of casein contracts and squeezes out a fluid—the **whey**.

(*b*) Repeat, but boil the rennet. No such result, because the ferment is destroyed by heat.

10. Tests for free mineral (HCl) and organic acids.

(*a*) Place in tube A 10 c.c. of a 0·2 p.c. HCl, and in tube B 10 c.c. of 2 p.c. acetic acid. To both add a few drops of **oo-Tropæolin** dissolved in alcohol. The very dilute mineral acid in A renders it rose-pink, while the organic acid does not affect its colour. Or, allow a drop of a saturated alcoholic solution to evaporate on a white porcelain slab at 40° C., and then add a drop of the acid solution. On evaporation a violet tint indicates an inorganic acid, ·005 HCl can be thus detected (*Langley*). It is stated not to be quite a reliable test in the presence of certain organic matters.

(*b*) Repeat (*a*), but add to the acids a dilute watery solution of **methyl-violet**, and note the change of colour produced by the mineral acid. It becomes blue and then green. If a strong solution of acid be used, the colour is discharged, but this is never the case with the percentage of acid in the stomach. The peptones in gastric juice may be precipitated by the previous addition of 10 p.c. tannic acid, and then the test can be applied. In the presence of proteids in gastric juice it does not give absolutely reliable results.

(*c*) Repeat (*a*) with the same acids, but use paper stained with **congo-red**, and observe the change of colour to blackish-blue or intense blue produced by the hydrochloric acid. Wash in ether; if the red colour reappears the acid is organic, if not, mineral. Organic acids make it violet, not blue.

(*d*) **Tœpfer's Test** (Di-methyl-amido-azobenzol) (0·5 p.c. alcoholic solution).—It gives a pink with dil. HCl or filtered gastric juice. Do the test on a white background. It is said to be the most delicate test for free HCl yet introduced.

(*e*) **Phloro-glucin and Vanillin** (*Günzburg*).—Dissolve 2 grms. phloro-glucin and 1 grm. vanillin in 100 c.c. alcohol. Mix equal quantities of this with the fluid to be tested, and evaporate the mixture in a watch-glass on a water-bath. Do not allow the fluid to boil. The presence of HCl is shown by the formation of a delicate rose-red tinge or stain, or, if there be much hydrochloric acid, of red crystals. This reaction will detect ·06 p.c. HCl, and is said not to be impeded by organic acids, albumin, or peptone.

(*f*) **Benzo-pupurin 6 B.**—Use filter paper soaked in a saturated watery solution of this fluid and dried. HCl (·4 grm. in 100 c.c.) makes it dark blue, while organic acids make it brownish-violet. If both HCl and organic acids be present, the stain is brownish-black; but if the stain be suspected to be partly due to HCl, wash the paper in a test-tube with sulphuric ether, which removes the stain due to the organic acid, leaving that due to the HCl unaffected. The sulphuric ether does not affect the mineral acid stain.

(*g*) **Mohr's test.**—Mix together 2 c.c. of a 10 p.c. solution of sulpho-cyanide of potassium, 0·5 c.c. of a neutral solution of ferric acetate, and 8·5 c.c. water. Place a few drops of this ruby-red fluid on a porcelain capsule, and allow a few drops of the gastric juice to mix with it = a light violet colour at the point of contact, and a mahogany brown when the fluids mix. It is less sensitive than the aniline tests.

(*h*) Shake up a mixture of dilute HCl and an organic acid—*e.g.*, lactic, with ether. Remove the ether, and on evaporating it, it will be found to have dissolved the organic acid, but not the mineral one. On this fact is based Richet's method of determining the amount of an organic acid in presence of a mineral acid.

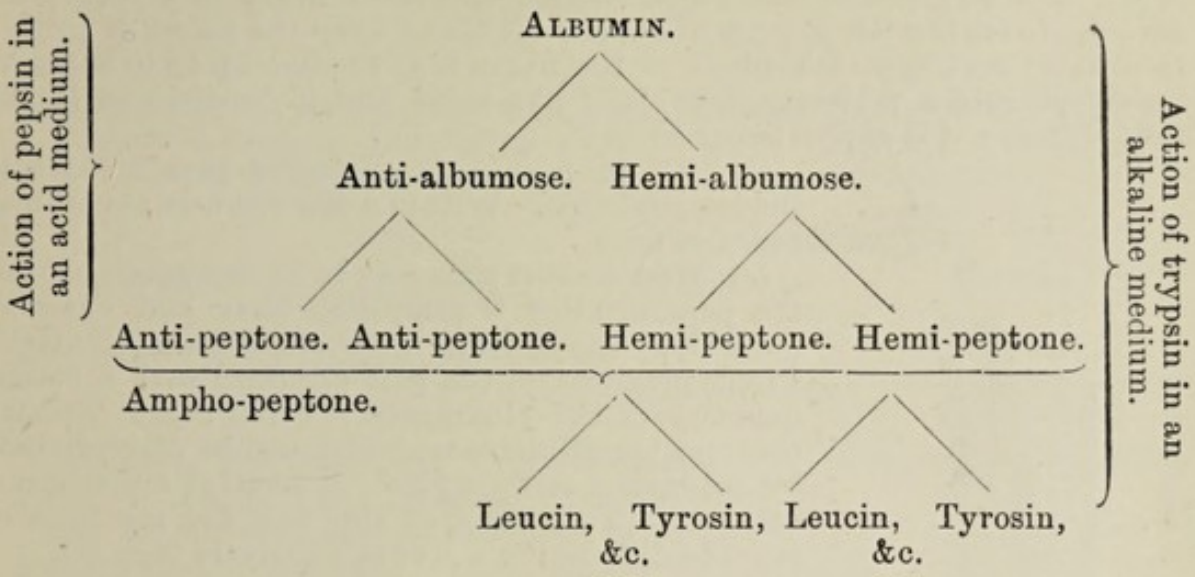
These reactions for a free mineral acid are important, as they are used clinically for ascertaining the presence or absence of HCl—*e.g.*, in a vomit. This acid is frequently absent from gastric juice in cancer of the stomach. In gastric catarrh the HCl may be greatly diminished and lactic acid abundant. The presence of peptones interferes with the delicacy of some of these reactions.

11. Carbolo-iron test for lactic acid (*Uffelmann*).—Prepare a fresh solution by mixing 10 c.c. of a 4 p.c. solution of carbolic acid with 20 c.c. of distilled water, and 1 drop of liquor ferri perchloridi. The amethyst-blue solution thus obtained is changed to *yellow* by lactic acid, while it is not affected by 0·2 p.c. HCl; but alcohol, sugar, and phosphate yield a similar reaction.

A faintly yellow-coloured solution of ferric chloride (2 to 5 drops to 50 c.c. water) is not affected by the addition of HCl, acetic, or butyric acid, but it is intensified in the presence of dilute lactic acid.

[Kühne suggested that proteids—*e.g.*, albumins—are split up by certain acids, and ferments into an anti-group and a hemi-group, as represented by the following scheme—it is nothing more :—

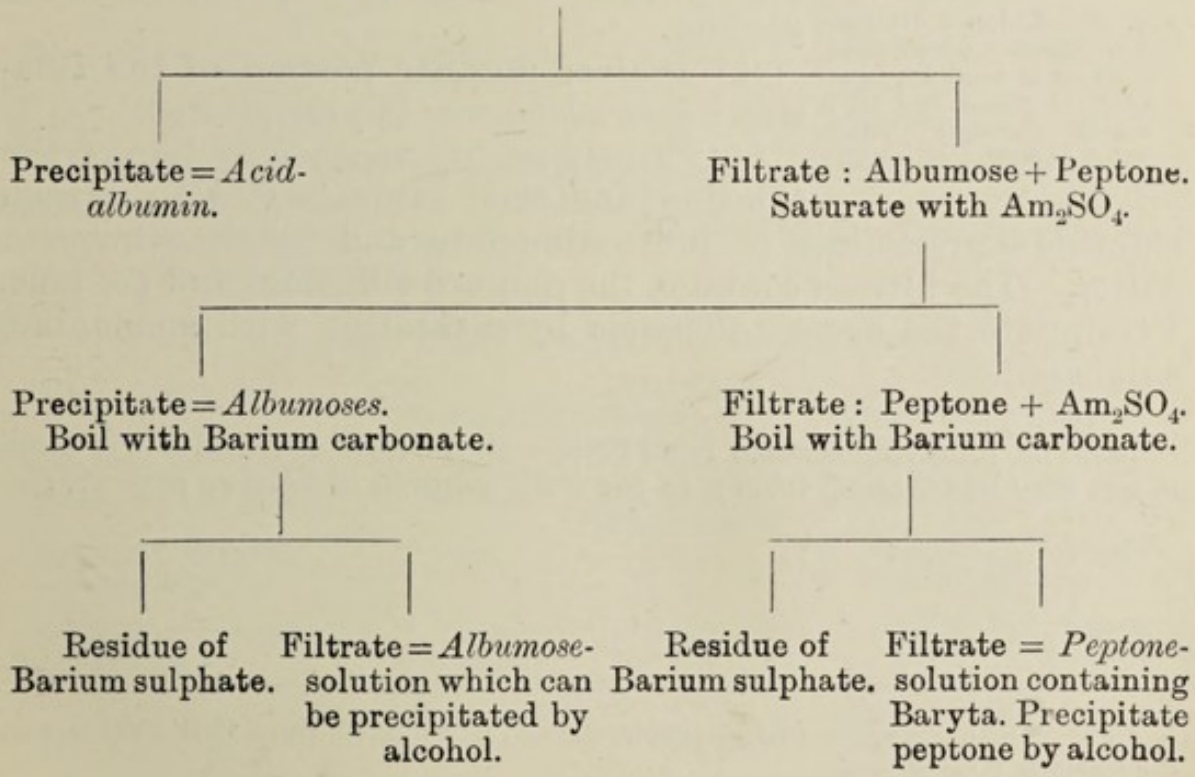
Action of Enzymes (Ferments).



The anti-group is not further acted on, but the hemi-group, although not split up by digestion, yields by tryptic digestion leucin, tyrosin, and other products.

The substance called hemi-albumose by Kühne has been shown to consist of three albumoses—viz., proto-albumose, hetero-albumose, and deutero-albumose. The first two are precipitated by NaCl, and the last by NaCl and acetic acid. For separation of these bodies—which can be obtained most easily from Witte's peptone—see 14.]

12. **Products of gastric digestion.**—To 25 grms. well-washed and boiled fibrin + 250 c.c. 0·2 p.c. HCl. Digest for several hours at 40° C. Neutralise with sodium carbonate (*Salkowski*).



13. Albumose and gastric peptones in quantity.

(*a*) Place 10 grms. of fresh, well-washed expressed fibrin in a porcelain capsule, cover it with 300 c.c. of 0·2 p.c. HCl, and keep the whole at 40° C. in a water-bath until the whole of the fibrin is so swollen up as to become converted into a perfectly clear, jelly-like mass, and it becomes so thick that a glass rod is supported erect in it.

(*b*) Stir in 1 or 2 c.c. glycerin pepsin extract (liquor pepticus). Within a few minutes the whole becomes fluid.

(*c*) After a short time—15 to 20 minutes—before the peptonisation is complete, filter and exactly neutralise the filtrate with ammonia or NaHO, which precipitates the acid-albumin with a small quantity of the albumoses. Filter; the filtrate contains the albumoses, which can be precipitated by saturation with crystals of neutral ammonium sulphate. To get rid of this salt the precipitate must be dialysed in a Kühne's dialyser (fig. 38).

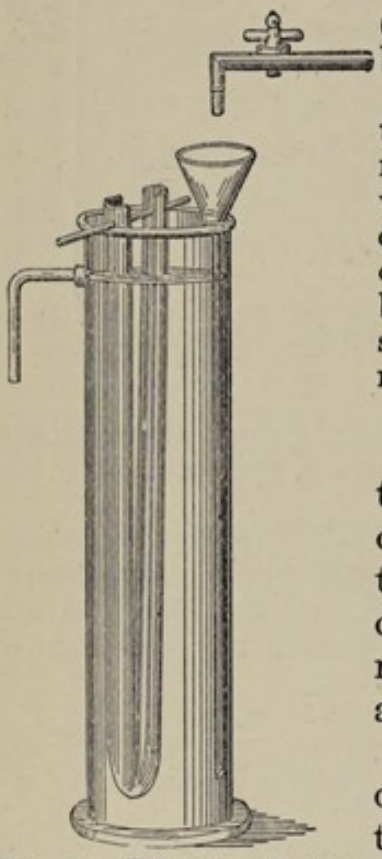


Fig. 38.—Kühne's dialyser. A parchment-paper tube, such as is used for sausages, is suspended in a vessel through which water is kept continually flowing.*

14. Albumoses.—Dissolve Witte's peptone—*i.e.*, "commercial peptone" consisting chiefly of albumoses—in 10 p.c. NaCl solution, and filter. This solution does not coagulate on heating, but gives the ordinary proteid reactions, together with biuret and nitric acid tests.

(*a*) Saturate with Am_2SO_4 = precipitate of albumoses. Filter. The peptone is in the filtrate and can be precipitated by alcohol.

(*b*) Dialyse another portion of the solution; *hetero-albumose* is precipitated.

(*c*) Faintly acidify another portion of the solution, and then saturate it with sodium chloride = precipitate of proto-albumose and hetero-albumose. Filter. The filtrate contains the deutero-albumose and peptone. Precipitate the *deutero-albumose* by saturating with ammonium sulphate.

(*d*) The following scheme from Schulz shows how the individual albumoses may be obtained from a 10 per cent. solution of Witte's peptone :—

* The parchment-paper tubing (Papier-därme) is obtained from Carl Brandegger, Ellenwagen, Würtemberg.

50 c.cm. of 10 p.c. Witte's peptone + 50 c.cm. saturated $(NH_4)_2S$.

- Precipitate I. = primary albumoses, dissolve in water and dialyse the adherent salt.
  - Precipitate. → Hetero-albumose.
  - Filtrate II. → Proto-albumose.
- Filtrate I. Saturate with Am_2SO_4 (acidify slightly).
  - Precipitate. → Secondary albumoses.
  - Filtrate III. → Peptone (Precipitate with iodine KI).

Primary Proteoses.—(*a*) Proto-albumose is soluble in pure salt-free water, so that it is not precipitated on dialysis.

(*b*) Hetero-albumose is insoluble in pure water, and therefore can be precipitated by dialysis.

Secondary Proteose.—(*c*) Deutero-proteose.

	Sat. $\hat{c}$ *NaCl in Neutral sol.*	*Half Sat.* $\hat{c}$ Am_2SO_4.	HNO_3.	$\bar{A}.\bar{A}a$ Fe_2CyK_6
Primary Proteoses, *v.*	↓	↓	↓	↓ at once.
Secondary ,,	not.	not. ↓	but only after +NaCl.	↓ only after standing.

	Sat. $\hat{c}$. $AmSO_4$	$\bar{A}.\bar{A}a$ F_2CyK_6		
Albumoses, *v.*	↓	↓		
Peptone,	not. ↓	not. ↓	Precipitated by Iodo.	KI from Am_2SO_4 sol.

The albumoses are (1) precipitated by alcohol, but not coagulated; (2) the nitric acid precipitate is soluble with heat, and returns on cooling—(to an albumose sol. + equal vol. sat. sol. NaCl + HNO_3); (3) the picric acid precipitate behaves as (2).

Some Tests for Proteoses and Peptones.

	PROTEOSES. Proto-alb.	Hetero-alb.	Deutero-alb.	PEPTONES. Peptones.
Biuret ($CuSO_4$, NaHO),	Pink	Pink	Pink	Pink
Water, hot and cold,	Soluble.	↓ Insoluble by dialysis.	Soluble.	Soluble.
Saturation $\hat{c}$ $MgSO_4$ or NaCl,	ppt.	ppt.	no ppt. (↓ by dil. HĀ).	no ppt.
Saturation $\hat{c}$ Am_2SO_4,	ppt.	ppt.	ppt.	no ppt.
Nitric Acid, . .	ppt. in cold, dissolved by heat, returns in cold.	do.	do. in presence of excess of NaCl.	no ppt.
$CuSO_4$, . . .	ppt.	ppt.	no ppt.	no ppt.

The products of gastric digestion of proteids are :—

(1) More soluble and diffusible than original proteid.

(2) Less easily precipitated by precipitants.

(3) Simpler than the mother proteid, and are said to gain in weight. Their elementary composition varies.

15. Effect of mechanical restraint on digestion.—Tie up rather lightly with thread a mass of fibrin, and subject it to an active artificial digestive fluid. Notice how much longer it takes to digest.

16. Influence of proteid on time for digestion.—Take two beakers (A and B), and place in each 40 c.c. of the digestive fluid. Place in A a piece of coagulated egg-albumin having a volume of 1 c.c., and in B a similar volume, which has been divided into 8 half-centimetre cubes. Note time in each case before digestion is complete.

17. Pepsin is destroyed by an alkaline salt.—Neutralise 5 c.c. of an effective artificial gastric juice. Filter. Add 5 c.c. 2 p.c. Na_2CO_3. Keep at 40° for half an hour. Then add HCl until the fluid is distinctly acid. Add fibrin, and keep at 40°. The fibrin is not digested, as the alkaline salt has destroyed the pepsin.

18. Comparative digestive power of pepsins—*e.g.*, the various pepsins found in the market, or the comparative digestive power of glycerin extracts of the stomach.

(*a*) Chop up well-washed and boiled fibrin, and stain it with ammoniacal carmine (24 hours). Wash thoroughly and preserve under ether or 1 p.c. $HgCl_2$. Place in the requisite number of beakers 0·2 p.c. HCl, equal amounts of the carmine fibrin, and then add the pepsin whose strength is to be tested; keep at 40° C. As the fibrin is digested the carmine is set free, so that the most deeply-stained liquid contains the most active pepsin (*Grützner's method*).

(*b*) Mette's method.—Fluid egg-albumin is packed into glass tubes of 1 to 2 mm. bore, and coagulated at 95° C. The tube is then cut into short pieces which are placed in a few c.cm. of the fluid, to be investigated for 10 to 12 hours at 38° C. The albumin is digested at the ends of the tubes. After a certain time the amount of undigested albumin is measured by means of a millimetre scale and lens. According to Pawlow and Ssamojloff, the digestion of albumin is during the first ten hours' proportion to the duration of the digestion. Moreover, the amount digested in a given time may be used as an index of the relative digestive powers of digestive fluid. The amount varies as the square root of the relative quantity of ferment present (*Schütz*). It is further stated that the length of the column digested is directly related to the amount of pepsin contained in the digestive fluid. (*Die Arbeit der Verdauungsdrüsen*, by J. P. Pawlow, p. 32, 1898.)

19. Chemical examination of the gastric contents—*e.g.*, Vomit.

(*a*) Test the reaction to litmus and Congo-red. The latter gives no change of colour with acid salts—*e.g.*, acid phosphate of soda—although they act on litmus.

(*b*) Determine the acidity (*e.g.*, of 10 c.c.) by means of a deci-normal solution of caustic soda. (See "Urine.")

(*c*) Test 10 c.c. for the presence of pepsin (digest with fibrin and HCl), and rennet (milk).

(*d*) Use the tests 10 (*c*), (*e*), (*f*), for determining the presence of free HCl.

(*e*) Make a rough estimate of the presence of lactic, butyric, and acetic acids by the method 9 (*h*).

(*f*) Examine for proteids—*e.g.*, albumin, albumoses, and peptone.
(*g*) Test for sugar and its digestive products.
(*h*) Distil some of the fluid, extract the remainder with sulphuric ether, and in the latter estimate the lactic acid which it contains.
(*i*) Test-meal.—When it is desired to know if digestion is normal, a test-meal is given. Ewald recommends a roll of stale bread taken on an empty stomach, with tea or water. After an hour the contents of the stomach are pumped out by means of a stomach pump, and examined as above. (See A. Lockhart Gillespie, *Manual of Modern Gastric Methods*, 1899, or v. Jaksch, *Clinical Diagnosis*, 1899).

20. Make experiments with each of the following by placing them—half an hour or longer—in an active gastric digestive fluid. Note and record the results :—
(*a*) Suet or fatty tissue of pig.
(*b*) Bread.
(*c*) Jelly of gelatin.
(*d*) Well-cooked lean meat.
(*e*) Cooked peas.
[(*f*) The hind legs of a frog with the skin removed.]
Test in each case the ultimate products (after Novy).

21. Drugs on gastric digestion.—As in Lesson VIII., 11, take 2 vols. HCl 0·2 p.c., ½ vol. gastric extract, 2½ vols. reagents. Pieces of carmine fibrin. Keep at 40°. The depth of the colour corresponds to the amount of digestion.

LESSON X.

PANCREATIC (INTESTINAL) DIGESTION.

Pancreatic juice is alkaline from the presence of sodium carbonate. It contains three or four ferments which occur in the gland in the form of inactive *zymogens*, which readily yield the corresponding active ferments after the addition of an acid, or after standing. It is for this reason that extracts of the gland can be used instead of the juice itself. A watery warm extract made from a gland may be acid from sarco-lactic acid, especially if the gland has been extracted some time after death.

1. Pancreatic extracts.

(*a*) Mince part of the pancreas of an ox or pig 24 hours after death, rub it up with pure quartz sand in a mortar, and digest it with cold *water*, stirring from time to time. Strain through linen and filter through paper. The filtrate has digestive properties, chiefly upon starch. A more potent solution is obtained by

digesting the pancreas at 40° C. for some hours with a 2 p.c. solution of *sodium carbonate*. To prevent the putrefactive changes, so apt to occur in all pancreatic fluids, add a little 10 p.c. alcoholic solution of thymol, or chloroform.

(*b*) Make a *glycerin* extract of the pancreas (pig) as directed for the stomach (Lesson IX., 1). This extract acts on starch and proteids. See that the glycerin does not reduce.

(*c*) For most experiments use the "liquor pancreaticus" of Benger, or of Savory & Moore, or Burroughs, Wellcome & Co., or the "trypsinum siccum" of Grübler.

(*d*) Weigh the pancreas taken from a pig just killed, rub it up with sand in a mortar, and add 1 c.c. of 1 p.c. acetic acid for every grm. of pancreas. Mix thoroughly, and after a quarter of an hour add 10 c.c. glycerin for every grm. of pancreas. After five days filter off the glycerin extract. Acetic acid is added to convert the "zymogen" into trypsin.

(*e*) Kühne's dry pancreas powder.—Extract a pancreas with alcohol and ether, and dry the residue. The extraction must be done in an ether fat-extracting apparatus; and as the process is somewhat tedious, it is better to buy it from Dr. Grübler of Leipzig.

(*f*) Other solvents of pancreatic enzymes.—Apart from the fat-splitting enzyme, the other ferments are readily extracted from the gland —under certain conditions by (i.) glycerin, (ii.) saturated watery solution of chloroform (*Roberts*), but the chloroform extract interferes with the reaction for grape-sugar. Harris and Gow find that a saturated solution of common salt extracts all the pancreatic enzymes (save the fat-splitting). Roberts found that by extracting the pancreas with a solution containing a mixture of boracic acid and borax a serviceable extract was obtained.

(*g*) Treat finely-minced pancreas with 8 to 10 vols. of water containing 0·03 ammonia. Let it stand for 24 hours. Filter and carefully precipitate with dilute acetic acid. Filter off the precipitate and dissolve the precipitate in dilute soda solution, and filter. The filtrate is principally tryptic (*Hammarsten*).

I. Action on starch (Amylopsin the ferment).

2. (*a*) To thick starch mucilage in a test-tube add glycerin extract of pancreas or liquor pancreaticus, and place it in a water-bath at 40° C. Rapidly the starch paste becomes fluid, loses its opalescence, and becomes clear. Within a few minutes some of the starch is converted through intermediate stages (as in salivary digestion) into maltose. Test for reducing-sugar (Lesson III.). Test rapidity of its action with 1 p.c. starch mucilage, as for saliva (Lesson VIII.).

(*b*) *Pancreatic juice on bile.*—Repeat A, but add a little bile; the starch disappears more quickly. Prove by testing on a white porcelain slab, as in Lesson VIII., 6.

II. Action on proteids.—Trypsin is the characteristic proteolytic enzyme of the pancreatic secretion, and also the chief

proteolytic agent in plants. Its general actions resemble those of pepsin, but it differs in at least two important respects, as it (1) causes a far more complete cleavage of the primary proteid molecule, so that even crystalline amides are formed; (2) it can act in a neutral—and even faintly acid—medium, although it acts better in an alkaline one—*e.g.*, weak carbonate of soda, but there is no definite relation between the alkali and enzyme, and it is not dependent on an "inorganic ally," such as HCl is to pepsin. It erodes the fibrin, so that its digests are turbid. With coagulated proteids no soluble globulins or alkali-albumin are produced; the first products that can be separated are deutero-albumoses.

The *vegetable trypsins—e.g.*, bromelin from the juice of the pine apple, papaïn from *Carica papaya*—act best in a faintly acid medium.

3. Proteolytic action and its conditions (Trypsin the ferment).

(*a*) Half-fill three test-tubes, A, B, C, with 1 p.c. solution sodium carbonate, and add 5 drops of glycerin pancreatic extract or liquor pancreaticus to each. Boil B, and make C acid with dilute HCl. Place in each tube well-washed fibrin, plug the tubes with cotton wool, and keep all at 40° C. [Previously soak the fibrin in the sodium solution.]

(*b*) Examine them from time to time. In A the fibrin is gradually *eroded*, and finally disappears, but it does not swell up, the solution at the same time becoming slightly turbid. After three hours no change is observable in B and C. The fibrin yields successively soluble proteid (globulins), alkali-albumin, deutero-albumoses, peptones, and some derivatives of the latter.

(*c*) Filter A, and carefully neutralise the filtrate with very dilute HCl or acetic acid = a precipitate of alkali-albumin. Filter off the precipitate, and on testing the filtrate, peptones are found. The intermediate bodies, the albumoses, are not nearly so readily obtained from pancreatic as from gastric digests.

(*d*) Filter B and C, and carefully neutralise the filtrates. They give no precipitate. No peptones are found.

(*e*) Make a digest of fibrin with *bromelin* and ·025 p.c. HCl for several hours. It yields products identical with those of tryptic digestion.

Putrefaction occurs rapidly in pancreatic digests. Prevent this either by the addition of an alcoholic solution of thymol or chloroform water (5 c.c. chloroform to 1 litre water).

The successive stages appear to be—

Fibrin.
|
(Globulins) soluble Proteid.
|
Alkali-albumin.
|
Deutero-albumoses.
|
Peptones.
|

Amido-acids.		Hexone bases.	Ammonia.	
Fatty.	*Aromatic.*	Lysin.		Antipeptone?
Leucin.	Tyrosin.	Arginin.		
Aspartic acid.		Histidin.		
Glutamic acid.				

This gives some insight into the great complexity of the proteid molecule.

Tryptic Digestion (*Salkowski*).

50 grms. fibrin + 200 c.c. alkaline (carbonate of soda 1 p.c.) chloroform water + liq. pancreaticus; digest for 36 hours; then boil and filter.

Residue; coagulated Proteid.	Filtrate (A) (reaction with bromine) concentrated by evaporation and allowed to stand.

Deposition (B) of Tyrosin.	Filtrate (C) further concentrated; Leucin and Peptone.

[See H. M. Vernon, "The conditions of action of trypsin on fibrin" (*Journ. of Phys.*, xxvi., 405, 1901).]

4. Other products.—(*a*) Digest at 40° C. (24 to 36 hours) equal parts of fresh moist fibrin and ox-pancreas in 1 litre of 1 p.c. sodium carbonate solution to which some thymol has been added, or an ox- pancreas is digested in the same way, the white of an egg being added every ten hours (Digest A). Make another digest, but do not add thymol. Digestion and putrefaction take place, the latter causing a most disagreeable smell (Digest B). Filter the digest A, and to some of it add Millon's reagent, which precipitates any albumin. Filter, boil the filtrate, a red colour indicates tyrosin.

(*b*) Concentrate some of the filtered digest A by boiling it to a small bulk on a water-bath. After several hours examine a

drop microscopically for leucin and tyrosin. Precipitate the peptones in some of the filtered digest A by alcohol. Filter. Concentrate the filtrate on a water-bath, when a sticky deposit of **leucin** is formed.

The digest A yields the chlorine or "bromine reaction."

Test the digest B for the products of putrefaction.

(*c*) Examine **leucin** and **tyrosin** microscopically (fig. 39). The former occurs as clear refractive balls, often with radiating lines, not unlike fat, and the latter consists of white shining needles arranged in sheaves or in a stellate manner. (See "Urine.") The deposit which is sometimes formed in Benger's liquor pancreaticus consists of tyrosin.

(*d*) *Tyrosin test* (Hofmann).—Dissolve some in boiling water, add Millon's reagent, and boil, which gives a red colour.

(*e*) *Indol test.*—Use either the watery solution of indol or the filtered offensive-smelling fluid before it is distilled.

(i.) Warm the liquid, and add first a drop or two of dilute H_2SO_4 to the filtered liquid, and then a *very dilute nitrite* solution. A red colour indicates the presence of indol. This test is very readily obtained with the products of digestion by Kühne's dry pancreas (Lesson X., 1, *e*).

(ii.) Acidify strongly with HCl a small quantity of the highly offensive fluid or the watery solution, and place in it a shaving of wood, or a wooden match with its head removed and soaked in strong HCl. The match is coloured red.

(*f*) *Chlorine reaction* (due to so-called tryptophan or protein-chromogen substance).—Add to some of the digest (4, (*b*), preferably digest B), drop by drop, chlorine water; it strikes a rosy-red tint. Or add very dilute bromine water (1 to 2 drops to 60 c.c. water); the fluid first becomes pale red, then violet, and ultimately deep violet (*Kühne*). This reaction is only obtained after the breaking down of part of the peptone molecule.

III. Action on fats.

5. (A) Emulsification.

(*a*) Rub up in a mortar, which has been warmed in warm water, a little olive or cottonseed oil or melted lard, and pieces of fresh pancreas. A creamy persistent emulsion is formed (microscope).

(*b*) Rub up oil as in (*a*), but use an extract of the fresh pancreas made with 1 p.c. sodium carbonate. A good emulsion is obtained.

(*c*) The presence of a little *free fatty acid* greatly favours emulsification. Take two samples of cod-liver oil, one *perfectly neutral* (by no means easily procured), and an ordinary brown oil—*e.g.*, De Jongh's. The latter con-

tains much free fatty acid. Place 5 c.c. of each in two test-tubes, and pour on them a little solution of sodium carbonate (1 p.c.). The neutral oil is not emulsified, while the rancid one is at once, and remains so. Many oils that do not taste rancid contain free fatty acids, and only some of them give up their acid to water, just according as the fatty acid is soluble or not in water.

(*d*) Proteids as emulsifying agents.—A solution of albumose, or egg-albumin shaken up with olive oil, yields an emulsion. Test how long each lasts. Emulsions may exist both with acid and alkaline reactions. Try the effect of acid-albumin and alkali-albumin in conserving emulsions (0·5 c.c. olive oil or 10 c.c.). The emulsions so formed last far longer than those with native proteid (Moore and Krumbholtz, *Journ. of Phys.*, xxii.).

B. Fat-splitting action of pancreatic juice (Steapsin, lipase, or pialyn, the ferment).—This ferment, to use the new terminology, is called lipase. In mammals it occurs chiefly in the pancreas, from which it can be extracted by neutral solvents, as it is very easily affected by acids. It occurs in some plants, where it plays an important part in decomposing fats stored up as reserve-products in many seeds. It is present in blood-serum (*Hanriot*). During germination of these seeds—*e.g.*, of *Ricinus*, the castor-oil plant—the fats are split up by it, and free fatty acids and glycerin are liberated.

$$\underset{\text{Tripalmitin (neutral fat).}}{C_3H_5(C_{16}H_{31}O_2)_3} + 3H_2O = \underset{\text{Glycerin.}}{C_3H_5(OH)_3} + 3\underset{\text{Palmitic acid.}}{H.C_{16}H_{31}O_2}.$$

(*a*) To an emulsion of almond oil add an extract of fresh pancreas, made with glycerin mixed with 1 p.c. Na_2CO_3, and some neutral litmus solution. Keep warm. The litmus becomes red from the liberation of free fatty acids by the lipase.

(*b*) A solution of monobutyrine (1 p.c.) is preferable. Add to it a piece of fresh pancreas and a trace of alkali—or even blood-serum—and a drop of phenolphthalein. Heat gently. In a few minutes the pink colour of the phenolphthalein is discharged, showing the formation of a free acid (*Hanriot*).

(*c*) Neutral oil.—A perfectly *neutral* oil is required, and as all commercial oils contain free fatty acids, they must not be used. Place olive or almond oil in a porcelain capsule, mix it with not too much baryta solution, and boil for some time. Allow it to cool. The unsaponified oil is extracted with ether, the ethereal extract separated from the insoluble portion, and the ether evaporated over warm water. The oil should now be perfectly neutral (*Krukenberg*).

IV. 6. Milk-curdling ferment.—Such ferments occur, both in the animal and vegetable kingdoms, the latter yielding the "vegetable rennets." They are often associated with a proteolytic ferment—*e.g.*, in pine-apple juice with bromelin—and are most frequently found in the fruit and seed.

(*a*) Add a drop or two of brine extract of the pancreas to

5 c.c. warm milk in a test-tube, and keep at 40° C. A solid coagulum forms, and then whey begins to separate.

(*b*) Repeat (*a*), but add a pinch of bicarbonate of soda. Coagulation occurs, so that this ferment acts in an alkaline medium. Its power is destroyed by boiling.

7. Pancreatic juice on milk.—(*a*) Add to cow's milk dilute (1 : 5) in a test-tube a drop or two of liquor pancreaticus. Keep at 40° C. for half an hour. The caseinogen is first curdled and then dissolved; the milk changes from a white to a yellowish colour, and has a peculiar taste and odour.

(*b*) Divide (*a*) into two portions, A and B. To A add dilute acetic acid; there is no precipitation of caseinogen, which has been converted into peptones. To B add NaHO and $CuSO_4$, which give a rose colour, proving the presence of peptones.

8. Peptonise milk.—A pint of milk is diluted with a quarter of a pint of water, and heated to a lukewarm temperature, about 140° F. (60° C.). Or the diluted milk may be divided into two equal portions, one of which may be heated to the boiling-point and then added to the cold portion; the mixture will then be of the required temperature. Two teaspoonfuls of liquor pancreaticus, together with about 1 grm., or half a level teaspoonful of bicarbonate of soda, are then mixed therewith. The mixture is next poured into a jug, covered, and placed in a warm situation to keep up the heat. In a few minutes a considerable change will have taken place in the milk, but in most cases it is best to allow the digestive process to go on for 10 or 20 minutes. The gradually increasing bitterness of the digested milk is unobjectionable to many palates; a few trials will, however, indicate the limit most acceptable to the individual patient; as soon as this point is reached, the milk should be either used or boiled to prevent further change. From 10 minutes to half an hour is the time generally found sufficient. It can then be used like ordinary milk (*Roberts*).

9. Succus entericus.—**Invertase,** or (invertin) sugar-splitting ferment, exists in succus entericus and the mucous membrane of the small intestine, which splits up cane-sugar into dextrose and levulose. A molecule of cane-sugar takes up a molecule of water, and splits into two molecules—one of glucose, the other of levulose (fructose)—

$$\underset{\text{Cane-sugar.}}{C_{12}H_{22}O_{11}} + H_2O = \underset{\text{Glucose.}}{C_6H_{12}O_6} + \underset{\text{Levulose.}}{C_6H_{12}O_6}.$$

A similar ferment can be extracted from yeast and many plants

(*a*) Make a watery extract of the mucous membrane of the small intestine. Digest it with 20 p.c. cane-sugar at 40° C. After a short time test for reducing sugars with Fehling's solution.

Additional Exercises.

10. Other products of tryptic proteolysis—Leucin ($C_6H_{13}NO_2$) and Tyrosin ($C_9H_{11}NO_3$).

(*a*) Place 300 c.c. of a 1 p.c. solution of sodium carbonate in a flask, add 5 grms. of fibrin, 5 c.c. of glycerin extract of pancreas, and a few drops of an alcoholic solution of thymol. Keep all at 38° C. on a water-bath for 10 to 16 hours, shaking frequently. After 16 hours take a portion of the mixture, filter, and to the filtrate cautiously add dilute acetic acid to precipitate any alkali-albumin that may be present in it. Filter, and evaporate the filtrate to a small bulk, and precipitate the peptones by a considerable volume of alcohol. Filter to remove the peptones, and evaporate the alcoholic filtrate to a small bulk, and set it aside, when tyrosin and leucin separate out. Examine them microscopically (fig. 39). These substances, as well as others—viz., aspartic acid and glutamic acid—have long been known as decomposition products of vegetable proteids—*e.g.*, as cleavage products—by boiling with dilute acids. Aspartic acid is amido-succinic acid, $COOH.CH_2CH(NH_2).COOH$, and is also a product of pancreatic digestion of fibrin, while glutamic acid, $COOH.C_3H_5(NH_2).COOH$, is amido-pyrotartaric acid. Both acids belong to the fatty acid series.

Drechsel has recently discovered two new nitrogenous bases—lysin and lysatinin or lysatin—products of the decomposition of proteids (*e.g.*, casein, gelatin, egg-albumin) when the latter are boiled with HCl and stannous chloride. These bodies result from the simple hydrolytic cleavage of the proteid molecule, and it has recently been shown by Hedin that they are also formed in trypsin-proteolysis.

(*b*) Lysin, $C_6H_{14}N_2O_2$, is a diamido-caproic acid, and is a representative of the fatty acid group, and has intimate chemical relationships with leucin.

(*c*) Lysatinin or lysatin, $C_6H_{13}N_3O_2$.—Its composition is less accurately known, but it has the composition of a kreatin. The special interest which attaches to this body is that, as a product of trypsin-proteolysis, it can by simple hydrolytic decomposition break down into urea. Thus, trypsin-proteolysis yields cleavage products, from one or more of which comes the substance lysatin, which behaves like kreatin in this respect—viz., that when boiled with baryta-water, it yields sarkosin and urea. Thus, chemists have found a series of cleavage products the result of hydrolytic decomposition between proteid and urea (Chittenden, *Digestive proteolysis*, p. 103, New Haven, 1895. Cartwright Lectures).

11. Putrefactive products of pancreatic digestion.—These include indol, skatol, phenol, volatile fatty acids, CO_2, H_2S, CH_4, and H.

$$\text{Indol } C_6H_4\begin{cases}CH = CH\\ \diagup\quad\diagdown\\ NH\end{cases} = C_8H_7N \text{ and}$$

$$\text{Skatol } C_6H_4\begin{cases}C.CH_3 = CH\\ \diagup\quad\diagdown\\ NH\end{cases} = C_9H_9N.$$

(*a*) Preparation of indol.—Place 1 kilogrm. of fresh fibrin in a 6-litre jar with 4 litres of water (to which 1 grm. KH_2PO_4 and ·5 grm. $MgSO_4$ are added). Mix this with 200 c.c. cold saturated solution of sodic carbonate, add to the whole a quantity of putrefying flesh-juice and some pieces of the putrid flesh as well. Cork the vessel, a vent-tube being placed in the cork, and place it aside for 5 to 6 days at a temperature of 40° to 42° C. Distil and acidify the strongly ammoniacal distillate with HCl, add some copper sulphate, and filter. Shake up equal volumes of the distillate and ether in a separation funnel. Allow the filtrate to settle, run it off, add some fresh filtrate, and shake again with the same ether. Distil the ethereal extract to about one-fourth of its volume, shake up the residue very thoroughly with caustic soda (to remove phenol and traces of acids). Distil the ether, and after the addition of caustic soda distil the oily indol. The distillate is shaken up with ether, and the ethereal extract is evaporated at a low temperature, when crystals or plates of indol separate. This preparation usually contains some skatol. (*Drechsel* after *Salkowski*.)

Leucin or *α*-Amido-isobutylacetic acid, $C_6H_{13}NO_2 = 2(CH_3)CH—CH_2—CH(NH_2)CO\,.\,OH$, and Tyrosin or Paraoxyphenyl-*α*-Amidopropionic acid, $C_9H_{11}NO_3 = C_6H_4{<}^{OH}_{C_2H_3}(NH_2)CO\,.\,OH$. — These two bodies are obtained together from nearly all proteids when the latter are decomposed by the action of acids. The former belongs to the fatty bodies, and tyrosin to the aromatic group, and is a derivative of benzene (C_6H_6).

12. Preparation of Leucin and Tyrosin.—Place 2 parts of horn shavings ($\frac{1}{2}$ to 1 kilo.) in a mixture of 5 parts of concentrated sulphuric acid and 13 parts of water. Boil for 24 hours in a vessel placed in connection with a condenser. Add thin milk of lime until a feebly alkaline reaction is obtained, filter through flannel, re-extract the residue with water, mix the filtrate and washings, and slightly acidulate them with oxalic acid. Filter to remove the oxalate of lime, and evaporate the filtrate until a scum forms on the surface. Cool and repeat the evaporation process until crystallisation ceases to take place in the mother-fluid. Collect the mass of crystals, dissolve them in boiling water with the addition of ammonia, and add lead acetate until the resulting precipitate is no longer brown, but becomes white. Filter, acidulate the acid filtrate feebly with dilute sulphuric acid, filter off the lead sulphate and allow the fluid to cool, when tyrosin in an almost pure form crystallises out.

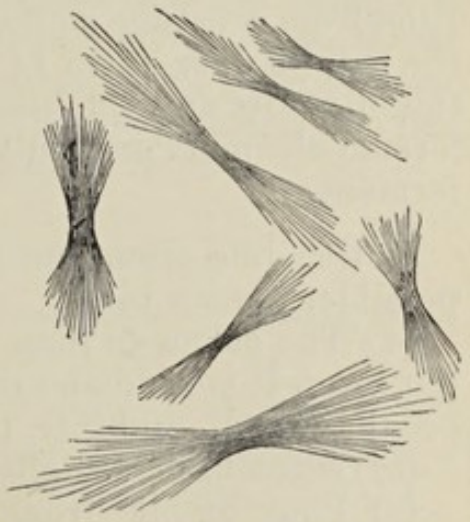


Fig. 39.—Crystals and sheaves of Tyrosin.

The mother-liquor, freed from tyrosin, is treated with sulphuretted hydrogen to get rid of the lead, filtered, evaporated, and boiled for a few minutes with freshly precipitated hydrated copper oxide, which forms a dark blue solution. The latter, when filtered and evaporated, yields blue crystals and an insoluble compound of leucin-copper oxide. This deposit and the crystals are decomposed in water by H_2S solution, the filtrate when necessary decolorised by boiling with animal charcoal, again filtered and evaporated to crystallisation, when leucin crystallises out. It is obtained pure by recrystallisation from boiling alcohol (*Drechsel*).

13. Tyrosin is insoluble in alcohol and in 1000 parts of cold water.

(*a*) Observe microscopically its crystalline form, as fine long silky needles arranged in sheaf-like bundles (fig. 39).

(*b*) Boil a hot watery solution with Millon's reagent (avoid excess) = a red colour (*Hofman's test*), due to the presence in the molecule of the group, C_6H_4OH.

14. Leucin.—(*a*) Under the microscope observe it in the form of refractive balls, with radiating and concentric lines. It is soluble in 27 parts of cold water, and much less soluble in alcohol.

(*b*) Heated in a tube it sublimes unchanged in very fine clouds with the odour of amylamine. A part is decomposed into CO_2 and $C_5H_{13}N$ (amylamine).

15. Drugs on proteolytic action.—As in Lesson VIII., 11, take 2 vols. of 5 p.c. Na_2CO_3, $\frac{1}{2}$ vol. panc. extract, $2\frac{1}{2}$ vols. of reagents, piece of fibrin. Keep at 40°. Add excess of NaHO, and then $CuSO_4$, until pink just begins to pass into blue. The depth of the pink colour roughly corresponds to the amount of tryptic digestion.

LESSON XI.

BILE.

Bile from a fistula contains 3 to 4 p.c. of solids, but that from the gall-bladder 13 to 14 p.c. Its characteristic substances are the bile-salts and the bile-pigments. It contains no proteids or formed elements and only a small amount—if any—of a diastatic ferment.

1. Use bile from the gall-bladder of an ox or sheep, and, if possible, human bile.

(*a*) The colour in man is brownish-yellow, in the ox greenish, but often it is reddish-brown when kept. Note its bitter taste, peculiar smell, and S.G. (1010 to 1020).

(*b*) If fresh, it is slightly alkaline or neutral to litmus paper.

(*c*) Pour ox-bile from one vessel to another, and note the thread of so-called mucin between the two vessels.

(*d*) Acidulate diluted bile with acetic acid = copious (yellow —*i.e.*, coloured with the bile-pigment) precipitate of mucinoid or mucoid substance. This substance is formed in the bile-ducts and gall-bladder, and is not present in bile secreted in the ultimate bile-capillaries. Filter off this precipitate. Test the filtrate.

(*e*) It gives no reactions for proteids.

(*f*) Add HCl and potassium ferrocyanide. A blue colour indicating presence of iron. Test for chlorides and other salts.

(*g*) Fresh human bile gives no spectrum, although the bile of the ox, mouse, and some other animals does.

2. Bile-salts or bilin (Glycocholate or taurocholate of sodium).—The bile-salts exist in bile in combination with alkalies (*e.g.*, Na). The respective proportion between the two salts differs in different animals. Dog's bile contains taurocholate, not the other. When bile is acidified the free bile acids are liberated; and as glycocholic acid is insoluble in water, if much be present, it is precipitated. Note that taurocholic acid contains sulphur.

$$\text{Glycocholic acid} = \underset{\text{Glycocoll.}}{NH_2 . CO_2 . COOH} + \underset{\text{Cholalic acid.}}{C_{24}H_{40}O_5}.$$

$$\text{Taurocholic acid} = \left(\underset{\text{Taurin.}}{NH_2 . CH_2 . CH_2S}\begin{matrix}O_2\\OH\end{matrix}\right) + \underset{\text{Cholalic acid}}{C_{24}H_{40}O_5}.$$

(*a*) Concentrate ox-bile to one-fourth of its bulk, mix with animal charcoal in a mortar to form a thick paste. Evaporate to complete dryness over a water-bath.

(*b*) To the dry charcoal-bile mixture, add 5 vols. absolute alcohol. Shake the mixture; after half an hour filter. To the filtrate add much ether, which gives a white precipitate of the bile-salts. If no water be present, sometimes the bile-salts are thrown down crystalline; but not unfrequently they go down as an opalescence which forms a resinous mass. Allow the mixture to stand for a day or two to obtain the glancing needles which constitute **Plattner's crystallised bile.**

Scheme for Bile-Salts, &c. (Salkowski).

200 c.c. of ox-bile, dried, mixed with animal charcoal, are extracted with absolute alcohol by the aid of heat; filter.

Residue, *mucin*, pigments, salts, charcoal.	Alcoholic solution treated with ether.
Precipitate, *Bile-salts.*	Solution contains *Cholesterin.*

3. Pettenkofer's test for bile-acids (salts) and cholic acid.

(*a*) To a nearly equal volume of bile add a drop or two of syrup of cane-sugar (10 p.c.). Pour in *concentrated* H_2SO_4, at the line of junction of the two fluids a *purple* colour is obtained.

Furfuraldehyde is formed by the action of sugar and H_2SO_4, and the purple compound is due to the aldehyde compound with cholalic acid. The white deposit seen above the line of junction is precipitated bile-acids. They are insoluble in water.

(*b*) Or, mix bile and syrup, shake until the upper part of the tube is filled with froth. Pour H_2SO_4 down the side. The froth becomes purple.

(*c*) To a film of bile on a porcelain capsule add a drop of syrup of cane-sugar, and then H_2SO_4 = purple colour.

(*d*) After mixing syrup with bile add strong H_2SO_4 drop by drop; mix them thoroughly. Heat gently, and the fluid becomes a deep purple colour. *Do not add too much syrup or overheat the tube.* If the requisite amount of H_2SO_4 be added the temperature becomes sufficiently high (70° C.).

(*e*) *Furfurol* (0·1 p.c.) may be used in place of sugar (*Mylius*).

(*f*) *Fluorescence of cholic acid in H_2SO_4.*—To bile add H_2SO_4, but no syrup. Or add cholic acid to H_2SO_4, heat slowly = golden red fluid in transmitted light, and green fluorescence by reflected light.

4. Similar purple colour reactions are obtained with many other substances—*e.g.*, albumin and α-naphthol, but the spectra differ.

Albumin and sulphuric acid.—To a solution of acid-albumin and syrup add H_2SO_4; a similar tint is obtained. The spectra, however, are different; the red-purple fluid from bile gives two absorption-bands—one between E and F, and another between D and E. In the albuminous solutions only one absorption-band exists, between E and F.

5. Bile or bile-salts precipitate sulphur (*M. Hay*).

(*a*) In one beaker (A) place diluted bile, and in the other (B) water. Pour flowers of sulphur on both. The sulphur falls in a shower through the fluid of A, while none passes through B.

(*b*) Test to what extent bile or bile-salts can be diluted before this effect ceases. It is due to diminution of surface tension by bile-salts.

Bile-pigments.—The chief are **bilirubin** (red) and **biliverdin** (green). Most probably they exist not as such in bile, but as bilirubinate and biliverdinate of soda. Bilirubin is the initial pigment, and from it biliverdin is derived by oxidation. This is the basis of Gmelin's test. The two pigments can be separated, for bilirubin is readily soluble in chloroform, and only slightly so in alcohol; while biliverdin is readily soluble in alcohol, and insoluble in chloroform. They contain no iron.

$$\text{Bilirubin} = C_{32}H_{36}N_4O_6.$$
$$\text{Biliverdin} = C_{32}H_{36}N_4O_8.$$

Ox-bile, from the gall-bladder, is usually yellowish, sometimes green. Pig = reddish and turbid; rabbit = green; and guinea-pig = green.

6. Gmelin's test for bile-pigments.

(*a*) To bile on a *white* porcelain slab add a drop or two of

strong *nitric acid yellow with nitrous acid.* Notice the succession of colours in zones, beginning with *green*, and passing through blue, violet, red, to dirty yellow (choletelin), due to oxidation.

Choletelin ($C_{32}H_{36}N_4O_{12}$) is produced from bilirubin by the oxidising action of nitric acid.

$$\underset{\text{Bilirubin.}}{(C_{32}H_{36}N_4O_6)} + O_2 = \underset{\text{Biliverdin.}}{(C_{32}H_{36}N_4O_8)} + 2O_2 = \underset{\text{Choletelin.}}{(C_{32}H_{36}N_4O_{12})}.$$

$$[2\underset{\text{Bilirubin.}}{(C_{32}H_{36}N_4O_6)} - O_2 + H_2O = 2\underset{\text{Hydrobilirubin.}}{(C_{32}H_{36}N_4O_5 \cdot 2H_2O.)}]$$

(*b*) Slant a tube containing impure nitric acid and pour in bile, a similar play of colours occurs—green above, blue, red, and yellow below. It is better to remove the mucin by acetic acid (Lesson XI., 1). Or add the nitric acid, and shake after the addition of every few drops; the successive colours from green to yellow are obtained in great beauty. For a modification applicable to urine (see "Urine").

(*c*) To yellow bile add alcoholic solution of iodine (oxidising agent = greenish tint.

(*d*) To green bile + amm. sulphide and shake = reduction to bilirubin.

(*e*) To yellow bile + KHO and heat, acidulate with HCl = green due to oxidation of bilirubin.

(*f*) *Bile putrefaction.*—Place some green bile in a tube and allow it to putrefy,—several days; observe a yellowish colour beginning below, the top layers remaining green.

(*g*) Extract acidified ($\bar{A}.\bar{A}$) ox-bile with chloroform = slight yellow tint of the chloroform due to bilirubin. Re-extract with absolute alcohol = greenish extract due to biliverdin.

(*h*) *Bile pigments.*—Use yellow or brown bile (or biliary calculi). Dilute bile slightly with water, add a little lime water, avoid excess, mix. Pass stream of CO_2 through the mixture to convert any excess of CaHO into calcium carbonate. The calcium compound of bilirubin is precipitated and filtered off. Wash the precipitate with water and suspend it in water. Decompose it with dilute HCl, and at once extract it with chloroform, which dissolves the free bilirubin. The bilirubin can be precipitated from the $CHCl_3$ solution by alcohol.

7. Cholesterin—Gall-stones.—(*a*) Powder a gall-stone and extract with ether or boiling alcohol. Heat the test-tube in warm water, and see that no gas is burning near it. Drop the extract on a glass-slide, and examine the crystals microscopically. They are rhombic plates, as if they had an oblong piece cut out of one corner (fig. 40). Ethereal solution yields needles, but a hot alcoholic solution gives the typical plates ($C_{25}H_{41}O \cdot OH$). It is insoluble in water, slightly soluble in bile-salts, but it is kept in solution mainly by the joint presence of bile-salts

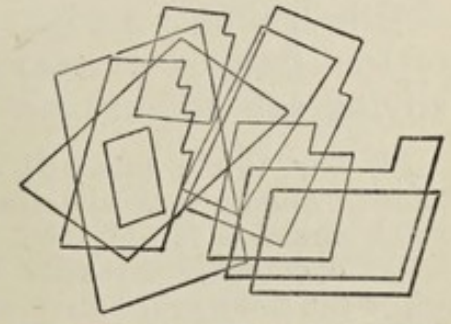


Fig. 40.—Crystals of cholesterin.

and lecithin. The latter also though insoluble in water is very soluble in bile-salts.

(*b*) Heat crystals in a watch-glass with a few drops of moderately strong H_2SO_4 (5 : 1), and then add iodine; a play of colours, passing through violet, blue, green, red, and brown, occurs.

(*c*) Dissolve crystals in chloroform—using a *dry* tube—add an equal volume of *concentrated* H_2SO_4, and shake the mixture. The chloroform solution floats on the top, becomes red, but changes quickly on exposure to the air, passing through violet and blue to green and yellow. A trace of water decolorises it at once. The layer of H_2SO_4 shows a green fluorescence.

(*d*) *Liebermann's reaction.* — Cholesterin dissolved in hot anhydrous acetic acid, cool + concentrated H_2SO_4 = rose-red, blue, bluish-green colour.

(*e*) Crystals acted on by strong H_2SO_4 become red (microscope).

(*f*) Examine microscopically crystals of cholesterin found in hydrocele fluid. The crystals may not be perfect, but their characters are quite distinct.

[Lanolin (fat from wool) gives (*c*) and (*d*). Instead of glycerin it contains cholesterin united to a fatty acid.]

8. Bile in digestion.

(*a*) **On starch.**—Test if bile (pig, sheep, or ox) converts starch mucilage into a reducing sugar (Lesson VIII.).

(*b*) **On fats.**—Mix thoroughly 10 c.c. bile with 2 c.c. almond oil, and observe both by the eye and the microscope to what extent emulsion occurs, and how long it lasts. Compare a similar mixture of oil and water. In the former case a pretty fair emulsion will be obtained. In the latter the oil and water separate rapidly.

(*c*) *Solvent action.*—To 10 c.c. bile, or 5 p.c. bile-salts, add oleic acid, shake, examine a drop microscopically = fatty emulsion. Heat mixture in water-bath for an hour, when few fat globules will be seen with the microscope, as the oleic acid has combined with the soda of the bile-salts to form a *soap*. Bile is a solvent of free fatty acids, and also of soaps, due chiefly to the bile-salts.

Bile has only a slight emulsifying power, but in the presence of free fatty acids soaps are formed which have a high emulsifying action, but its chief action is as a solvent.

(*d*) Mix 10 c.c. bile with 2 c.c. almond oil, to which some oleic acid is added. Shake well, and keep the tube in a water-bath at 40° C. A very good emulsion is obtained. The bile dissolves the fatty acids, and the latter decompose the salts of the bile-acids; the bile-acids are liberated, while the fatty acid unites with the alkali of the bile-salts to form a soap. The soap is soluble in the bile, and serves to increase the emulsifying power, as an emulsion, once formed, lasts much longer in a soapy solution than in water.

(*e*) Action on proteid products of gastric digestion.—To a filtered gastric fibrin digest add ox-bile, or solution of bile-salts. It causes a white precipitate of peptones and acid-albumin. The acid of the gastric juice splits up the bile-salts, so that the bile-acids are also thrown down.

(*f*) Action on acid-albumin.—To acid-albumin in solution (Lesson I.) add bile or bile-salts = curdling of the mass.

[Bile as a solvent.—Bile has practically no specific action on any of the three classes of food-stuffs; in this respect it is unlike the other digestive fluids. Its presence in the intestine is necessary for the complete absorption of fats in normal quantity, although fats are absorbed to a less extent in its absence.

It has been shown that bile acts as a *solvent* (1) for lecithin and cholesterin, two bodies which occur in bile in greater quantity than in any other fluid in the body, and the bile seems to be the only channel by which these substances are removed from the body; (2) for both free fatty acids and soaps in the intestines; in fact the solubility of the former entirely depends on its presence. These solvent powers are chiefly due to the bile-salts, but in the case of the fatty acids and soaps, the action is greatly increased by the presence of lecithin. These solvent powers help to explain the reabsorption of the bile-salts and the precipitation of lecithin and cholesterin, as the bile-salts are absorbed into the columnar epithelium of the villi. Cholesterin has but a low solubility in bile, while lecithin is readily soluble. This explains why gall-stones are so frequently almost entirely composed of cholesterin. This solvent action of bile on insoluble substances is obviously of primary, perhaps major, importance in the absorption of fats and in the excretion of otherwise insoluble substances. ("Bile as a solvent," Moore and Parker, *Proc. Roy. Soc.*, lxviii., 64, 1901.]

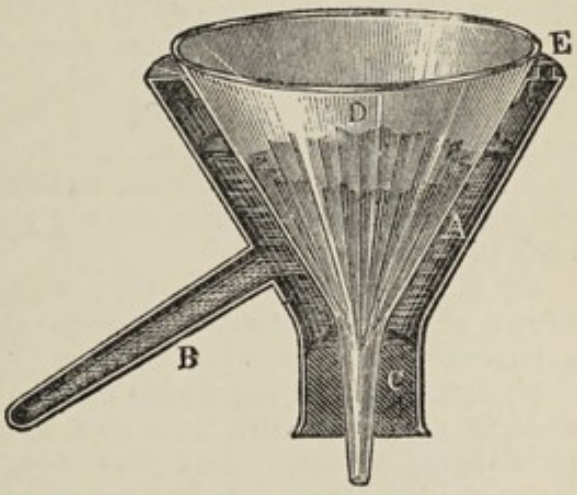


Fig. 41.—Double-walled filter for filtering hot solutions.

Additional Exercises.

9. Cholesterin.—(*a*) Boil powdered pale gall-stones in water, and then extract them with boiling alcohol. Filter through a double-walled filter kept hot with boiling water (fig. 41). The filtrate, on cooling, precipitates impure cholesterin. Recrystallise it from boiling alcohol containing potash, wash with alcohol and water, and dry the residue in a desiccator (fig. 13).

Scheme for Gall-Stones (Salkowski).

Powdered gall-stones are extracted with ether; filter.

Solution evaporated *Cholesterin* (A).	Residue (B) treated on the filter with dilute HCl.
Solution (C) *Lime salts.*	Residue (D) washed with water, dried, treated with chloroform; *Bilirubin.*

(*b*) Make a 5 p.c. solution of bile-salts (OX) and test at 39° C. to what extent cholesterin or cholesterin gall-stones are soluble. It has an excessively low solubility, as was shown by E. M. Brockbank in Owens College in 1893 ("Gall-Stones," Diss. for M.D., Victoria University), a fact recently confirmed by Moore.

10. Glycocholic acid (Hüfner's method).—To every 100 parts of ox bile in a stoppered bottle add 4 vols. of ether and 4HCl. "Sow" in it a few crystals of glycocholic acid, and shake vigorously. Let the vessel stand for some time in a cool place. The mixture may become almost solid from the formation of crystals of glycocholic acid. Filter, and wash mass in filter with cold distilled water, until all green pigment is removed. Boil the crystals in distilled water, and cool. Acicular crystals separate out. It succeeds also with rabbits' bile.

11. Micro-chemical reactions for iron in liver cells.—(*a*) Harden the liver or spleen of a young animal in alcohol. Make sections, and place them in a freshly-prepared solution consisting of equal parts of 1·5 p.c. solution of potassium ferrocyanide and 0·5 p.c. HCl. The granules of iron become blue (*Tizzoni*).

(*b*) Harden the liver in 65 p.c., then in 90 p.c. alcohol to which a few drops of sulphuretted hydrogen are added. After 24 hours the iron granules become green (*Zaleski*).

(*c*) Organic and inorganic iron.—The sections of alcohol-hardened tissue are placed for a short time in a watery solution of hæmatoxylin, 0·5 p.c. in distilled water. Any *free* iron, or iron in inorganic combination, is stained blue-black. It has no effect upon true organic compounds of iron. Artificial "ferratin" gives the reaction for inorganic iron. To show iron in organic combination previously treat the sections with 96 p.c. alcohol containing 10 parts HCl (Macallum, *Journ. of Phys.*, xxii., 92, 1897).

(*d*) Toluylenediamin if injected into the blood-vessels of a chloroformed animal, after 4 to 5 hours causes much iron to appear in the cells around the hepatic vein in the centre of a liver lobule.

12. Micro-chemical reaction for phosphorus in cells.—Place sections of the fresh organ, or organ fixed in OsO_4, for half an hour in a strong solution of ammonium molybdate, and then transfer them to a 20 p.c. solution of pyrogallic acid dissolved in ether. After a few minutes pass them through spirit and clove oil, and mount in Canada balsam. A compound containing phosphorus is stained yellow or brown, and such compounds are usually found in the nuclei. It is stated that by this method nucleo-albumin may be distinguished from mucin (Lilienfeld and Monti, *Zeit. f. physiol. Chem.*, xvii., 410, 1893).

N.B.—Macallum (*Proc. Roy. Soc.*, vol. lxiii., 1898) uses the following method for localising P in tissue elements. The sections of tissue are acted on for some hours at 35° C. by a nitro-molybdate mixture, and are then transferred to 1 to 4 p.c. freshly-prepared aqueous solution of phenyl-hydrazin chloride, when the presence of "masked" phosphorus is shown by the dark-green oxide of molybdenum. Mount in balsam. Use fresh tissue, but alcohol-hardened material is the best. The "*nitro-molybdate reagent*" is made by dissolving 1 part of pure molybdic acid (MoO_3) in 4 parts of strong ammonia, and adding slowly 15 parts HNO_3 (S.G., 1·2), which gives a yellowish colouring fluid. Decant off from any precipitate.

The inorganic phosphates are first and rapidly affected, then lecithin, and lastly, the organic phosphorus, which is much more slowly converted into orthophosphate. To exclude lecithin, extract the tissues frequently with hot alcohol. This method has shown the presence of "masked" phosphorus in the chromatin of all animal and vegetable nuclei—in anisotropous substance of muscle, zymogen of pancreas, liver cells, Nissl's granules in nerve cells, &c.

13. Mucin and Mucinoid:—

	True Mucin.	*"Mucinoid" from bile.*
Viscidity,	caused by both.	
Alcohol, Caustic alkalies,	↓ Soluble.	↓ Soluble.
Acetic acid,	↓ Insol. in excess.	↓ Sol. in excess.
Elemen. comp.	C.H.O.N.S.	C.H.O.N.S.P.
Boiled with mineral acid, yields,	Proteid and reducing sugar. ∴ Comp. of a "Glyco-proteid."	No reducing body. Digested ĉ gastric juice yields P-containing body. Nuclein + proteid. "Nucleo-proteid."

LESSON XII.

GLYCOGEN IN THE LIVER.

Glycogen, or animal starch, is an amylose, soluble in water, insoluble in alcohol and ether. Its aqueous solutions are opalescent and non-dialysable. It does not reduce Fehling's solution, nor does it ferment with yeast (Lesson III.).

1. Preparation.—(*a*) Feed a rabbit on carrots and bran for a day, or a rat on oats, and 5 or 6 hours after the last meal decapitate it or kill it by bleeding; or, by means of a tube, introduce into the stomach 10 to 15 grms. of cane-sugar dissolved in water 5 to 6 hours before the rabbit is killed. *Rapidly* open

the abdomen, remove the liver, cut one-half in pieces, and throw it into one hundred times its bulk of *boiling* water slightly acidulated with acetic acid. Lay the other half aside, keeping it moist in a warm place for some hours, and then extract it by any of the methods for the preparation of glycogen. It may yield little, the glycogen being replaced by grape-sugar. After boiling the first portion for several minutes until the cells are killed, their proteids coagulated and the ferment destroyed, pound it in a mortar with sand, and boil again for a few minutes. Filter while hot. The filtrate is *opalescent*, and is a watery solution of glycogen and other substances. The acetic acid coagulates the proteids, while the boiling water destroys either a ferment in the liver or the liver cells, which would convert the glycogen into grape-sugar.

(*b*) *Brücke's method.*—Feed and kill a rabbit, as in foregoing paragraph. Open the abdomen, rapidly remove the liver.

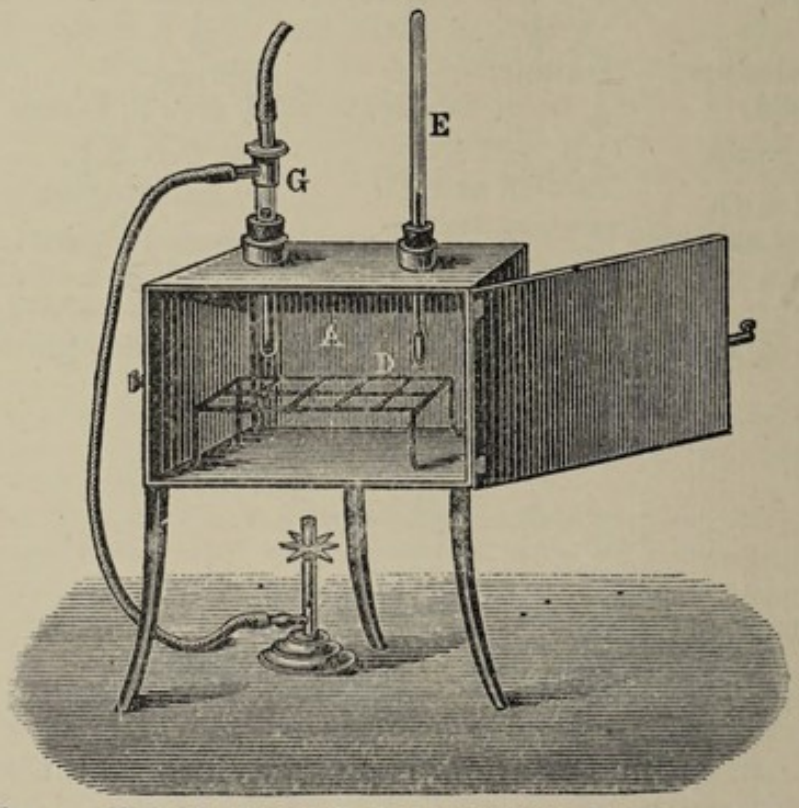


Fig. 42.—Hot-Air Oven. G, Gas regulator; E, Thermometer.

Wash out blood-vessels with normal saline; divide it into two; cut one-half as rapidly as possible into small pieces, and throw them into boiling water. Boil, and afterwards pound them in a mortar and boil again. Filter while hot into a cooled beaker, observe the *opalescent* filtrate, which is a solution of glycogen and proteids. Precipitate all the proteids by adding alternately HCl and potassio-mercuric iodide. Filter off the

proteids, and the opalescent filtrate is an imperfect solution of glycogen. *Separate the glycogen.* Evaporate the fluid to a small bulk, and precipitate the glycogen by adding 96 p.c. alcohol until the solution contains over 60 p.c. of alcohol. The glycogen is precipitated as a white flocculent powder, which is collected on a filter, washed with alcohol and ether, and then dried in an oven at 100° C. (fig. 42).

(*c*) *Külz's method.* — Feed a rabbit and kill it as above. Open the abdomen, rapidly remove the liver (weigh), cut it quickly into pieces, and throw the latter into a large porcelain capsule (400 c.c. water to 100 grms. liver) of water boiling briskly. Boil for half an hour. Remove them, rub them up into a pulp in a mortar with well-washed white sand. Replace the pulp in the boiling water and add 3 to 4 grms. of KHO (*i.e.*, for 100 grms. liver). Heat on a water bath and evaporate until about 200 c.c. of fluid remains for 100 grms. liver. If a pellicle forms on the surface, heat the whole in a beaker covered with a watch-glass until the pellicle is dissolved. Cool. Neutralise with dilute HCl and precipitate the proteids by adding alternately HCl and potassio-mercuric iodide in small quantities, until no further precipitation occurs. Filter through a thick filter to remove proteids. Remove the deposit from the filter with a spatula, and rub it up in a mortar with water containing HCl and potassio-mercuric iodide, and again filter the pulp. Repeat this process several times to get out all the glycogen. Mix the filtrates and add 2 volumes of 96 p.c. alcohol, stirring briskly; this precipitates the glycogen. Allow it to stand in a cool place for a night; filter, and wash the precipitate thoroughly, first with 62 p.c. and then with 96 p.c. alcohol. Usually the glycogen contains traces of albumin. To remove the latter, redissolve the moist glycogen in warm water, and after cooling, reprecipitate with HCl and potassio-mercuric iodide and proceed as above. Wash the glycogen with alcohol and then with ether, and dry it by exposure to the air. This method gives the most satisfactory results.

(*d*) *Fränkel's method.*—Rub up the liver with $2\frac{1}{2}$ volumes of 2 to 4 p.c. solution of trichloracetic acid—which precipitates the proteids—filter, wash with trichloracetic acid, and precipitate the filtrate with alcohol as above.

(*e*) Instead of a rat or rabbit's liver, use oysters or the edible mussel, and prepare a solution of glycogen by methods (*a*) or (*b*).

N.B.—Nerking (*Pflüger's Archiv.*, lxxxv., 313, 1901) finds that boiling with water does not extract all the glycogen either from liver or muscle. More may be extracted—after boiling water—by potash

(*f*) Use the other half of the liver of the rat or rabbit that has been kept warm, and make a similar extract of it.

2. **Precipitate the glycogen with alcohol.** — Evaporate the filtrate of (*a*) or (*b*) to a small bulk, add alcohol to 60 p.c. to precipitate the glycogen as a white powder. Filter; wash the precipitate with absolute alcohol and ether, and dry it over H_2SO_4 or in a hot-air oven (fig. 42).

3. **Glycogen tests.**—(*a*) To the opalescent filtrate add iodine

solution = a port wine red or mahogany-brown colour (like erythro-dextrin). If much glycogen be present the colour disappears, and more iodine has to be added. Heat to 70° C.: the colour disappears, but returns on cooling.

N.B.—Make a *control-experiment.* Take two test-tubes, A and B. In A place glycogen solution; in B, an equal volume of water. To both add the same amount of iodine solution. A becomes red, B faint yellow.

(*b*) Add lead acetate = a precipitate (unlike dextrin). The solution must be free from proteids and mercuric salts.

(*c*) Add lead acetate and ammonia; the glycogen is precipitated (like dextrin).

(*d*) Saturate either the solution or its iodide with Am_2SO_4 = white precipitate.

(*e*) Test a portion of the glycogen solution for grape-sugar. There may be none, or only the faintest trace.

(*f*) To a portion (A) of the glycogen solution add saliva or liquor pancreaticus, and to another portion (B) add blood, and keep both at 40° C. After 10 minutes test both for sugar. (A) will be transparent, and give no reaction with iodine. Perhaps both will give the sugar reaction; but certainly (A) will, if care be taken that the solution is not acid after adding the saliva. The ptyalin converts the glycogen into a reducing sugar.

(*g*) Boil glycogen solution with one-third its volume of HCl in a flask; the opalescence disappears, with the formation of glycogen, dextrin, and dextrose; neutralise with NaHO, and test with Fehling's solution for sugar.

(*h*) To a solution add solution of commercial peptones, and test with iodine. The reaction only occurs after much I is added, and may be absent if the peptones—*i.e.*, albumoses—greatly predominate. Hence impure glycogen solutions give bad iodine reaction.

(*i*) Glycogen does *not* dialyse. (Try.)

4. Test the watery extract of the other half of the liver. It is clearer, perhaps only slightly opalescent.

(*a*) Perhaps no glycogen reaction, or only a slight one.

(*b*) It contains much reducing sugar.

5. Extract of dead liver.—(*a*) Mince a piece of liver from an animal which has been dead for 24 hours. Boil either in water or a saturated solution of Na_2SO_4. Filter; the filtrate is clear and yellowish in tint, but not opalescent. It is acid to litmus paper.

(*b*) Test with iodine = little or no glycogen.

(*c*) Tests for grape-sugar = much reducing sugar.

After death the glycogen is transformed into grape-sugar unless precautions be taken to prevent this transformation.

6. Hydrolysis of glycogen.—Boil a 2 p.c. solution of glycogen with an equal volume of 2 p.c. HCl. The opalescence disappears, and if the solution be tested from minute to minute as for starch it will be found to show

successively soluble glycogen, an erythrodextrin, achroodextrin, and finally dextrose. The first two are readily "salted out" by Am_2SO_4 (Tebb, *Journ. of Phys.*, xxii. 421, 1898).

7. Microchemical—Glycogen in liver cells.—The essential part of this process is that as glycogen is soluble in water, the liver or other tissue supposed to contain the glycogen must *not be placed in water*. Feed a rabbit on carrots, and 5 to 6 hours afterwards kill it; cut part of the liver into small pieces and harden them in absolute alcohol. Cut hand sections, moistening the razor with alcohol, or embed and cut in paraffin. Get rid of the paraffin by means of turpentine, and treat both the paraffin and alcohol sections with chloroform in which iodine is dissolved, and mount in chloroform balsam containing some iodine. The brown stain in the liver cells indicates glycogen, which is deposited chiefly in the cells around the hepatic vein. Use an autumn frog. The sections may be mounted in a syrupy solution of lævulose.

LESSON XIII.

MILK, FLOUR, AND BREAD.

I. **Cow's milk** on an average contains water 87 and solids 13 p.c., consisting of caseinogen (and little lact.-alb.) 4, fats 3·8, lactose 3·8, and salts 0·7 p.c. Human milk contains more lactose, 5 p.c., and less proteid, 2·7 p.c.

1. **Milk.**—Use fresh cow's milk. Examine

(*a*) Its "naked-eye" characters,

(*b*) Noting numerous small, highly-refractive **oil-globules** floating in a fluid **lactoplasm** (fig. 43).

(i.) Add dilute caustic soda. The globules run into groups.

(ii.) Add osmic acid; globules first become brown and then black.

(iii.) In colostrum observe the "colostrum corpuscles" (fig. 43, *C*).

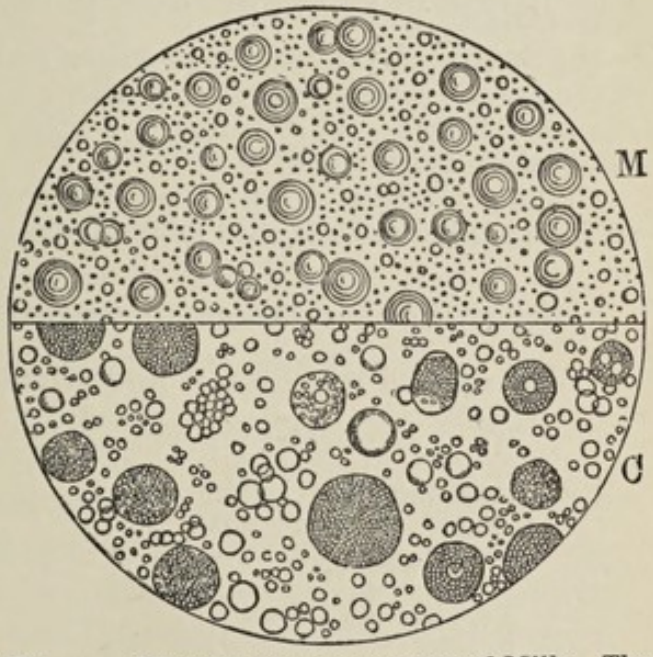


Fig. 43.—Microscopic Appearance of Milk. The upper half, *M*, is milk; the lower half, colostrum, *C*.

(iv.) Add Soudan iii., yellowish colour of fat globules, or "Schlarch R" [or "Scarlet R"] red colour of globules.

(*c*) Its **reaction** is neutral or slightly alkaline to litmus paper. If stale or "turned" it is acid (due to lactic acid from lactose).

(*d*) Take the **specific gravity** (1028 to 1034) of perfectly fresh unskimmed milk with the lactometer. Take it next day, after the cream has risen to the surface, or after the cream is removed. The S.G. is increased (1035 to 1037) by the removal of the lightest constituent—cream. The S.G. is raised by the addition of water. Why?

(*e*) Dilute milk with 10 volumes of water, neutralise it with *dilute* acetic acid, and observe that at first there is no precipitate, as the caseinogen is prevented from being precipitated by the presence of alkaline phosphates (Lesson I.). Cautiously add more until there is a copious granular precipitate of **caseinogen**, which, as it falls, entangles the greater part of the fat in it. Precipitation is hastened at 70° C.

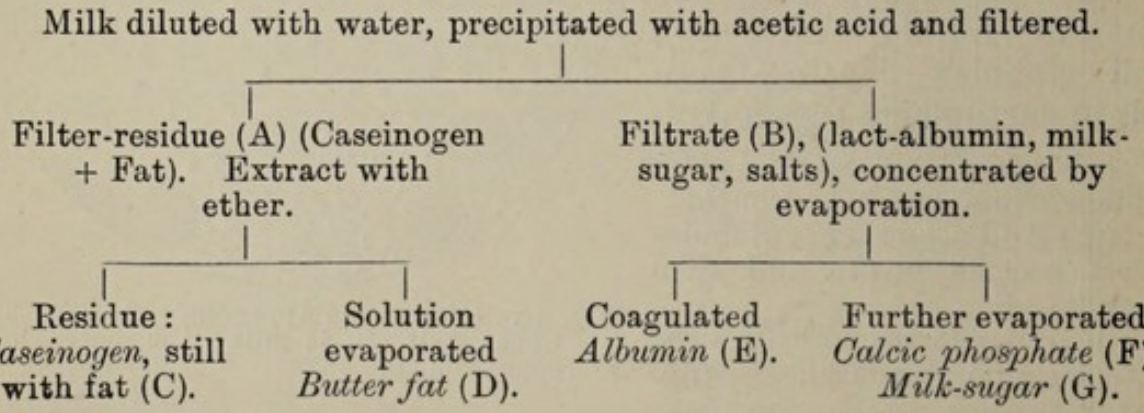
(*f*) *Proteids.*—Filter (*e*) through a moist plaited filter. Keep the residue on the filter. Divide the fairly clear filtrate into two portions. Take one portion, divide it into two, and boil one = a precipitate of **lactalbumin** (serum-albumin). Filter, and keep the filtrate to test for sugar. To the remainder add potassium ferrocyanide, which also precipitates the albumin.

(*g*) Test the second half of the filtrate for **milk-sugar**. Instead of proceeding thus, test for the presence of a reducing sugar with the filtrate of (*f*) after the separation of the serum-albumin.

(*h*) Scrape off the residue of casein and fat from the filter (*f*); wash with water, and exhaust the residue with a mixture of ether and alcohol. Place some of the ethereal solution on a slide, and allow it to evaporate, a greasy stain of fat is obtained.

(*i*) To *fresh* milk add a drop of tincture of guaiacum, which strikes a blue colour; boiled milk is said not to do so.

Separation of the chief constituents of milk (Salkowski).



2. **Separation of caseinogen by salts—Whey.**—Saturate milk with crystals of $MgSO_4$ or NaCl, or half saturate with Am_2SO_4. The caseinogen and fat separate out, rise to the surface, and leave a clear salted whey beneath. Caseinogen, like globulins,

is precipitated by saturation with NaCl or $MgSO_4$, but it is not coagulated by heat. It was at one time supposed to be an alkali-albumin, but the latter is not coagulated by rennet. It appears to be a nucleo-albumin—*i.e.*, a compound of a proteid with nuclein, the latter a body rich in phosphorus. It is not coagulated by boiling or by alcohol.

Precipitation of Caseinogen by $MgSO_4$ or half sat. with Am_2SO_4.

Filter residue Fat + Caseinogen.	Filtrate : Milk, sugar, albumin, salts.

Collect the precipitate of caseinogen and fat on a filter and wash it with a saturated solution of $MgSO_4$. Add distilled water, which in presence of the $MgSO_4$ dissolves the caseinogen, which passes through the filter and is collected. From the solution of caseinogen in weak $MgSO_4$, precipitate the caseinogen by excess of acetic acid. To get the caseinogen quite pure it must be redissolved in weak alkali or lime water, and precipitated and redissolved several times.

The filtrate after precipitation of caseinogen contains lactalbumin, and can be completely precipitated by saturation with Na_2SO_4. It coagulates between 70° and 80° C.

The fluid contains lactose, salts, and serum-albumin. Filter.

3. **Butter.**—Place milk in a narrow, cylindrical, stoppered bottle; add half its volume of NaHO and ether; shake the mixture. Put the bottle in a water-bath at a low temperature; the milk loses its white colour, and an ethereal solution of the fats floats on the surface. On evaporating the ethereal solution the butter is left behind.

Milk fats consist chiefly of palmitin, stearin, and olein, mixed with fats derived from fatty acids lower in the series—*e.g.*, butyrin and caproin. The proportion is given as olein $\frac{3}{7}$, palmitin $\frac{1}{3}$, stearin $\frac{1}{6}$, butyrin, caproin, and caprylin $\frac{1}{14}$.

4. **Salts.**—(*a*) To the filtrate of 6 (*a*), add magnesia mixture—Lesson XVII. (*g*), *i.e.*, ammonio-sulphate of magnesia, which gives a precipitate of *phosphates*. Calcium phosphate is the most abundant salt. There is a little magnesium phosphate. Test with HNO_3 and ammonium molybdate and heat = yellow precipitate.

(*b*) Silver nitrate gives a precipitate insoluble in nitric acid, indicating *chlorides* (chiefly potassium and sodium).

(*c*) Add ammonium oxalate = precipitate due to calcium salts.

5. Boil milk in a porcelain capsule. No coagulation. Remove the surface pellicle formed; boil again; another pellicle or skin is formed (Jamieson and Herz, *Journ. of Phys.*, xxvii., 26, 1901, for formation of films on proteid solutions and its relation to fats).

6. **Curdling of milk—Whey**—(*a*) *Acid.*—Place milk in a flask; warm to 40° C., and add a few drops of acetic acid. The mass clots or curdles, and separates into a solid **curd** (caseinogen and fat), and a clear fluid, the **whey**, which contains the lactose and inorganic salts. Filter.

(*b*) *Rennet ferment.*—To 5 c.c. of fresh milk heated to 40° C. add extract of rennet, or glycerin (Rennin) extract of gastric mucous membrane, which has been neutralised with dilute sodium carbonate, and keep in the warm bath. The whole mass curdles, so that the tube can be inverted without the curd falling out. By-and-by the curd shrinks, and squeezes out a clear slightly-yellowish fluid, the **whey**. Filter.

(*c*) Repeat (*b*), but boil the rennet. Its action is destroyed, and no change occurs.

(*d*) Boil milk, allow it to cool, then add rennet; in all probability no coagulation will take place.

(*e*) Dissolve one part of the curd from 6 (*a*) in NaHO and the other in lime water. Add rennet to both, warm to 40° C. The lime solution coagulates, the soda solution does not.

(*f*) *Calcium necessary for clotting decalcified milk.*—To milk add a drop or two of saturated solution of ammonium oxalate, mix, add rennet, and keep at 40° for half an hour or longer = no coagulation. Add a drop or two of 2 p.c. $CaCl_2$ = rapid coagulation.

As in the case of blood, calcium is necessary for milk-clotting.

Note the analogy between blood-clotting and clotting of milk.

Blood,	Plasma.		Milk,	Lactoplasma.	
	Corpuscles.			Oil globules.	
Coagulated Blood,	Clot,	Fibrin.	Milk Clotted,	Clot (curd).	Casein.
		Corpuscles.			Oil globules.
	Serum.			Lactoserum (whey).	

There are numerous points of resemblance.

7. **Souring of milk.**—Place milk in a vessel in a warm place for several days. It turns sour and curdles, becomes acid—test this (Lesson IX. 10)—having undergone the **lactic acid fermentation**, the lactose being split up by a micro-organism into lactic acid.

8. **Umikoff's reaction for human milk.**—By this one can determine the probable period from the beginning of lactation. To 5 c.c. of human milk add half its volume (2·5 c.c.), 10 p.c. watery solution of ammonia, and heat 15 to 20 mins. in a water-bath (60° C.). The milk becomes violet-red in colour, and the tint is deeper the longer the period of lactation. Cow's milk gives only a yellow or yellowish-brown reaction (N. Sieber, *Zeit. f. physiol. Chem.*, xxx., 101, 1900).

II. EGGS, *e.g.*, of hen, apart from the shell which is made up chiefly of calcium carbonate, consist of (i.) the white and (ii.) the yolk or yellow. The white, as already stated, consists of a fluid containing about 12 to 13 p.c. of a specific proteid—egg-albumin—with minute traces of ovo-globulin and ovo-mucoid, enclosed in a delicate fibrous membrane, grape-sugar (0·5 p.c.), traces of some other bodies and ash (0·6 p.c.). In the *yolk* are suspended particulate "yolk-spherules," one variety is coloured yellow by fat or lipochrome, and the other is proteid, and consists of *vitellin* (nucleo-proteid?). There are also small quantities of lecithin, cholesterin, and inorganic salts.

9. Grape-sugar in egg.—To uncoagulated white of egg add 10 vols. of water, mix, heat, add diluted acetic acid, and boil till all the proteids are coagulated. Filter. Evaporate the filtrate to 15 c.c. Test for grape-sugar with Trommer's test or by fermentation = positive result.

10. Vitellin.—Shake the yolk with water and ether, as long as the washings show a yellow colour. Dissolve the residue in a minimal amount of 10 p.c. NaCl solution. Pour it into a large quantity of water, slightly acidulated with acetic acid = white precipitate of impure vitellin.

(*a*) Dissolve some of the precipitate in a very weak saline solution, and observe that it is not reprecipitated by saturation with NaCl.

(*b*) Test some of the weak saline solution = coagulation about 75° C.

(*c*) Precipitate is soluble in ·1 p.c. HCl, and also in weak alkalies.

III. WHEATEN FLOUR contains 13 to 14 p.c. water and 87 to 86 p.c. solids, the chief constituents of the latter being starch 68 p.c. and proteids 12 to 13 p.c., with cellulose and fats, and almost no sugar. The gluten of dough does not exist in flour as such. The two proteids which it contains (globulins), when mixed with water, are converted into the insoluble proteid gluten.

11. Gluten.—(*a*) Moisten flour with water to form a tough dough; tie it in muslin, and knead it in a vessel containing water until all the starch is separated. (Test the washings for starch and sugar.) There remains on the muslin a greyish-white sticky, elastic mass of "crude gluten," consisting of the insoluble albuminoids, some of the ash, and the fats (basis of diabetic bread). Draw out some of the gluten into threads, and observe its tenacious characters. Test its proteid characters.

(*b*) Dry some gluten, heat it strongly in a test-tube = an ammoniacal odour of burned feathers. Water, which is alkaline (due to ammonia), condenses in the upper part of the tube.

(*c*) Extract for several hours 10 grms. wheat flour with 50 c.c. water in a flask. Filter; a clear filtrate is obtained. Heat a part of the filtrate = coagulation of *vegetable albumin*.

(*d*) With portions of the filtrate (*c*) apply (i.) xanthoproteic, (ii.) acetic acid and ferrocyanide, (iii.) NaHO and $CuSO_4$ tests for proteids.

(*e*) Extract wheat flour with 10 p.c. solution of NaCl for 12 hours. Filter, and drop some of the clear filtrate into water; a milky precipitate of a *globulin* is obtained.

(*f*) Saturate the filtered saline extract (*e*) with powdered NaCl or $MgSO_4$ = a precipitate of a globulin.

(*g*) The chief salt is potassium phosphate. The watery extract gives a yellow precipitate with platinic chloride (presence of potassium); heating it with molybdate of ammonium and HNO_3 gives a canary-yellow precipitate (presence of phosphates).

12. **Pea-meal.**—(*a*) Make corresponding watery and saline extracts, and perform the same experiments with them as with wheat flour.

(*b*) Observe the copious precipitate on boiling the watery extract.

(*c*) Note specially the copious deposit of globulin on adding the saline extract to water.

IV. WHEATEN BREAD contains, approximately, carbohydrates 55, proteids 7 to 9, fats 1, salts 2, and the rest water.

13. (*a*) Make a watery extract of the crumb with warm water, filter, and test the filtrate. Its reaction is alkaline.

(*b*) Test for starch and sugar.

(*c*) The insoluble residue gives the xanthoproteic and other proteid reactions.

(*d*) Test the "crust." It contains dextrin.

Additional Exercises.

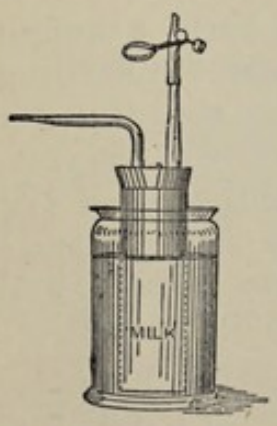


Fig. 44.—Porous cell for filtration of milk.

14. **Separation of caseinogen and fat by filtration.**—Using a Bunsen's pump, filter milk through a porous cell of porcelain. The particulate matters—caseinogen and fat—remain behind, while a clear filtrate containing the other substances passes through. The porous cell is empty, and fitted with a caoutchouc cork with two glass tubes tightly fitted into it. One tube is closed with a clip (fig. 44), and the other is attached to the pump. Place the porous cell in an outer vessel containing milk. On exhausting the porous cell, a clear watery fluid slowly passes through. Test it for proteids and sugar. Notice the absence of fat and caseinogen.

15. Coagulation of milk.—Calcium salts seem to play an important part in this process.

(i.) *Halliburton's method.*—Prepare caseinogen by saturating milk with powdered $MgSO_4$. Allow it to stand for a few hours and filter. Keep the

filtrate (A). The filter residue consists of caseinogen + fat; wash this with saturated solution, $MgSO_4$, until the washings contain no albumin. On adding water to the precipitate it dissolves, the fat remaining in the filter.

Precipitate the solution of caseinogen in weak $MgSO_4$ by acetic acid. Collect the precipitate on a filter and wash the acid away with distilled water. Dissolve the precipitate in lime water, rubbing it up in a mortar, filter = opalescent solution of caseinogen.

Place some of this opalescent solution of caseinogen in two tubes, A and B.

To A add rennet, and keep at 40° C. = no coagulation.

To B add rennet and a few drops of phosphoric acid (·5 p.c.). Heat to 40° C. = coagulation—*i.e.*, casein is formed from caseinogen in the presence of calcic phosphate.

(ii.) *Ringer's method* to show the conversion of caseinogen into casein.—Precipitate caseinogen (+ fat) with acetic acid. Collect and wash the precipitate, and grind it up in a mortar with calcium carbonate. Throw the mixture into excess of distilled water. The fat floats, the excess of calcium carbonate falls to the bottom, while the very opalescent solution contains the caseinogen. Divide the fluid into three tubes, A, B, C. Keep all at 40° C.

To A add rennet = no clot of casein.

To B a few drops of 10 p.c. solution of $CaCl_2$ = no clot of casein.

To C rennet and $CaCl_2$ = clot of casein.

16. Opacity of milk—Vogel's Lactoscope.—A graduated cylindrical c.c. measure to hold 200 c.c.; lactoscope, with parallel glass sides, 5 mm. apart (fig. 45); burette, finely graduated; stearin candle.

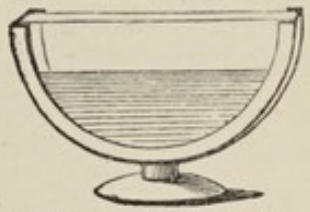


Fig. 45.—Lactoscope.

Process.—(*a*) Be certain, by microscopical examination, that the milk contains no starch, or chalk, or other granular impurity.

(*b*) Mix 3 c.c. milk with 100 c.c. water in the cylindrical measuring-glass.

(*c*) In a dark room place the lactoscope on a table, and 1 metre distant from it a lighted stearin candle. Fill the lactoscope with the diluted milk, and look at the candle flame through the glass. If the contour of the flame can be seen distinctly, pour back the diluted milk into the bottle, and add another c.c. of milk. Mix again. Test the mixture again, and repeat until, on looking through the glass, the outline of the candle flame can no longer be recognised. Add together the quantities of milk used. An empirical table constructed by Vogel gives the percentage of fat.

17. Place diluted milk in a sausage-paper dialyser, and next day examine the diffusate. Test for (1) inorganic salts, (2) lactose, (3) milk proteids, and (4) fats. Record results.

18. Butter fats.—Dissolve some in alcohol (+NaHO) to saponify them. Dissolve the soaps in water, and acidify with weak H_2SO_4. The fatty acids separate, with the characteristic odour of free *butyric* acid.

19. Casein.—That it contains P can be shown by incinerating some and testing the ash for the presence of P. It gives the ordinary proteid reactions, but it has certain special reactions.

It is soluble in very dilute alkali, and precipitated by acetic acid. Test (1) its solubility in sodium carbonate solution, (2) how it reacts

to boiling with alkali and lead acetate; (3) add $CaCO_3$ + water. It is dissolved as a lime salt.

	Lactose	*v.*	Glucose.
(*a*)	*Alcoholic fermentation.*—Not at all, or very slowly.		Rapidly.
(*b*)	*Rubner's reaction.*—Add ¼ vol. of powdered neutral lead acetete, and boil. + NH_4HO = reddish-white precipitate.		With same treatment gives a similar precipitate which rapidly becomes yellow.
(*c*)	H_2SO_4.—Dissolves without coloration.		Dissolves with brown coloration.
(*d*)	*Boiled with HCl.*—Split into glucose and galactose, both right rotatory, and therefore the specific rotatory power is increased.		Rotatory power not changed. (Lesson III.)
(*e*)	HNO_3.—By oxidisation yields mucic acid, $C_6H_{10}O_8$. Heat 5 grms. lactose + 20 c.c. HNO_3 (S.G., 1·13). Cool; crystals of mucic acid separate.		With same treatment yields oxalic acid. (*Fr. N. Schulz.*)

LESSON XIV.

MUSCLE, SKELETAL AND SMOOTH.

1. Reaction.—(*a*) Arrange on a varnished surface several red and blue strips of glazed litmus-paper, side by side, or allow one strip to slightly overlap the other. Pith a frog; cut out the gastrocnemius, remove as much blood as possible, divide the muscle transversely, and press the cut ends on the litmus-paper; a faint blue patch is produced on the red paper, showing that the muscle is *alkaline* during life. The blue paper may not be affected, but, as a rule, both papers are affected so that the reaction is "amphichromatic."

(*b*) Similarly test a piece of butcher's meat; it is acid, due to

sarco-lactic acid. Make a watery extract of lean butcher meat, and test for this acid by Uffelmann's reaction (Lesson IX.).

(*c*) Dip the other gastrocnemius into water at 50° C. until *rigor caloris* sets in. Test its reaction; now it is *acid*.

(*d*) Boil some water, and plunge into it any other muscle of the same frog; it is *alkaline*.

(*e*) Tetanise a muscle for a long time; its reaction becomes acid (Lesson XL.). The colour is retained after the paper is dry.

2. **Watery and saline extracts** (skeletal muscle).

(*a*) *Watery.*—Mince perfectly fresh muscles from a rabbit or dog. Extract with water, stirring from time to time. After half an hour, filter and keep. Re-extract the remainder with water until the extract gives no proteid reactions. For the purposes of this exercise, half an hour is sufficient. Pour off the water, which contains the substances *soluble in water*.

(*b*) *Saline.*—Collect the water-washed muscle on muslin, rub it up with sand in a mortar, and extract it with a large volume of 13 p.c. solution of NH_4Cl, or 10 p.c. NaCl. Stir occasionally, and allow it to extract for an hour or more. A stronger extract is obtained if it be left until next day. Pour off the somewhat viscid fluid, keep it, as it contains the substances *soluble in saline* solutions—the globulins, chiefly in the form of myosin. Myosin is a globulin, and as such is insoluble in water and in saturated neutral solutions, but soluble in weak neutral solutions. It is precipitated by saturation with neutral salts. Its solutions coagulate on boiling.

3. With the filtrate of 2 (*a*)—

(*a*) Test for proteids—*e.g.*, albumin.
(*b*) Test the coagulating point of its proteids (45° and 75° C.).
(*c*) Saturate with crystals of Am_2SO_4 = all proteids precipitate.

4. With the filtrate of 2 (*b*)—

(*a*) Pour a few drops into a large quantity of water; observe the milky deposit of **myosin**. Allow the deposit to settle, pour off the fluid and collect the myosin. The precipitate is redissolved by adding a strong solution of common salt (20 p.c.) = a saline solution of myosin.

(*b*) Test the coagulating point of the dissolved myosin. Four proteids are coagulated by heat at 47°, 56°, 63°, and 73° C., an albumose being left in solution. The fluid is acid in reaction.

(*c*) Saturate some of the dissolved myosin with crystals of NaCl or NH_4Cl. The myosin is precipitated.

(*d*) Dissolve some of the precipitate of 4 (*c*) in weak solution of NaCl. Test for proteids. Repeat 3 (*c*).

(*e*) Suspend in the fluid a crystal of rock salt; the latter soon becomes coated with a deposit of myosin.

5. Extractives of muscle.—Prepare Kreatin ($C_4H_9N_3O_2 + H_2O$).

(*a*) Make a strong watery solution of Liebig's extract of meat. Cautiously add lead acetate until precipitation ceases, avoiding excess of the lead. Filter, pass sulphuretted hydrogen through the filtrate to get rid of the lead. A pellicle is very apt to form on the surface. Filter, and evaporate the filtrate to a syrup on a water-bath, and set it aside in a cool place to crystallise. Crystals of kreatin separate out.

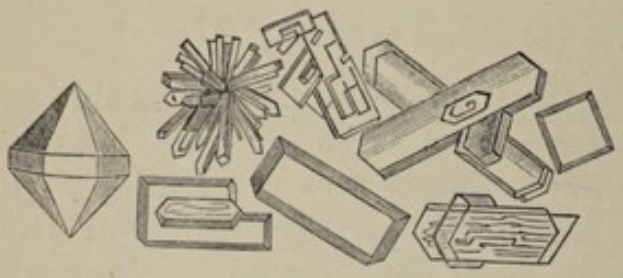


Fig. 46.—Crystals of Kreatin.

(*b*) After several days, when the kreatin has separated, pour off the mother-liquor, add to it 5 volumes of 90 per cent. alcohol to precipitate more kreatin. Filter, wash the crystals with alcohol, redissolve them in boiling water, allow them to recrystallise, and examine them with the microscope (fig. 46).

Sarkin and Xanthin may be prepared from the alcoholic filtrate of (*b*).

Preparation of Kreatin (Salkowski).

Minced flesh, digested with water, strained.

- Filtrate heated to boiling, filter.
  - Filtrate + lead acetate, filter.
    - Filtrate + H_2S to remove lead, filtrate concentrated = *Kreatin*.
    - Deposit = phosphate chloride and sulphate of lead.
  - Residue = coagulated albumin.
- Residue.

6. Liebig's extract of meat. *Test for*—

(*a*) Proteids; they are absent. Xanthoproteic test may give a faint yellow colour.

(*b*) Glycogen, doing a control test. It usually contains a small quantity.

(*c*) Kreatinin (see "Urine"). Weyl's test usually succeeds. Add sodium nitroprusside and NaHO = a red colour; boil, the colour fades; while boiling add acetic acid = a blue or green colour.

(*d*) Ether extract gives no fat.

(*e*) Microscopically; besides crystals of NaCl (cubes), few clear knife-rest forms, there are numerous crystals of kreatin.

7. Beef-tea.—Prepare by boiling meat and bones. If much bone and connective-tissue be present, the extract gelatinises on cooling.

(*a*) Xanthoproteic reaction, faint yellow colouring. Little, if any, proteid is present.

(*b*) Test for kreatinin. Usually present.

Additional Exercises.

8. Muscle-plasma.—Kill a rabbit by bleeding from the carotids, open the abdomen, insert a cannula in the aorta, and wash out all the blood from the lower limbs with cold (iced) saline solution (0·6 p.c. NaCl). Skin the limbs quickly, cut off pieces of muscle and plunge them into a mixture of salt and ice (1° to 2° C.), where they quickly become quite hard and frozen. When frozen remove them from the mixture, wipe them with blotting-paper, and place them on a plate kept cold by ice and salt mixture. Cut them into fine slices (cutting parallel to the direction of the fibres). Wrap the slices in linen and squeeze them in a pair of cooled enamelled iron lemon-squeezers; a yellowish, viscid alkaline plasma is obtained, which sets in the course of an hour or so into a solid jelly, with the simultaneous appearance of an acid reaction. By-and-by a clear clot of myosin and a fluid muscle-serum is obtained, just as in a blood-clot. The muscle-plasma contains several proteids. (See Halliburton, *Journal of Physiology*, viii. p. 133, 1887.)

9. Proteids of muscle.—Wash out the blood-vessels of a rabbit as above. Remove the muscles quickly, chop them up and extract for a day with a 5 p.c. solution of $MgSO_4$. Use this fluid.

(*a*) It is probably acid due to lactic acid. Test for this (p. 84).

(*b*) *Coagulation.* Dilute some with 4 vols. of water, divide it into two parts; keep one at 40° C. (rapid coagulation), and the other at the ordinary temperature (coagulation, but slower). Clot of myosin is formed in both.

(*c*) Remove the clotted myosin from (*b*); it is readily soluble in 0·2 p.c. HCl, forming syntonin; and also in 10 p.c. sodium chloride.

(*d*) Add a few drops of 2 p.c. acetic acid to some of the extract = stringy precipitate of myosinogen.

(*e*) Perform fractional heat coagulation.

> "(i.) With the original extract coagula are obtained at 47°, 56°, 63°, 73° C.
>
> "(ii.) With liquid (salted muscle-serum) from (*b*), after separation of the clot, coagula are obtained at 63° and 73° C.
>
> "(iii.) With muscle-extract which has been saturated with $MgSO_4$ and filtered. The globulins are thus separated. Coagulation now occurs at 73° C., but the coagulum is small" (*Halliburton*).

The following table from Halliburton shows these results :—

Name of Proteid.	Coagulation Temp.	Action of $MgSO_4$	Is it globulin or albumin?	Fate.
Myosinogen.	47° C.	Precipitated.	Globulin.	These form muscle-clot or *Myosin*.
Myosinogen.	56° C.	,,	,,	
Myo-globulin.	63° C.	,,	,,	These are left in *muscle-serum*.
Myo-albumin.	73° C.	Not precipitated.	Albumin.	

10. Pigments of muscle.

(*a*) Notice the difference between the red (semi-tendinosus) and pale muscles (adductor magnus) of the rabbit.

(*b*) The muscular part of the diaphragm shows the spectrum of oxy-hæmoglobin, even after the blood-vessels have been washed out by salt solution.

(*c*) A piece of the great pectoral muscle of a pigeon, either fresh or which has been placed in glycerin to render it more transparent, on being pressed between two pieces of glass shows absorption bands of myo-hæmatin (*MacMunn.*) Map out their position with the spectroscope.

(*d*) Red colour of salted meat.—Boiled fresh meat has a brown colour due to the splitting up of HbO_2 into hæmatin and coagulated proteid. "Salted" meat has a characteristic red colour when cooked, the colour depending on the nitre which is used.

(1) Boil meat in a watery solution of a nitrite = red colour (*Kisskalt*).

(2) Extract *freshly*-exposed salted meat with water. It gives a spectrum unaltered by $(NH_4)_2S$, and two absorption bands slightly nearer D than those of HbO_2. It appears to be identical with nitric oxide – Hb. The colour of *cooked* salt meat is due to NO – hæmochromogen (Haldane, *Journ. of Hygiene*, i., 1901).

11. Smooth muscle obtained from the stomach of sheep and calf, and rectum of the latter animal. Extracts are made as above with 5 p.c. $MgSO_4$, or 0·9 p.c. NaCl (*v. Fürth*), and their coagulation point determined. The saline extracts are usually neutral, sometimes alkaline, while those of skeletal muscle are acid. They contain but little paramyosinogen (myosin of v. Fürth, coag. 47° to 50°), and much myosinogen (myogen coag. 55° to 65°). The 0·9 NaCl extract coagulates spontaneously. Smooth muscle shows slight rigor mortis (*Munk;* also *Swale Vincent and Lewis*).

LESSON XV.

SOME ORGANIC SUBSTANCES—NERVE TISSUE—THYROID GLAND.

ENZYMES.

FERMENTATION is due either directly to cell life (I.) *organised ferments*, or indirectly as the result of soluble substances secreted by cells—*i.e.*, (II.) *unorganised ferments*, *soluble ferments* or *enzymes* (Kühne, 1879). Fermentation is decomposition of complex organic material into simpler bodies by the agency either of protoplasm itself or of a secretion (Enzyme) prepared by it.

Organised ferments (unicellular micro-fungi—*e.g.*, yeast, various bacilli, &c.—connected with the life of the cell). Their action is arrested by protoplasmic poisons, such as antiseptics (dil. sols. of $HgCl_2$, phenol, thymol, formalin, sodic fluoride, $CHCl_3$, &c.). They grow and multiply in certain media.

Enzymes are non-living, soluble bodies, whose actions—chemical or physical—are not arrested by certain antiseptics. They can transform an almost indefinite amount of material. Are they altered in the process? They do not seem to be quantitatively involved in the reaction. Destroyed by boiling.

No sharp distinction can be drawn between the action of cellular ferments and the enzymes—*e.g.*, yeast yields a soluble inverting ferment, and yeast cells change sugar into alcohol and CO_2,, but Buchner (1896) has shown that the latter action is due to a soluble *zymase*, which can be extracted from yeast cells. *Micrococcus ureæ* yield a soluble enzyme which splits up urea.

Most of the chemical changes in the digestive processes are brought about by enzymes in a fluid medium.

Some of the methods for extracting and isolating these enzymes have already been referred to. The most common solvents are water and glycerin, and these solutions retain their activity. They are precipitated by $(NH_4)_2SO_4$, and also by alcohol. They have never been obtained in a pure state, so that their chemical nature is unknown. As to their modes of action, most probably they are hydrolytic—*i.e.*, some cause the substance acted on to take up the elements of water—but others have quite a different action. Are they proteid or nucleo-proteid in nature?

The rapidity of action (zymolysis) depends on—(1) Temp. [each has an optimum temp. usually about 40° C.]; (2) accumulation of products of action; (3) reaction of liquid; (4) concentration of enzyme; (5) condition of material to be acted on.

Groups of Enzymes—1. *Amylolytic.*—Those which transform insoluble carbohydrates ultimately into soluble sugars—*e.g.*, *Diastase* of plants (1833) acts on starch; *ptyalin* on starch; *cytase* on cellulose.

2. *Inversive.*—Transform sugars (bioses) into simpler hexoses—*e.g.*, *invertase* acts on cane-sugar; *glucase* on maltose in small intestine and blood; *lactase* in the small intestine.

3. *Proteolytic*, which decompose various insoluble proteids, forming proteoses and peptones—*e.g.*, *pepsin*, *trypsin.* Vegetable trypsins. *Bromelin* (in pine-apple). *Papaïn.*

4. *Coagulative* or *clotting* enzymes which convert soluble into insoluble proteids—*e.g.*, *rennet* on milk; *thrombase* (coag. of blood); *myosin-ferment*; *pectase* (producing vegetable jellies); *vegetable rennet.*

5. *Lipolytic* or *fat-splitting*, which decompose fats and oils into fatty acids and glycerin—*e.g.*, steapsin or *lipase* or pialyn. It occurs in pancreatic juice, blood, and is common in oily seeds.

6. *Oxidising* enzymes—*i.e.*, *oxidases*—which assist oxidation of various substances—*e.g.*, *laccase* (crude sap of lac tree), *tyrosinase.* Animal oxidases, present in blood and many tissues. (Glycolysis in blood.)

7. Others—*e.g.*, *urease*—acts on urea and brings about ammoniacal fermentation—*e.g.*, in urine. *Histozyme* on hippuric acid. *Zymase* (alcohol producing enzymes).

[8. *Glucoside-splitting—e.g.*, *emulsin.*]

Consult *The Soluble Ferments and Fermentations*, by J. Reynolds Green (1899); *Les Oxydases*, by P. Portier, 1897.

Examples of most of the above have already been referred to, so that only one group is given here.

1. Oxidases have all the properties of enzymes. They can be extracted by water or glycerin, are precipitated by alcohol, and are destroyed by heat. They promote direct oxidation of various substances—*e.g.*, aromatic compounds and sugar, and occur both in the animal and vegetable kingdoms. They are most conveniently extracted by macerating an organ in chloroform water. They have the power of oxidising various substances in the presence of free oxygen (gaseous or in solution), and their action is accompanied by absorption of oxygen.

The reagents used are chiefly (1) Tincture of guaiacum (1·5 p.c. in alcohol); (2) guaiacol in distilled water; (3) hydroquinone (1 p.c. in distilled water).

(*a*) Make a solution of fibrin in 10 p.c. NaCl, or 2 p.c. NaFl. It strikes a blue with guaiacum. Probably the ferment is due to the leucocytes entangled in the fibrin. If the solution be boiled the result is negative.

(*b*) Globulins of blood have no oxidising action. It is doubtful if oxidases exist in living blood or tissues. It seems to be produced from the dead leucocytes.

(*c*) The spleen extract acts powerfully (see *Appendix*).

2. **Zymogens.**—The term zymogen, or "mother of ferment," is applied to the bodies from which ferments are derived—*i.e.*, they are the precursors of ferments. Most frequently they exist in the form of granules in the protoplasm of secretory gland or other cells—*e.g.*, pepsin is derived from *pepsinogen*, rennin from a separate zymogen. The zymogen granules of the pancreas exist in the inner zone of the secretory cells of the organ. The zymogen of the fibrin ferment exists in the white blood-corpuscles.

(*a*) Microchemical—Zymogen granules—*e.g.*, of pancreas.—Expose a small piece of the pancreas to the vapour of osmic acid. Tease a piece in normal saline or glycerin, and observe the inner zone of the cells crowded with granules. Other glands may be similarly treated. The number of granules varies with the condition of physiological activity of the gland under observation.

3. **Nervous tissue.**—(*a*) Make a *saline* extract (*e.g.*, 5 p.c. $MgSO_4$), *e.g.*, of the cerebrum, and apply the usual tests for proteids. Positive results, the proteids being derived chiefly from the *grey* matter—*i.e.*, from its nerve cells.

(*b*) Make an *ethereal* extract of the brain or spinal cord. Such substances as lecithin and cholesterin are dissolved out, chiefly from the *white* matter. The latter can readily be obtained in crystals, while the ethereal solution on adding H_2SO_4 carefully gives a red colour at the junction of the two fluids indicating cholesterin. This substance is often found in large plates floating in anatomical preparations of the brain preserved in alcohol. It is widely distributed also in plants.

4. **Thyroid gland.**—(*a*) Extract a fresh gland of a sheep freed of fat and connective tissue with 5 p.c. $MgSO_4$ or 0·1 p.c. NaHO for several hours, when a reddish fluid will be obtained. Add acetic acid carefully, until a copious white precipitate falls; it consists chiefly of the "colloid matter" of the gland acini. Collect the precipitate, redissolve it in dilute alkali, and reprecipitate with acetic acid. This body contains *iodine* (0·7 p.c.).

(*b*) Iodine is present.—Incinerate at a dull red heat several thyroid tablets, mixed with excess of pure NaHO. Dissolve the residue in water, and add HNO_3 = brown fluid. Extract with chloroform, which shows a violet tint due to the presence of iodine.

5. Thymus gland—Nucleo-proteids.—(see Lesson II., VI).

This gland is chosen because it consists essentially of leucocytes, and the nucleo-proteids are abundant in its nuclei. By gastric digestion nuclein is split off, is not digested, and can thus be obtained. It is insoluble in acids, but soluble in alkalies. When heated with acids, it yields what are called the "purin bases"—*e.g.*, xanthin, adenin, &c.—bodies allied to uric acid. In the head of spermatozoa is a compound of nucleic acid and protamines (*Kossel*). See Appendix.

6. Glycosamin hydrochloride.—Free the chitinous parts of crabs and lobsters as much as possible from their soft parts, dry, and divide into small pieces, and decalcify in dilute HCl. Gently boil the decalcified parts for 3 to 4 hours with HCl, then evaporate and allow to crystallise. On cooling, a dark brown humus substance and crystals separate out. Filter, dissolve the crystals in water, and re-evaporate until crystallisation takes place. The glycosamin hydrochloride ($C_6H_{13}NO_5HCl$) separates in colourless glancing crystals about the size of a pea, which reduce Fehling's solution on boiling. They have a somewhat sweet taste like sugar.

7. Nuclein of yeast.—Mix 1 part fresh German yeast with 4 parts water; allow the deposit to subside. Pour off the turbid fluid from the slimy deposit of yeast, place the latter in ·5 p.c. KHO, stir, and filter directly into dilute HCl. The deposit is filtered off, washed with dilute HCl, and then with alcohol. It is then boiled with alcohol and dried over H_2SO_4.

(*a*) It is an amorphous powder, insoluble in water and dilute acids, but readily soluble in alkalies.

(*b*) Fuse a little with sodium carbonate and nitrate of potash = a mass with a strongly acid reaction due to phosphoric acid.

8. Lecithin.

$$C_3H_5\begin{cases} O.R^* \\ O.R \\ O.PO\begin{cases} OH \\ O.CH_2.\underset{(CH_3)_3}{CH_2}\end{cases}\end{cases}N.OH.$$

Extract the fresh yellow of eggs free from white, with ether, until the latter takes up no more. Distil off the ether, dissolve the residue in petroleum ether, and filter. Extract the filtrate in a separation filter several times with 75 p.c. alcohol. Mix the alcohol extracts, let them stand until they become clear, separate any petroleum ether, and filter. The rest of the petroleum ether is got rid of by distillation, and the residue is exposed for several days to the air in a cool place, whereby a deposit separates. The clear fluid is decanted and filtered. Decolorise it by boiling with animal charcoal, filter and evaporate to a thick syrup at 50° to 60°. Dissolve the syrup in ether and evaporate, and the nearly pure lecithin remains behind (*Drechsel*).

The three chief characteristics of the lecithins are *solubility in alcohol and in ether, dark cross in globules in polarisation microscope, acidity of residue after charring.*

(*a*) It is a soft doughy indistinctly crystalline body. Place a little under a microscope, add a drop of water, and observe the oil-like drops assuming worm-like forms, so-called "myelin-forms."

---

* R = radical of palmitic acid ($C_{15}H_{31}CO$), stearic acid ($C_{17}H_{35}CO$), or oleic acid ($C_{17}H_{33}CO$).

(*b*) Heat some on platinum, either alone or with sodium carbonate and potassium nitrate = a residue, strongly acid, in which phosphoric acid is readily detected.

(*c*) Polarised light.—Examine a little under a polarisation microscope. With crossed Nicol's each granule of the substance shows a dark cross on a white ground, just like starch (*Dastre*).

9. Glycocoll.—$C \left\{ \begin{matrix} H_2 \\ NH_2 \end{matrix} . CO . OH \right\} = C_2H_5NO_2$ or amido-acetic acid.

Preparation.—Boil 1 part of hippuric acid with 4 parts of dilute H_2SO_4 (1 : 6 water) for 10 to 12 hours, using a condenser. Pour the mass into a capsule, and let it stand for 24 hours. Filter, wash the benzoic acid in the filter with cold water, concentrate the filtrate by evaporation, and free it from the last traces of benzoic acid by shaking it with ether. Dilute strongly the acid solution, and neutralise it exactly with baryta water. Allow the precipitate to subside, decant, wash the precipitate with warm water, again concentrate the filtrate until crystals begin to separate on its surface. Allow it to stand 24 hours, pour off the mother-liquid, and again evaporate the latter until other crystals are formed. The crystals are recrystallised from water.

Glycocoll forms clear colourless crystals, with a sweet taste, readily soluble in water, and insoluble in alcohol.

10. Guanin reaction.—Guanin occurs in very considerable quantity in the skin of fishes and frogs. Heat with HNO_2, in a porcelain capsule, a piece of skin from the belly of a frog. Add NaHO = orange to cherry-red colour. There is no reaction with ammonia. If there be very little guanin, add dilute KHO, and blow on the stain, when the latter will pass through several *nuances* from blue to orange.

LESSON XVI.

URINE.

1. **Urine** is a transparent light-straw coloured watery secretion derived from the kidneys, containing nitrogenous or azotised matters, salts, and gases. It contains about 96 p.c. water and 4 p.c. solids; of the latter urea makes up over 2, and NaCl nearly 1 p.c. It has a peculiar odour, bitter saltish taste, and acid reaction.

2. **Quantity in 24 hours,** about 2½ pints (50 ounces) or 1500 c.c. There are considerable variations even in health, the quantity being regulated by the amount of fluid taken, and controlled by the state of the tissues, the pulmonary and cutaneous excretions. It should be collected in a tall graduated glass cylinder of a capacity of 2500 c.c. with a ground glass top to exclude impu-

rities. Samples of the mixed urine of the 24 hours are used for examination.

Increased by drinking water (*urina potus*) or diuretics; when the skin is cool, its blood-vessels are contracted, and the cutaneous secretion is less active; after a paroxysm of hysteria, and some convulsive nervous diseases; in *diabetes insipidus* and *d. mellitus;* some cases of hypertrophy of the left ventricle, and some kidney diseases. The increase may be temporary or persistent, the former as the effect of cold, diuretics, or nervous excitement; the latter in diabetes and certain forms of kidney-disease.

Diminished after profuse sweating, diarrhœa; early stage of acute Bright's disease; some forms of Bright's disease; the last stages of all forms of Bright's disease; in general dropsies; in acute febrile and inflammatory diseases.

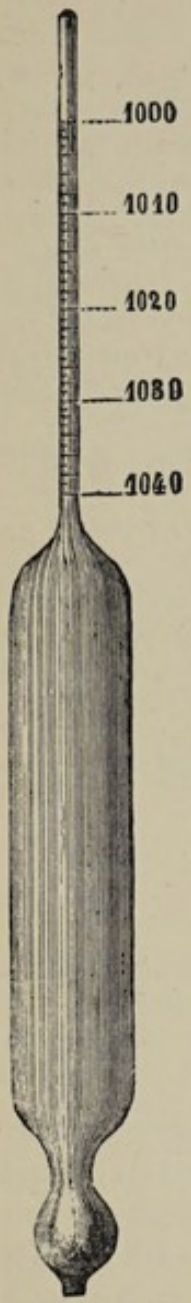


Fig. 47. Urinometer.

3. **Colour.**—Light-straw to amber-coloured. It varies greatly in health, and is chiefly due to the presence of normal urobilin or urochrome, an iron-free derivative of the blood pigment.

Pale after copious drinking and after paroxysmal nervous attacks (hysteria), in diabetes, anæmia, and chlorosis. *N.B.*—Pale urines indicate absence of fever.

High-coloured after severe sweating, violent muscular exercise, diarrhœa, or during febrile conditions.

Pathological pigments, purpurine or uro-erythrine in febrile disorders; bile pigments; blood.

Medicinal substances.—Creosote and carbolic acid make urine nearly black (hydrochinon), rhubarb (gamboge-yellow), senna (brownish).

4. **Specific Gravity.**—Average 1020 (1015 to 1025). It is taken by means of a **urinometer** (fig. 47), which is really a hydrometer with a stem graduated from 1000 (zero) above to 1040 below.

(*a*) Place a urinometer in a tall cylindrical vessel filled with urine. Bring the vessel to the level of the eye, and as soon as the instrument comes to rest, read off the mark on its stem opposite the lower surface of the meniscus against a bright background.

Precautions.—1. The vessel must be wide enough to allow the urinometer to float freely and not touch the sides. 2. The instrument must be dry. 3. The urine itself must be free from air-bubbles on the surface. *N.B.*—Always take the S.G. of the "mixed urine" of 24 hours.

Low S.G.—Normally the S.G. varies inversely as the quantity of urine passed. Causes which increase the water of the urine only—*e.g.*, drinking on an empty stomach; hysteria; in *diabetes insipidus* or *polydipsia*. *N.B.*—If continually below 1015, suspect *diabetes insipidus* or chronic Bright's disease.

High S.G.—When the urine contains much solid matter—*e.g.*, in *Diabetes mellitus*—due to a large amount of grape-sugar; first stages of acute fevers; rapid wasting of the tissues, especially if associated with sweating or diarrhœa. It is highest normally three to four hours after a meal; and as it varies during the day it is necessary to mix the urine of the 24 hours, and test the S.G. of a sample of the "mixed urine." *N.B.*—If above 1025 and the urine be pale, suspect saccharine diabetes.

5. Estimation of amount of solids from S.G.—By *Christison's formula* ("*Häser-Trapp's coefficient*"), "multiply the last two figures of a specific gravity expressed in four figures by 2·33. This gives the quantity of solid matter in every 1000 parts"—*i.e.*, the number of grms. in 1000 c.c. ($33\frac{1}{3}$ oz.).

Example.—Suppose 1200 c.c. are passed in 24 hours, S.G. 1022.

$$22 \times 2{\cdot}33 = 51{\cdot}26 \text{ grms. in 1000 c.c.}$$

To ascertain the amount in 1200 c.c.

$$1000 : 1200 :: 51{\cdot}26 : x = \frac{51{\cdot}26 \times 1200}{1000} = 61{\cdot}51 \text{ grms.}$$

This formula is not applicable in diabetes and some cases of Bright's disease.

The normal total solids are about 70 grms. (2 to $2\frac{1}{2}$ ozs.) in 24 hours—*i.e.*, 1000 to 1050 grains. Of the solids about $\frac{2}{3}$ are organic and $\frac{1}{3}$ inorganic.

6. Odour is "peculiar," "characteristic," and "aromatic."

Certain substances influence it—turpentine (violets); cubebs, copaiba, and sandal-wood oil give a characteristic odour, and so do asparagus, valerian, assafœtida, garlic, &c. In *disease*, note the ammoniacal odour of putrid urine and the so-called "sweet" odour in saccharine diabetes.

7. Reaction.—*Normal.*—Slightly *acid*, it turns blue litmus-paper slightly red, and does not affect red litmus-paper. The acidity is due not to free acid, but to that of acid salts, chiefly acid sodium phosphate (NaH_2PO_4). A *neutral* urine does not alter either blue or red litmus-paper. A *very acid* urine turns blue litmus-paper very red. It may be "amphoteric" (rare) when the amount of neutral and acid phosphates is the same.

(*a*) Test with appropriate litmus-paper a normal, very acid, neutral, and alkaline urine.

(*b*) That the acidity is not due to a free acid is shown by its giving no precipitate with sodium hyposulphite, and also by the fact that it has no action on congo-red. The colour of the latter body becomes dark violet on adding solution of hippuric acid 1 : 50,000 (*Brücke*).

(*c*) The acidity of normal urine decreases on standing. The urate of sodium is decomposed by the acid sodium phosphate whereby acid sodium

urate and then uric acid are thrown down as a sediment owing to diminished acidity.

I. $NaH_2PO_4 + C_5H_2Na_2N_4O_3 = Na_2HPO_4 + C_5H_3NaNO_3$.

II. $NaH_2PO_4 + C_5H_3NaN_4O_3 = Na_2HPO_4 + C_5H_4N_4O_3$.

8. **Variations in acidity during the day.**—*Digestion*—*i.e.*, two or three hours after a meal—the urine becomes neutral or alkaline ("*alkaline-tide*"). The cause of the alkalinity is a fixed alkali, probably derived from the basic alkaline phosphates taken with the food (*Roberts*). According to others, the formation of free acid in the stomach liberates a corresponding amount of bases, which pass into the urine, and diminish its acidity or even render it alkaline. The "*acid-tide*" occurs after fasting.

Nature of food.—With a vegetable diet the excess of alkali may cause an alkaline urine. In herbivora it is alkaline, in carnivora very acid. Herbivora (rabbits) whilst fasting have a clear acid urine, because they are practically living on their own tissues. It is very acid during starvation.

Acids slightly increase the acidity. *Alkalies* and their carbonates are more powerful than acids, and soon cause alkalinity; alkalies—*e g.*, the alkaline salts of citric, tartaric, malic, acetic, and lactic acids—appear as carbonates (*Wöhler*).

9. **Alkalinity, fixed or volatile alkali.**—In the former case the blue colour of the litmus-paper does not disappear on heating; in the latter it does, and the paper assumes its original red colour.

(*a*) Test with red litmus-papers two urines, one of which is alkaline from a fixed alkali, the other from a volatile one. Both papers become blue.

(*b*) Heat both side by side on a glass slide. The blue colour of one disappears (volatile alkali), the red being restored, while the blue of the other remains (fixed alkali).

(*c*) Fix sensitive red litmus-paper to the cork of the bottle containing the urine. If the alkali is volatile, the paper becomes blue, and changes again to red on exposure to air. Fixed alkalies only cause such change when the paper is dipped in the urine.

The alkalinity may be due to ammonium carbonate (volatile), derived from the decomposition of urea; the urine may be ammoniacal when passed, in which case there is always disease of the urinary tract; or it may become so on standing—from putrefaction—when it is always turbid, and contains a sediment consisting of amorphous phosphate of lime and triple-phosphate, and sometimes urate of ammonium; it has an offensive ammoniacal odour.

The acidity is increased during the resolution of febrile diseases; excessive in gout, acute rheumatism, and whenever much uric acid is given off (uric acid diathesis); in saccharine diabetes the amount of the acidity may be determined by using a standard solution of caustic soda (p. 131).

10. Transparency.—Observe if it contains suspended particles, rendering it more or less turbid, either when it is passed or some time afterwards.

11. Fermentation.—When freely exposed to air it undergoes two fermentations—(1) *acid;* (2) *alkaline.* At first it becomes slightly *more acid,* from the formation of lactic and acetic acids (although this is denied by some observers), then it gradually becomes *neutral,* and finally *alkaline* from putrefaction. It becomes lighter in colour, turbid, and a whitish heavy precipitate falls; it swarms with bacteria; a pellicle is formed on

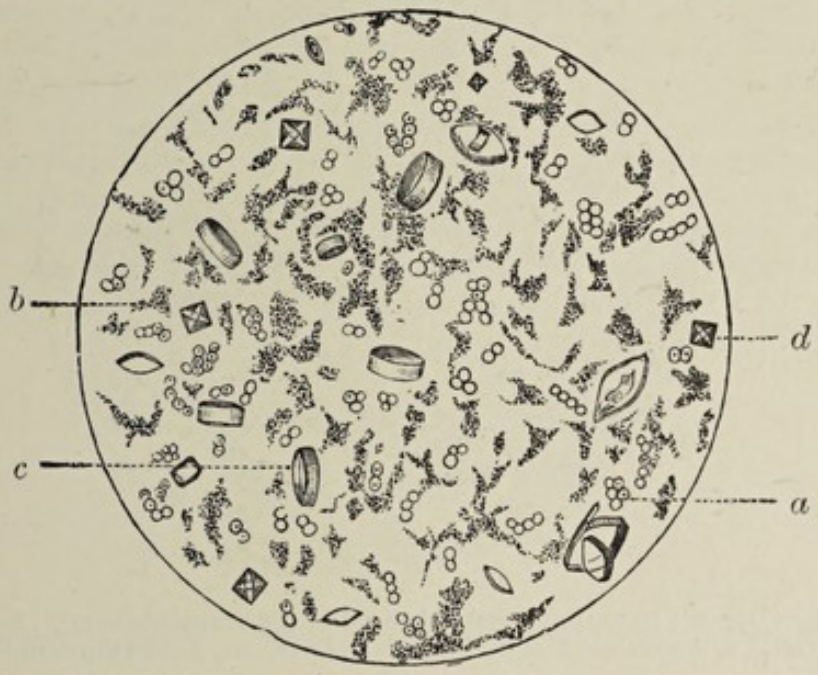


Fig. 48.—Deposit in "Acid Fermentation" of Urine. *a*, Fungus; *b*, Amorphous sodium urate; *c*, Uric acid; *d*, Calcium oxalate.

the surface; its odour is strongly ammoniacal, which is due to the splitting up of the urea, thus—

$$CON_2H_4 + 2H_2O = (NH_4)_2CO_3.$$

$$(NH_4)_2CO_3 = 2NH_3 + H_2O + CO_2.$$

The urea is split up by a ferment or enzyme—*urease*—formed by the *Micrococcus ureæ.* Carbonate of ammonium makes the urine alkaline; the earthy phosphates are precipitated because they are insoluble in an alkaline urine. Phosphate of lime is precipitated ($Ca_3(PO_4)_2$, amorphous), but phosphate of magnesia unites with the ammonia and is precipitated as ammonio-magnesic phosphate or triple phosphate ($MgNH_4PO_4 + 6H_2O$). Part of the ammonia escapes, and some unites with uric acid to form later on urate of ammonium, $C_5H_2(NH_4)_2N_4O_3$.

N.B.—Urine may be kept "sweet" for a long time in perfectly clean vessels, but on being mixed with decomposing matter it rapidly putrefies. All urinary vessels should be scrupulously clean, and all instruments introduced into the bladder be thoroughly purified by an efficient antiseptic.

(*a*) Place normal urine in a warm place (for days). Observe it daily; note reaction, change of colour, transparency, odour, and any deposit. Examine the deposit microscopically (figs. 48, 49).

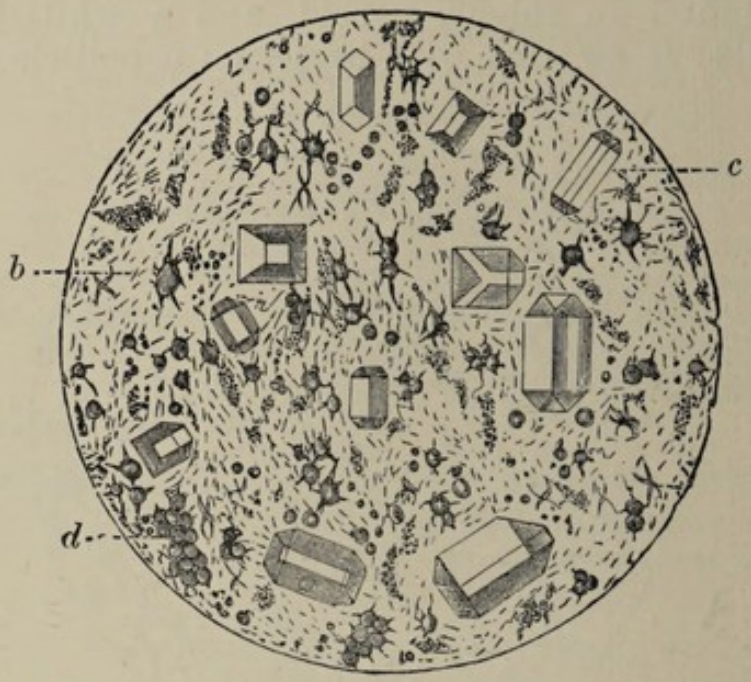


Fig. 49.—Deposit in ammoniacal urine (alkaline fermentation). *b*, Bacterium ureæ. *c*, Ammonio-magnesium phosphate. *d*, Acid ammonium urate.

Fermentation is *hastened* by rise of temperature, more especially if the urine be passed into a contaminated vessel, or the urine itself contains blood or pus. It is *retarded* in a very acid urine.

Additional Exercise (Acidimetry).

12. Estimation of acidity.—Ascertain the amount of caustic soda required to exactly neutralise 100 c.c. of urine. As the soda solution cannot be prepared by weighing the soda, because of the varying amount of water contained in it, the soda solution must be titrated with a standard solution of oxalic acid. Make a *normal* solution of oxalic acid by dissolving 63 grms. of dry crystallised oxalic acid in 1000 c.c. water, $C_2H_2O_4 + 2H_2O = 126$ (*i.e.*, half the quantity is taken because the acid is dibasic). A normal solution of caustic soda would contain 40 grms. per litre (NaHO)—*i.e.*, Na = 23, H = 1, O = 16 (= 40). 1 c.c. = 40 mgrms. or ·04 grm. Dissolve 150 grms. of caustic soda in about 1000 c.c. water.

(*a*) Normal caustic soda.—To 10 c.c. normal oxalic acid solution in a beaker add a few drops of alcoholic solution of rosolic acid (orange solution). Allow the NaHO solution to drop from a burette until the rosolic acid gives a rosy-red tint. Suppose that to saturate the acid 9·2 c.c. of the soda solution are added, then to every 9·2 c.c. 0·8 c.c. must be added to obtain a solution 1 c.c. of which will correspond to 1 c.c. of acid, so that for 1000 c.c. of NaHO 9·2 : 1000 : : 0·8 : x $\left(\frac{1000 \times 0{\cdot}8}{9{\cdot}2} = 86{\cdot}9\right)$ 86·9 c.c. water must be added.

(*b*) Determine acidity of urine.—To 100 c.c. of urine in a beaker add from a burette the normal soda solution (1 c.c. = 0·063 oxalic acid); or dilute the soda solution to obtain a *deci-normal solution* $\frac{N}{10}$ (*i.e.*, one-tenth as strong). In this case 1 c.c. = ·0063 oxalic acid. Place strips of red litmus-paper in the fluid, drop in NaHO, stir, and add caustic soda until the litmus begins to turn blue. Suppose 15 c.c. of the dilute $(\frac{N}{10})$ solution are used, then the acidity of 100 c.c. urine = 15 × 0·0063 = 0·0945; and suppose the total quantity of urine passed to be 1500 c.c., then the total acidity of the urine passed in 24 hours expressed as oxalic acid = 1·417 grms. The result is merely approximative.

In Freund's method recently introduced the principle is to determine the acidity from the diacid sodium phosphate, which is the exclusive cause of the acidity of the urine.

LESSON XVII.

INORGANIC CONSTITUENTS OF URINE.

The constituents of urine may be classified as follows :—

(1) *Water* and *inorganic salts.*

(2) *Urea* and *related nitrogenous bodies;* uric acid, xanthin, guanin, kreatinin, allantoin, oxaluric acid.

(3) *Aromatic substances;* ether-sulpho-acids of phenol, cresol, pyrocatechin, hippuric acid, &c.

(4) *Fatty non-nitrogenous bodies;* oxalic and lactic acids.

(5) *Pigments.*

(6) *Gases.*

The ratio of inorganic to organic constituents is 1 to 1·2 to 1·7. The amount of salts excreted in 24 hours is 16 to 24 grms. (normally).

1. **Water** is derived chiefly from food and drink, a small quantity being formed in the body (normally 1500 c.c., or about 50 ozs.).

2. **Chlorides**, chiefly of sodium (the most abundant), with a little potassium and ammonium, derived chiefly from the food, amount to 10 to 13 grms. (150 to 195 grains), or a mean of 12 grms. (180 grains)—*i.e.*, less than half the amount of urea excreted. NaCl crystallises in cubes and octahedra.

(*a*) Test with a few drops of $AgNO_3$ (1 pt. to 8 distilled water) = white, cheesy, or curdy precipitate in lumps, insoluble in HNO_3. The phosphate of silver is also thrown down, but it is soluble in HNO_3. It is better to add the HNO_3 and then the $AgNO_3$. The deposit is normally about $\frac{1}{4}$ volume of the urine. The AgCl is soluble in ammonia. A rough estimate may be formed of the amount by allowing the precipitate to subside, and comparing its bulk from day to day.

$$AgNO_3 + NaCl = AgCl + NaNO_3.$$

Variations, increased when the urine is secreted in excess, although the NaCl usually remains very constant ($\frac{3}{4}$ p.c.); lessened in febrile affections, and where a large amount of exudation has taken place, as in acute pneumonia, when chlorides may be absent from the urine. Their reappearance in the urine is a good symptom, and indicates an improvement in the condition of the lung. *N.B.*—Test urine daily for chlorides in pneumonia.

(*b*) Evaporate a few drops of urine on a slide = octahedral or rhombic crystals, a compound of NaCl and urea.

(*c*) Test urine from a case of pneumonia, and compare the amount of the precipitate with that of a normal urine.

3. Quantitative estimation of chlorides.—(1) *Standard silver nitrate.*—Dissolve 29·075 grms. fused silver nitrate in 1000 c.c. distilled water. 1 c.c. = 0·01 NaCl.

(2) *Saturated solution of neutral potassic chromate* (K_2CrO_4).

(*a*) Dilute 10 c.c. of not too dark-coloured urine with 100 c.c. water, and place it in a beaker; add a few drops of (2). Allow the silver solution to drop in, stirring all the time until a faint orange tint indicates that there is an end of the reaction. Deduct 1 from the number of c.c. of the silver solution added.

4. **Sulphates** exist in urine in two forms.

A. *Preformed sulphates* (Inorganic)—viz., Na and K.

B. *Combined or ethereal* (see *Indican*, Lesson XX., 16, 6).

The daily quantity is 45 to 60 grains, or 3 to 4 grms. Only a small amount is taken with the food, so that they are chiefly formed from the metabolism of proteids in the body. Most have an unpleasant taste. Only a very small part of the inorganic sulphates is derived from the mineral constituents of the food. Sulphuric acid exists in urine not only in combination with alkalies, as indicated above, so-called "preformed

sulphuric acid," but also with organic radicles, phenol, skatol, and other aromatic substances forming aromatic ether-sulpho-compounds, or **"ethereal sulphates,"** the **"combined sulphuric acid."** The latter form about one-tenth of the total sulphates, and originate from putrefactive processes in the intestine. The chief ethereal sulphates are phenol-sulphate of potassium and indoxyl-sulphate of potassium or indican, $(C_8H_6N)KSO_4$. The amount of these organic sulphates rises or falls with the degree of putrefaction; hence their importance, clinically, as an index of the amount of putrefaction in the intestine.

(*a*) **Test** with a soluble salt of barium (the nitrate or chloride) = white heavy precipitate of barium sulphate, insoluble in HNO_3; or, add a few drops of HCl to keep the phosphates in solution, and then the barium salt—

$$K_2SO_4 + BaCl_2 = BaSO_4 + 2KCl.$$

(*b*) **Separate combined (ethereal) sulphuric acid.**—Mix 50 c.c. urine with an equal volume of "baryta mixture." Stir and filter. This removes the ordinary H_2SO_4 as sulphate of barium. Add 10 c.c. HCl and keep in a water-bath at 100° C. for an hour, and then allow the ethereal or combined sulphates to settle.

Phosphoric acid is always present as orthophosphoric acid (H_3PO_4 or $3H_2O, P_2O_5$) and exists in two special combinations:—

(1) Phosphates of alkaline metals, or *alkaline phosphates*—chiefly of sodium (NaH_2PO_4).

(2) Phosphates of the alkaline earths, or *earthy phosphates*—chiefly acid salts of magnesium ($Mg(H_2PO_4)_2$) and calcium ($Ca(H_2PO_4)_2$). If the reaction changes, these salts undergo changes and re-arrangement.

The phosphates are in

A. *Natural urine,*	Na_2HPO_4. $CaHPO_4$. $MgHPO_4$.	B. *Acid urine,*	NaH_2PO_4. $Ca(H_2PO_4)_2$.
C. *Alkaline urine,*	Na_3PO_4. $Ca_3(PO_4)_2$. $Mg_3(PO_4)_2$.	D. *Decomposing urine,*	$NH_4MgPO_4 + 6H_2O$ (Ammonio-magnesium phosphate).

5. **Phosphates** consisting of *alkaline* and *earthy* salts in the proportion of 2 to 1. The latter are insoluble in an alkaline medium, and are precipitated when the urine becomes alkaline. They are insoluble in water, but soluble in acids; in urine they are held in solution by free CO_2. The phosphates of the *alkalies* are very soluble in water, and they *never form urinary deposits.*

Varieties.—The composition of the phosphates in urine varies. In acid urine the acid salts, NaH_2PO_4 and $Ca(H_2PO_4)_2$, are generally present. In neutral urine in addition Na_2HPO_4, $CaHPO_4$, and $MgHPO_4$. In alkaline urine there may be also Na_3PO_4, $Ca_3(PO_4)_2$, and $Mg_3(PO_4)_2$. Thus, it is obvious that the phosphates of the urine can readily change from one form to another. Sometimes on heating urine in testing for albumin in a faintly acid urine, cloudiness, due to earthy phosphates, may be thrown down. It is soluble in acetic acid; the albumin coagulum is not. The dicalcium phosphate is decomposed by heat into soluble acid calcium phosphate and insoluble tri-calcium phosphate, which is precipitated: $4\ CaHPO_4 = Ca(H_2PO_4)_2 + Ca_3(PO_4)_2$.

6. **Earthy phosphates** are phosphates of calcium, $(Ca_3PO_4)_2$ (abundant), and magnesium (scanty), $MgHPO_4 + 7H_2O$. Quantity 1 to 1·5 grms. (15 to 23 grains). They are precipitated when the urine is alkaline, although not in the form in which they occur in the urine (Lesson XVI.). They are insoluble in water, readily soluble in acetic and carbonic acid, and are precipitated by ammonia.

(*a*) To filtered urine add HNO_3, boil, and add barium chloride, and boil again = a precipitate of barium sulphate. Filter, cool, and to filtrate add ammonia = a precipitate of barium phosphate.

Clinical significance.—They are *increased* in osteomalacia and rickets, in chronic rheumatoid arthritis, after prolonged mental fatigue, and by food and drink, and *diminished* in renal diseases and phthisis.

7. **Phosphates** *of alkalies* are chiefly acid sodium phosphate (NaH_2PO_4), with traces of acid potassium phosphate (KH_2PO_4); they are soluble in water, and not precipitated by alkalies, and never occur as urinary deposits. The quantity is 2 to 4 grms. (30 to 60 grains). They are chiefly derived from the food, and perhaps a small amount from the oxidation of phosphorus.

(*a*) To clear urine add ammonia, caustic soda, or potash, and heat until the phosphates begin to separate; let it stand for some time = a white precipitate of earthy phosphates. Estimate approximately the proportion of the deposit. [If a high-coloured urine be used, the phosphates may go down coloured.]

(*b*) To urine add half-volume HNO_3, and then 3 volumes of ammonium molybdate and boil = a canary-yellow crystalline precipitate of ammonium phospho-molybdate—a reaction for phosphoric acid (P_2O_5). *N.B.*—The molybdate decomposes on keeping.

(*c*) To urine add half its volume of ammonia, and allow it to stand = a white precipitate of *earthy phosphates*. Filter and test the filtrate as in 7 (*b*). It gives the reaction for phosphates. This method separates the alkaline from the earthy phosphates.

(*d*) To urine add half its volume of baryta mixture [Lesson XIX. 12 (*c*)] = a copious white precipitate. Filter and test the filtrate as in 7 (*c*). It gives no reaction for phosphoric acid, showing that all the phosphates are precipitated.

(*e*) To urine add excess of NH_4Cl and ammonia = a white precipitate of *earthy phosphates* and oxalate of lime. Filter, and to the filtrate add a solution of $MgSO_4$ = a precipitate of the alkaline phosphates as *triple phosphate*. If the filtrate be tested for phosphoric acid by 7 (*c*), no precipitate will be obtained.

(*f*) Instead of 7 (*e*), use magnesia mixture, composed of $MgSO_4$ and NH_4Cl, each 1 part, distilled water 8 parts, and liquor ammoniæ 1 part. It gives the same results as in 7 (*e*).

(*g*) To urine add a few drops of acetic acid, and then uranium acetate or nitrate = bright yellow or lemon-coloured precipitate of uranium and ammonium double phosphate—$2(U_2O_3)NH_4PO_4$. This reaction—and the fact that a uranium salt added to potassium ferrocyanide gives a reddish-brown colour—forms the basis of the volumetric estimation of phosphoric acid.

(*h*) Uranium acetate solution + K_4FeCy_6 = a brown colour.

8. In some pathological urines earthy phosphates are deposited on boiling, due to the acid being changed into the basic form.

(*a*) Boil such a urine = a precipitate. It may be phosphates or albumin. An albuminous precipitate falls before the boiling-point, and phosphates when the fluid boils. Add a drop or two of NHO_3 or acetic acid. If phosphates, the precipitate dissolves; if albumin, it is unchanged.

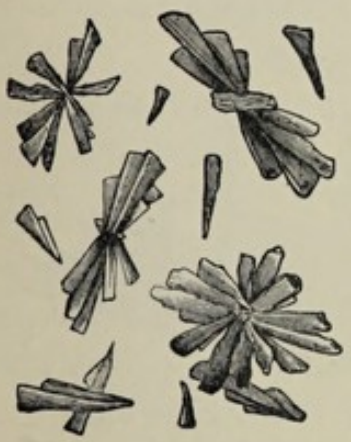


Fig. 50.—Stellar Phosphate.

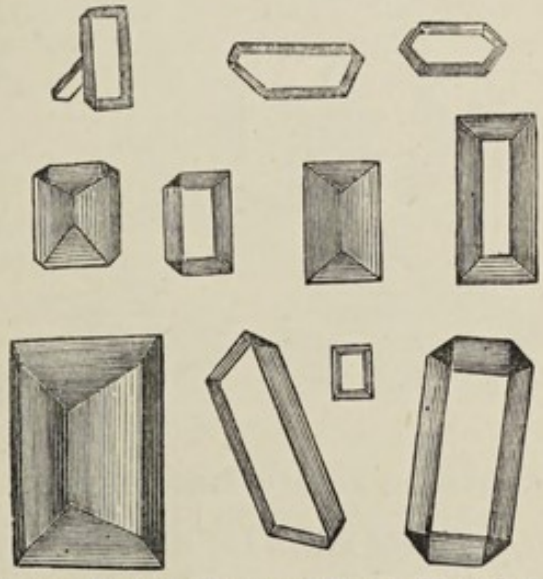


Fig. 51.—Various Forms of Triple Phosphate.

9. **Microscopic examination.**—*N.B.* Alkaline phosphates are soluble in water, and never occur as urinary deposits.

(*a*) Examine a preparation or a deposit of **calcic phosphate**, which may exist either in the *amorphous* form or the crystalline condition, when it is known as "*stellar phosphate*" (fig. 50).

(*b*) Prepare "stellar phosphate" crystals by adding $CaCl_2$ to normal urine, and then nearly neutralising. On standing, crystals like the rare clinical form of stellar phosphates are obtained.

(*c*) **Triple phosphate**, or ammonio-magnesic phosphate $Mg(NH_4)PO_4 + 6H_3O$, never occurs in normal urine. Its presence indicates decomposition of urea, which yields the ammonia necessary to combine with magnesium phosphate to form this compound. It forms large, clear "knife-rest" crystals (fig. 51).

(*d*) Add ammonia to urine; the ammonio-magnesic phosphate is thrown down in a *feathery* form, which is very rarely met with in human urine clinically (fig. 52).

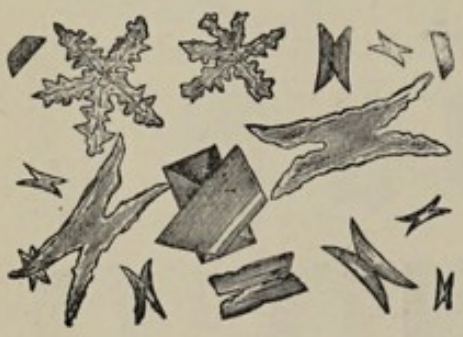


Fig. 52.—Feathery Forms of Triple Phosphate.

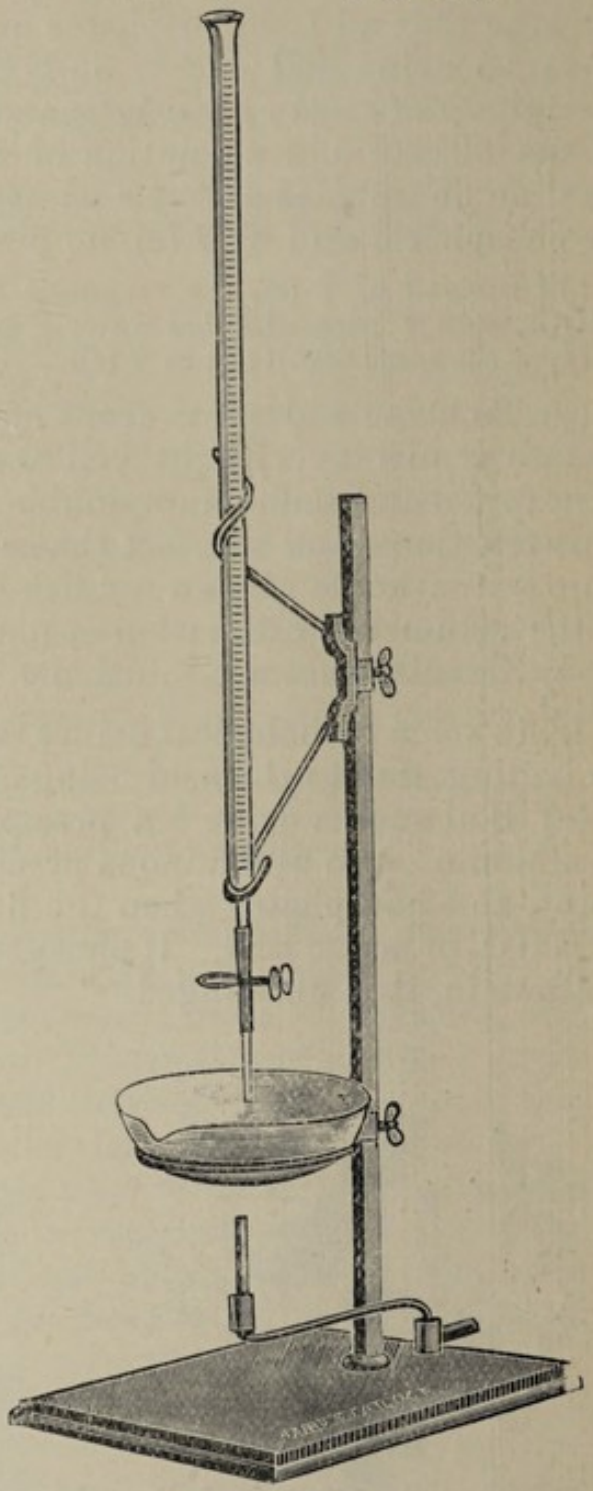


Fig. 53.—Burette and Stand.

10. General rules for all volumetric processes.

[**Burette and stand** (fig. 53), designed by Dr. F. J. Allen. It combines a retort stand, a Bunsen burner, and a spiral burette holder. The burner and the burette can be turned aside after

use, the burner always moving to the right, the burette to the left, so that the burette cannot be accidentally injured by the flame. (Made by Baird & Tatlock.)]

(*a*) The burette must be carefully washed out with the titrating solution, and fixed vertically in a suitable holder.

(*b*) Air-bubbles must be removed from the burette and outflow tube. The latter must be quite filled with the titrating solution.

(*c*) Fill the burette with the solution up to zero, and always remove the funnel with which it is filled.

(*d*) Read off the burette always in the same manner, and allow a short time to elapse before doing so, to allow the fluid to run down the side of the tube.

(*e*) The titrating fluid and the fluid being titrated must always be thoroughly well mixed.

(*f*) Make two estimations, the first approximate, the second exact.

11. Volumetric process for phosphoric acid (P_2O_5) (*Ferrocyanide of potassium as indicator*).—1 c.c. of the SS. (Uranium acetate) = ·005 grm. phosphoric acid.

Solutions required—Sodium acetate solution.—Dissolve 100 grms. of sodium acetate in 100 c.c. pure acetic acid, and dilute the mixture with distilled water to 1000 c.c.

Potassium ferrocyanide solution.—1 to 20 parts in water.

Uranium nitrate solution (1 c.c. = ·005 grm. P_2O_5).—Dissolve 35·5 grains of uranium nitrate in strong acetic acid, and dilute the solution to 1 litre.

Apparatus required.—Mohr's burette, fitted in a stand, and provided with a Mohr's clip; white porcelain or drop paper; tripod stand and wire gauze; small beaker; two pipettes, one to deliver 50 c.c., the other 5 c.c.; glass rod.

(*a*) Measure amount of urine passed during 24 hours.

(*b*) By means of a pipette place 50 c.c. of the mixed and filtered urine in a beaker. Place it under a burette.

(*c*) To the urine add 5 c.c. of the sodium acetate solution; mix.

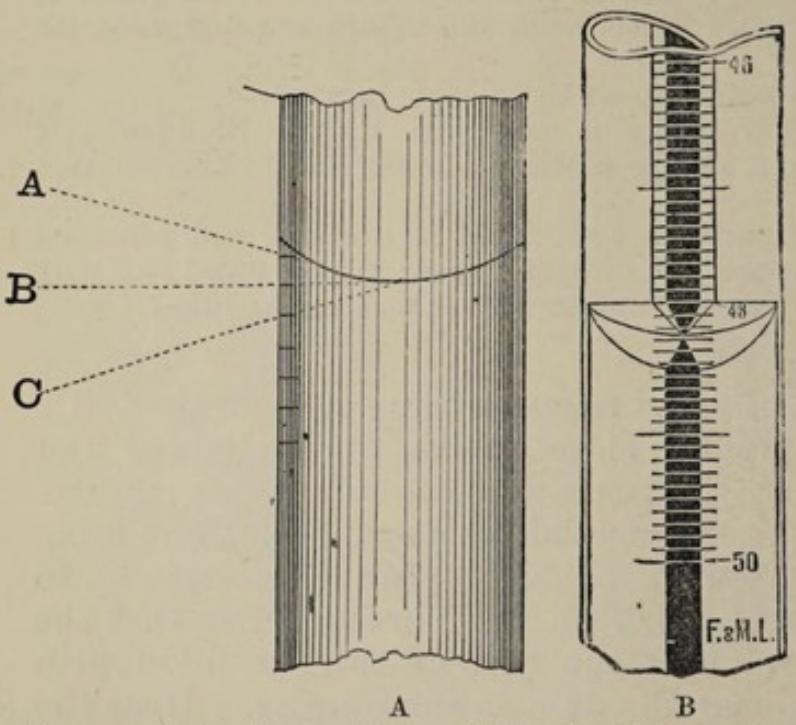
(*d*) Fill Mohr's burette with the SS. of uranium acetate up to zero, or to any convenient mark on the burette. See that the Mohr's clip is tight, and that the outflow tube is filled with the SS. Note the height of the fluid in the burette. Heat the urine in the beaker to about 80° C. Drop in the SS. of uranium acetate from the burette. Mix thoroughly. Test—on drop-paper or on a white plate—a drop of the mixture from time to time, until it gives a faint brown colour when mixed with a drop of potassium ferrocyanide.

(*e*) Boil the mixture and test again. If necessary add a few more drops of the SS., until the brown colour reappears on testing with the indicator. [Paper may be dipped in the indicator solution and tested with a drop of the mixture.] Read off the number of c.c. used.

Example.—Suppose 17 c.c of the SS. are required to precipitate the phosphates in 50 c.c. of urine; as 1 c.c. of SS. = ·005 grm. of phosphoric acid, then ·065 × 17 = ·185 grm. of phosphoric acid in 50 c.c of urine. Suppose the patient passed 1250 c.c. of urine in 24 hours, then

$$50 : 1250 :: \cdot 085 : x \quad \frac{1250 \times \cdot 085}{50} = 2 \cdot 12 \text{ grms. of phosphoric in 24 hours.}$$

12. Reading off the burette.—In the case of the burette being filled with a watery fluid, note that the upper surface of the water is concave. Always bring the eye to the level of the same horizontal plane as the bottom of the meniscus curve. Fig. 54 shows how different readings may be obtained if the eye is placed at different levels, A, B, C.



A B

Fig. 54.—A, Burette meniscus; B, Schellbach's burette with white enamelled back and black and white stripe for accurate reading (*F. and M. Lautenschläger*).

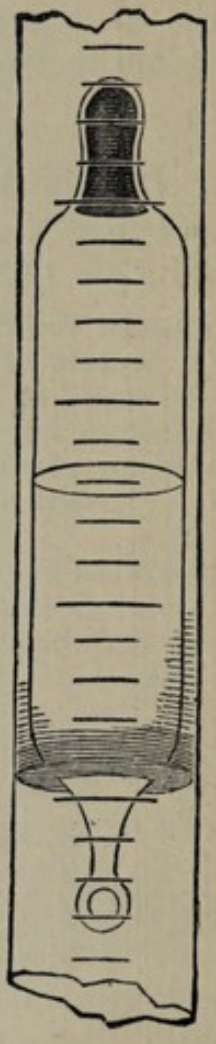


Fig. 55. Erdmann's float.

13. Erdmann's float (fig. 55) consists of a glass vessel loaded with mercury, so that it will float vertically. It is used to facilitate reading the burette. It has a horizontal line engraved round its middle, and must

be of such a width as to allow it just to float freely in the burette. Read off the mark on the burette which coincides with the ring on the float.

14. Carbonates and bicarbonates of the alkalies are generally present in alkaline urine, and are most abundant in the urine of herbivora and vegetarians. They are derived from oxidation of organic vegetable acids. Carbonate of lime is not normally present in human urine, though it is sometimes found as a deposit.

15. Iron.—0·001 to ·01 grm. excreted daily, so that the body holds very tenaciously to its iron.

16. Calcium and magnesium occur to the extent of 0·2 to 0·3 grm. per day. *They are chiefly* combined with phosphoric acid.

(*a*) *Calcium.*—Add a drop or two of acetic acid, then ammonium oxalate solution = precipitate of calcium oxalate. Filter.

(*b*) To filtrate of (*a*) + NH_4HO and sodium phosphate. Ammonio-magnesium phosphate soluble in acetic acid.

17. Oxalate of lime occurs in very small quantity as a constituent of normal urine (oxalic acid = 10 to 15 mgrs. per day). It is said to be increased by eating rhubarb and tomatoes. It is often present in excess in diabetes. When it occurs in undue amount the condition is called *Oxaluria.* Oxalates may occur in acid, neutral, or alkaline urine. In normal urine oxalates are held in solution by the acid phosphates. On standing, the acid become neutral phosphates, and oxalates are deposited.

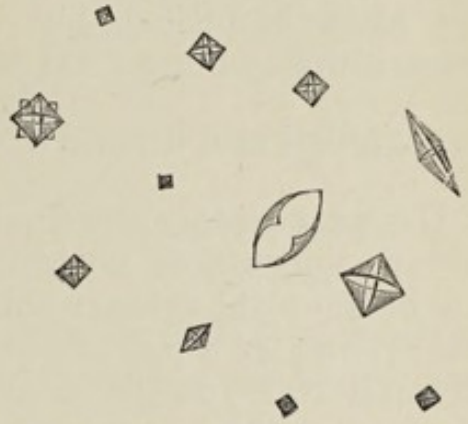


Fig. 56.—Oxalate of lime. Octahedra and hour-glass forms.

(*a*) To 200 c.c. urine add a few drops of saturated solution of oxalic acid solution. Set aside for 24 hours. Examine the sediment for octahedra (fig. 56), of oxalate of lime, and perhaps other forms. Ascertain their solubility in acetic acid and in hydrochloric acid.

(*b*) To 500 c.c. urine add $CaCl_2$ (10 p.c.) and ammonia. Acidify with acetic acid = precipitate of calcium oxalate and uric acid. After 24 hours filter and wash the precipitate in the filter with dilute HCl, which dissolves the oxalate, but not the uric acid. To the filtrate add ammonia when oxalate of lime is precipitated (microscope, fig. 56), but owing to its lightness it sinks slowly.

18. Ammonia.—A little exists in urine combined as a salt, some of it in combination with uric acid. Amount excreted daily 0·7 grm. (12 grains). About 2 to 4 p.c. of the waste N of the urine is given off in the form of ammoniacal salts.

(*a*) To 25 c.c. urine in a beaker, add 10 c.c. milk of lime. Cover with a glass-cover having on its under side a strip of moistened red litmus. The lime liberated turns the red litmus blue.

LESSON XVIII.

ORGANIC BODIES IN URINE—UREA—KJELDAHL'S ESTIMATION OF TOTAL N.

1. Urea (CON_2H_4) is the most important organic constituent in urine, and is the chief end-product of the oxidation of the nitrogenous constituents of the tissues and food. It crystallises in silken four-sided prisms, with obliquely-cut ends (rhombic system), and, when rapidly crystallised, in delicate white needles. It has no effect on litmus; odourless, weak cool-bitter taste, like saltpetre. It is very soluble in water and in alcohol, and almost insoluble in ether. It is isomeric with—*i.e.*, it has the same empirical, but not the same structural formula as ammonium cyanate $(NH_4)CNO$. It may

be regarded as a diamid of CO_2 or as carbamid = $CO\begin{cases} NH_2. \\ NH_2. \end{cases}$

Urea represents the final stage of the metamorphosis of albuminous substances within the human body. It forms about 2 p.c. of the total urinary solids. This equals 0·5 per kilo. of body-weight and 45·8 p.c. of the total urinary solids. Urea contains 46·6 p.c. N. 100 grms. of proteid are represented by 33 grms. of urea in the urine (blood contains less than 0·02 p.c.). About nine-tenths (86 p.c.) of all the N taken in is excreted in the form of urea. While this is true for man and mammals, it is not so for amphibians, reptiles, and birds, where uric acid is the chief nitrogenous waste product.

2. Preparation.—To 20 c.c. of filtered human urine add 20 c.c. baryta mixture—Lesson XIX. 12 (*c*)—to precipitate the phosphates. Filter, evaporate the filtrate to dryness in an evaporating chamber, and extract the residue with boiling alcohol. Filter off the alcoholic solution, place some of it on a slide. The urea separates out spontaneously, usually in long, fine, transparent needles. Examine them microscopically (fig. 57, *a*).

3. Combinations.—Urea combines with acids, bases, and salts. Evaporate urine to one-sixth its bulk; divide the residue into two portions (for the preparation of nitrate and oxalate).

4. **Urea nitrate** (CH_4N_2O, HNO_3).—(*a*) To the concentrated urine add strong *pure* HNO_3 = a precipitate of glancing scales

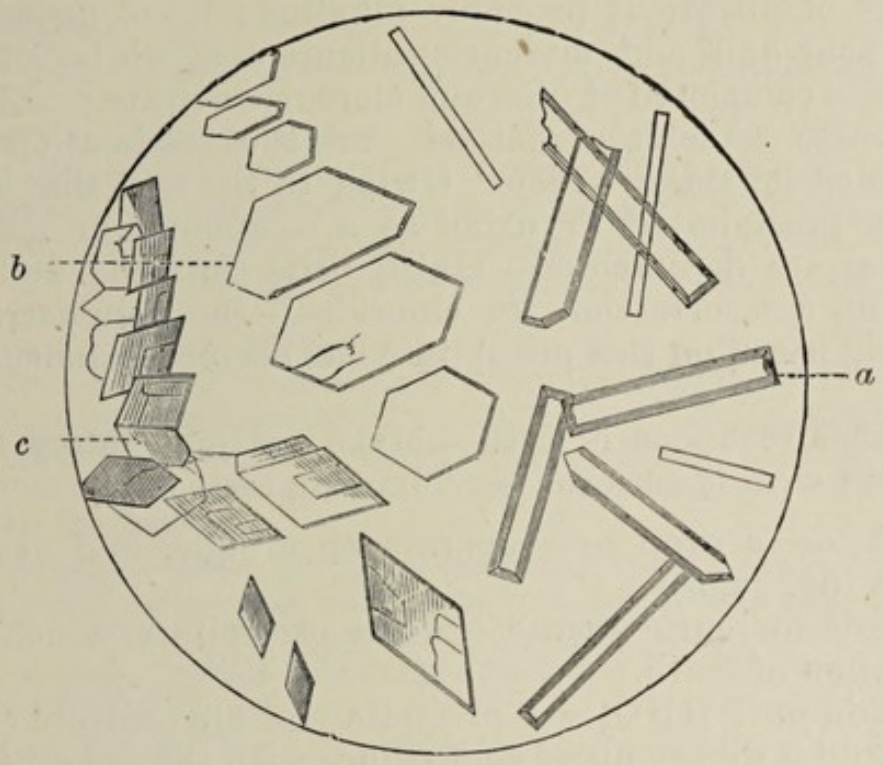


Fig. 57.—*a*, Urea; *b*, Hexagonal plates; and *c*, Smaller scales, or rhombic plates of urea nitrate.

of urea nitrate, which, being almost insoluble in HNO_3, separate out in rhombic plates or six-sided tables, with a mother-of-pearl lustre, and often imbricate arrangement. Best if kept cool. Examine with microscope (fig. 57, *c*).

[(*b*) If dealing with traces of urea, concentrate the fluid, place a drop on a slide, put into the drop one end of a thread, apply a cover-glass, and put a drop of *pure* HNO_3 on the free end of the thread. The acid passes into the fluid, and microscopic crystals of urea nitrate are formed on the thread (Microscope)].

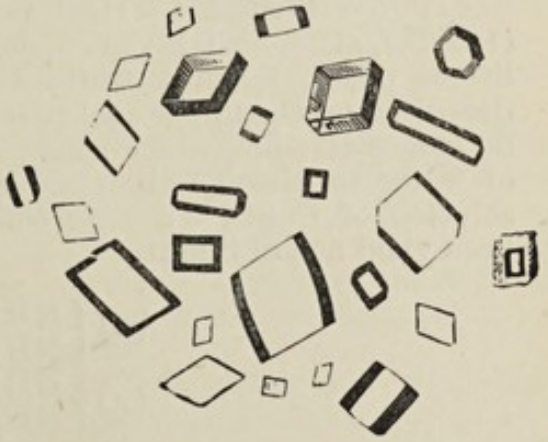


Fig. 58.—Crystals of oxalate of urea from urine.

5. **Urea oxalate** $(CH_4N_2O)_2\ C_2H_2O_4 + H_2O$.—(*a*) To the concentrated urine add a strong solution of oxalic acid. Crystals of oxalate of urea separate (Microscope, fig. 58).

6. Urea and mercuric nitrate ($2CON_2H_4 + Hg(NO_3)_2 + 3HgO$).—(*a*) To urine add one-third volume of baryta mixture to remove the phosphates and sulphates. Filter this off, reaction of filtrate is probably alkaline; if so, neutralise with acetic acid and add mercuric nitrate = a white, cheesy precipitate, a compound of urea and mercuric nitrate. Add NaCl, it disappears. Liebig's method for the estimation of urea is founded on this reaction. Owing to the fact that before the urea is precipitated in urine as a compound of mercury the chlorides are decomposed—$HgCl_2$ being formed—and therefore involving a "correction for chlorides," the volumetric process of Liebig based on this precipitate has been abandoned.

7. Other reactions of urea.—Make a strong watery solution of urea, and with it perform the following tests :—

(*a*) Allow a drop to evaporate on a slide, and examine the crystals (fig. 57, *a*).

(*b*) Add mercuric nitrate = white precipitate, which dissolves on addition of NaCl.

(*c*) Add *pure* HNO_3 = a precipitate of urea nitrate (fig. 57, *b*).

(*d*) Add ordinary nitric acid yellow with *nitrous acid;* bubbles of gas (CO_2 and N) are given off.

(*e*) Add a solution of sodium hypobromite = rapid evolution of gas (N and CO_2).

$$CO(NH_2)_2 + 3NaBrO = 3NaBr + CO_2 + N_2 + H_2O$$

(*f*) *Biuret reaction.*—Heat some *crystals* in a hard dry tube; they melt (130° C.), ammonia is given off, and is recognised by its smell and its action on litmus paper, while a white sublimate of cyanuric acid ($C_3H_3N_3O_3$) is deposited on the upper cool part of the tube. Heat the tube until there is no longer an odour of ammonia. Allow the tube to cool, add a drop or two of water to dissolve the residue, a few drops of NaHO and very dilute solution of $CuSO_4$ = a *pink* colour (biuret reaction). Two molecules of urea yield one of biuret.

$$\begin{matrix} CO\{NH_2 \\ \quad\ NH_2 \end{matrix} \quad \begin{matrix} CO\langle NH_2 \\ \quad\ NH \\ \quad\ NH_2 \end{matrix} + NH_3$$
$$CO\{NH_2 \quad =$$

(*g*) Add caustic potash, and heat. The urea is decomposed, ammonia is evolved, and ammonium carbonate is formed :—$CON_2H_4 + 2H_2O = (NH_4)_2CO_3$.

(*h*) Cover crystals of urea in a porcelain capsule with a saturated freshly-prepared concentrated watery solution of *furfurol*, and add a drop of HCl = a rapid play of colours, beginning with yellow and passing through green, purple, to violet or brown. This test requires care (*Schiff*).

(*i*) When split up by the ferment of *micrococcus ureæ* it takes up water and yields carbonate of ammonia :—$CO(NH_2)_2 + 2H_2O = (NH_4)_2CO_3$.

8. **Quantity.**—An adult excretes 30 to 40 grms. (450 to 600 grains) daily; children relatively more. It varies, however, with

(*a*) *Nature and quantity of the food.*—Increased when the nitrogenous matters are increased in the food, and is diminished by vegetable diet. On a purely meat diet a person may excrete 60 grms. per day, and on a bread diet about 20 grms. hence it is most abundant in the urine of carnivorous animals. It is increased by copious draughts of water, salts. It is still excreted during starvation.

(*b*) *Muscular exercise* (ordinary) has little effect on the amount.

(*c*) *Disease.*—In the acute stage of fevers and inflammation there is an increased formation and discharge, also in diabetes. Diminished in anæmia, cholera, in acute and chronic Bright's disease. If it is retained within the body it gives rise to uræmia, when it may be excreted by the skin, or be given off by the bowel.

9. **Occurrence.**—Urea occurs in the blood, lymph, chyle, liver, lymph glands, spleen, lungs, brain, saliva, amniotic fluid. The chief seat of its formation is very probably the liver. It also occurs in small quantity in the urine of birds, reptiles, and herbivora, but it is most abundant in that of carnivora.

10. **Kjeldahl's method of estimating nitrogen.**—This method, when once the standardised solutions are prepared, and the apparatus set up, can be carried out in about an hour, and several estimations can be carried out simultaneously. (*a*) *Incineration.*—The organic matter is destroyed by prolonged heating of the substance with sulphuric acid, until the originally blackish fluid becomes clear and yellow coloured—ammonium sulphate which contains all the N being formed. (*b*) *Neutralisation.*—After it cools, caustic soda is added in excess to liberate the ammonia. (*c*) *Distillation.*—The flask is corked, and the mixture is distilled, whereby the ammonia passes over into a standardised solution of sulphuric acid. (*d*) *Titration.*—The ammonia is calculated by titrating the sulphuric acid with standard caustic soda.

(*a*) **Destruction of organic matter.**—Place in a long-necked boiling flask of 200 c.c. capacity 0·1 to 1 grm. of the powdered dry substance, or 10 c.c. urine. To destroy the organic matter add 10 to 20 c.c. of the following mixture :—15 c.c. pure H_2SO_4, 10 grms. potassium sulphate and 0·5 grm. $CuSO_4$, all free from ammonia. Heat on a wire gauze with a Bunsen burner, but keep the temperature below boiling. Heat for 1 to 2 hours until the fluid becomes clear and greenish.

(*b*) **Neutralisation.**—Cool the flask, add a little water, and wash the contents, with as little water as possible, into a large flask of 700 c.c. capacity. Neutralise with pure caustic soda or potash (S.G. 1·13). Add a little metallic zinc to prevent bumping during the subsequent distillation.

(*c*) **Distillation.**—Rapidly close the flask with a perforated caoutchouc stopper through which passes a tube with two 1-inch bulbs blown upon it. The bulbs are to collect and prevent the passage of soda spray. The tube above the bulbs passes through a condenser, and the delivery tube end of the condenser tube passes into a flask containing a measured excess

of standard acid (H_2SO_4). Distil the mixture about an hour in the flask, and the ammonia passes over into the acid.

(*d*) Titration.—Determine the amount of acidity in the distillate by titration with a standard solution of caustic soda or potash, methyl orange being used as an indicator of the end of the reaction. Methyl orange gives a pink with an acid, and yellow with an alkali.

The apparatus used in the Physiological Laboratory of Owens College is that made by Messrs. Baird & Tatlock, and is so arranged that several estimations can be made simultaneously. Other modifications are in use.

Example.—Suppose 0·15 grm. of the N-substance has been treated with acid, neutralised, and the ammonia distilled over and received by 100 c.c. of a decinormal solution of HCl (= 10 c.c. normal acid). The distillate is then treated with decinormal caustic soda, and suppose it is found that the neutral point is reached when 60 c.c. of the decinormal soda has been added. The remaining 40 c.c. must therefore have been neutralised by the ammonia obtained from the nitrogenous substance investigated. This 40 c.c. of decinormal acid = 4 c.c. of normal acid = 4 c.c. of normal ammonia = $4 \times 0{\cdot}017 = 0{\cdot}068$ grm. of ammonia; 0·15 grm. of the substance, therefore, yields 0·068 grm. of ammonia, and this amount contains 0·056 grm. of nitrogen; 100 grms. of the substance investigated will therefore contain $\frac{100 \times 0{\cdot}056}{0{\cdot}15} = 37{\cdot}3$ grms. of nitrogen.—(From Sutton's *Volumetric Analysis*, by Warington.)

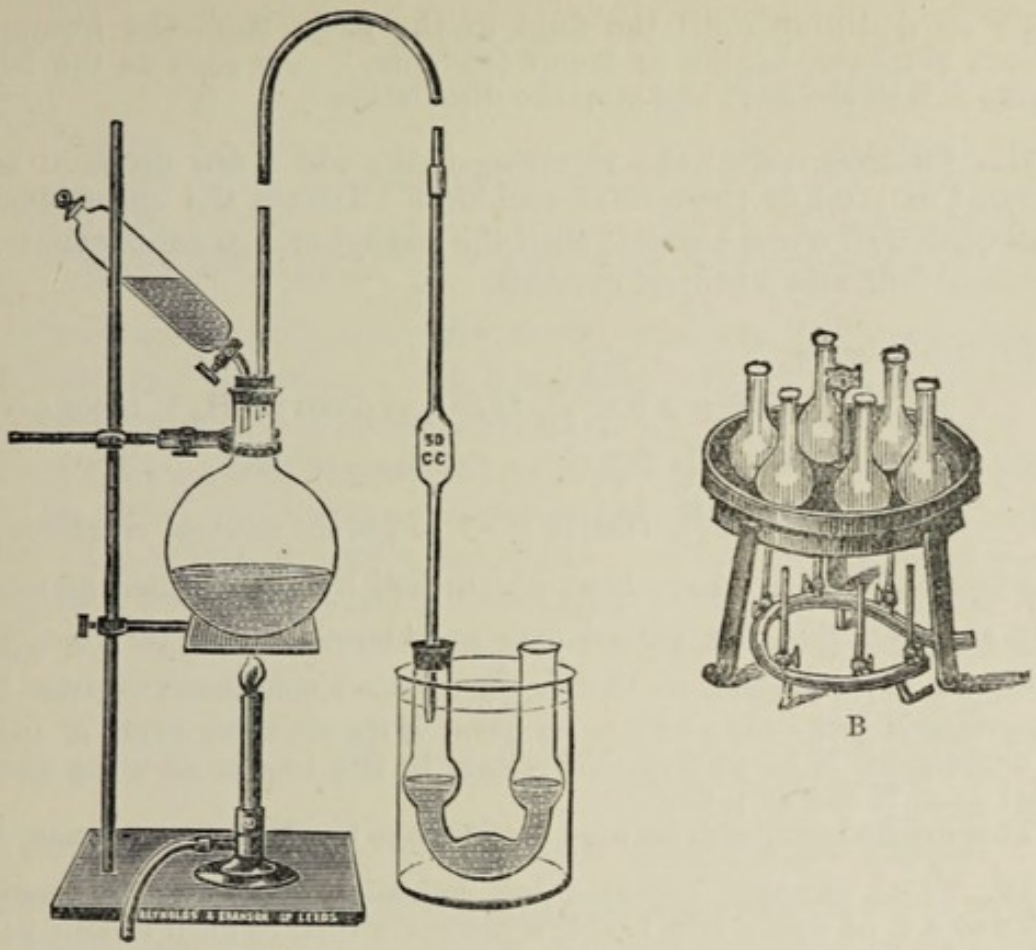
There are various modifications of this method, both as regards apparatus and procedure. In Willfarth's apparatus a globule of metallic mercury is added to assist oxidation, while talc is added during distillation to avoid bumping. Others add dry potassium sulphate and copper sulphate (0·5 grm.). The former (12 grms.) raises the boiling point, and the copper aids oxidation. Decinormal sulphuric acid is used as the standard acid.

For a method of distillation *without a condenser*—by means of tubes with wide bulbs in their course—see *Leather Industries*, by Proctor, and a somewhat similar apparatus in v. Jaksch, *Clinical Diagnosis*, 4th edit., 1899, fig. 141, p. 368.

In the more recent apparatus, as shown in fig. 59, the distillate is not cooled by a water condenser; all that is required is wide bulbs in the course of the distillation tube.

11. Kjeldahl-Gunning method (for urine).—I. *Oxidation.*—From a burette measure 10 c.c. urine into a hard glass flask. Add the ingredients suggested by Gunning—*i.e.*, 15 c.c. H_2SO_4 and 0·5 grm. $CuSO_4$; heat, and then add the powdered potassium sulphate. In an evaporating chamber boil about an hour—inclining the flask—on a sand-bath or wire gauze, as shown in fig. 59, until the mixture becomes almost clear (greenish).

II. *Distillation.*—Cool, and pour the fluid into the flask of the distillation apparatus. Wash out thoroughly with water, and add this to the original fluid. Cool again. Add some talc and about 40 c.c. of caustic soda, S.G. 1·34, which neutralises all the H_2SO_4 and leaves an excess of soda sufficient to liberate the NH_3. Connect the distillation flask as quickly as possible with the distilling apparatus previously arranged (fig. 59). The vessel receiving the distillate consists either of a U-shaped tube with bulbs or, preferably, an Erlenmeyer flask (300 c.c.). Into the latter is run in beforehand from a burette, say, 80 c.c. $\frac{N}{10}$ (decinormal) H_2SO_4.



A

B

C

Fig. 59.—A, Kjeldahl apparatus, as suggested by Proctor. This shows the distillation apparatus. Where gaps are shown in the tubes glass bulbs occur. Instead of the bent-receiving tube an Erlenmeyer's flask is more suitable. B, Incinerating apparatus. C, Apparatus for distillation of many samples at one time (Reynolds & Branson, Leeds).

Go on distilling until the fluid in the flask, from the formation of sodium sulphate, begins to bump (20 mins.). As soon as the bumping begins detach the flask and stop the distillation.

III. *Titration.*—Cool the receiving flask, add a few drops of methyl-orange (·01 p.c.) or tincture of cochineal. Titrate the uncombined acid with $\frac{N}{10}$ NaOH (from burette) until the methyl-orange turns yellow or the cochineal indicates a neutral reaction.

IV. *Calculation.*

1 c.c. $\frac{N}{10}$ NaOH = 1 c.c. $\frac{N}{10}$ H_2SO_4 = 0·0017 NH_3 = 0·0014 N.

Suppose (1) 80 c.c. $\frac{N}{10}$ H_2SO_4 in Erlenmeyer flask,

(2) 10 c.c. $\frac{N}{10}$ NaOH used to obtain neutral reaction.

The 30 c.c. $\frac{N}{10}$ H_2SO_4 have been neutralised by the distilled ammonia, so that as much NH_3 has passed over as is contained in 30 c.c. $\frac{N}{10}$ NH_3. Ammonia is monobasic, so that a $\frac{N}{10}$ solution contains 1·7 grms. NH_3 or 1·4 grms. N per litre—*i.e.*, 0·017 grm. NH_3 = 0·0014 grm. N in 1 c.c. or 0·042 grm. N in 30 c.c. Therefore, in the 10 c.c. of urine there was 0·042 grm. N = 0·42 p.c. N.

This method may also be used to estimate the N in fæces, meat, &c.

12. Total nitrogen (*Pflüger and Bohland's approximative method*).—Take 10 c.c. of urine, add Liebig's mercuric nitrate until a faint yellow is obtained with a drop of the mixture when the latter is tested with sodium carbonate. The number of c.c. of the SS. used multiplied by 0·04 gives the total N.

LESSON XIX.

VOLUMETRIC ANALYSIS FOR UREA.

1. Before performing the volumetric analysis for urea practise the following reactions, which form the basis of this process :—

(*a*) To a solution of sodic carbonate add mercuric nitrate = a yellow precipitate of mercuric hydrate.

(*b*) To urine add sodic carbonate, and then mercuric nitrate = first of all a white cheesy precipitate ; on adding more mercuric nitrate, a yellow is obtained—*i.e.*, no yellow is obtained until the mercuric nitrate has combined with the urea—and there is an excess of the mercuric salt.

(*c*) To urine add *hypobromite of soda.* At once the urea is decomposed, and bubbles of gas—N and (CO_2)—are given off.

2. Estimation of urea by Hüfner's hypobromite method.

The principle of this method depends on the fact that urea is decomposed by alkaline solution of sodium hypobromite, yielding water, CO_2 and N. The CO_2 is absorbed by the excess of NaHO, the N, which is disengaged in bubbles, is collected and measured in a suitable apparatus.

	Remain in Solution.				Escapes and is measured.
	Sodium	Carbon		Sodium	
Urea.	Hypobromite.	Dioxide.	Water.	Bromide.	Nitrogen.

$$CON_2H_4 + 3NaBrO = CO_2 + 2H_2O + 3NaBr + N_2$$

Every 0·1 grm. of urea contains ·046 grm. N ; this at the ordinary temperature and pressure = 37·3 c.c. of nitrogen. In practice 35·4 c.c. are obtained at ordinary temperature (18° C.) and pressure, owing to the difficulty of disengaging all the gas. The urates and kreatinin also yield a little N, so that in the empirical graduating of instruments this error is taken into account. It is the method generally used for clinical purposes.

3. Apparatus and solutions required.

(i.) A 40 p.c. solution of NaHO.

(ii.) Tubes (already prepared) containing 2 and 4 c.c. of bromine. This is more convenient than fluid bromine.

(iii.) Strong glass cylinder with a glass stopper.

(iv.) 5 c.c. pipette.

(v.) Urea apparatus—*e.g.*, of Dupré, or Gerrard.

4. *Hypobromite solution.*—Place 23 c.c. of the NaHO solution in the glass-stoppered cylinder, drop into it gently a tube containing 2 c.c. of bromine. Shake the cylinder so as to break the tube; the soda combines with the bromine. Make the solution fresh each time.

5. Dupré's apparatus.—In this apparatus (fig. 60) the graduation on the collecting tube represents either the percentage of urea or c.c. of N. The collecting tube, which is clamped above, is placed in a tall vessel of water, and connected with a small glass flask containing a short tube.

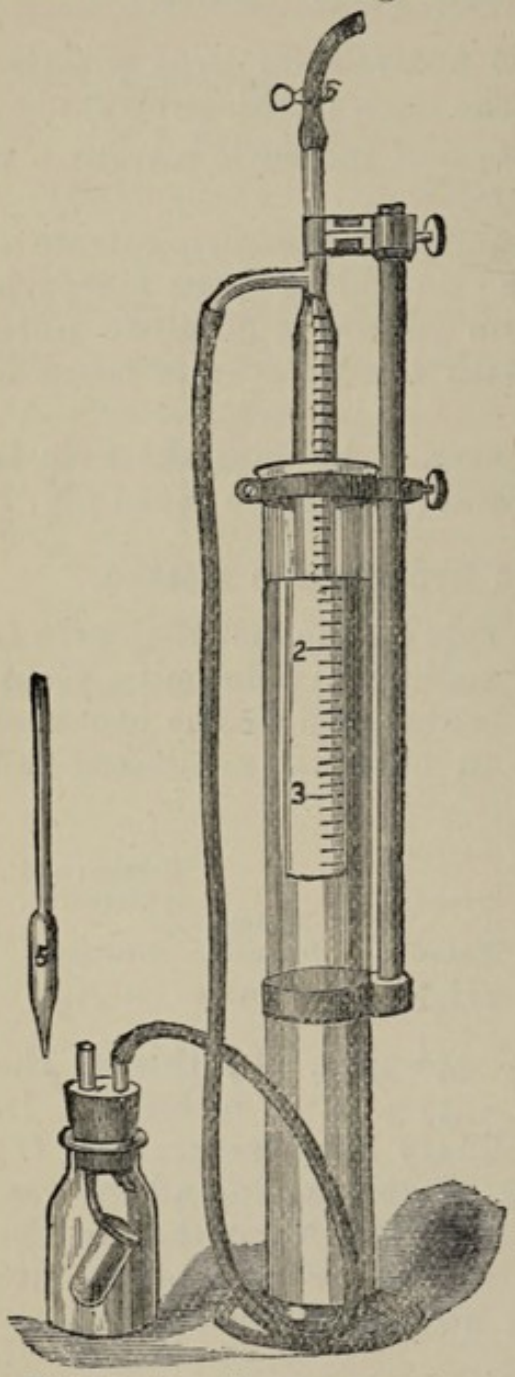


Fig. 60.—Dupré's urea apparatus.

(*a*) Remove the short tube from the flask, and in the latter place 25 c.c. hypobromite solution.

(*b*) With a pipette place 5 c.c. of filtered urine in the short tube attached to the india-rubber stopper. Replace the caoutchouc stopper.

(*c*) Test if all the connections are tight. Open the clamp at the upper end of the collecting tube, depress the tube in the water until the water inside and outside the tube is at zero of the graduation. Close the clamp, and raise the collecting tube. If the apparatus be tight no air will pass in, and on lowering the collecting tube the water will stand at zero inside and outside the tube.

(*d*) Gently tilt the flask to mix the urine and hypobromite solution. Gases are rapidly given off, but the CO_2 is absorbed by the excess of NaHO, while the N collects in the graduated measuring tube.

(*e*) Place the flask in a jar of water, and slightly lower the measuring tube. After all effervescence has ceased, and when the N collected in the collecting tube has cooled to the temperature of the room—*i.e.*, in 5 to 10 minutes—raise the collecting tube until the fluid inside and outside stands at the same level.

Read off the graduated tube; this gives the percentage of urea. Or, if the burette be graduated in c.c., read off the number of c.c., and calculate the amount of urea from the amount of N evolved.

Other bodies in urine—*e.g.*, uric acid (urates) and kreatinin, but not hippuric acid—also yield nitrogen by this process; further note, that only about 92 p.c. of the N of urea is given off. These sources of fallacy are, however, taken into account in graduating the apparatus.

6. **Steell's apparatus** (fig. 61).—The collecting tube is a

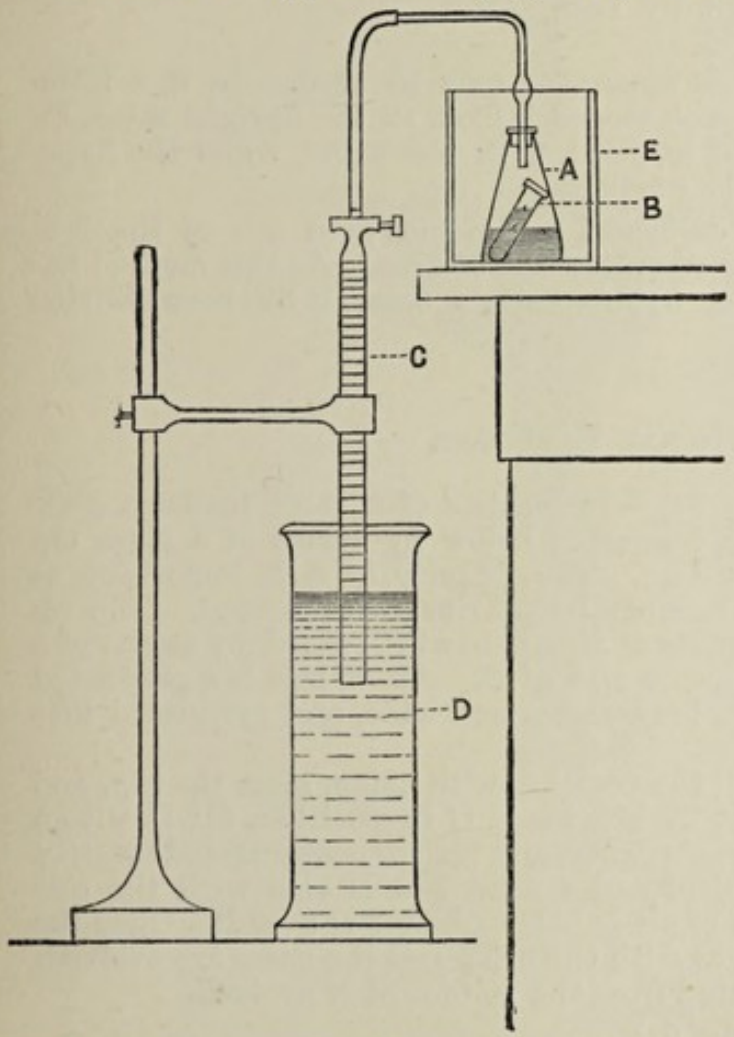


Fig. 61.—Steell's apparatus for urea. A, Flask for hypobromite. B, Tube for urine. C, Burette. D, Vessel with water. E, Vessel with water to cool A.

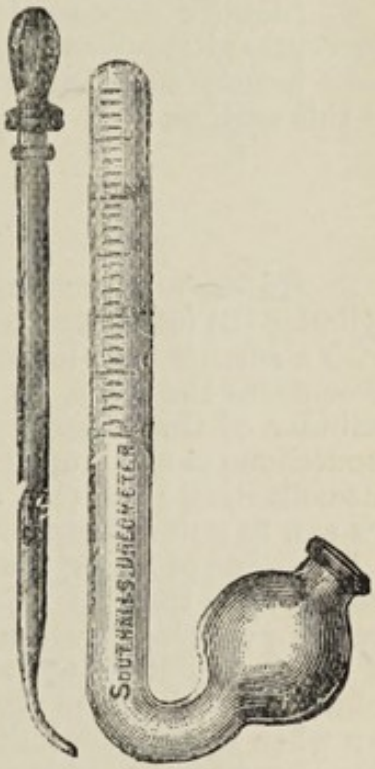


Fig. 62.—Ureameter of Doremus, with pipette.

burette graduated in c.c.—(*a*) Use this apparatus in a similar manner. The tube B is introduced into the flask A by means of a pair of forceps.

(*b*) Read off the number of c.c. of N evolved, and from this calculate the amount of urea. Every 35·4 c.c. N = 0·1 grm. urea.

7. **Ureameter of Doremus** (fig. 62).—It consists of a graduated

bulb-tube, closed at one end. The hypobromite solution is poured into the tube up to a certain mark, and diluted with water to fill the long arm and bend. The urine to be tested is drawn into the pipette to the graduation. The tip of the pipette is then passed into the ureameter, as far as the bend, and the nipple is compressed slowly. The urine rises through the hypobromite solution, and the gas evolved collects in the upper part of the tube.

Each division indicates ·001 grm. of urea in 1 c.c. of urine. The percentage of urea present in the urine is found by multiplying the result of the test by 100.

A recent modification of this apparatus may be used. In it a knee-shaped, graduated open tube, with stopcock fixed to the upright stem, replaces the pipette. This tube is filled with the urine, while the hypobromite solution is placed in the ureameter.

8. Liebig's process (*Sodic carbonate as indicator*).—1 c.c. of the S.S. (mercuric nitrate) = ·01 grm. or 10 milligrams of urea. As this method has been largely supplanted by the hypobromite process, it has been omitted in this edition.

Additional Exercises.

9. Hüfner's apparatus (fig. 63).—It consists of a stout fusiform glass cylinder (B) (capacity 100 c.c.), connected below by means of a glass tap with a smaller tube (capacity 5 c.c.). The capacity of A is important, as it contains the urine, so that it must be previously calibrated. The remainder of the apparatus consists of a glass bowl (C) fitted by means of a caoutchouc stopper upon the upper end of B. Above this is a graduated gas-collecting tube (D), 40 cm. long and 2 cm. wide, and graduated into 0·2 cm. in units of capacity.

By means of a long funnel fill the vessel A with urine, close the tap, and wash every trace of urine out of B. Place C in position, fill B with a freshly-prepared solution of hypobromite, and place a concentrated solution of common salt in C to the depth of 1 cm. Fill D also with the salt solution, avoiding the presence of air-bubbles. Insert D over B. Open the tap when the hypobromite mixes with the urine and the gases are evolved. The quantity of urea is calculated from the volume of N evolved.

10. Gerrard's apparatus (fig. 64).

Method of using.—Pour into the tube 5 c.c. of the urine, and in the bottle (*a*) 25 c.c. or 6 fluid drachms of sodium hypobromite solution. Place the tube carefully inside the bottle, as shown in the illustration, avoiding spilling any of the contents. Fill the glass tubes (*b*, *c*) with water, so that the level reaches the zero-line, taking care that when this is done the tube (*c*) contains only a little water by being placed high—it having to receive what is displaced from (*b*) by the nitrogen evolved. Now connect the india-rubber tubing to the bottle, and noting lastly that the water is exactly at zero, upset the contents of the tube into the hypobromite solution. Nitrogen is evolved, and depresses the water in (*b*). When this ceases, lower (*c*) until the level of the water in both tubes is

equal. To be exact, dip (*a*) into cold water to cool the gas before taking a reading, and note the result, which shows percentage of urea.

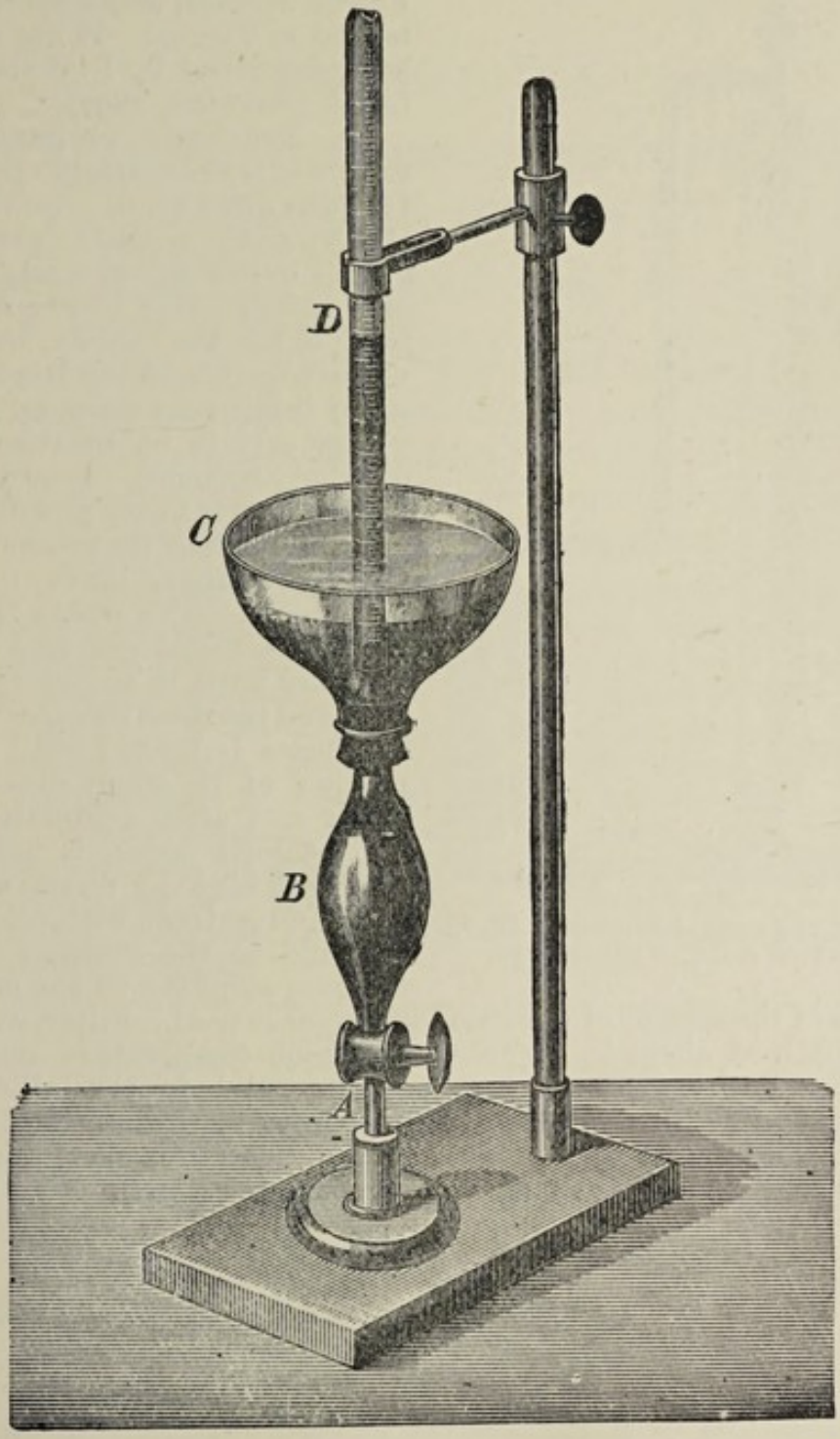


Fig. 63.—Hüfner's urea apparatus.

The solution of hypobromite of soda is made by dissolving 100 grms. of caustic soda in 250 c.c. of water, then adding 22 c.c. of bromine.

To avoid the danger of the bromine vapour, the bromine is sold in hermetically sealed glass tubes, containing 2·2 c.c.; one of these placed in the large bottle with 25 c.c. of the soda solution gives, when broken with

a sharp shake, the exact quantity of hypobromite for one estimation of urea, and all bad odour is avoided.

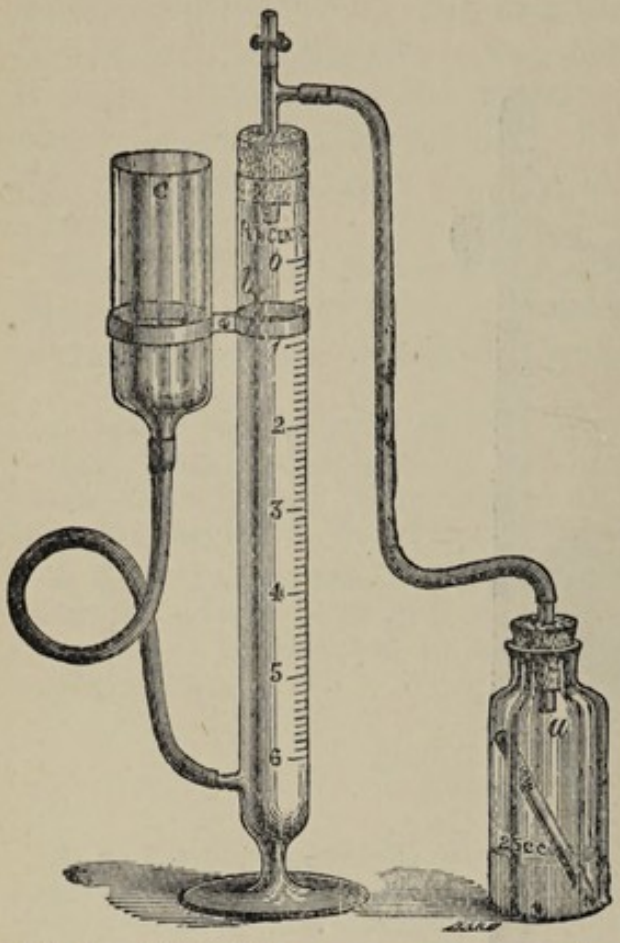


Fig. 64.—Gerrard's urea apparatus, as made by Gibbs, Cuxson & Co., Wednesbury.

11. Other forms are used in the French schools, and a special form is used in Vienna. It has recently been described by I. Walker Hall (*Med. Chronicle*, 1899).

12. **Synthetic preparation of urea.** — Heat coarsely-powdered ferrocyanide of potassium ($FeCy_2.4KCy + 3H_2O$, about 250 grms.) over a fire in a large porcelain vessel. Stir constantly, and heat until the whole assumes a white colour, and the larger pieces when broken up show no trace of yellow. If it be overheated the powder becomes brown. The white mass is finely powdered and mixed with half its volume of dry, finely-powdered, black oxide of manganese. The whole is heated in a black metal pot in a draught chamber until it begins to scintillate, and the mass becomes doughy. The mass is heated until a small portion of it, when dissolved in water and after acidulation with hydrochloric acid, is no longer rendered blue by ferric chloride. Cool and extract with cold water, and add to the solution dry ammonium sulphate to the extent of three-fourths of the weight of potassic ferrocyanide used. Filter, evaporate on a water-bath at about 60° to 70° C. (at which temperature ammonium cyanate passes into urea). At first potassic sulphate crystallises out; remove it from time to time. Lastly, evaporate to dryness, and extract the urea from the residue by absolute alcohol. The urea crystallises from the alcoholic solution at a moderate temperature (*Drechsel*).

LESSON XX.

URIC ACID—URATES—HIPPURIC ACID—KREATININ—PIGMENTS.

1. **Uric acid** ($C_5H_4N_4O_3$) contains 33·33 p.c. of N, and as a carrier of waste N in the urine it ranks third—kreatinin is second—carrying off 3 p.c. of the total waste N. In birds, reptiles, amphibians, and insects it forms the chief nitrogenous excretion. The proportion of urea to uric acid is 45 : 1. It is regarded by some as a specific decomposition product of the nucleins, and of those containing nucleinic acid.

2. Quantity and solubility.—0·5 grm. (7 to 10 grains) daily. It is said to be increased by sodium salicylate and in most cases of leukæmia, and diminished by quinine. It is dibasic, colourless, and crystallises, chiefly in rhombic plates, and when the obtuse angles are rounded the "whetstone" form is obtained. It often crystallises spontaneously in rosettes from saccharine diabetic urine. It is tasteless, reddens litmus, is very insoluble in water (15,000 parts of cold and 1900 of warm water), insoluble in alcohol and ether. Its low solubility is important clinically, so that it occurs frequently as a deposit in urine. In the urine it occurs chiefly in the form of *acid* urates of soda ($C_5H_2N_4O_3$, HNa) and potash.

Its amount is influenced by the proteids of the food, but to a much less extent than urea (*Bunge*).

	Urea.	Kreatinin.	Uric Acid.
Meat diet, . .	67 grms.	2·16 grms.	1·40 grms.
Bread diet, .	21 ,,	0·96 ,,	0·25 ,,

3. **Preparation** (*Heintz*).—(*a*) In a conical glass add 5 parts of HCl to 20 parts of urine; keep in a cool place for 24 hours. Yellow or brownish-coloured crystals of uric acid—visible to the naked eye—are deposited on the sides of the glass, or form a pellicle on the surface of the fluid like fine grains of cayenne-pepper. Both uric acid and its salts (urates), when they occur as sediments in urine, are coloured, and the colour is deeper the more coloured the urine. The slow separation of the uric acid is probably due to the presence of phosphatic salts.

(*b*) Examine the crystals **microscopically**. They are chiefly

rhombic, whetstone, lozenge-shaped, in rosettes, quadrilateral prisms, &c. They are *yellowish* in colour, although their tint may vary from yellow to red or reddish-brown, depending on the depth of the colour of the urine (figs. 65, 66).

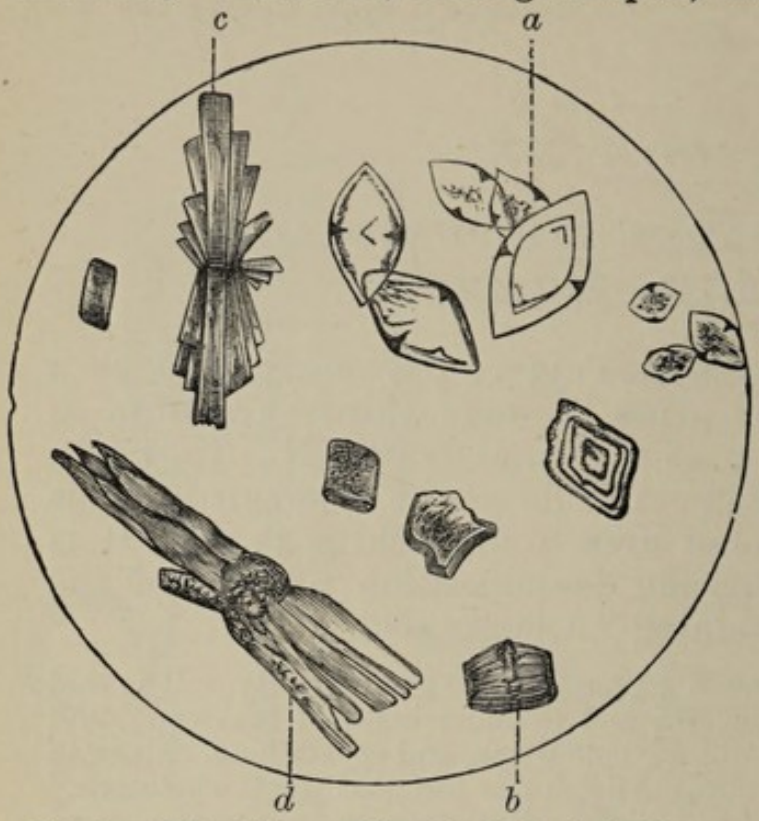


Fig. 65.—Uric Acid. *a*, Rhombic tables (whetstone form); *b*, Barrel form; *c*, Sheaves; *d*, Rosettes of whetstone crystals.

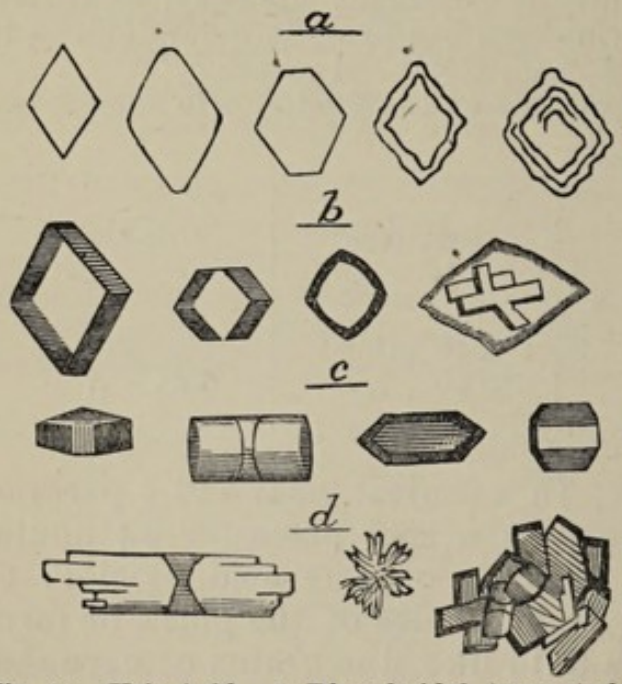


Fig. 66.—Uric Acid. *a*, Rhomboidal, truncated, hexahedral, and laminated crystals; *b*, Rhombic prism, horizontally truncated angles of the rhombic prism; *c*, Prism with a hexahedral basic surface, barrel-shaped figure, prism with a hexahedral basal surface; *d*, Cylindrical figure, stellate and superimposed groups of crystals.

(*c*) They are soluble in NaHO or KHO, or 10 p.c. piperazin solution (microscope). After solution, add HCl, when characteristic spindle-shaped crystals separate.

(*d*) With the aid of heat dissolve some serpent's urine—which is solid, and consists chiefly of ammonium urate — in 10 p.c. NaHO. Add water, and allow it to stand. Pour off the clear fluid, and precipitate the uric acid with dilute hydrochloric acid. Collect the deposit and use it for testing.

(*e*) *Hopkins' method* (see Lesson XX., 5).

4. Tests—(*a*) *Murexide test.*—To uric acid in a porcelain capsule add HNO_3, and heat gently, taking care that the temperature is not above 40° C. Disagreeable fumes are given off, while a yellow or reddish stain remains.

Cool, and bring a rod dipped in ammonia near the stain, or moisten it with ammonia, when a *purple-red* colour of *murexide*, $C_8H_8(NH_4)N_5O_6$, appears.

[The uric acid is oxidised by the HNO_3 to alloxantin, which is composed of alloxan and dialuric acid ($CO<^{NH-CO}_{NH-CO}>CH-OH$). Ammonia converts the latter into dialuramide, $CO<^{NH-CO}_{NH-CO}>CH-NH_2$, which with alloxan yields purpuric acid, and the excess of ammonia yields purpurate of ammonia or murexide.]

(*b*) Repeat the test, but act on the residue with NaHO or KHO, when a violet-blue colour—discharged by heat—is obtained. The latter distinguishes it from guanin (p. 125). When uric acid is acted on by HNO_3, alloxantin ($C_8H_4N_4O_7$) is formed, which, on being further heated, yields alloxan ($C_4H_2N_2O_4$); the latter strikes a purple colour—murexide—with ammonia.

(*c*) Dissolve uric acid in liquor potassæ, and place a drop on a slide; add HCl or HNO_3 just to excess, and examine with the microscope the crystals of uric acid which form—transparent rhombs with obtuse angles, dumb-bells, or rosettes.

(*d*) Dissolve uric acid in NaHO, add Fehling's solution—or dilute $CuSO_4$ and NaHO—and boil = a white precipitate of cupric urate, which after a time becomes greenish.

(*e*) *Schiff's test.*—Dissolve uric acid in a small quantity of sodium carbonate. Place a drop of solution of $AgNO_3$ on filter-paper, and on this place a drop of the uric acid solution. A dark brown or black spot of reduced silver appears.

(*f*) Heat sharply uric acid in a test-tube. It blackens and decomposes into ammonia, hydrocyanic acid (recognised by odour), urea, and cyanuric acid.

(*g*) To uric acid, dissolved with addition of ammonia, add picric acid = copious yellow precipitate (*Jaffé*).

(*h*) To uric acid as in (*g*) add the magnesia mixture and then $AgNO_3$ = a gelatinous precipitate of urate of silver and magnesia.

(*i*) *Garrod's microscopic test.*—Add 6 to 8 drops of glacial acetic acid to 5 c.c. urine in a watch-glass, put into it a few silk threads, and allow the whole to stand for 24 hours. Prevent evaporation by covering it with a watch-glass. Examine the threads microscopically for the crystals of uric acid, which are soluble in KHO. A similar reaction may be carried out on a microscopic slide.

5. **Estimation of uric acid** (*Hopkins' method*).—This depends on the fact that uric acid is completely precipitated—as ammonium urate—on saturation with NH_4Cl, and it can be titrated in warm H_2SO_4 solution with $KMnO_4$.

A. (*a*) *Separation.*—To 100 c.c. urine in a beaker add about 25 grms. ammonium chloride; stir till dissolved. Add 2 c.c. strong ammonium hydrate—*i.e.*, to distinct alkaline reaction. Allow the mixture to stand until the precipitate of ammonium urate falls (about 2 hours). Filter, using vacuum. After complete separation of the precipitate, wash the precipitate on the filter with saturated NH_4Cl solution, and then dissolve it in weak alkali or warm water. Add a little HCl and the uric acid crystallises out.

(*b*) *Titration.*—The uric acid is titrated with a standard solution ($\frac{N}{20}KMnO_4$) of permanganate of potash. The precise details are omitted here.* Folin has somewhat modified the original method.

B. For clinical work the following modified process may be adopted:—The yellowish precipitate on the filter is thoroughly washed with a saturated solution of $(NH_4)_2SO_4$ to remove every trace of the NH_4Cl. The deposit is then washed into a vessel by the aid of a jet of hot water. Add a little carbonate of soda to get the deposit in solution. To this add, when cool, 20 c.c. H_2SO_4 = evolution of heat. Titrate the hot acid liquid with a one-twentieth normal solution of $KMnO_4$ (1·578 grms. per litre) until a pink colour—remaining permanent for thirty seconds—is obtained. It disappears on standing—*i.e.*, uric acid is oxidised by the permanganate to a colourless compound. Read off the c.c. of the SS. used. Each c.c. = 0·00375 grm. of uric acid.

Example.—Suppose 17·5 c.c. of permanganate solution are used:—17·5 × ·0375 = ·065 grm. of uric acid in 100 c.c. of urine—*i.e.*, 0·065 p.c. If the patient passes 1500 c.c. urine in 24 hours, then the total would be 0·065 × 15 = 0·975 grm. of uric acid.

C. This is sometimes done by the method (3, *a*), but it is not accurate. *Haycraft's method* depends on the formation of urate of silver, which is practically insoluble in water or acetic acid (*British Medical Journal*, 1885). The urate of silver is of a slimy nature and must be washed on an asbestos filter. The titration of the silver compound is done by means of Volhard's ammonium thio-cyanate method (Sutton's *Volumetric Analysis*, 5th edit., 1886, pp. 116, 324).

6. **Uric acid salts (Urates, "Lithates").**—Uric acid forms salts (chiefly acid), with various bases, which are soluble with difficulty in cold, but readily soluble in warm water. HCl and acetic acid decompose urates, and then the uric acid crystallises.

Urine, which is clear when passed, may on standing for a time become turbid, and a copious reddish-yellow—sometimes like pea-soup—or purplish precipitate falls, because urates are more soluble in warm water than in cold. When there is only

* *Journ. of Path. and Bact.*, i., p. 451, 1893.

a small quantity of water to hold the urates in solution, on the urine cooling they are precipitated. Their occurrence is favoured by an acid reaction, a concentrated condition of the urine, and a low temperature. Ammonium urate occurs in ammoniacal urine.

The urates deposited in urine consist chiefly of sodic urate mixed with a small amount of ammonium urate.

Urates form one of the commonest deposits in urine. There is usually a copious precipitate, varying in colour from a light pink or brick-red to purple. They occur in catarrhal affections of the intestinal canal, after a debauch, in various diseases of the liver, in rheumatic and feverish conditions. They frequently occur as the "milky" deposit in the urine of children. Urates constitute the "lateritious" deposit or "critical" deposit of the older writers. Urates frequently occur even in health, especially when the skin is very active (in summer), or after severe muscular exercise; when much water is given off by the skin and a small quantity by the kidneys. The following are the formulæ of the more common urates:—

[Uric acid, ...	$C_5H_4N_4O_3$.]
Acid sodic urate, ...	$C_5H_3N_4O_3Na$.
Neutral (normal) sodic urate, . . .	$C_5H_2N_4O_3Na_2$.
Acid ammonium urate, ...	$C_5H_3N_4O_3(NH_4)$.
Acid potassic urate, ...	$C_5H_3N_4O_3K$.

Acid sodium urate is the chief constituent in a deposit of "urates."

7. **Tests for "Urates" or "Lithates" in urine.**—(*a*) The deposit is usually copious = yellowish-pink, reddish, or even shading into purple due to uro-erythrin. The deposit moves freely on moving the vessel, and its upper border is fairly well defined.

(*b*) Heat gently the upper stratum of the turbid urine. It becomes clear, and on heating the whole mass of fluid it also becomes clear, as the urates are dissolved by the warm liquid.

(*c*) To some of the deposit on a glass slide add a drop of HCl. Uric acid is deposited in one or more of its many crystalline forms. (Microscope).

(*d*) *Microscopic.*—The urates are generally "amorphous," but urate of soda may occur as small spheres covered with spines, and the ammonium urate, as spherules often united together (fig. 70).

(*e*) Make a saturated solution of uric acid in NaHO. Allow a drop to evaporate on a slide, and examine it microscopically. Urate of soda in the form of spheres covered with spines will be obtained.

(*f*) The same result as in (*e*) is obtained by dissolving the ordinary deposit of urates in caustic soda, and allowing some of it to evaporate on a slide.

8. **Uric acid from serpent's excrement.**—This consists of acid urate of ammonium without pigment. Heat the powdered excrement in a porcelain vessel with 15 to 20 vols. of water just to boiling, add carefully small quantities of caustic potash or soda until the whole is dissolved and there is no further odour of ammonia given off. Filter, and saturate the filtrate with CO_2, which causes at first a gelatinous and then a finely-granular precipitate of acid alkaline urate. Separate the latter by syphoning off the fluid, wash it with small quantities of iced water, place it in boiling dilute HCl, and boil the mixture for some time. After it cools, uric acid crystallises out, the latter is washed with cold water and dried.

9. **Quadriurates.**—According to Roberts these are the normal urates in urine. They tend to break up in water into acid urates and uric acid. This result is prevented in the urine by the chlorides and phosphates.

(*a*) Add water to a trace of serpent's urine on a slide, examine microscopically, and observe small, short needles of uric acid and granules of ammonium urate.

(*b*) Collect a deposit of urates on a filter, wash with absolute alcohol, and when dry examine a little on a slide microscopically, after adding distilled water. The amorphous granules decompose, and yield rhombic crystals of uric acid (*Roberts*).

(*c*) Place urine in a test-tube (add a drop or two of chloroform to prevent decomposition). Cork. After a few days crystals of uric acid are deposited owing to the influence of the acid phosphates.

Deposit of Urinary Urates	*v.*	Urinary Phosphates.
1. Usually pink or reddish. Precipitated when urine cools. They may be white in children.		1. Always white, heavy-looking deposit.
2. Soluble on heating below boiling point.		2. Not soluble on heating.
3. Insoluble in acid.		3. Soluble in acid.
4. Soluble in KHO.		4. Not soluble in KHO. May be increased.

10. **Xanthin or nuclein bases** are closely related to uric acid.

Hypoxanthin, ...	$C_5H_4N_4O$.
Xanthin, ...	$C_5H_4N_4O_2$.
Uric acid, ...	$C_5H_4N_4O_3$.

They also include guanin ($C_5H_5N_5O$) and adenin ($C_5H_5N_5$), and are sometimes called "alloxur" or "purin" bases. They are probably derived from nuclein. They occur in very small amount in the urine, and seem to be greatly increased in leukæmia (see *Appendix*).

11. **Hippuric acid,** $C_9H_9NO_3$ (benzoyl-amido-acetic acid or benzoyl-glycin), so-called because it occurs in large quantity in horse's urine and in that of many herbivora, chiefly in the form of alkaline hippurates (sodium hippurate). It belongs to the aromatic series, as it contains the benzene nucleus, like phenol and cresol. Dissolves readily in hot alcohol, but is sparingly soluble in water.

Quantity in man ·5 to 1 grm. daily. It is a conjugate acid, which, when boiled with alkalies and acids, takes up water and splits into benzoic acid and glycin. It occurs in colourless four-sided prisms, usually with two or four bevelled surfaces at their ends. It has a bitter taste. Benzoic acid, oil of bitter almonds, benzamid, cinnamic acid, and toluol reappear in the urine as hippuric acid. The benzoic acid unites with the elements of glycocoll (glycin), and is excreted as hippuric acid in the urine.

Benzoic Acid.		Glycocoll.		Hippuric Acid.		Water.
$C_7H_6O_2$	+	$C_2H_5NO_2$	=	$C_9H_9NO_3$	+	H_2O.

The amount is *increased* by eating pears, apples with their skins, cranberries, and plums. Nothing is known of its clinical significance. It seems to be formed chiefly from the husks or cuticular structures.

Tests and reactions.—(*a*) Heat crystals in a dry tube. At first they dissolve without decomposition, but above 187° C. oily red drops are deposited in the tube, while a sublimate of benzoic acid and ammonium benzoate are given off. The latter is decomposed, with the odour of ammonia and an aromatic odour of oil of bitter almonds (benzonitril, C_6H_5CN and CNH). The red colour is due to the decomposition of glycocoll or glycin.

(*b*) Examine colourless four sided prisms, which are insoluble in acetic acid (fig. 67).

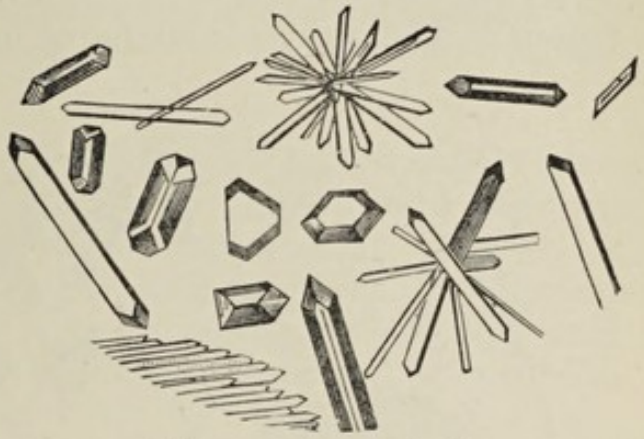


Fig. 67.—Hippuric Acid.

(*c*) *Lacke's reaction.*—Heat with HNO_3, mix the residue with sand, and heat to dryness = odour of nitro-benzol ($C_6H_5NO_2$). Benzoic acid, and many acids of the aromatic series, give a similar reaction.

12. Preparation of hippuric acid.—(*a*) Evaporate 100 c.c. of cow's or horse's urine to one-sixth its bulk; add HCl, and set it aside. The brown mass is collected, dried between folds of blotting-paper, redissolved in a very small quantity of water, and mixed with charcoal, then filtered and set aside to crystallise. It is not quite pure and contains a brownish colouring-matter.

(*b*) Boil horse's urine with milk of lime = a copious precipitate, and alkaline reaction. Filter off the bulk of the precipitate through flannel, and filter again through paper. Concentrate the filtrate to one-sixth of its volume and add HCl = a copious precipitate of prismatic crystals of hippuric acid. After 24 hours decant the fluid from the crystals, redissolve the latter in hot water, and filter through animal charcoal.

13. Kreatinin ($C_4H_7N_3O$) is related to kreatin ($C_4H_9N_3O_2$) of muscle, and seems to be derived chiefly from the kreatin in the meat of the food, and not from the kreatin in the muscles of the body. If kreatin be boiled with acids or with water for a long time, it loses water, and becomes converted into a strong base—kreatinin.

Quantity, 0·5 to 1 grm. (7 to 15 grains), so that it ranks second as the form in which waste N is excreted by the urine. Soluble in water and alcohol; forms colourless oblique rhombic crystals; unites with acids, and with salts, chiefly $ZnCl_2$. The kreatinin-zinc-chloride is used as a microscopic test for its presence. Rare as a deposit, and nothing is known of its clinical significance. Its amount is increased by meat diet, but with a milk diet it is at a minimum.

14. Tests for kreatinin—(*a*) *Weyl's test.*—Add a very dilute solution of sodium nitro-prusside, and very cautiously NaHO = a ruby-red colour, which is evanescent, passing into a straw colour. The ruby colour fades on boiling, and if while boiling acetic acid be added, Prussian blue is formed, but the colour struck is green.

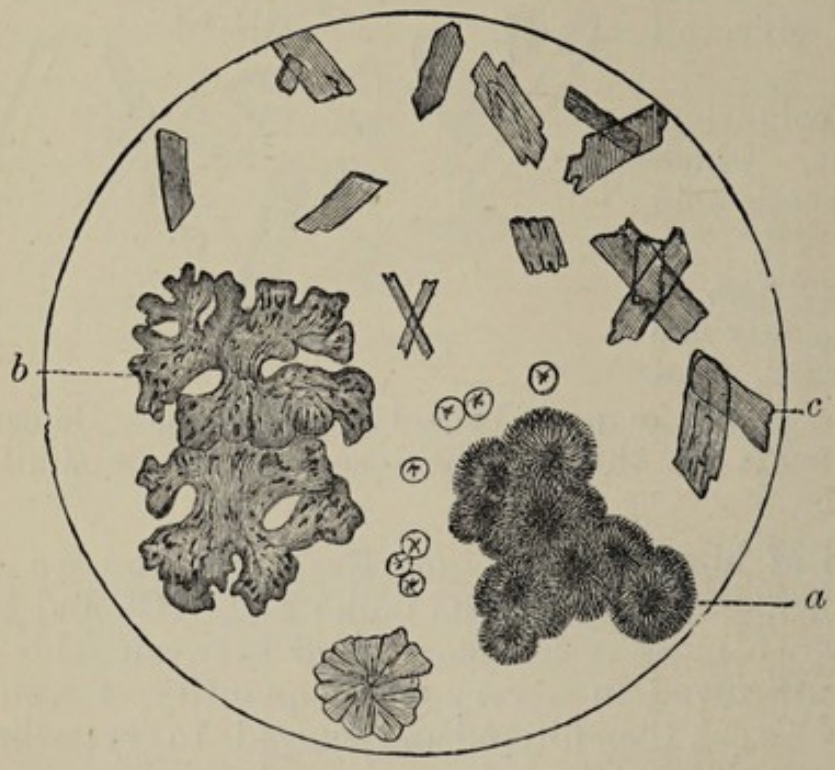


Fig. 68.—Kreatinin-zinc-chloride. *a*, Balls with radiating marks; *b*, Crystallised from water; *c*, Rarer forms from an alcoholic extract.

(*b*) *Jaffé's test.*—Add to 10 c.c. of urine some drops of saturated solution picric acid, and then 2 c.c. NaHO = deep red colour.

(*c*) Kreatinin reduces an alkaline solution of cupric oxide—*e.g.*, Fehling's solution—and might be mistaken for a reducing sugar.

(*d*) *Microscope.*—It forms definite crystalline compounds with $ZnCl_2$ (fig. 68) and $HgCl_2$.

(*e*) *Obermayer's test.*—Add strong HCl and ferric chloride (500 c.c. to 1 to 2 pts. Fe_2Cl_6), and shake with chloroform.

(*f*) The injection subcutaneously of 0·1 grm. neutral sodium oxalate in a dog, next day and for several days gives strongly the indigo reaction. (E. Harnack, *Zeit. f. physiol. Chemie*, xxix., 205, 1900.)

15. **Preparation of kreatinin.**—(*a*) Precipitate 250 c.c. of urine with milk of lime, and filter. Evaporate the filtrate to a syrupy consistence, and extract it with alcohol. Filter, and to the filtrate add a drop or two of a neutral solution of $ZnCl_2$, and set the vessel aside. After a time kreatinin-zinc-chloride ($C_4H_7N_3O$, $ZnCl_2$) is deposited on the sides of the vessel. (Microscope, fig. 68.)

(*b*) To half a litre of urine add baryta mixture until no further precipitation takes place; filter, and evaporate the filtrate to a thin syrup on a water-bath; add to this an equal volume of alcohol, and allow it to stand for 24 hours in the cold. The salts separate; filter, and to the filtrate add 1 to 2 c.c. of a concentrated alcoholic solution of $ZnCl_2$. Kreatinin-zinc-chloride separates as a yellow crystalline powder. After two to three days filter, wash with alcohol, dissolve in warm water, and decompose it by boiling for half an hour with hydrated lead oxide or carbonate of lead. Filter while hot, decolorise the filtrate with animal charcoal, filter again, evaporate to dryness, and extract the kreatinin from the residue with alcohol in the cold. A small quantity of kreatinin remains undissolved.

16. **Pigments of the urine.**—(1) **Urochrome** is now regarded as the most abundant urinary pigment. It is a yellow pigment without absorption bands. Garrod has separated it from urine. The urine is saturated with Am_2SO_4 and filtered; the pigment is in the filtrate, from which it is extracted by shaking with alcohol. It is probably an oxidation product of urobilin [*Thudichum*, 1864].

(2) **Urobilin** was formerly regarded as the principal pigment, but normal urine contains very little urobilin. A chromogen urobilinogen is present, which when exposed to air yields urobilin. Add to urine neutral and basic lead acetate = a precipitate of lead salts, which carry down with them the colouring matter, leaving the solution nearly colourless. Filter. Extract the pigment from the filtrate by alcohol acidulated with H_2SO_4. Filter = alcoholic extract of deep yellow colour, which can be extracted by chloroform. On evaporation of the chloroform it is deposited as a yellow-brown mass, which in an acid solution shows with the spectroscope one absorption band close to and enclosing F at the junction of the blue and green. On adding an alkali the band disappears (*MacMunn*). Its spectrum and composition are practically identical with choletelin, $C_{16}H_{18}N_2O_3$, and it is regarded as an iron-free derivative of hæmoglobin on the supposition that it is modified bile-pigment absorbed from the intestinal canal and excreted by the urine [*Jaffé*, 1868].

(3) **Uroerythrin** is the pigment which gives the pink or pinkish-red colour to urates or "lateritious deposits" in urines.

(4) **Hæmatoporphyrin** occurs normally in exceedingly small

amount, but after the use of sulphonal it is greatly increased. It probably exists chiefly as a chromogen.

[Febrile urobilin.—A substance of this nature has been described. This gives the dark colour to urines in fever. It seems to be a less oxidised form of urobilin, is isolated in the same way, its spectrum shows the band near F, and two additional bands, one near D and one between D and E.]

(5) **Chromogens in urine.**—Besides those of urobilin and hæmatoporphyrin there are the

(6) **Indigogens or indigo-yielding bodies.**

Indigo-forming substance—Indoxyl (Indican).—This is derived from indol, C_8H_7N, which is developed in the intestinal canal from the pancreatic digestion of proteids, and also from the putrefaction of albuminous bodies. In urine it is a yellow pigment, and is more plentiful in the urine of the dog and horse. It exists in urine as a conjugated sulpho-acid salt of potassium—viz., as indoxyl-sulphate of potassium ($C_8H_6NSO_4K$).

(*a*) *Jaffé's test.*—The urine must be free from albumin. To 5 c.c. urine add 5 c.c. HCl, whereby indoxyl is liberated from the sulphate. Then add cautiously by means of a pipette solution of hypochlorite of soda or bleaching powder (1 : 20), when a blue colour appears. Add chloroform; this absorbs the blue, and forms a layer at the bottom.

$$\underset{\text{Potassium indoxyl sulphate.}}{2C_8H_6NKSO_4} + O_2 = 2KHSO_4 + \underset{\text{Indigo blue.}}{C_{16}H_{10}N_2O_2}.$$

Weber's test.—Heat equal volumes of urine and HCl. Add a little HNO_3, and boil = violet colour. Cool; add ether and shake. The ether—bluish—rises to the surface, while the rest of the fluid is reddish-brown—indigo-red.

(*c*) Place a crystal of chlorate of potash in a dry tube, add urine, and then let HCl run down the side, whereby the urine is raised. At the junction of the acid and urine a purple band is seen, due to oxidation by the chlorate in the presence of HCl.

[See A. E. Garrod, "Urinary pigments in their pathological aspects," *Lancet*, 1900.]

17. General reactions for urine pigments.

(*a*) Add to normal urine a quarter of its volume of HCl, and boil = a pink or yellow colour.

(*b*) Add HNO_3 = a yellowish-red colour, usually deeper than the original colour.

(*c*) Drop into 2 volumes of H_2SO_4 1 of urine. The mixture becomes more or less garnet-red if indican be present.

(*d*) Add acetate of lead = a precipitate of chloride, sulphate, and phosphate of lead. Filter; the filtrate is colourless. This substance is used to decolorise urine for the saccharimeter.

(*e*) Filter urine through animal charcoal = decolorised.

(*f*) With a dark yellow coloured urine perform the following test:—

To 40 drops of urine + 3 to 4 c.c. of strong HCl and 2 to 3 drops of HNO_3; on heating, a *violet-red* colour with the formation of true rhombic crystals of indigo-blue indicates the presence of indican.

(*g*) Test for indican.—Mix equal volumes of urine and HCl, add, drop by drop, a saturated solution of chloride of lime (*i.e.*, bleaching powder, which also contains hypochlorite of lime) = a blue colour. Shake up with chloroform and the blue colour is absorbed by the latter.

The pathological pigments—bile, blood, &c. (Lesson XXII.)

18. Aromatic compounds are chemical derivatives of the benzene molecule, C_6H_6. Several are excreted in combination with H_2SO_4, and constitute *conjugated* or *ethereal sulphates.* The H_2SO_4 in these organic combinations forms about one-tenth of that excreted in the urine (Lesson XVII.). They have a certain clinical importance, as they are increased in several diseases of the intestinal canal, and as they arise chiefly from putrefactive processes in the intestine. The aromatic organic bodies so formed in the intestine—*e.g.*, phenol, kresol, skatol—are poisonous, but when converted into ethereal sulphates they are innocuous.

19. Phenol (carbolic acid), C_6H_6O (hydroxy-benzol), occurs in the urine as phenol-sulphate of potassium, $C_6H_5O - SO_3 - OK$. There is a corresponding salt of **Cresol** (hydroxy -toluol, C_7H_7OH), most abundant in the urine of herbivora, and chiefly in the form of para-cresol. Add H_2SO_4 to 200 c.c. urine until the latter contains 5 p.c. of the acid. Distil as long as the distillate becomes cloudy with bromine water. Test the distillate as follows:—

(*a*) Bromine water = precipitate of tri-bromo-phenol ($C_6H_2Br_3OH$).
(*b*) Neutralise and add neutral ferric chloride = violet colour.
(*c*) Heated with Millon's reagent it gives a red colour. (See also p. 179).

20. Mucus.—A trace occurs normally. Collect fresh urine in a tall conical vessel, and allow it to stand; fine clouds ("mucous clouds") like delicate cotton-wool appear. These consist of mucus entangling a few epithelial scales.

(*a*) If the urine contain an excess of mucus, on adding a saturated solution of citric acid to form a layer at the bottom of the test-tube, a haziness at the line of junction of the urine and acid indicates mucus. There is no deposit with healthy, freshly-passed urine. Citric acid is used because it is heavier than acetic.

21. Ferments.—Urine contains pepsin. Some observers state that it also contains trypsin and a sugar-forming ferment; but the latter statement is denied.

(*a*) Place in morning urine fresh washed and boiled fibrin, which absorbs the ferment (2 hours). Digest the fibrin with 0·2 p.c. HCl at 40° C.; the fibrin is dissolved and peptones are formed. Test for the peptones by the biuret reaction.

22. Reactions of normal urine towards reagents.

(1) Add 5 c.c. of HCl to 100 of urine. After 24 hours crystals of uric acid separate out.

(2) Add NaHO or NH_4HO = precipitate of phosphates of the alkaline earths, partly in an amorphous state, partly in acicular crystals.

(3) Acidulate with NHO_3 and heat with phospho-molybdic acid = blue coloration due to urates.

(4) Add mercuric nitrate = white cloudiness, which disappears on shaking. This is a precipitate due to the formation of sodium nitrate and mercuric chloride ($Hg(NO_3)_2 + 2NaCl = 2NaNO_3 + HgCl_2$), soluble in acid urine. After all the NaCl is decomposed—but not till then—a permanent precipitate, a compound of urea and the mercury salt, forms.

(5) Silver nitrate = white precipitate of AgCl and Ag_3PO_4; the latter falls first, and afterwards all the silver combines with the chlorine. The precipitate is insoluble in NHO_3, but soluble in NH_4HO.

(6) Barium chloride = white precipitate of $BaSO_4$ and $Ba_3(PO_4)_2$.

(7) Lead acetate = whitish precipitate of $PbSO_4$, $PbCl_2$, $Pb_3(PO_4)_2$, and the pigments.

(8) Ferric chloride after acidulation with acetic acid = precipitate of $Fe_2(PO_4)_2$.

(9) An ammoniacal solution of cupric oxide is decomposed and decolorised at the boiling-point by the urates.

(10) Tannic acid = no precipitate (*Krukenberg*).

23. Average amount of the several urinary constituents passed in 24 hours by a man weighing 66 kilos.—*Parkes.*

	Grms.
Water,	1500
Total solids,	72

Organic solids—	Grms.	*Inorganic solids*—	Grms.
Urea,	33·18	Sulphuric acid,	2·01
Uric acid,	·55	Phosphoric acid,	3·16
Hippuric acid,	·40	Chlorine,	7·00
Kreatinin,	·91	Ammonia,	0·77
Pigment and other substances,	10·00	Potassium,	2·50
		Sodium,	11·09
		Calcium,	0·26
		Magnesium,	0·21

LESSON XXI.

ABNORMAL CONSTITUENTS—ALBUMIN.

Some of the substances referred to are present in excessively minute traces in normal urine—*e.g.*, sugar; and in the urine of a certain percentage of persons *apparently* enjoying perfect

health, minute traces of albumin are sometimes present. When, however, these substances occur in considerable quantity, then their presence is of the utmost practical and diagnostic value, and is distinctly abnormal. Practically, healthy urine contains no proteids.

1. Albumin.—When albumin occurs in notable quantity in the urine, it gives rise to the condition known as **albuminuria.** One of the most frequent causes of its appearance is disease of the kidneys (Bright's disease). This so-called "albumin" of the urine is most frequently a mixture of *serum-albumin* and *serum-globulin.* [Other proteids, albumose, peptone, &c., may be found.] The admixture of albuminous fluids with the urine is here excluded. As regards the amount of albumin that may be present pathologically (*Tappeiner*), a *small* amount is 0·1 p.c. (under 2 grms. daily), *moderate* 0·5 p.c. (8 grms.), *large* 0·5 to 1 p.c. (8 to 15 grms.). Albuminous urine is not unfrequently of low S.G., and froths readily on shaking.

2. Tests.—The urine must be clear before testing (filter).

(*a*) *Coagulation by heat.*—If the urine is acid, place 10 c.c. in a test-tube and boil. Near the boiling-point, if albumin be present in small amount, it will give a haziness; if in large amount, a distinct coagulum. On standing, the coagulum is deposited. Some prefer to boil the top of a long column of urine in a test-tube. If the urine be acid, then any haziness formed is readily seen against the clear subnatant fluid. If the urine is alkaline, acidify with a few drops of dilute acetic acid, then boil.

Precautions.—(i.) Always test the reaction of the urine, for albumin is only precipitated by boiling in a neutral or acid medium. Hence if the urine be alkaline, boiling will not precipitate any albumin that may be present. (ii.) Boil the upper stratum of the fluid first of all, holding the tube obliquely, taking care that the coagulum does not stick to the glass, else the tube is liable to break. (iii.) Heat, by driving off the CO_2, also precipitates *earthy phosphates* if they are present in large amount, hence a turbidity on boiling is not sufficient proof of the presence of albumin. The points of distinction are that albumin goes down before the boiling-point is reached (coagulated at 75° C.), while phosphates are precipitated at the boiling-point. Again, the phosphatic deposit is soluble in an acid—*e.g.*, acetic or nitric—while the albuminous coagulum is insoluble in these fluids.

(*b*) If the urine is alkaline acidulate with a little dilute acetic or nitric acid, and then boil. If nitric acid be used, add one-tenth

to one-twentieth of the volume of urine; or boil the suspected urine, add HNO_3 (5 to 10 drops) till the reaction is strongly acid. If a precipitate appears on adding the acid, or if a precipitate already formed does not disappear, then albumin is present.

Precautions.—If the urine contain only very minute traces of albumin, the latter may not be precipitated if too much nitric acid be added, as the acid albumin is kept in solution. If too little acid be added, the albumin may not be precipitated, as only a part of the basic phosphates are changed into acid phosphates, and the albumin remains in solution as an albuminate (a compound of the albumin with the base). On heating the urine of a person who is taking copaiba, a deposit may be obtained, but its solubility in alcohol at once distinguishes it from coagulated albumin. This test acts with serum-albumin and globulin, and if the deposit occurs only after cooling, also with albumose, but not with peptone.

(*c*) *Heller's cold nitric acid test* (Ring test).—Place 15 c.c. urine in a conical test-glass; incline it, and pour slowly down its side strong HNO_3 = a white cloud at the line of junction of the fluids. Or, better still, add 10 c.c. pure HNO_3 by means of a pipette which is carried to the bottom of the conical glass, where the acid is slowly allowed to escape by diminishing the pressure of the finger on the end of the tube or pipette (*Simon*). This is also a useful method of applying HNO_3 in connection with colour tests in urine.

Precautions.—A *crystalline* deposit of urea nitrate is sometimes, though very rarely, obtained with a very concentrated urine, and after some time. It may be prevented by previous dilution of the urine. If the urine contain a large amount of urates, they may be deposited by the acid, but the deposit in this case occurs above the line of junction, and disappears on heating. It is not obtained if the urine be diluted beforehand. *Coloured rings* occur, especially in highly-coloured urines, owing to the oxidation of the urinary pigments by HNO_3. These rings are readily distinguished from an albuminous cloud.

(*d*) Acidulate 10 c.c. of urine with acetic acid, add one-fifth of its bulk of a saturated solution of magnesium or sodium sulphate, and boil = a precipitate.

(*e*) Acetic acid and potassium ferrocyanide.—Acidify strongly with acetic acid, and add a solution of potassium ferrocyanide = a white precipitate, varying in amount with the albumin present. The reaction may be done as follows:—Mix a few c.c. of moderately strong acetic acid with some solution of potassium ferrocyanide, and pour this over some urine in a test-tube by the contact method (*d*). The presence of albumin is indicated by a white deposit in the form of a ring at the line of junction of the fluids. A solution of platino-potassium cyanide may be used instead of the ferrocyanide. The solution of the former is colourless. This test precipitates serum-albumin, globulin, albumose, but not peptone.

(*f*) *Picric acid.*—Use a saturated watery solution by the contact method of Heller (*c*). The urine is below, and the picric

acid on the top. A rapidly-formed deposit at the line of junction of the fluids indicates the presence of a proteid; the deposit is not dissolved by heat.

N.B.—Picric acid precipitates all forms of proteid which occur in urine. It also precipitates mucin, but in this case the deposit usually forms slowly and after a time. If a person be taking quinine, a haziness is obtained in the urine on adding picric acid, but it disappears on heating.

(*g*) **Metaphosphoric acid** completely precipitates albumin, but it must be freshly prepared, and is difficult to keep. Hence it is not satisfactory.

(*h*) **Acidulated brine**, as suggested by Roberts, consisting of a saturated solution of sodium chloride with 5 p.c. dilute HCl, may be used, but it sometimes gives a precipitate with normal urine. Nor is potassio-mercuric-iodide satisfactory (*Tanret*). In case of doubt, use several tests, especially 2 (*b*), (*c*), (*e*), and (*f*).

(*i*) **Trichloracetic acid** precipitates albumin in urine.

(*j*) **Salicyl-sulphonic acid** gives a white precipitate with proteids, which is soluble on heating in the case of albumose and peptone (*M'William*).

3. **Dry tests.**—(*a*) Use the ferrocyanic pellets introduced by Dr. Pavy.

(*b*) Use the test-papers—citric acid and ferrocyanide of potassium—introduced by Dr. Oliver.

4. **Globulinuria.**—**Serum-globulin** is present in nearly every albuminous urine. It gives the reactions described under 2.

(*a*) Drop the urine into a tall glass of distilled water, and observe if a milkiness is seen, indicating the presence of a globulin. This body is insoluble in pure water, but soluble in weak saline solutions (Lesson I., II.), hence on diluting the urine it is precipitated.

(*b*) Test by the contact method with a saturated solution of $MgSO_4$.

(*c*) It is precipitated by saturation with Am_2SO_4.

If globulin be present along with serum-albumin add an equal volume of a saturated solution of Am_2SO_4. A white flocculent precipitate indicates globulin.

5. **Albumosuria.**—The albumoses have been found in cases of osteomalacia. If such a urine can be procured, do test 2 (*b*), using nitric acid; the deposit only takes place after a long time or on cooling, and in fact the urine sometimes becomes almost solid, but is dissolved by heat. If there is a deposit, filter and test the filtrate for proteid reactions—*e.g.*, the biuret test. It will give a precipitate with acetic acid and potassic ferrocyanide. Then saturate a portion of the urine with sodium chloride, and acidify with acetic acid = a precipitate, which dissolves on adding much acetic acid and heating, and reappears on cooling.

6. **Peptonuria.**—**Peptone** may be present in albuminous urine, most

frequently in urine in cases where there is an accumulation and breaking up of leucocytes or pus-corpuscles, as in the stage of resolution of pneumonia and suppurative processes. Get rid of the albumin by acidification with acetic acid and boiling.

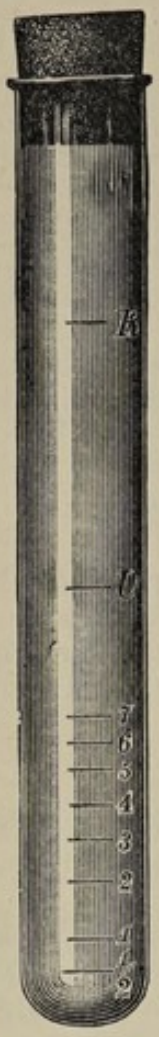


Fig. 69.
Esbach's Tube.

(*a*) Put some urine in a test-tube, and by the contact method pour on Fehling's solution. At the line of junction a phosphatic cloud is formed, and, if peptones be present, above it a rose-pink colour. If albumin also be present a violet colour is obtained. Albumoses give the same (pink) reaction.

7. Quantitative estimation of albumin.—This can only be done accurately by precipitating the albumin, drying and weighing it; but as this is a tedious process, and requires much time, it is not suitable for the physician.

8. Esbach's albuminimeter (fig. 69).—A. *Reagent.*—Dissolve 10 grms. picric acid and 20 grms. dry citric acid in 800 c.c. of boiling water, and make up the solution to a litre. It precipitates not only albumin but also uric acid, kreatinin, peptones, and some resinous acids.

B. *Process.*—Pour urine into the tube (6 inch × $\frac{5}{8}$ inch) up to the mark U, then the reagent up to the R. Cork; mix thoroughly by tilting the tube several times. Set the tube aside (vertical) for 24 hours, and then read off on the scale the height of the coagulum. The figures indicate grms. of dried albumin per litre of urine —*i.e.*, the percentage is obtained by dividing by ten. If the amount of albumin be less than 0·5 grm. per litre, it cannot be accurately estimated by this method.

LESSON XXII.

BLOOD, BILE, AND SUGAR IN URINE.

1. Blood in urine (Hæmaturia).—The blood may come from any part of the urinary apparatus.

If from *kidney* it is usually small in amount and well mixed with the urine, and the microscope may reveal the presence of "blood-casts"—*i.e.*, blood-moulds of the renal tubules. Large coagula are never found, and the urine not unfrequently is "smoky." From the *bladder* or *urethra*, usually the urine is red, and relatively large coagula are frequently present. In all forms blood-corpuscles are to be detected by the microscope, and albumin by its tests.

(*a*) The colour may vary from red to brown, or the urine may have a "smoky" appearance.

(*b*) *Microscope.*—Examine any deposit for blood-corpuscles. They are often discoloured or misshapen.

(*c*) *Spectrum.*—HbO_2 or met-hæmoglobin (Lesson VI.).

(*d*) *Guaiacum test* (Almén's test).—Add fresh prepared tincture of guaiacum, and then ozonic ether; a blue colour indicates the presence of hæmoglobin. This may be done on filter-paper.

(*e*) *Heller's blood test.*—Make the urine strongly alkaline with NaHO, and boil. On standing, a deposit of earthy phosphates, coloured red or brown by hæmatin, occurs, the deposit carrying down the altered colouring-matter of the blood with it. This is not a satisfactory test.

(*f*) The urine gives the reactions of serum-albumin.

2. In **hæmoglobinuria** the urine gives the same reactions as in hæmaturia, but no blood-corpuscles are detected by the microscope.

3. **Bile.**—Test for the *bile-pigments* and *bile* (salts), *e.g.*, in jaundice. Bile-pigments in urine constitutes "choluria."

A. Bile-pigments.—(*a*) *Colour.*—The urine is yellow or yellowish-green, and the urine froths readily when shaken. Filter paper dipped into it gives a yellow stain on drying.

(*b*) *Gmelin's test* (nitric acid containing nitrous acid).—(1) Place a few drops of the urine on a white porcelain plate, and near it a few drops of the impure nitric acid; let the fluids run together and the usual play of colours is observed (Lesson XI.). (2) Add *impure* HNO_3 to urine in a test-tube until it forms a stratum at the bottom; if bile-pigments be present at the line of junction of the fluids a play of colours takes place—from above downwards—*green*, blue, violet or dirty red, and yellow. Nearly all urines give a play of colours, but *green* is the necessary and characteristic colour to prove the presence of bile-pigments.

(*c*) *Rosenbach's modification.*—Filter the urine several times through the same filter-paper (Swedish), dry paper, and on it place impure nitric acid, when the same play of colours is observed.

(*d*) A solution of methyl-violet poured on icteric urine by the contact method gives a bright carmine ring at the point of contact.

(*e*) If much bile-pigment be present, the following test succeeds:—Mix the urine with caustic potash (1 KHO to 3 water), and add HCl. The fluid becomes green, due to the formation of biliverdin.

(*f*) *Maréchal or Trousseau iodine test.*—On freshly passed diluted urine (if not fresh heat it) pour tincture of iodine diluted (1 in 10) with alcohol. The tincture floats, a green ring at the junction indicates biliverdin.

(*g*) Extract the bile-pigment with chloroform 2 c.c. to a test-tube of urine. Separate the chloroform, add an equal volume of water and a little NaHO, and shake. The bile-pigment becomes soluble in water. Apply Gmelin's test.

(*h*) *Huppert's test.*—Precipitate 15 c.c. urine with milk of lime (or $BaCl_2$), which forms salts carrying with them the bile-pigment = yellowish precipitate. Filter, and treat the precipitate with H_2SO_4 until acid, and absolute alcohol. Boil; the sediment parts with its colour, which tinges the fluid green.

(*i*) *Hammarsten's modification of Huppert's test.*—Mix 19 vols. of 25 p.c. HCl and 1 vol. 25 p.c. HNO_3. Let the mixture stand for a few hours until it becomes yellow, then add 5 vols. 95 p.c. alcohol. It is better to mix the alcohol with the acid just before use.

A few drops added to a urine that does not contain other pigment at once gives the characteristic green.

If there be much other pigment and little bile add a few drops of $BaCl_2$, and centrifugalise the deposit. Pour off the fluid, add the reagent, and centrifugalise for half a minute. Above the nearly colourless deposit is a green ring. It is said to detect $\frac{1}{50}$ milligram of bilirubin (*Scand. Arch. f. Phys.*, ix., 313).

B. Bile-acids (glycocholic and taurocholic acids—*i.e.*, salts).—There is little doubt that they occur in the urine in jaundice, but in so small an amount that often it is difficult to detect their presence. Usually they require to be isolated by a tedious process.

(*a*) *Pettenkofer's test.*—Add to urine a few drops of syrup of cane-sugar (8 p.c.), mix, and pour strong H_2SO_4 down the side of the tube until it forms a layer at the bottom. The temperature must not rise above 70° C., and the urine must not contain albumin. At the line of junction a *cherry-red* or *purple-violet* colour indicates the presence of the bile-acids. Or shake the urine and syrup to get a froth; when the H_2SO_4 is added the froth shows the colour. *N.B.*—The test in this simple form often fails with urine; in fact, there is no satisfactory simple test for minute quantities of these acids in urine.

(*b*) *Strasburger's modification.*—Dissolve cane-sugar in the suspected urine, dip filter-paper into it, and allow this to dry. Touch the paper with a glass rod dipped in strong H_2SO_4, a purple-violet colour indicates the presence of the bile-pigments.

(*c*) *Sulphur test of M. Hay.*—Try this (Lesson XI. 5). See Chauffard and Gournaud, *Journ. de Phys.*, iii., 461, 1901, where this reaction is ascribed to Haycraft. It should be Hay.

4. Sugar in urine (glycosuria).—Brücke maintains that the merest trace of *glucose* or *grape-sugar* is normally present in urine. In *diabetes mellitus*, however, it occurs persistently in considerable amount, and is, of course, then quite abnormal. In large quantity it is easily detected, but with traces this may be troublesome. Do not depend on a single test. Apply several.

Characters of diabetic urine.

(1) The patient usually passes a very large *quantity* (to 10,000 c.c.), and although the quantity is large

(2) The *specific gravity* is high—1030 to 1045. *N.B.*—When the quantity is above normal, and the S.G. is 1030, suspect the presence of grape-sugar.

(3) The *colour* is pale straw, from the dilution—not diminution—of the urine pigments. The urine may be somewhat turbid.

(4) It has a peculiar *smell*, and usually froths when poured from one vessel into another.

5. Tests for grape sugar.—In all cases remove any albumin present—*i.e.*, acidulate with acetic acid, boil, and filter.

(*a*) *Moore's test.*—Add an equal volume of NaHO or KHO, and boil the upper stratum of the fluid. If much sugar be present, a dark sherry or bistre-brown colour is obtained (due to the formation of glucic and melassic acids), according to the amount of sugar present. This is not a delicate test.

(*b*) *Trommer's test.*—Add one-third its bulk of NaHO, and then a *few drops* of 5 p.c. solution of $CuSO_4$; a clear blue solution of the hydrated oxide is obtained. Boil the *upper* stratum of the fluid. If sugar be present, a yellow or yellowish-red ring of reduced cuprous oxide is obtained.

(*c*) *Fehling's solution* is alkaline potassio-tartrate of copper ($K_2Cu_2C_4H_4O_6$). Boil Fehling's solution in a test-tube. If no discoloration (yellow) takes place, it is in good condition. Add a few drops of the suspected urine and boil, when the mixture suddenly turns to an opaque yellow or red colour, indicating the presence of a reducing sugar. For most cases no other test is required. It is better perhaps only to warm and not to boil the Fehling after adding the urine. Should a green appear—no previous precipitate being obtained in the blue fluid—after the test has stood for some time, this is probably due not to glucose, but to some other reducing agent.

(*d*) *Böttger's test.*—Mix urine with an equal volume of sodium carbonate solution, add a little basic bismuth nitrate, and boil for a short time. A grey or black deposit indicates the presence of a reducing sugar.

(*e*) *Picric acid.*—Add an equal volume of a saturated watery solution of picric acid, and then KHO. Boil, an intensely deep red or reddish-brown colour, due to picramic acid, indicates a reducing sugar. The larger the amount of sugar the deeper the tint.

(*f*) *Phenyl-hydrazine.*—Repeat this as described in Lesson III. This is a reliable test.

(i.) The *simple method* of performing the phenyl-hydrazine test (p. 22), requires only 2 minutes' boiling, and gives no crystals with normal urine. It succeeds even when much albumin is

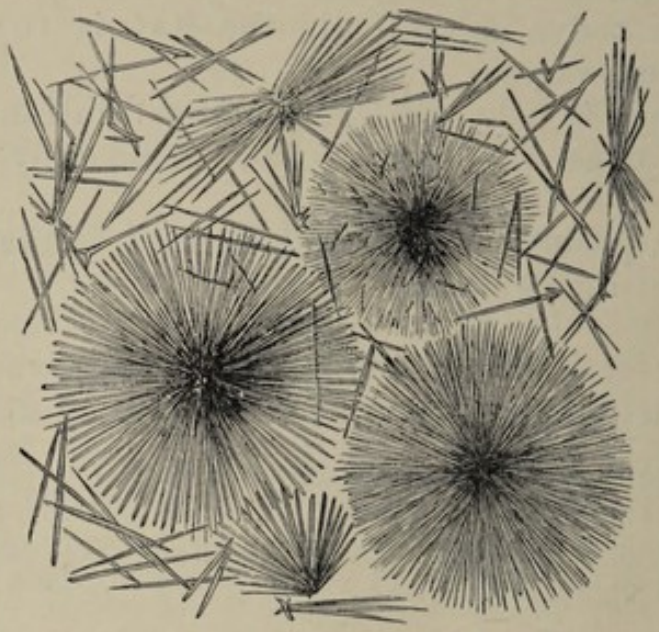


Fig. 70.—Yellow-coloured Phenyl-glucosazone Crystals from Diabetic Urine (× 300).

present. It gives no reaction with uric acid, kreatinin, hippuric acid, which substances, if in excess in urine, may reduce Fehling's solution, especially if the boiling be long continued. It will detect 0·015 p.c. dextrose in diabetic urine. (R. T. Williamson, *Medical Chronicle*, Aug., 1895, and *Diabetes Mellitus*, 1898.)

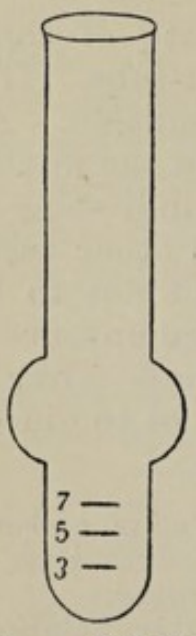


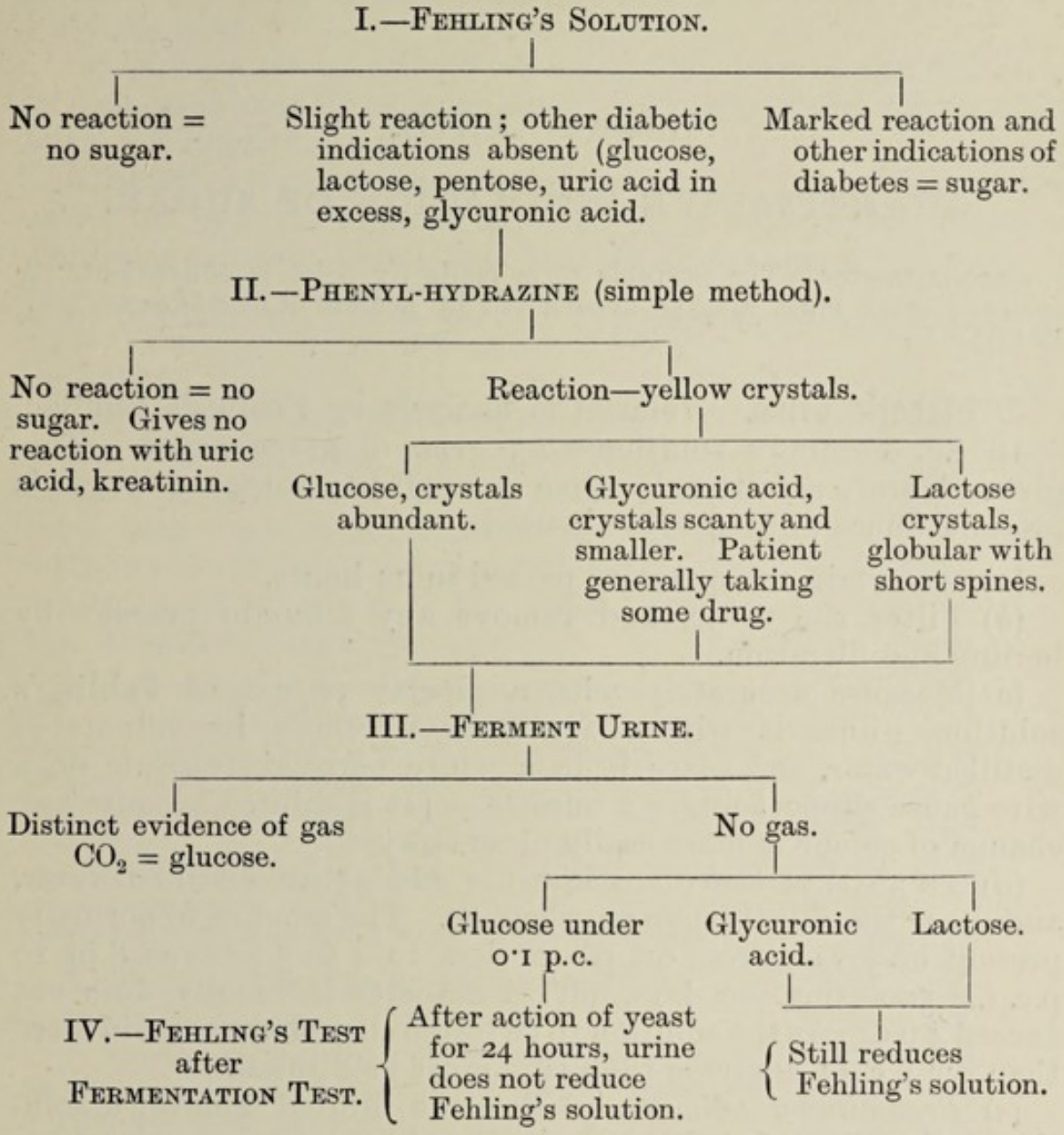
Fig. 71.—Bulb Test-tube for Osazone Test.

(ii.) *Neumann's modification.*—The sensitiveness of the test can be regulated by using a small amount of phenyl-hydrazine and controlling the strength of the acetic acid. An excess of the latter delays crystallisation.

Place 5 c.c. diabetic urine in a bulb-tube (fig. 71), add 2 c.c. of 50 p.c. acetic acid saturated with acetate of soda. Evaporate—holding the tube nearly horizontal—to 3 c.c. Cool; diminish the acidity by adding NaHO, but leave the fluid still acid. Boil and allow to cool. Crystals separate out. The whole process occupies 3 minutes.

These methods bring this most useful test well within the range of those tests capable of being rapidly carried out by the practitioner. (Walter Hall, *Brit. Med. Journ.*, Feb., 1901.)

The following table from Williamson gives a comprehensive summary of the relative values of the chief tests employed clinically :—



(*g*) *Indigo-carmine test.*—Add sodium carbonate solution and indigo-carmine solution until a blue colour appears. Boil, and a yellow colour is obtained, if sugar be present, owing to the reduction of indigo-blue to indigo-white. Pour the fluid into a cold test-tube, when the blue colour is restored, a play of colours intervening between the yellow and the blue. This is not a satisfactory test. (Mulder's test, see p. 20.)

(*h*) Repeat Molisch's test (Lessons I., II., III.).

The consideration of occurrence of *lactose* in the urine of some women during lactation is here omitted, as well as what is called "Alimentary glycosuria" and the occurrence of the rare pentoses.

[Nitro-propiol test for glucose (p. 20).]

LESSON XXIII.

QUANTITATIVE ESTIMATION OF SUGAR.

1. Saccharimeter.—The portable form made by Zeiss is convenient. A coloured urine must first be decolorised by acetate of lead [Lesson XX., 17 (*d*)].

2. **Diabetic urine. Volumetric analysis by Fehling's solution.** —10 c.c. Fehling's solution = ·05 grm. of grape sugar. This gives accurate results only when a small percentage of sugar is present, hence diluted urine is used.

(*a*) Ascertain the quantity passed in 24 hours.

(*b*) Filter the urine, and remove any albumin present by boiling and filtration.

(*c*) Measure accurately with a pipette 10 c.c. of Fehling's solution, dilute it with about 5 to 10 times its volume of distilled water, and place it in a white porcelain capsule on a wire gauze support under a burette. [It is diluted because any change of colour is more easily observed.]

(*d*) To 5 c.c. of the diabetic urine add 45 c.c. distilled water, and place the *diluted urine in a burette.* The amount abnormally present may vary from 0·5 p.c. or less to 4 to 5 or even 8 or 10 p.c. (= 500 grms. per day), but in diabetes it usually does not exceed 4 p.c. As the solution to be tested should not contain more than 0·5 p.c. of dextrose the urine must be diluted.

(*e*) *Preliminary estimation.*—Boil the diluted Fehling's solution, and whilst it is boiling gradually add the diluted urine from the burette—stirring—until all the cuprous oxide is precipitated as a reddish powder, and the supernatant fluid has a straw-yellow colour, not a trace of blue remaining. This is best seen when the capsule is tilted. It is not advisable to spend too much time in determining when the blue colour disappears, as it is apt to return on cooling. It is sometimes difficult to determine when all the blue colour has disappeared. The following process is useful:—Filter a little of the hot fluid, acidulate with acetic acid, and add potassic ferrocyanide. If copper is present a brown colour or precipitation is produced. If this be so, add more urine until no brown colour is produced.

(*f*) Read off the number of c.c. of *dilute* urine employed. If 18 c.c. were used, this, of course, would represent 1·8 c.c. of the original urine.

(*g*) Make a *second estimation*, using the data of the first, and in this case run in at once about 1 c.c. less of the dilute urine than was required at first.

Example.—Suppose the patient passes 8550 c.c. of urine, then as 1·8 c.c. of urine reduced all the cupric oxide in the 10 c.c. of Fehling's solution, it must contain 0·5 grm. sugar; hence

$$1{\cdot}8 : 8550 :: {\cdot}05 \therefore \frac{8550 \times {\cdot}05}{1{\cdot}8} = 237{\cdot}5$$ grms. of sugar passed in 24 hours.

N.B.—When the urine contains more than 1 p.c. and less than 0·5 p.c. of sugar the method is inaccurate. If the urine contains more than 1 p.c. of sugar dilute it accurately with water to bring its strength within the limits indicated above. The second estimation should be made quickly.

[**Pavy's modification** of Fehling's solution is sometimes used. In it ammonia holds the copper in solution, so that there is no yellow or red precipitate formed, as the ammonia holds the oxide in solution. The reduction is complete when the blue colour disappears. 10 c.c. Pavy's Fehling = 1 c.c. Fehling = 5 milligrams of dextrose. For the modification of the apparatus required see fig. 14. It is identical with that used by Bernard. See *Appendix*.]

3. Picro-saccharimeter of G. Johnson.

Solutions required.

1. Ferric acetate, the colour of which is equal to that yielded by a solution of sugar containing ¼ grain per fluid ounce.
2. Saturated solution of picric acid.
3. Liquor potassæ (B.P.).

(*a*) Measure 1 fluid drachm of urine into the boiling tube, add 30 minims of liquor potassæ and 80 minims of the saturated solution of picric acid. Make up to the 4-drachm mark on the tube with distilled water. Boil for 1 minute.

(*b*) Dip the tube in cold water to cool it. The volume must be exactly 4 drachms. If it is less, add water; if more, evaporate it. If the colour of the boiled liquid is the same as that of the ferric acetate ¼-grain standard, or paler, the urine contains 1 grain of sugar per fluid oz., or less.

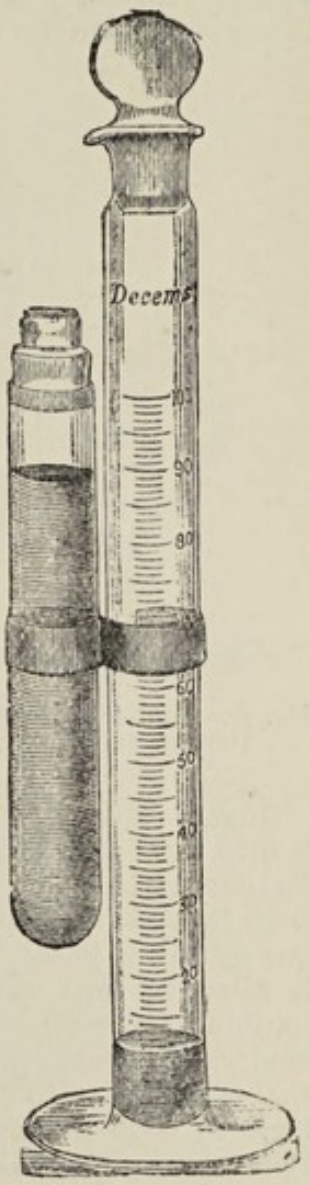


Fig. 72.
Picro-saccharimeter.

(*c*) Should the colour be *darker* than the standard, place some of the boiled liquid in the graduated stoppered tube (fig. 72) to fill 10 divisions of

the scale, while the stoppered tube affixed to the former is filled with the SS. of ferric acetate. Fill up the graduated tube with distilled water until the dark red liquid has the same colour as that of the SS. These tints are best compared in the flat-bottomed tubes supplied with the apparatus.

(*d*) Read off the level of the fluid in the saccharimeter, each division above 10 = 0·1 grain per fluid oz. Thus, 13 divisions = ·3 grain per fluid oz.

(*e*) If more than 8 grains per oz. are present further dilution is required. Full instructions are supplied with the apparatus.

4. **Fermentation method.**—Sir William Roberts' method depends on the diminution of the S.G. which the fluid undergoes during fermentation. Every degree lost in the S.G. corresponds to 1 grain of sugar in a fluid ounce. Recently a modification of this method has been introduced in Germany under the title of **Einhorn's fermentation saccharimeter** (fig. 73). Estimate the S.G. of the urine, which is diluted according to the S.G. as follows:—If the urine have a

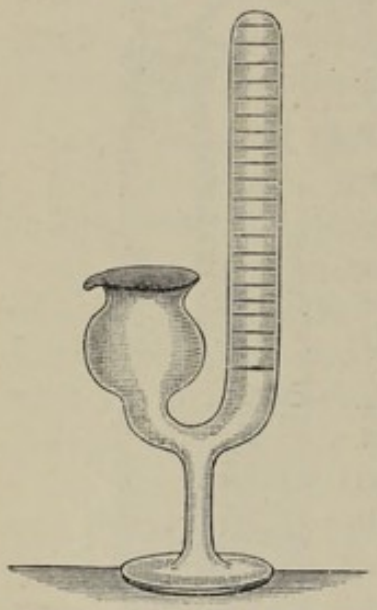


Fig. 73.—Einhorn's fermentation saccharimeter.

S.G. 1018 to 1022, dilute it with 2 vols. water.
,, 1022 to 1028, ,, ,, 5 ,,
,, 1028 to 1038, ,, ,, 10 ,,

Place 10 c.c. urine in the apparatus. Add 1 grm. of yeast to the urine in the tube, incline the latter until the fluid flows into the limb of the latter. Let the apparatus stand at the ordinary temperature for 15 hours, and then the quantity of CO_2 given off is read off. The scale on the tube is empirical, and indicates directly the percentage of sugar in the urine.

5. **Acme sacchar-ureameter** (fig. 74).—This is a simple apparatus for the direct estimation of sugar and urea in urine—the former by the fermentation test, the latter by the hypobromite method.

Sugar.—Measure 1 volume of the urine in the tube so marked, and pour it into the bottle *a*. Wash out with water, and add to the urine. Dilute further with water if the urine contains much sugar. Acidify the urine with tartaric acid until acid to test-paper ($\frac{3}{4}$ to 1 p.c. of free acid). Add a few grains of yeast, and connect up the apparatus. The measuring tube *b* is filled to zero with a saturated solution of common salt (the CO_2 is soluble in water). When *b* is full *c* must be empty. Place the whole in a moderately warm place—the surrounding temperature should be such as to enable it to rise to 92° to 94° F. When the fermentation ceases—or from time to time during the time of fermentation—lower *c* until the levels of brine are equal. Allow it to cool, and read off the result.

6. Other sugars may be found in urine abnormally—*e.g.*, **Lactose** in women suckling their children, and in some rare cases **pentoses** ($C_5H_{10}O_5$), but details regarding these are omitted here.

7. **Glycuronic acid**, which is not a sugar, is sometimes found in urine,

especially after the use of such drugs as chloral and chloroform. It reduces Fehling's solution, but does not ferment with yeast (Lesson XV., 6).

8. Acetone (C_3H_6O or CH_3COCH_3) very frequently occurs in the urine in *Diabetes mellitus*, more especially towards the termination of such cases,

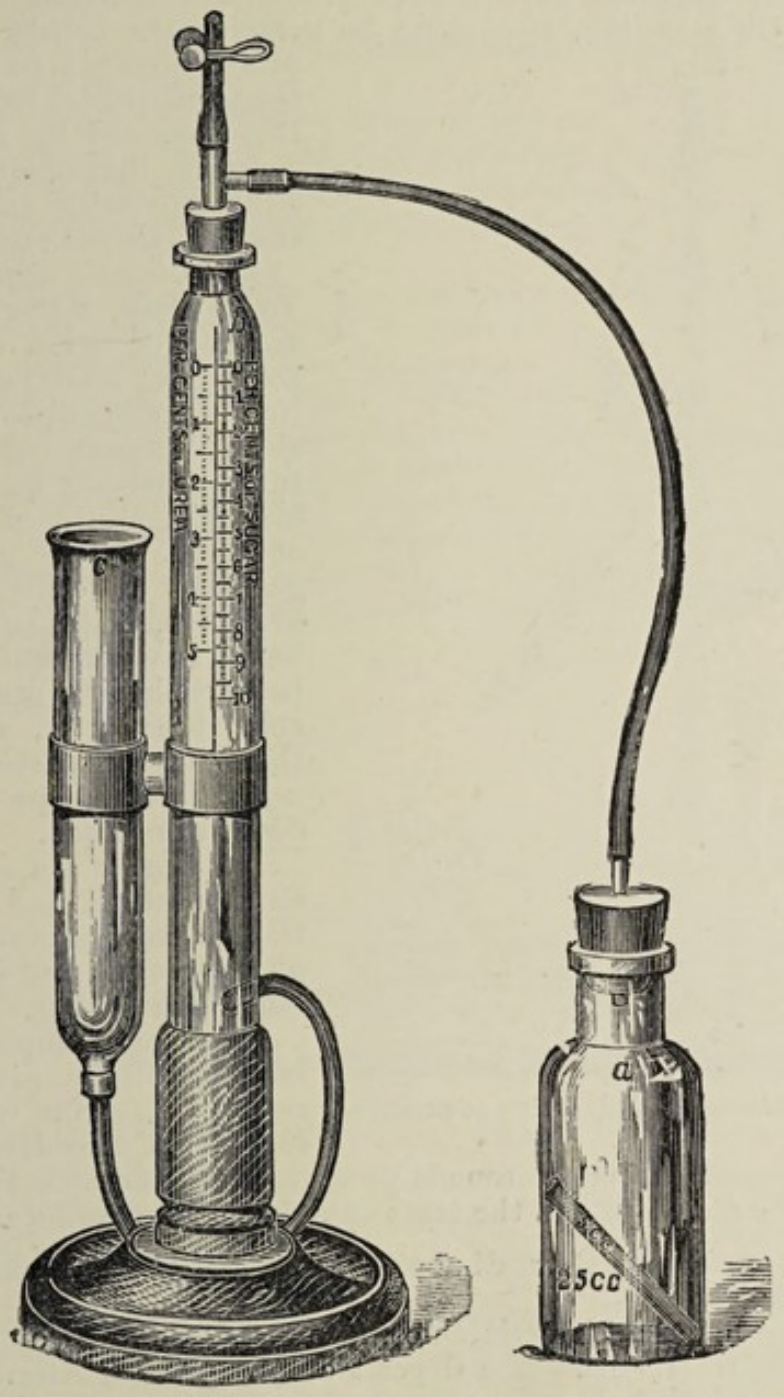


Fig. 74.—Sacchar-ureameter, made by Messrs. Gibbs, Cuxson & Co., Wednesbury.

also in some cases of carcinoma of the digestive tract. It gives rise to "acetonuria." The urine may have a fruity characteristic odour. It is said to occur normally in minute traces. Although it may be detected directly in the urine, as it is a volatile body it is better to distil it off by

the simple method shown in fig. 75, by using a fractional distillation tube.

Tests.—To obtain the acetone, acidulate half a litre of urine with phosphoric acid to prevent frothing. The distillate will give the following reactions :—

(*a*) *Iodoform test of Gunning.*—To the urine or distillate, add tincture of iodine and ammonia until a dark precipitate of iodide of nitrogen is obtained. On standing, if acetone be present, the colour of the precipitate changes to yellow, from the formation of iodoform, which is recognised by its smell and crystals (hexagons and stars). Sahli specially commends this test for clinical purposes.

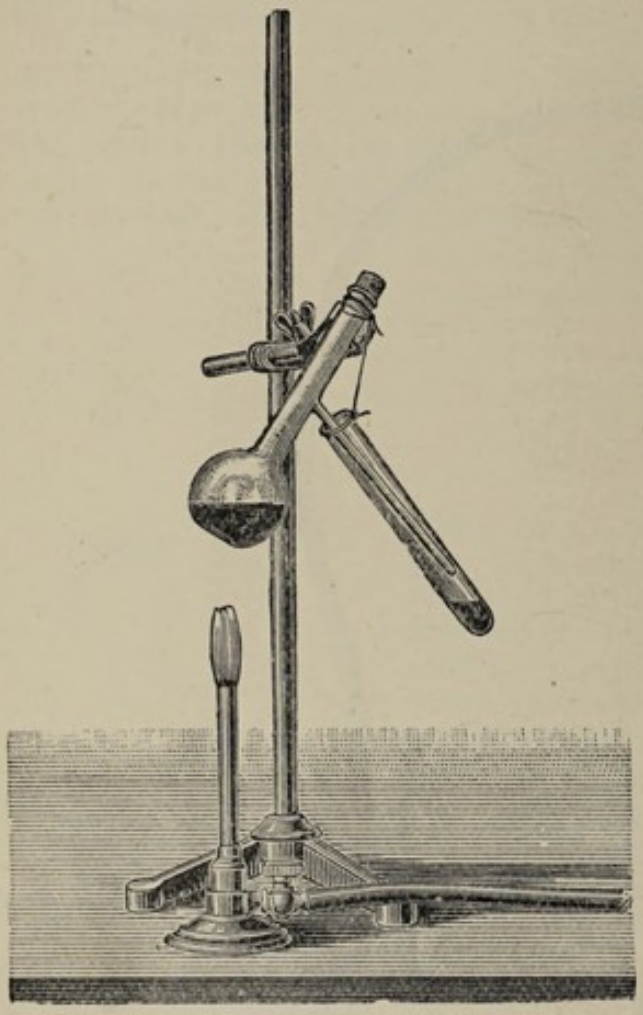


Fig. 75.—Apparatus for distillation of acetone.

(*b*) *Lieben's iodoform test.*—To a weak, watery solution of acetone or urine distillate add solution of NaHO and then solution of iodine dissolved with the aid of potassic iodide (not tincture), warming gently. A yellow precipitate of iodoform is obtained. The precipitate is generally described as forming hexagonal plates or radiate stars, but I have generally found it to be amorphous or granular. Other substances—*e.g.*, alcohol—give the iodoform reaction.

(*c*) Smell the peculiar ethereal odour of acetone.

(*d*) *Legal's test.*—Add caustic soda solution, and then a solution of freshly-prepared sodium nitro-prusside and acetic acid = a red colour.

In all cases employ both tests, but they only give a decided reaction in urine when the acetone is in considerable amount. To be quite certain that acetone is present, a considerable amount of the urine must be distilled, and the tests applied to the distillate.

9. Aceto-acetic acid or di-acetic acid is found in certain diabetic urines, but not in all.

(*a*) To the urine add ferric chloride; a *red* colour is obtained if this acid be present. If there is a deposit of phosphates, filter. The colour disappears on heating.

If a diabetic urine containing aceto-acetic acid be distilled, this acid is decomposed, and acetone is obtained.

10. Carboluria.—The urine is generally greenish-brown or greenish-black, changing on exposure to air. (Salol gives a similar result.) The phenol does not exist as such, but as an organic compound. The colour of the urine is due to pyrocatechin and hydroquinone—derived from phenol—

which become oxidised. The condition might be mistaken for bile in urine, but it does not give Gmelin's reaction.

Phenol.—The method of obtaining phenol from its compound in the urine is given at p. 163. To a watery solution of phenol—

(*a*) Add ferric chloride = a bluish-violet colour.

(*b*) Add bromine water = a yellow (or rather white) precipitate of bromine compounds.

(*c*) Add Millon's reagent = a beautiful *red* colour or deposit. This reaction is aided by heat.

11. **Pyrocatechin** is sometimes found in urine.

(*a*) To a dilute solution add ferric chloride = a *green* colour, which becomes *violet* on the addition of sodium bicarbonate.

(*b*) Add ammonia and silver nitrate, which gives a *black* precipitate of reduced silver.

12. **Salicylates** rapidly pass into the urine and appear as salicylic and salicyluric acids. Add perchloride of iron; filter to get rid of precipitated phosphates. Add some Fe_2Cl_6 = violet colour.

13. **Ehrlich's Diazo-Reaction.**—Make (A) 100 c.c. of a 5 p.c. solution of HCl, and add 1 grm. sulphanilic acid. (B) Sodium nitrite 0·5 p.c. solution. Mix 40 of A and 1 of B immediately before using. Mix with an equal volume of urine, add ammonia so as to form a layer above the urine mixture. In certain pathological urines—that of typhoid fever, and some cases of phthisis—a red colour is obtained. The nature of the body giving this reaction is unknown, but it is related to bilirubin. (Modified by Simon).

LESSON XXIV.

URINARY DEPOSITS—CALCULI—GENERAL EXAMINATION OF URINE.

1. Collecting urinary deposits.—(i.) Place the urine in a conical glass, cover it, and allow it to stand for 12 hours. Note the reaction before and after standing. With a pipette remove some of the deposit and examine it microscopically.

(ii.) Dr. Harris has published the following (*Brit. Med. Journ.*, 1894, vol. i., p. 1356):—The urine is placed in a tube drawn to a fine point, and fixed in a vertical position in a clamp. The pointed end is down, and after being filled it is corked tight. After the deposit subsides and collects in the lower pointed end of the tube, a small quantity of it may be obtained by clasping the tube with the warm hand or by pushing in the cork slightly.

(iii.) **Centrifuge.**—The rapid sedimentation of urine by means of a small centrifuge has proved of great value. By means of

a hand centrifuge (figs. 76, reduced to $\frac{1}{5}$, and 77) any deposit in urine is readily collected at the bottom of a test-tube. Add some antiseptic to prevent the growth of bacteria—*e.g.*, a few drops of formol. There are many other forms of centrifugal machines

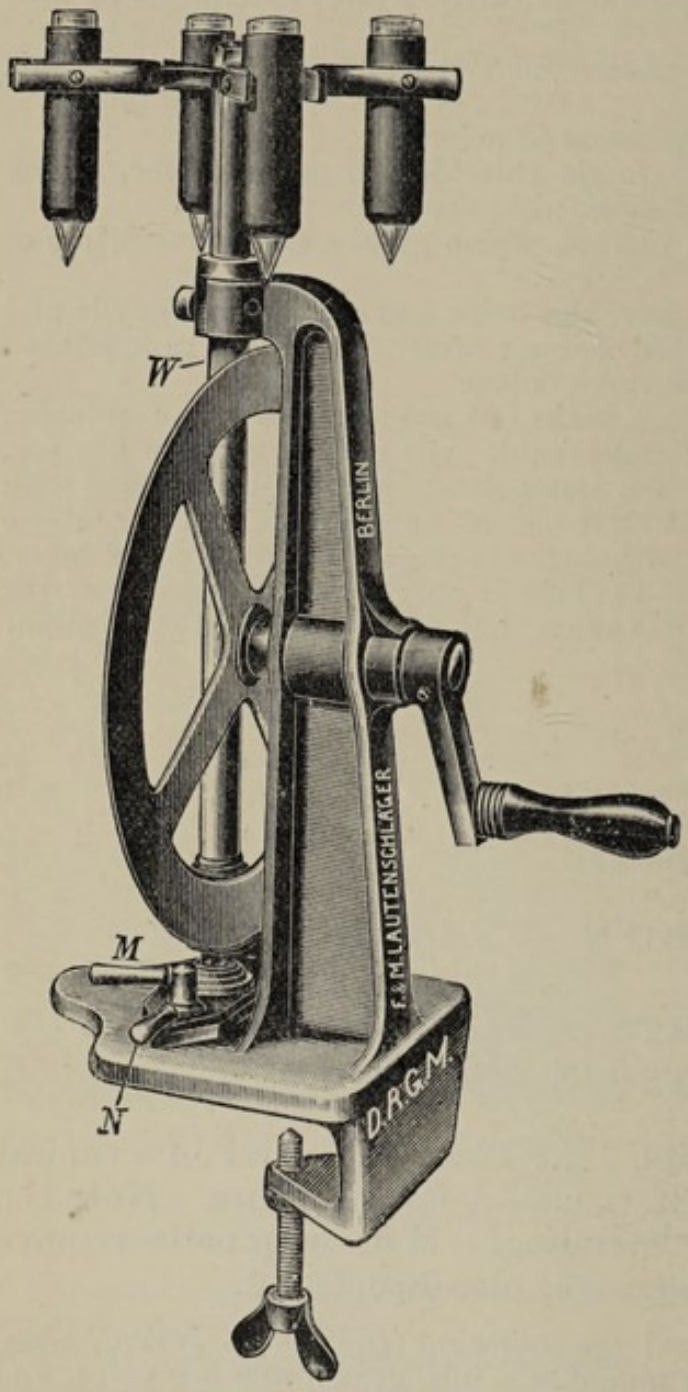


Fig. 76.—Hand centrifuge.
Lautenschläger, Oranienburgerstrasse 54. Berlin.

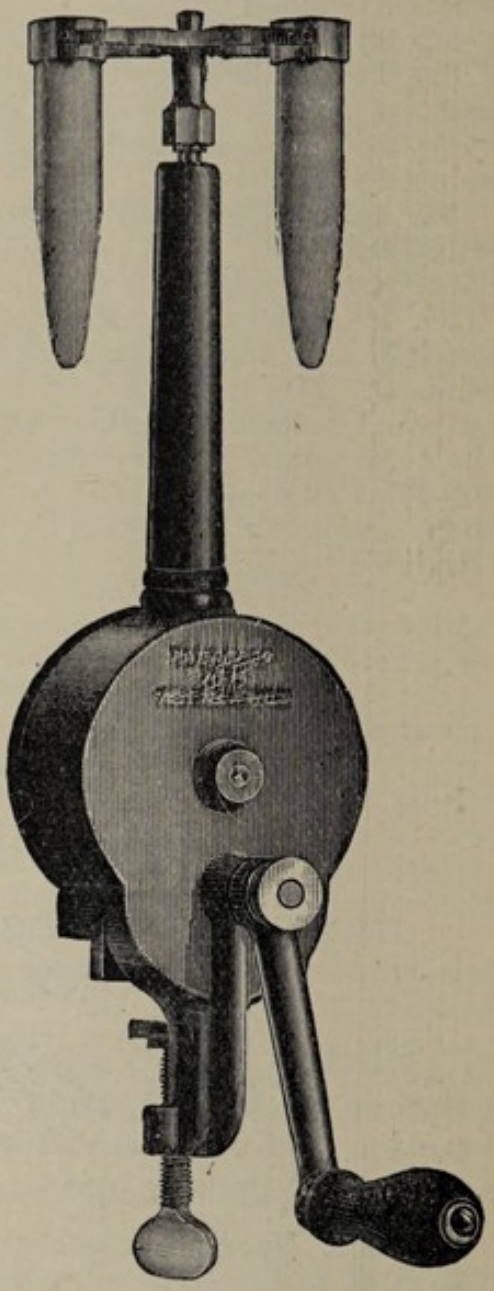


Fig. 77.—High-speed medical centrifuge.

used for separating urinary sediments, and which can be adapted as "hæmatocrits." Amongst these is the high-speed medical centrifuge (fig. 77) made by Metzger, and sold in this country by Messrs. R. & J. Beck, Ltd.

The separate revolving arm, called a **hæmatocrit**, takes the place of the ordinary test-tube cradles, and carries two small capillary tubes for sedimenting blood or fluids when a small quantity only is employed.

The hæmatocrit attachment consists of a metallic frame for carrying two graduated capillary blood cylinders or other tubes. The glass tubes, seated in rubber cushions, are held in position securely by spring cups, so that there is no danger of losing the tubes during rotation. By drawing back the milled heads the tubes are instantly released, and as quickly clamped again into position.

Examining blood with hæmatocrit.—The finger of the patient is thoroughly cleansed with water, and then punctured by means of a spear-pointed needle. The first drop of blood is rejected, and a second drop is secured by very slight pressure. The blood is then drawn by suction into the blood tubes, and placed in the hæmatocrit frame, revolved for one minute, making 5000 revolutions. The rapidity and simplicity of this process is apparent at once. The blood has not time to coagulate. The red corpuscles, having the greatest specific gravity, are thrown to the distal extremity of the tube, and will occupy about one-half of the tube or to about mark 50. The white corpuscles, next in specific gravity, will occupy a position between the red corpuscles and the liquor sanguinis, which is found in the proximal end of the tube, quite clear and free from corpuscles.

Examining milk.—To obtain absolutely reliable test of milk the graduated milk bottle has been devised, and gives results accurate to within one-fifth of 1 p.c. of fat.

5 c.c. of the sample is introduced into the milk bottle by means of a pipette, 1 c.c. of alcoholic solution No. 1 is added and shaken by hand. Then by means of another pipette, solution No. 2 is added little by little with shaking, until the bottle is filled to the neck. It is then placed in the centrifuge and whirled from 1 to 2 minutes, when the fat rises to the top in a clear, yellowish layer, and can be read off in direct percentages. If the milk should be richer than 5 p.c., add 5 c.c. of water to 5 c.c. of milk, mix thoroughly; take 5 c.c. for analysis and multiply the result by two. For cream, add 20 c.c. of water to



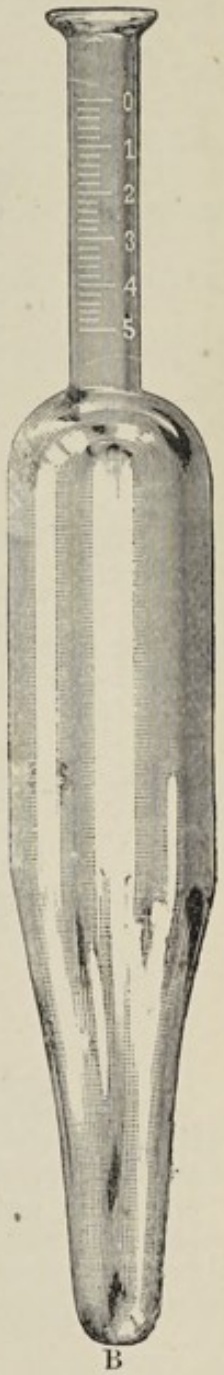


Fig. 78.—Hæmatocrit attachment. B, Milk bottle.

5 c.c. cream, mix; take 5 c.c. for analysis and multiply the result by five.

Solution No. 1 consists of fusel oil, 37 parts by volume; wood alcohol, 13 parts by volume; hydrochloric acid, 50 parts by volume. Solution No. 2, sulphuric acid, S.G. 1·832.

The centrifuge is also used for bacteriological examinations and for sputum. Another form is made by William Petzoldt of Leipzig, the driving arrangement being a pedal attached to a large sewing-machine belt. (Catalogue, 1897, fig. 135.)

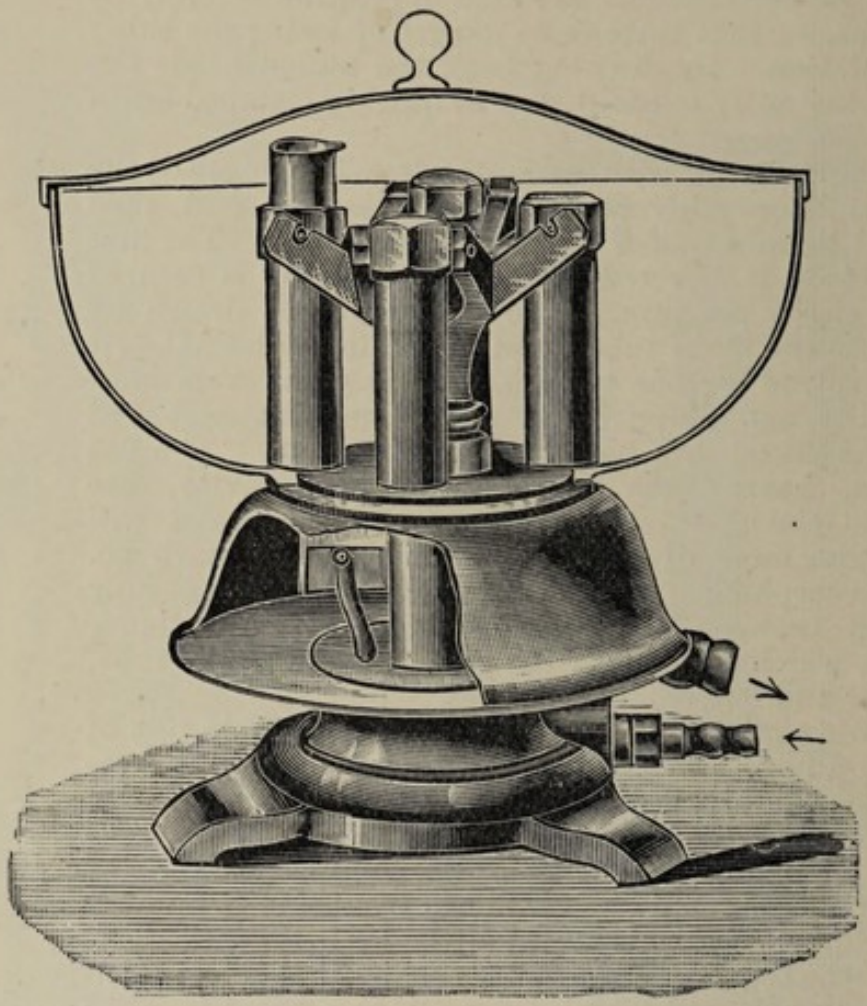


Fig. 79.—Centrifuge. Arranged for water-pressure.

When larger quantities of fluid have to be dealt with, as in the case of the separation of the blood-corpuscles in blood-plasma, a centrifuge, such as Fig. 79, driven by means of water-pressure is essential. Its motor power is unimportant so long as the rate of revolution is obtained, 3000 per min.

There are two classes of deposits, *organised* and *unorganised*.

ORGANISED DEPOSITS.

1. Pus (p. 183).
2. Blood (p. 168).
3. Epithelium.
4. Renal tube casts.
5. Spermatozoa.
6. Micro-organisms.
7. Elements of morbid growths.
8. Entozoa.

2. Pus in urine (**Pyuria**) produces a thick creamy yellowish-white sediment after standing, although its appearance varies with the reaction of the urine. If the urine be *acid* the precipitate is loose, and the pus-corpuscles discrete; if *alkaline*, and especially from ammonia, it forms a thick, tough, glairy mass. The urine is usually alkaline, and is always albuminous, and rapidly undergoes decomposition. Pus is found in the urine in leucorrhœa in the female, gonorrhœa, gleet, cystitis, pyelitis, from bursting of an abscess into any part of the urinary tract, &c.

(*a*) *Donné's test.*—Filter off the fluid, add to the deposit a few drops of strong solution of KHO: the deposit becomes ropy and gelatinous, and cannot be dropped from one vessel into another—due to the formation of alkali-albumin; the deposit is pus. With *mucus* acted on by KHO the deposit becomes more fluid and limpid, clears up, and looks like unboiled egg-white.

(*b*) Microscopically pus-corpuscles are seen; add acetic acid, they show a bi- or tri-partite nucleus. This test is not conclusive.

(*c*) Urine containing pus gives the reactions for albumin, while, if mucus alone be present, it gives only those for mucin.

UNORGANISED DEPOSITS.

A. In Acid Urine.

1. *Amorphous.*

(*a*) **Urates.**—Soluble when heated, redeposited in the cold; when hydrochloric acid is added microscopic crystals of uric acid are formed = urates.

(*b*) **Tribasic phosphate of lime.**—Not dissolved by heat, but disappears without effervescence on adding acetic acid. It is probably tribasic phosphate of lime ($Ca_3 2PO_4$).

(*c*) **Oil globules.** — Very small highly refractive globules, soluble in ether (very rare).

2. *Crystalline.*

(*a*) **Uric acid.**—Recognised by the shape and colour of the crystals and their solubility in KHO.

(*b*) **Oxalate of lime.**—Octahedral crystals, insoluble in acetic acid (fig. 80.

(*c*) **Cystin** (very rare).—Hexagonal crystals, soluble in NH_4HO (fig. 82).

(*d*) **Leucin and tyrosin** (very rare). (Fig. 83.)

(*e*) **Cholesterin** (very rare). (Fig. 40).

B. In Alkaline Urine.

1. *Amorphous.*

(*a*) **Tribasic phosphate of lime** dissolves in acids without effervescence.

(*b*) **Carbonate of lime.** (See (*c*) below.)

2. *Crystalline.*

(*a*) **Triple phosphate.**—Shape of the crystals (knife-rest or coffin-lid), soluble in acids.

(*b*) **Acid ammonium urate.** — Small dark balls, often covered with spines, and also amorphous granules (fig. 81).

(*c*) **Carbonate of lime.** — Small colourless balls, often joined to each other; effervescence on adding acids (microscope).

(*d*) **Crystalline phosphate of lime.**

(*e*) **Leucin and tyrosin** (very rare). (Fig. 83.)

3. Urinary calculi.

They are composed of urinary constituents which form urinary deposits, and may consist of one substance or of several, which are usually deposited in layers, in which case the most central part is spoken of as the "*nucleus.*" The nucleus not unfrequently consists of some colloid substance—mucus, a portion of blood-clot, or some albuminoid matter—in which crystals of

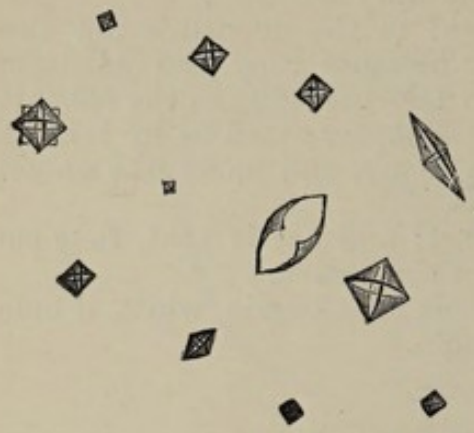


Fig. 80.—Oxalate of lime. Octahedra and hour-glass forms.

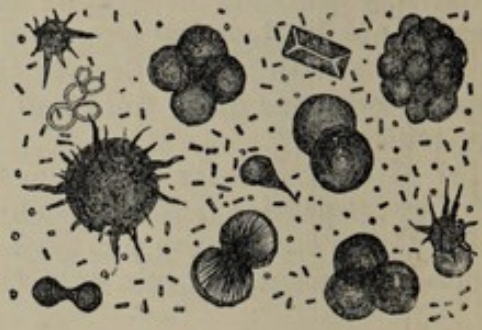


Fig. 81.—Acid urate of ammonium.

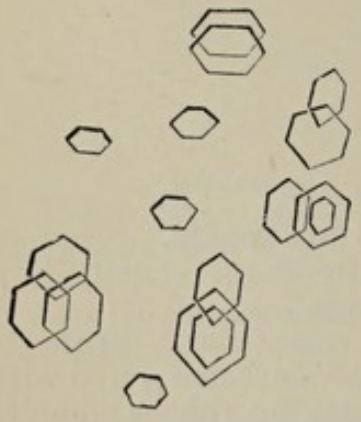


Fig. 82.—Cystin.

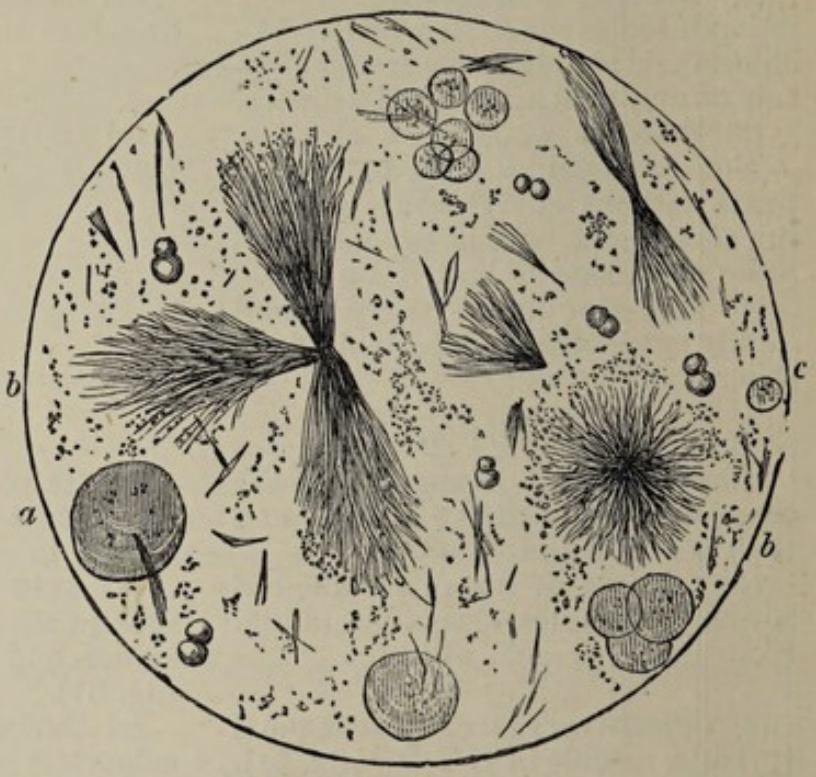


Fig. 83.—*a*, *a*, Leucin balls; *b*, *b*, Tyrosin sheaves; *c*, Double balls of ammonium urate.

oxalate of lime or globular urates become entangled. Layer after layer is then deposited. In certain cases the nucleus may consist of a foreign body introduced from without. Calculi are sometimes classified as *primary* and

secondary; the former are due to some general alteration in the composition of the urine, whilst the latter are due to ammoniacal decomposition of the urine, resulting in the precipitation of phosphates on stones already formed. This of course has an important bearing on the treatment of calculous disorders. Calculi occur in acid and alkaline urine. A *highly acid* urine favours the formation of *uric acid* calculi, because that substance is most insoluble in very acid urine. A *highly alkaline* urine favours the formation of calculi consisting of *calcium phosphate* or *triple phosphate*, as these substances are insoluble in alkaline urine.

4. Method of examining a calculus.

(*a*) Make a section; it may consist of one or more substances; examine it with the naked eye and a portion microscopically.

(*b*) Heat some to redness on platinum foil.

A. If *entirely combustible*, or almost so, it may consist of uric acid or urate of ammonium, xanthin, cystin, coagulated fibrin or blood, or ureostealith.

B. If *incombustible*, or if it leaves *much ash*, it may consist of urates with a fixed base (Na, Mg, Ca), oxalate, carbonate, or phosphate of lime, or triple phosphate.

5. **A. Combustible.**—Of this group, uric acid and urate of ammonium give the *murexide test.*

(i.) **Uric acid** is by far the most common form, and constitutes five-sixths of all renal concretions. Concretions the size of a split-pea, or smaller, may be discharged as *gravel.* When retained in the bladder, they are usually spheroidal, elliptical, and somewhat flattened; are tolerably hard; the surface may be smooth or studded with fine tubercles; the colour may be yellowish, reddish, reddish-brown, or very nearly white. When cut and polished, they usually exhibit a concentric arrangement of layers. Not unfrequently a uric acid calculus is covered with a layer of phosphates, and some calculi consist of alternate layers of uric acid and oxalate of lime. Its *chemical relations:* nearly insoluble in boiling water; soluble in KHO, from which acetic acid precipitates uric acid crystals (microscopic); gives the murexide test (Lesson XX.).

(ii.) **Urate of ammonium calculi** are very rare, and occur chiefly in the kidneys of children; they form small irregular, soft, fawn-coloured masses, easily soluble in hot water.

(iii.) If it is combustible and gives no murexide test, it may consist of **xanthin**—very rare, and of no practical importance.

(iv.) **Cystin** is very rare, has a smooth surface, dull yellow colour, which becomes greenish on exposure to the air; a glistening fracture with a peculiar soapy feeling to the fingers; soft, and can be scratched with the nail; is soluble in ammonia, and after evaporation forms regular microscopic hexagonal plates (fig. 82). It occurs sometimes in several members of the same family.

The other calculi of this group are very rare.

6. (A) Group.—*Murexide test.*

It is obtained { Treat the original powder with potash. { No odour = *Uric acid.* / Odour of NH_3 = *Ammonium urate.*

The residue is not coloured, but becomes yellowish-red on adding caustic potash . . . . . = *Xanthin.*

The residue is not coloured either by KHO or NH_4HO; the original substance is soluble in ammonia, and on evaporation yields hexagonal crystals . . = *Cystin.*

On heating, it gives an odour of burned feathers: the substance is soluble in KHO, and is precipitated therefrom by excess of HNO_3 . . . . = *Proteid.*

7. B. Incombustible.

(i.) Urates (Na, Ca, Mg) are rarely met with as the sole constituent. They give the murexide test.

(ii.) **Oxalate of lime** or mulberry calculi, so called because their surface is usually tuberculated or warty; are hard, dark brown, or black. They cause great irritation of the urinary mucous membrane. When in the form of gravel the concretions are usually smooth, variable in size, pale grey in colour. Layers of oxalate of lime may alternate with uric acid. When heated they blacken, but do not fuse, and then become white, being converted into the carbonate and oxide. The white mass is alkaline to test-paper, and when treated with HCl effervesces (CO_2). Oxalate of lime is not dissolved by acetic acid.

(iii.) **Carbonate of lime.**—Rare in man; usually multiple; dissolve with effervescence in HCl; sometimes crystals occur as a deposit; and they are common in horse's urine.

(iv.) **Basic phosphate of lime** are very rare—white and chalky.

(v.) **Mixed phosphates (fusible calculi)** consist of triple-phosphate and basic phosphate of lime. They indicate that the

urine has been ammoniacal for some time, owing to decomposition of the urea. They are usually of considerable size, and whitish. When triple-phosphate is most abundant, they are soft and porous, but when phosphate of lime is in excess, they are harder. A *whitish* deposit of phosphates is frequently found coating other calculi. This occurs when the urine becomes ammoniacal, hence in such cases regard must always be had to the condition of the urinary mucous membrane. Such calculi are incombustible, but, when exposed to a strong heat, fuse into a white enamel-like mass, hence the name, fusible calculi.

8. (B) Group.

(i.) *The substance gives the murexide reaction, indicates urates.*

The residue is treated with water.

- It is soluble, and the solution is alkaline.
  - Neutralise; add platinic chloride, a yellow precipitate . . . . . = *Potassium.*
  - The residue yields a yellow flame . = *Sodium.*
- Scarcely soluble; the solution is scarcely alkaline; soluble in acetic acid.
  - Ammonium oxalate gives a white crystalline precipitate . . . = *Calcium.*
  - Ammonium oxalate gives no precipitate, but on adding ammonium chloride, sodium phosphate, and ammonia, there is a crystalline precipitate of triple-phosphate . . . . = *Magnesium.*

(ii.) *The original substance does not give the murexide test.*

Treat the original substance with hydrochloric acid.

- It dissolves with effervescence . . . . = *Calcium carbonate.* / *Magnesium carb.*
- It dissolves without effervescence. Heat the original substance, and treat it with HCl.
  - It dissolves with effervescence . = *Calcium oxalate.*
  - There is no effervesc'nce. Heat in a capsule.
    - It melts. The original stone treated with KHO
      - Evolves NH_3 . = *Triple-phosphate.*
      - Evolves no NH_3 . = *Neut. calc. phosp.*
    - It does not melt on heating. . . = *Acid. calc. phosp.*

The following *résumé* by Sahli shows the chief non-organised urinary deposits :—

Readily soluble on heating—*Urates.*

Not soluble on heating :—

Soluble in acetic acid.
- *Phosphates*—without evolution of gas.
- *Carbonates*—with escape of CO_2.
- *Ammonium urate* } with formation of crystals of uric acid.

Insoluble in acetic acid.
- *Oxalate of lime, Leucin, Tyrosin, Xanthin, Cystin,* } Soluble in HCl.
- *Uric acid,* insoluble in HCl.

9. General examination of urine.

(i.) **Quantity** in 24 hours (normal 50 ozs. or 1500 c.c.).

(ii.) **Colour, odour,** and **transparency** (if bile or blood be suspected, test for them).

(iii.) **Specific gravity** of the mixed urine, normal 1015 to 1025. If above 1030, test for sugar.

(iv.) **Reaction** (normally slightly acid ; if alkaline, is the alkali volatile or fixed ?)

(v.) **Heat.**

(*a*) If turbid and becomes clear = *urates.*

(*b*) If it becomes turbid = *earthy phosphates* or *albumin.* Albumin is precipitated before the boiling-point is reached (73° C.), while phosphates are precipitated about the boiling-point. It is necessary, however, to add HNO_3, which dissolves the phosphates, but not the albumin. A case may occur where both urates and albumin are present ; on carefully heating, the turbid urine will first become clear (urates) and then turbid. The turbidity does not disappear on adding HNO_3 (albumin). Estimate approximately the amount of albumin present.

(vi.) Test for **chlorides** with HNO_3 and $AgNO_3$ (if albumin be present, it must be removed by boiling and filtration).

(vii.) If sugar be suspected, test for **sugar** (Trommer's or Fehling's test), and if albumin be present, remove it.

(viii.) Test for **albumin** (and its allies).—Heat *clear* urine. If precipitate, either albumin (globulin) or phosphates. Add acetic acid, precipitate ; dissolves if phosphates ; if not, albumin.

(ix.) Make naked-eye, microscopical, and chemical examinations of the **sediment,** using a centrifuge if necessary.

The following scheme modified from Novy may serve as a basis for a report on a given sample of urine :—

REPORT ON EXAMINATION OF URINE, No.....

Received..... Reported on.....

Physical and Chemical Examination.

Total quantity in 24 hours,.....

Specific gravity, ... Total solids,.....

Reaction,..... Equivalent to.....

Colour,..... ..... Odour,.....

General appearance,.....

Sediment, colour and characters,..... Quantity, ...

	Qualitative test.	Quantitative (grms. in 24 hrs.).		Qualitative test.
Albumin, . . .			Bile acids, . . .	
Glucose, . . .			,, pigments, . .	
Urea, ...			Blood, . . .	
Uric acid (urates), .			Indoxyl, . . .	
Oxalates, . . .			Phenol, . . .	
Chlorides, . . .			Acetone, . . .	
Phosphates, . .			Ammoniacal fermentation, . . .	
Sulphates, total, . .				
,, ethereal, .				

Microscopical Examination.

Sediment unorganised—

Crystalline,..... Amorphous,.....

Sediment organised—

Signed.....

TABLE OF URINARY TESTS.

	Serum-albumin.	Serum-globulin.	Proteoses.	Peptones.	Blood.[1]	Bile.	Dextrose.	Deposit of Urates.	Deposit of Phosphates
Heat, ...	coag.	coag.	—	—	coag. (?)	—	—	clear	—
Ac. Ac. + heat, . .	coag.	coag.	—	—	coag. (?)	—	—	clear	clear
HNO_3 cold, . .	coag.	coag.	pp. (*a*)	—	coag. (?)	Colour (*b*)	—	—	clear
Ac. Ac. + K_4FeCy_6, .	pp.	pp.	pp. (*a*)	—	pp. (?)	—	—	—	—
Picric Acid, . .	pp.	pp.	pp. (*c*)	pp. (*c*)	?	—	+ KHO + Heat = red colour.	—	—
Dil. c. H_2O, . .	—	pp.	—	—	—	—	—	—	—
Sat. $MgSO_4$, . .	—	pp.	—	—	?	—	—	—	—
Sat. Am_2SO_4, . .	pp.	pp.	pp.	—	?	—	—	—	—
*$CuSO_4$ + NaHO, .	Brown-red or violet.	Brown-red or violet.	Pinkish (?)	Pinkish (?)	?	—	Boil = Red pp.	—	—
*Fehling, . . .	,,	,,	,,	,,	,,	—	,,	—	—
Guaiacum + O_2,. .	—	—	—	—	blue	—	—	—	—

(*a*) Disappear on heating; reappear on cooling. (*b*) Impure HNO_3 for bile pigments. (*c*) Soluble on heating.

* Colour reactions apt to be obscured by urine pigments.

[1] Proteid reactions in blood largely depend on amount of blood. In all cases remove coagulable proteids by acidification and heat, before testing for non-coagulable proteids and dextrose.

Exercises on the foregoing.

A. The student must practise the **analysis of fluids** containing one or more of the substances referred to in the foregoing Lessons.

No hard and fast rule can be laid down for the examination of the fluids met with in physiological work at all comparable with the method employed in inorganic chemistry. To begin with, the student must be largely guided by the **physical characters**—colour, smell, taste, &c.—of the fluid he is dealing with, and these will usually give him a clue as to the chemical tests he should employ.

N.B.—In all cases concentrate some of the fluid for subsequent use if required, and complete the concentration on a water-bath to avoid overheating or charring.

A *colourless* solution should be examined for proteids and carbohydrates by the method described in Lesson IV. Marked opalescence indicates milk or glycogen, less distinct opalescence may suggest the presence of starch or certain proteids. Colourless solutions may also contain proteids, urea, bile-salts, leucin, tyrosin, or ferments.

Colour.—A *red* colour will suggest blood, *green* bile, *yellow* urine, *brown* methæmoglobin or hæmatin. If blood-pigment or one of its derivatives is suspected, use the spectroscope at once, and observe the spectrum of (*a*) the original solution, (*b*) the same shaken with air, and (*c*) after the addition of $(NH_4)_2S$.

The *smell* may give an indication as to the presence of bile or urine. Do chemical tests accordingly.

Taste.—If *salt*, examine for globulins or urea, if *bitter* for bile-salts, if *sweet* for sugars.

Following the indications obtained from the physical characters, select from the following **chemical tests** those applicable to the fluid which is being examined :—

1. Test for *proteids* by xanthoproteic and Millon's tests, and for *carbohydrates* by iodine and Trommer's test. The tests for special proteids and carbohydrates have been already described.

2. *Blood.*—Test chemically for proteid constituents, the specific spectrum of Hb or its compounds.

3. *Bile.*—Do Gmelin's test for bile-pigments, and, if proteids are absent, Pettenkofer's test for bile-acids. If proteids (not proteoses and peptones) are present, neutralise, boil, filter, and test filtrate for bile-salts. Remove proteoses and peptones, if present, by precipitation with alcohol, filter and test filtrate for bile-salts.

4. *Tyrosin.*—Add Millon's reagent and boil. A red colour in the solution indicates the presence of tyrosin.

5. *Urea.*—(1) Add sodium hypobromite or impure nitric acid (containing HNO_2). If no bubbles of gas, no urea is present. If gas given off (2) remove phosphates and sulphates by addition of baryta mixture and filtration, and remove proteids (see 3), concentrate the filtrate if necessary, place a drop on each of two slides, allow one to evaporate slowly under a cover-glass, and to the other add a drop of strong pure HNO_3 and cover. Examine the former for crystals of urea, and the latter for crystals of urea nitrate. (For other tests see Lesson XVIII.)

6. *Uric acid.*—If in solution, is in the condition of a urate. (1) Add a drop of HCl and allow to stand for 24 hours. Examine deposit for crystals of uric acid. (2) Concentrate original solution (after removal of any proteids present), and apply the murexide test to a small quantity.

7. *Kreatinin.*—Add a drop of dilute solution of nitroprusside of sodium and excess of caustic soda. A burgundy-red colour indicates kreatinin.

8. *Ferments*—(*a*) *Digestive ferments.*—Place 5 c.c. of the suspected fluid in each of four test-tubes. Label these A, B, C, and D. Neutralise the fluid in C and D, if necessary. To A add 5 c.c. 4 p.c. HCl and a thread of boiled fibrin, to B 5 c.c. of 2 p.c. sodium carbonate solution and a thread of boiled fibrin, to C 5 c.c. starch solution, and to D 5 or 10 c.c. milk. Place the four tubes, along with four control tubes A′, B′, C′, D′ (the contents of which are the same as those of A, B, C, and D, but without the suspected solution), on a water-bath at 40° C. After a time (10 to 30 mins.) examine the tubes. Digestion in A, B, or C, or coagulation of the milk in D, indicates, if there is no corresponding change in the control tube, the presence of *pepsin*, *trypsin*, *amylolytic* ferment or *rennin* respectively.

(*b*) *Blood ferment.*—If the solution is suspected to be salted plasma, or if it be oxalate plasma, in the former case dilute with water and place in a water-bath (Lesson V., 15). In the latter add calcium chloride (Lesson V., 14), and observe if coagulation occurs. This will also show presence of fibrinogen.

N.B.—In all cases make a note of what you do, the result thereof, and your inferences. The following form is convenient:—

Experiment.	*Observation.*	*Inference.*

B. Examination of solid substances.

Physical characters.

1. The *colour* may suggest blood-pigment, or one of its derivatives, or bile-pigment.

2. *Taste* may indicate bile-salts, urea, or sugar.

3. Examine deposit **microscopically**—amorphous or crystalline. If the latter, the substance may be recognised by its crystalline form—*e.g.*, uric acid, leucin, tyrosin, cholesterin, &c.

4. Burn some in a tube; note smell. Does it leave an ash?

5. Examine its *solubility* in cold and warm water, caustic soda, dilute acid, saline solutions, alcohol, and ether. Test the solution in the first four reagents as directed under examination of fluids. Examine the ethereal solution for *fats* and *cholesterin*.

Cholesterin.—(1) Evaporate a little of the ethereal solution in a watch-glass, and add a drop of strong H_2SO_4. A red colour indicates cholesterin. (2) Examine microscopically. Cholesterin crystallises from ethereal solution in colourless needles, from solution in boiling alcohol in its characteristic plates.

C. Analysis of urine.—The student must also practise the analysis of urines containing one or more abnormal constituents, and he must also practise the estimation of the quantity of the more important substances present. Both sets of processes must be done over and over again, in order that he may perfect himself in the methods in common use. (See scheme, p. 188.)

For a list of the reagents and solutions required for Part I. see *Appendix*.

PART II.—EXPERIMENTAL PHYSIOLOGY.

Each student must **provide himself with** *a large and a small pair of scissors; a large and a fine-pointed pair of forceps; scalpel; blunt needle or "seeker" in handle; watch-glasses, narrow glass rod drawn out at one end to act as a "seeker"; two camel-hair brushes of medium size; duster.*

The students work in pairs at separate tables. Each table is provided with recording drum, muscle chamber, and myograph, two Leclanché cells (quart), induction coil, two du Bois or other keys, a rod on which to wind the wires, basin, frog-plate, bottle of normal saline, in a box—wires, and a wooden scale, showing inches and centimetres.

MUSCLE AND NERVE.

LESSON XXV.

GALVANIC BATTERIES AND GALVANOSCOPE.

1. Daniell's Cell is the standard cell for physiological work on account of the constancy of the current it yields. It consists of a glazed earthenware pot (18 × 9 cm.) (fig. 84), containing a saturated solution of copper sulphate. Crystals of $CuSO_4$ are placed in it to keep the solution saturated (depolarising agent). In the copper solution is placed a roll of sheet-copper, provided with a binding screw. Within is a porous unglazed cylindrical cell containing 10 p.c. H_2SO_4. A well amalgamated rod of zinc, provided with a binding screw, is immersed in the acid. The zinc is the negative pole or **Kathode** (–), and the copper the

positive pole or **Anode** (+). E.M.F. (electromotive force) or voltage is about 1·07 volts. The H liberated at the zinc plate combines with the sulp-ion from the $CuSO_4$, and fresh metallic copper is deposited in the copper plate, hence the constancy of action of this cell.

N.B.—After using a battery the zincs must be washed and dried, the porous cells must be carefully washed, and completely immersed in a large quantity of water, frequently renewed.

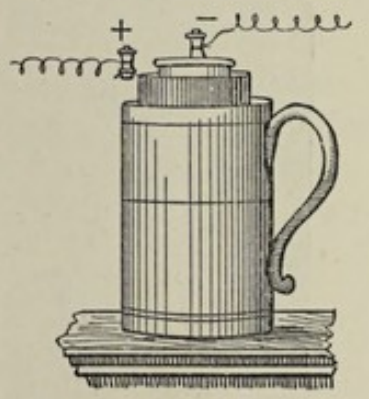


Fig. 84.—Daniell's cell.

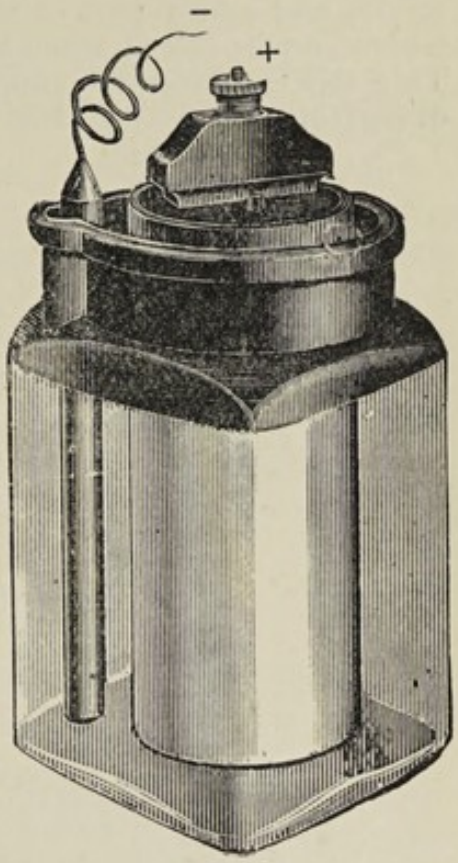


Fig. 85.—Leclanché cell.

2. A Leclanché cell (fig. 85) consists of a glass vessel containing a saturated solution of ammonium chloride, which replaces the acid of the other batteries, and in it dips a rod of amalgamated (zinc – pole). The + pole consists of a carbon plate fixed in a porous pot packed round with granulated carbon and manganese dioxide—the whole filled up with ammonium chloride solution. The MnO_2 yields oxygen slowly, which combines with the nascent hydrogen to form water, but as the hydrogen collects the cell polarises if the circuit be kept closed for a long time, so that it is used when a current is required only for short periods. It must not be used too long in a primary coil, nor must it be left "short-circuited." E.M.F. about 1·5 volts. It has many advantages—no fumes, no acids, always ready—which make it particularly useful for the work of the student.

3. Dry batteries.—Most are modified Leclanché cells. They are convenient for many purposes—*e.g.*, Obach dry battery, manufactured by Siemens.

4. Grenet or Bichromate cell (fig. 86) is a one-fluid battery. It consists of a glass bottle containing one zinc and two carbon plates immersed in the following mixture:—Dissolve 1 part of potassic bichromate in 8 parts of water, and add 1 part of sulphuric acid. The zinc is attached to a rod, which can be raised when it is desired to stop the action of the battery. This cell is convenient enough when it is not necessary to use a current of perfectly constant intensity. E.M.F. about 1·8 volts.

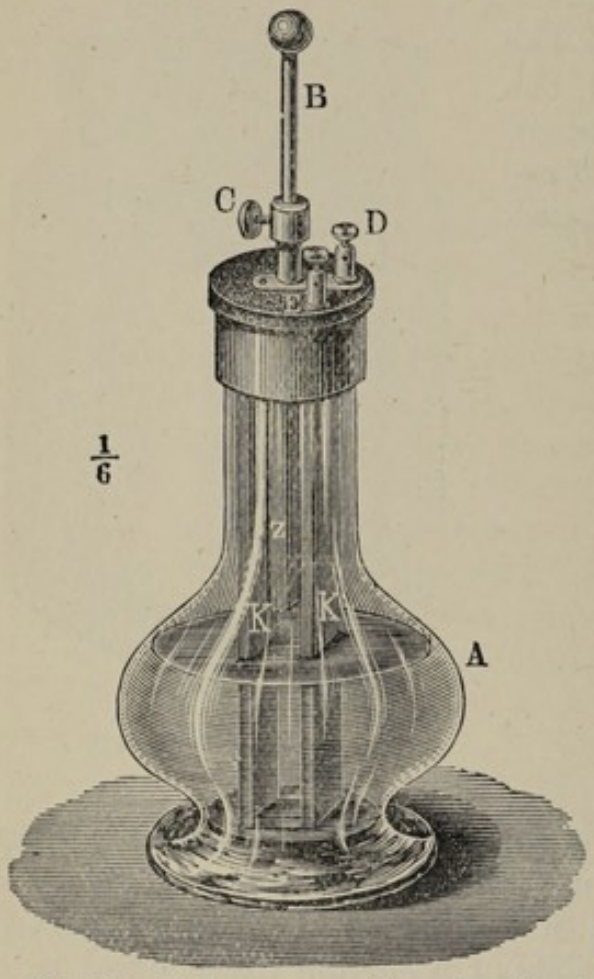


Fig. 86.—Bichromate or Grenet cell. *A*, Glass vessel; *K K*, Carbon; *Z*, Zinc; *D*, *E*, Binding screws for wires; *B*, Rod to raise or depress zinc in the fluid; *C*, Screw to fix *B*.

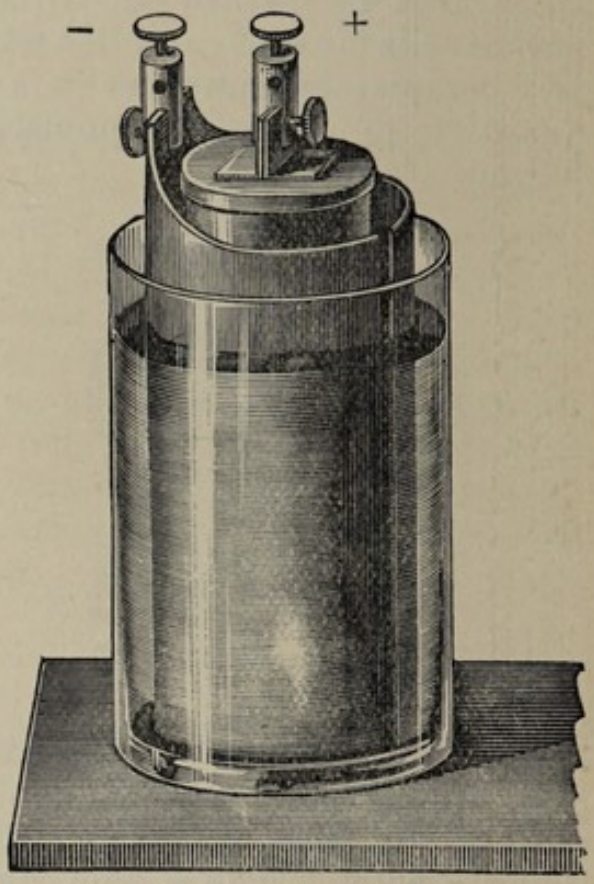


Fig. 87.—Large Grove's element.

5. Grove's cell (fig. 87) consists of an outer glazed earthenware, glass, or ebonite jar, containing amalgamated zinc and 10 p.c. H_2SO_4. In the inner porous cell is platinum foil with strong nitric acid. The platinum is the + positive pole or anode, the zinc the – negative pole or kathode. Sometimes for physiological purposes small Grove's cells are used. When in use the battery ought to be placed in a draught chamber to prevent the nitrous fumes from affecting the experimenter.

6. Wires, connections, or leads.—Cotton-covered wire (No. 18) is used to connect up the batteries. The ends of the wire must be bared and polished by scraping with a knife. See that there are no flaws or cracks in wire. Roll the wires into spirals on the wooden rod provided, and then when the apparatus is set up

the whole will present an orderly appearance, and the course of the current can then be readily traced. Keys can be inserted to control the course of the current.

7. **Galvanoscope or detector, or current indicator.**

(*a*) Charge a Daniell's cell and attach a copper wire to the negative pole (zinc), and another to the positive pole (copper). On bringing the free clean ends of the two wires together the circuit is made, and a current of **continuous, galvanic,** or **voltaic electricity** circulates outside the battery from the + to the − pole. Prove the existence of this current by its effect on a magnetic needle.

(*b*) Use a vertical **galvanoscope** or **detector** (fig. 88), in which the magnetic needle is so loaded as to rest in a vertical position. It consists of a magnetised needle surrounded by a coil of wire. It indicates the *passage* and *direction* of a current. If properly graduated it would represent a galvanometer. A needle attached to this moves over a semicircle graduated in degrees. Connect the wires from the + and - poles of the Daniell's battery with the binding screws of this instrument, and note that when the circuit is made the needle is deflected from its vertical position, but the angle of deflection is not directly proportional to the current passing in the instrument. Break the circuit by disconnecting one wire, and notice that the needle travels to zero and resumes its vertical position. Introduce a commutator (fig. 99) with cross bars to show how the commutator reverses the direction of the current. The detector made by Stöhrer, of Leipzig, is a convenient form.

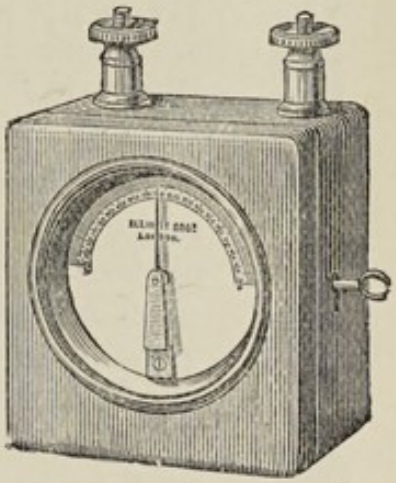


Fig. 88.—Detector.

8. **Effect of constant or voltaic current on tongue.**—Apply the free ends of the wires to the tongue, and note the effect of the current; or a key may be placed in the circuit. The physiological effects of a moderate constant current are but slight on the sensory nerves of the tongue, there being perhaps a slight metallic taste.

9. **Electrical units.**—The unit of current is an **ampère,** the unit of resistance an **ohm,** and the unit of pressure a **volt.** The electromotive force (E.M.F.) is the force or pressure which causes the electricity to move from a point of higher to one of

lower potential, and its unit is the volt. The pressure or potential of a Daniell's cell is about 1 volt. One ampère current is obtained by 1 volt *pressure* through 1 ohm *resistance;* through 20 ohms $\frac{1}{20}$ ampère. The internal resistance of an ordinary cell varies from 1 to 10 ohms.

Current is measured in *ampères.*

$$\text{Current (ampères)} = \frac{\text{Total E.M.F. (in volts)}}{\text{Total resistance (in ohms)}}, \textit{ i.e., } C = \frac{E}{R.}$$

Resistance is measured in *ohms.* For practical purposes 1 ohm resistance is = that of a pure silver wire 1 mm. diameter and 1 metre long.

Electromotive force is measured in *volts.* A volt is the amount of electrical energy which will produce 1 ampère current through 1 ohm resistance.

The "ions," which are supposed to wander from the point of highest electrical potential or anode towards the point of lowest potential or kathode, are called "kations"—*e.g.*, H^+, NH_3^+; those moving from lowest to highest potential are "anions"—*e.g.*, Cl^-, Br^-, NO_2^-, SO_4^-; the quicker the ions move the more electricity transferred by them in the unit of time.

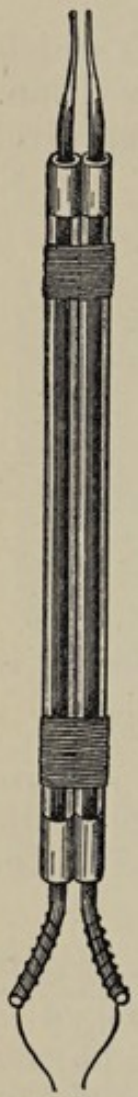


Fig. 89. Hand-electrodes.

10. Make hand-electrodes (every student must make a pair for himself).—(*a*) Take a piece of double or twin wire (No. 16) enclosed in gutta-percha (that used for electric bells), about 6 to 7 cm. long ($2\frac{1}{2}$ to 3 inches). Remove the gutta-percha from the ends. By means of a file taper one pair of ends to blunt points, to the other ends solder pieces 60 to 90 cm. long (2 to 3 feet) of thin copper wire. Coil the thin wire round a glass or wooden rod to make them into a spiral, and to their free unattached ends solder thicker copper wire 1 inch long.

(*b*) Another form is shown in fig. 89, where the copper wires are thrust through two glass tubes.

11. Glass-seeker or nerve hook.—Take 20 cm. of a glass rod 4 to 5 mm. in diameter. Heat and draw in centre to $1\frac{1}{2}$ mm. in diameter. Cut across the drawn-out part, fuse ends in Bunsen flame, and bend the end over to form a hook or an obtuse angle. This is used for dissecting out or lifting up nerves.

12. Amalgamation of zinc.—(*a*) The zinc should always be well amalgamated. When a cell hisses the zinc requires to be amalgamated. Dip the zinc in 10 p.c. H_2SO_4 until effervescence commences. Lift it out and place it on a shallow porcelain plate. Pour some mercury on the zinc, and with a piece of cloth or coarse brush rub the mercury well over the zinc. Dip

the zinc in the acid again, and then scrub the surface with a rag under a stream of tap water.

(*b*) "**Amalgamation mixture.**"—Heat gently to dissolve 4 parts of mercury in 5 parts of nitric acid and 15 parts of hydrochloric acid; then add 20 parts HCl. The zincs, after being well cleaned, as directed above, are dipped into this mixture, or the mixture may be applied to the clean zinc by means of a brush.

13. **Wilke's pole-reagent paper.**—(*a*) This is a convenient method for determining the (−) pole in any combination. Moisten one of the papers, place it on a clean piece of glass, and touch the surface with the two wires coming from the battery; a red spot indicates the negative pole.

14. **Electrolysis of potassium iodide.**—(*b*) Filter paper is dipped in starch paste and dried. This starch paper is preserved, and can be used from year to year. A strip is moistened with KI solution, and the electrodes applied. The iodine-starch reaction—dark-blue iodide of starch—indicates the anode (*L. B. Mendel*).

LESSON XXVI.

KEYS—COMMUTATOR—RHEOCHORD.

It is convenient to make or break—*i.e.*, close or open—a current by means of **keys**, of which there are various forms.

1. **Du Bois-Reymond key** (figs. 90, 91) consists of a plate of vulcanite, attached to a wooden or metallic framework (fig. 91) which can be screwed to a table. Two oblong brass bars (II. and III.), each provided with two binding screws, are fixed to the ebonite, while a movable brass bar (IV.) with an ebonite handle is fixed to one of the bars, and can be depressed so as to touch the other brass bar. The complete contact is gradually, though rapidly, made, hence the key is sometimes called a "friction key." The classic form of the du Bois key has disadvantages in some respects over those keys which are not screwed upon the table edge, but have a heavy weighted base. A very convenient and useful key, with heavy metallic base and suitable for "short circuit" arrangement, is made by Runne. Mercury connections.

2. **Two ways of using du Bois key** :—

1st. As a simple contact or "in-circuit" key (fig. 92).

2nd. As a short-circuiting key (fig. 93).

(1) *When the key is closed the current is made, and when it is opened the current is broken* (fig. 92). Apparatus.—Daniell or Leclanché cell, detector, three wires, and a du Bois key.

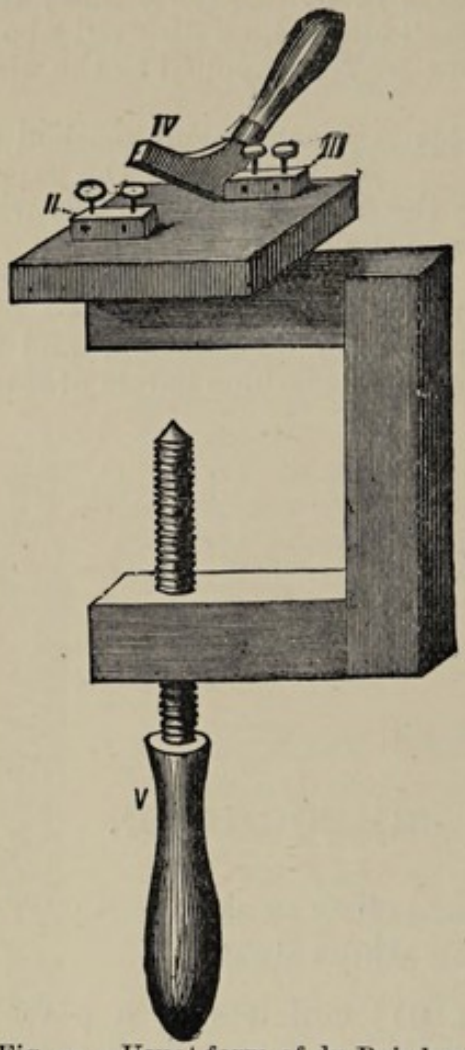


Fig. 90.—Usual form of du Bois key.

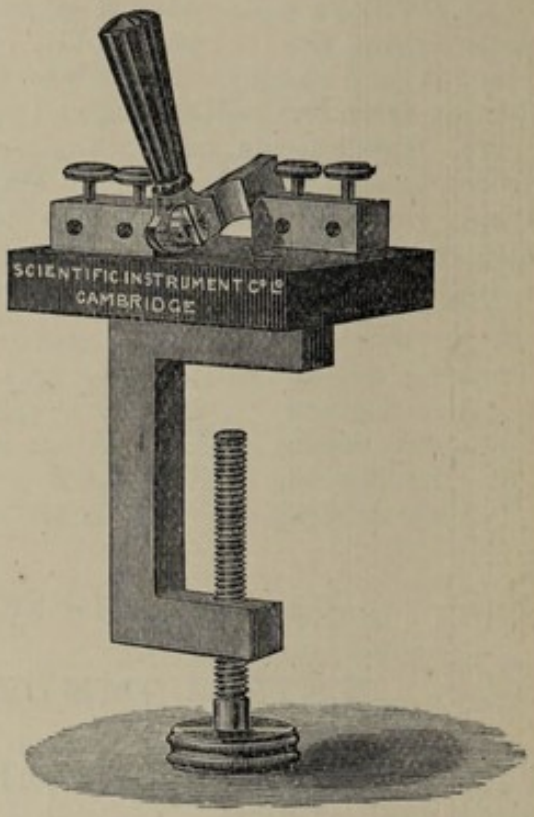


Fig. 91.—Newer form of du Bois key.

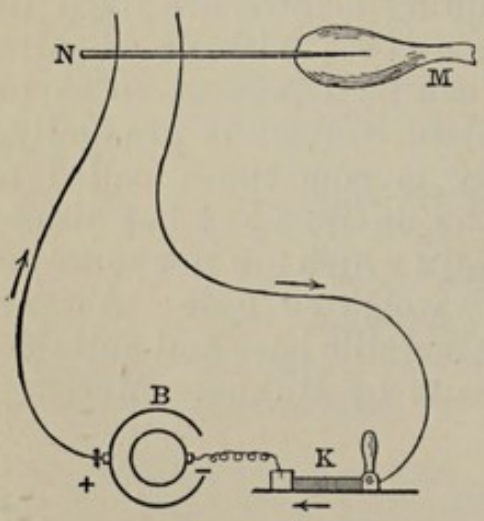


Fig. 92.—Scheme of du Bois key. *B*, Battery; *K*, Key; *N*, Nerve; *M*, Muscle.

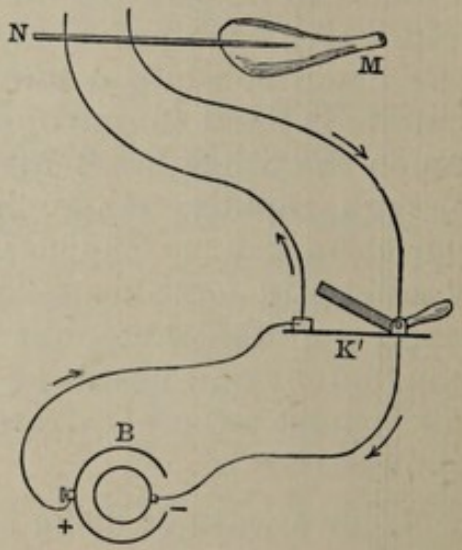


Fig. 93.—Scheme of du Bois key for short-circuiting. *N*, Nerve; *M*, Muscle; *B*, Battery; *K'*, Key.

(*a*) As in the scheme (fig. 92), connect one wire from – pole of the battery to one brass bar of the key, and the other brass bar with one binding screw of the detector. Connect the other binding screw of the detector with the + pole of the cell.

(*b*) On depressing the key (*i.e.*, making the circuit) the needle is deflected, on raising it (*i.e.*, breaking the circuit) the needle passes to zero. This method of using the key we may call that for "**making** and **breaking** a current," but it does not make a "clean" make and break on account of the gradual contact and friction. For this purpose a spring contact or mercury key is preferable. The du Bois key is always used in this manner "in circuit" with a battery, or in the primary circuit of an induction machine.

(2) *When the key is closed the current is said to be* "**short-circuited.**" **Apparatus.**—Cell, detector, four wires, and du Bois key.

(*a*) As in scheme (fig. 93) connect the + pole of the cell to the outer binding screw of one brass bar of the key, and the – pole to the outer binding screw of the other brass bar. Then connect the inner binding screws of both brass bars with the detector.

(*b*) Observe when the key is depressed or closed, there is no deflection of the needle—*i.e.*, when the current is cut off from the long circuit beyond the key or bridge; when the key is raised the needle is deflected. When the key is depressed, the current is said to be "**short-circuited,**" for the key acts like a bridge, "bridging a circuit," and so a large part of the current passes through it back to the battery, while only an excessively feeble current passes through the wires beyond the key; so feeble is it that it does not affect a nerve. On raising the key, the whole of the current passes through the detector or nerve, as the case may be. This method of using the key is called the method of "short-circuiting."

(*c*) Test the effect of a galvanic current by applying the electrodes to the tip of the **tongue.**

N.B.—In using the key to apply an induction current to excite a nerve or muscle, always use this key by the second method—*i.e.*, always place a short-circuiting key in the secondary circuit.

3. Mercury key.—Where a *fluid* contact is required the wires dip into mercury. It is used in the same way as a du Bois key (fig. 94) for make and break shocks, but here the whole contact is made practically at the same moment.

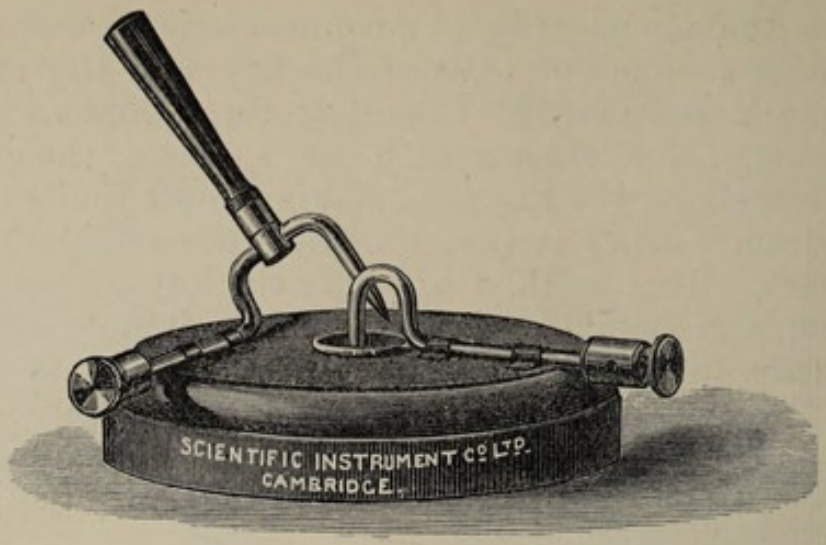


Fig. 94.—M. and B. Mercury key.

4. Morse key (fig. 95).—If it is desired to make or break a current rapidly, this key is very convenient. If this key be used to make and break the primary circuit, connect the wires to B and C; when the style of the lever, *l*, is in contact with *c*, the current does not pass in the primary circuit. On depressing the handle, K, the primary circuit is made. If, however, the wires be connected to A and B, the current passes and is broken on depressing K. To use this key as a short-circuiting key, connect the wires from the battery to A and B, and those of the electrodes to A and C. The current is short-circuited until K is depressed, when the current passes from C to A through the electrode wires.

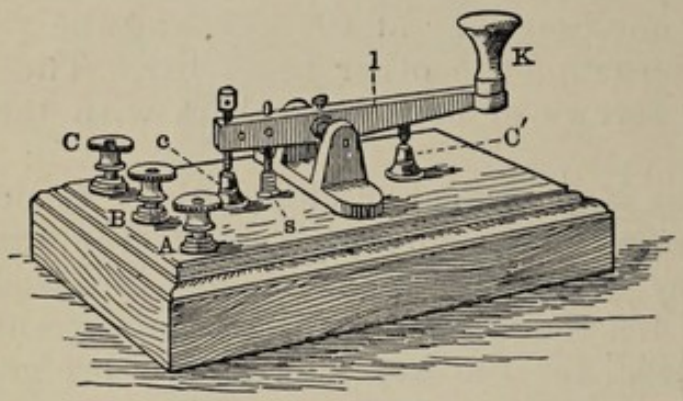


Fig. 95.—Morse key. The connections are concealed below, but are *B* to *l*, *A* to *c*, *C* to *C′*.

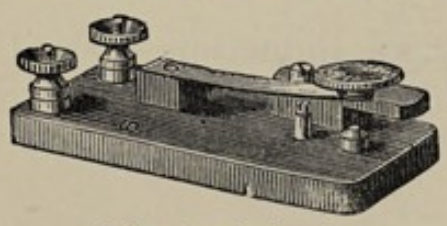


Fig. 96.—Spring key.

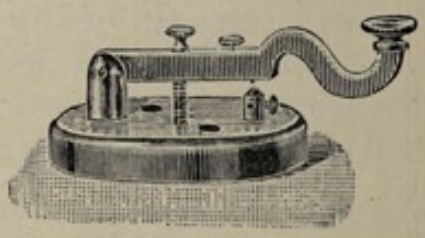


Fig. 97.—Contact key made by Zimmermann, chiefly used for "reaction-time."

5. Contact or Spring key (figs. 96, 97) is also very useful for rapidly making and breaking a circuit, or for giving a single

shock when a constant strength of stimulus is not necessary. As in the Morse key, the whole contact is practically made at the same moment. The current can only pass between the binding screws when the metallic spring is pressed down. The left end of the spring is in metallic contact with the upper binding screw, while the second binding screw is similarly connected with the little metallic peg at the right-hand end of the fig.

6. **Plug-key** (fig. 200).—Two brass bars are fixed to a piece of vulcanite. The circuit is made or broken by inserting a brass plug between the bars. Each brass bar is provided with two binding screws, to which one or two wires may be attached so that it can be used like a du Bois key, either by the first or second method.

7. "**Trigger or Turn-over key**" is referred to in Lesson XXXV.

8. **Ostwald's key** gives a dry platinum contact and is useful for many purposes. The electrical current is made and opened by means of a screw. It is less in size than a du Bois key (fig. 98.)

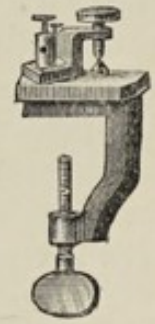


Fig. 98.—Ostwald's key.

9. **Tumbler key.**—This is used in the electric light trade. It is cheap, and is really a friction key like the du Bois form. It can be used either to interrupt or bridge a circuit. A keyboard with several tumbler keys joined in a series forms a useful arrangement when one wishes to pass a current into one or other of several circuits.

10. **Simple switch.**—This is used in the electrical trade, and is cheap. It can be fixed to a frame and be clamped to the table.

11. **Double keys** are sometimes useful—*e.g.*, where it is desired to make or break two separate currents; *e.g.*, to close one and open the other simultaneously.

12. **Polarisation of electrodes.**—When a constant current is led through a nerve for some time it causes electrolysis where the metallic wires come into contact with the liquids of the nerve. The excitability of the nerve is altered by the secondary electromotive changes thus produced, so that the nerve is thereby excited, and the muscle is thrown into contraction.

Apparatus.—Electrodes (fig. 89), two wires, du Bois key, cell, frog.

(*a*) Pith a frog (Lesson XXIX., 1), lay it belly downwards on a frog-plate, and expose one sciatic nerve.

(*b*) Screw the du Bois key to the table, place the copper electrodes under the sciatic nerve, and connect their other ends each with the outer binding screw of the brass bars of the du Bois key. Close the key, and observe that no contraction of the leg muscles occurs.

(*c*) Connect a cell with a du Bois key. Open the key to allow the current to pass through the nerve for 3 or 4 minutes, and observe that there is no contraction as long as the current is passing. Close the key—*i.e.*, short-circuit the battery, and at once a contraction occurs. Remove the battery, close and open the key. Contractions occur, but they gradually get feebler as the polarisation ceases. The contractions are due to polarisation of the electrodes.

(*d*) If non-polarisable electrodes are used, this does not happen.

(*e*) See Lesson XLVIII. for another method.

13. Non-polarisable or unpolarisable electrodes, Lesson XLV. For Ostwald's "Normal Electrode" as modified by Oker-Blom, see *Pflüger's Archiv.*, vol. lxxix., 543, 1900.

14. **Pohl's commutator** (fig. 99) is used for sending a current along two different pairs of wires—**deflecter**, or for reversing the direction of the current in a pair of wires—**reverser**. [It may also be used as a simple Hg-key.] It consists of a round or square wooden or ebonite block with six cups, each in connection with a binding screw. Between two of these stretches a bridge insulated in the middle. The battery or *leading-in* wires are attached to the cups connected with this (1 and 2).

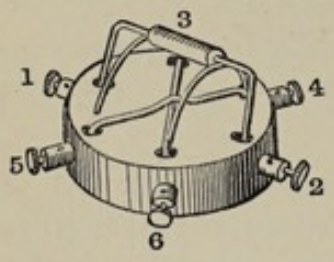


Fig. 99—Pohl's commutator with cross-bars.

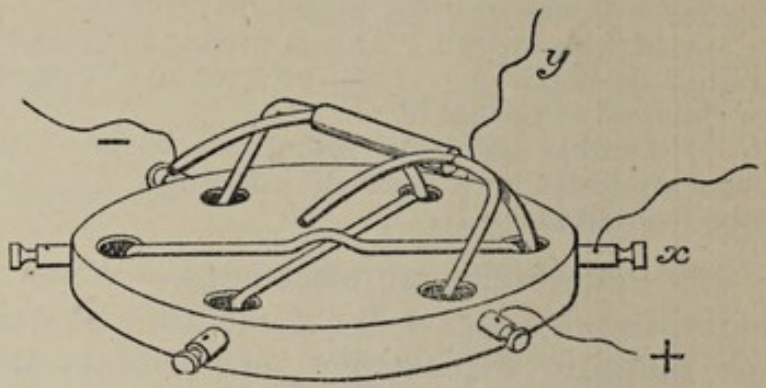


Fig. 100.—Pohl's commutator with cross-bars, arranged for reversing the direction of a current.

When it is used as a deflecter to pass a current through different wires, the cross-bars are removed and the *leading-out* wires are attached to 3 and 4, 5 and 6. On turning the bridge to one side or other, the current is sent through one or other pair of wires. To reverse the direction of a current, only one pair of leading-out wires, besides the battery wires, is attached to the binding screws of the mercury cups—*e.g.*, to 3 and 4, or 5 and 6, the cross-bars remaining in.

It can be used—

(1) To change the *course* of a current—*i.e.*, as a **deflecter**.

(2) To change the *direction* of a current—*i.e.*, as a reverser (fig. 100).

[(3) As a simple mercury key.]

(1) When it is desired to send a current through either of two pairs of wires, connect the commutator *without the cross-bars* — *i.e.*, as a deflecter, as shown in fig. 124. The *leading-in* wires are connected to the Hg-cups at the ends of the rocker, and the *leading-out* wires to the other cups. As the rocker is turned to the one side or the other, so is the current made to pass along the one or other set of wires. The positive pole remains on the same side. Trace course of current.

(2) For reversing the direction of a current, *insert the cross-bars.* The *leading-in* wires are attached as before, but only one pair of *leading-out* wires is attached to the cup on one side only. When the rocker dips into the cup connected with the leading-out wire, the + pole is on the same side, but when the rocker is reversed the other leading-out wire becomes positive. Trace the course of the current.

[(3) As a Hg-key. Connect the wire to a cup at one end of the rocker bridge, and the other wire to the Hg-cup. It then acts as a simple M. and B. key.]

Graduating a galvanic current.—Besides altering the number, arrangement, or size of the cells, we can use a simple rheochord to divide the current, the battery remaining constant, so that weak constant currents of varying strength can thus be easily obtained. From the formula, $C = \frac{E}{R}$, it is obvious that one can decrease the current by increasing the resistance. For this purpose various rheochords and rheostats are used.

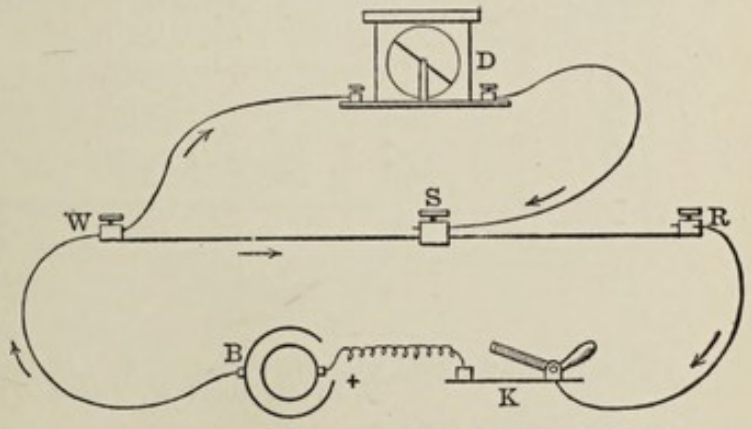


Fig. 101.—Scheme of simple rheochord. *B*, Battery; *K*, Key; *W*, *R*, Wire; *S*, Slider; *D*, Detector.

15. A Monochord is in principle a simple rheochord, and consists of a brass or German-silver wire, about 20 ohms resistance and 1 metre in length, stretched longitudinally along a board, with its ends connected to binding screws and insulated (fig. 101). On the wire there is a "slider," which can be pushed

along as desired. Apparatus.—Monochord, Daniell's cell, detector, du Bois key, five wires.

(*a*) Arrange the experiment as in fig. 101—*i.e.*, with the rheochord as a deriving circuit. When the slider S is hard up to W, practically all the electricity passes along the wire (W, R) back to the battery.

(*b*) Pull the slider away from W, and, in doing so, the resistance in the detector circuit is diminished, and some of the electricity passes along the detector circuit or the "deriving circuit" and deflects the needle. The deflection is greater—but not proportionally so—the further the slider is removed from W. The deflection is nearly proportional to the distance of the slider from W, when the resistance in the detector circuit is great compared with that of the rheochord, which is, of course, the case when a tissue occupies the place of the detector.

(*c*) Make a table showing the extent of deflection of the needle of the detector according to the distance of S from W.

By it one can obtain a small electromotive pressure. It is used to neutralise the "current of injury" in living tissues by the compensation method.

16. The **simple rheochord** may be arranged as in fig. 102; a slider, S, S, consisting of an ebonite cup filled with mercury, can be moved along the wires. Make connections as in fig. 102.

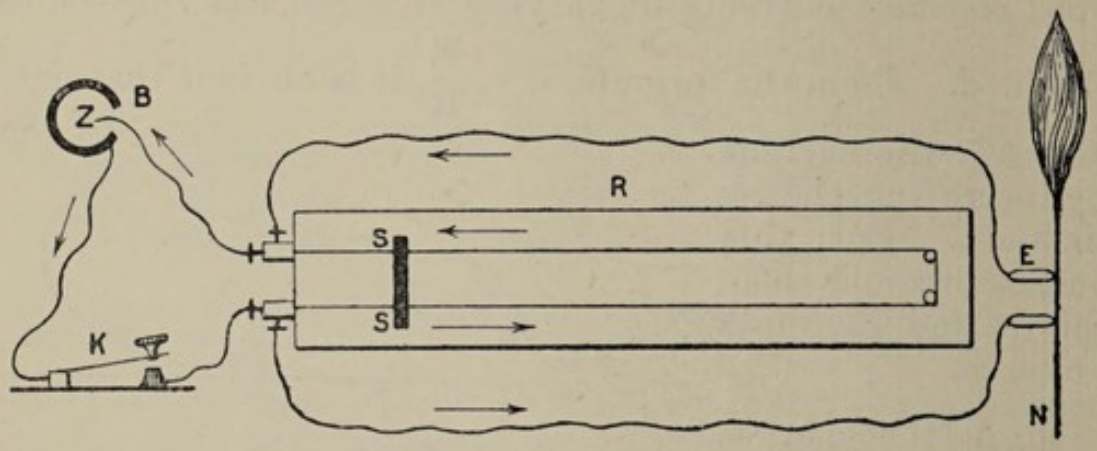


Fig. 102.—Rheochord with Hg-slider, *S*, *S*. *B*, Battery; *K*, Contact spring key; *E*, Electrodes; *N*, Nerve or detector.

Observe, as the mercury cup is pulled away from the binding screws, there is a greater deflection of the needle, but the deflection is not in proportion to the distance of the cup. Make a table of your results.

Distance of Hg-Bridge in cm.	Deflection of Galvanometer.	Distance of Hg-Bridge in cm.	Deflection of Galvanometer.
1	1	10	9·5
2	2·5	15	11
3	4	20	12·5
4	6	30	14

The resistance in the rheochord circuit is low as compared with that in the principal circuit. By means of the slider the resistance in the deriving circuit can be increased or diminished, and, consequently, the magnitude of the current diverted into the principal circuit. The rheochord also affords a means of dividing a current into two parts, according to the respective resistances in the two circuits. A rheochord is also used to compensate any current of injury in nerve and muscle in rheotomic experiments.

17. **Simple rheochord.**—A form used in Oxford consists of a German-silver wire about 20 ohms resistance, wound round ebonite pegs fixed at equal distances at the opposite ends of a wooden board. The board is divided into oblongs, so that each division represents $\frac{1}{100}$ part of the whole length of the wire, which ends in two block terminals, A, B, each provided with two binding screws. One of the terminals of the electrodes is attached to one terminal of the wire (A), and the other to the movable block S, which represents a slider, and which can be applied to any part of the wire at any distance from A. Owing to the great resistance of the nerve as compared with that of the wire, the current through the nerve or muscle is in proportion to the length of wire between the slider S and the block.

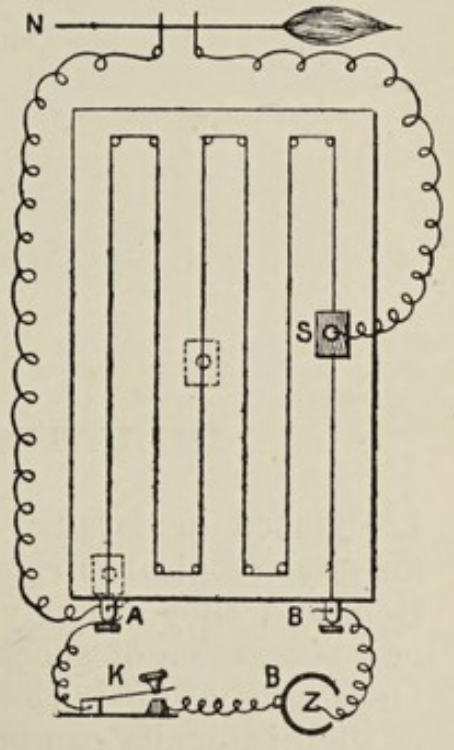


Fig. 103.—Simple rheochord as used in Oxford. *B*, Battery; *K*, Spring key; *A*, *B*, Terminals of rheochord wire; *S*, Slider; *N*, Nerve.

(*a*) Connect a cell, as in fig. 103, with the two block terminals (A, B), interposing a spring key (K). Of the electrode wires, one is connected to A, and the other to the slider S.

Expose the sciatic nerve of a frog, and place the electrodes under it, or make a nerve-muscle preparation and stimulate the nerve. Place the slider close to A, there is no response either at make or break. Place the slider at different distances from A, and note when contraction occurs at make.

18. **Du Bois-Reymond rheochord.**—For some physiological experiments, it is necessary to cause a *gradual* increase or decrease of the current; then this form is useful (fig. 225). In the rheostat, where plugs are inserted or pulled out, the variation of the current is step by step. When the slider L is pushed hard up to 1 and 2, and all the plugs are in, we have the same condition as in a rheostat with all the plugs in. When the slider is pulled away from zero to 100, 1 ohm resistance is gradually thrown into the short circuit. **Apparatus.**—One cell, du Bois-Reymond rheochord, detector, five wires, du Bois key.

(1) Set up apparatus as shown in fig. 102, substituting a detector for the nerve-muscle preparation—*i.e.*, connect battery wires to A and B, introducing a du Bois key in one wire. From A and B connect the other two wires with a detector.

(2) Push up bridge or slider (L) to zero, close key, and note angle of deflection, if any.

(3) Leave key closed and gradually slide the bridge to 1, and then slowly and evenly to 100, noting the deflection of needle.

(4) Open key, remove plug representing 1 ohm, slide bridge to zero, close key, note angle when needle is at rest. It should be the same as at 100 if the R is equal. The angle may be increased by using slider.

LESSON XXVII.

INDUCTION COIL—ELECTRODES.

1. **Induced or faradic electricity** is most frequently employed for physiological purposes. Induction shocks are of short duration, and they are physiologically very active. A single shock or a succession of shocks may be applied. As successive shocks but slightly impair the physiological activity of the tissues, and as their intensity can be accurately graduated, induced electricity is largely used as a stimulus in physiological experiments.

2. **Induction apparatus of du Bois-Reymond.**—In fig. 104 the **primary coil** (R′) consists of 150 to 500 turns of **thick** insulated copper wire, wound on a wooden bobbin with a core of soft iron wires. The wire is thick to offer slight resistance to the galvanic

current. The ends of the wire of the primary coil are connected to the binding screws S″ and S‴. The **secondary** coil (R″) consists of 6000 turns (or more) of **thin** insulated copper wire coiled on a larger wooden bobbin—*secondary coil;* the whole spiral can be moved along the board (B), to which a millimetre scale (I) is attached, so that the distance of the secondary (S) from the primary spiral (P) can be read off, while the cavity of the secondary coil is such that it can be pushed right over the primary coil. The ends of the wire of the secondary coil are connected with the terminals on the secondary coil opposite I in fig. 104. At one end of the apparatus is a **Wagner's hammer**

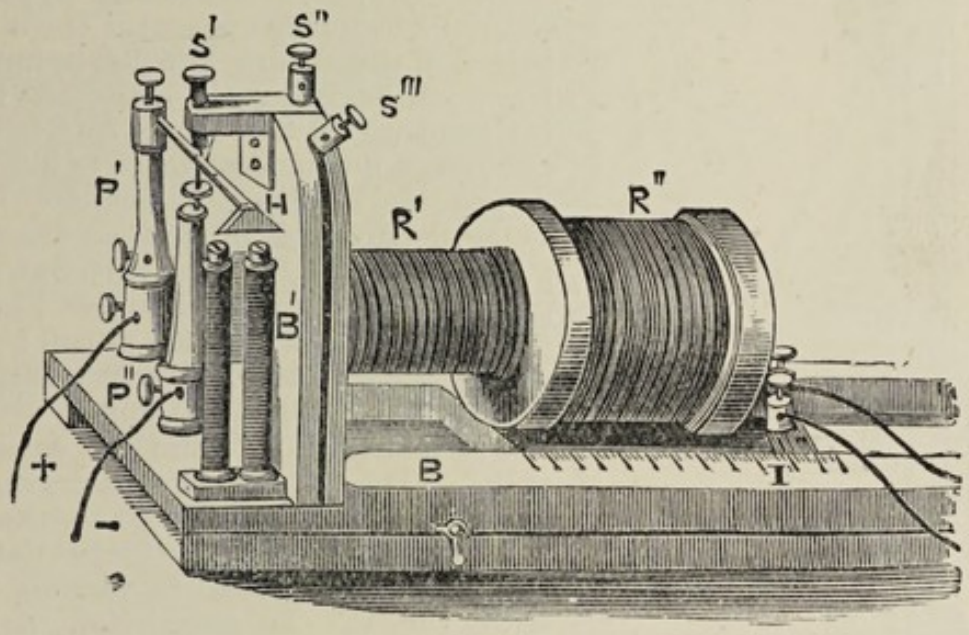


Fig. 104.—Induction apparatus of du Bois-Reymond. *R′*, Primary; *R″*, Secondary spiral; *B*, Board on which R″ moves; *I*, Scale; + −, Wires from battery; *P′*, *P″*, Pillars; *H*, Neef's hammer; *B′*, Electro-magnet; *S′*, Binding screw touching the steel spring (*H*); *S′* and *S‴*, Binding screws to which are attached wires when Neef's hammer is not required.

as adapted by **Neef**—*Neef's hammer*—which is an automatic arrangement for making and breaking the primary circuit. When Neef's hammer is used to obtain what is called an **interrupted current** or "**repeated shocks**," the wires from the battery are connected as in the figure, but when **single shocks** are required, the wires from the battery are connected with a key, and this again with the two terminals of the primary spiral, S″ and S‴ (fig. 104).

Suppose we push the secondary coil right up over the primary, and take this as zero, then an index on the side of the slot will give the distance in millimetres of the S from the P coil, the

current being strongest when the S coil is completely over the P, and diminishing as the S is removed from the P.

In the du Bois coil the strength of the induced shock is conveniently modified by altering the distance between S and P. The strength of the induced shock, however, is not inversely proportional to the distance of S from P; the increase in strength of the induced shock increases in a greater ratio as S approaches P.

3. **Vertical inductorium**, where the secondary spiral moves vertically in a slot, and is compensated by means of a counterpoise, so that it moves easily (fig. 105). It is used in the same way as the other form.

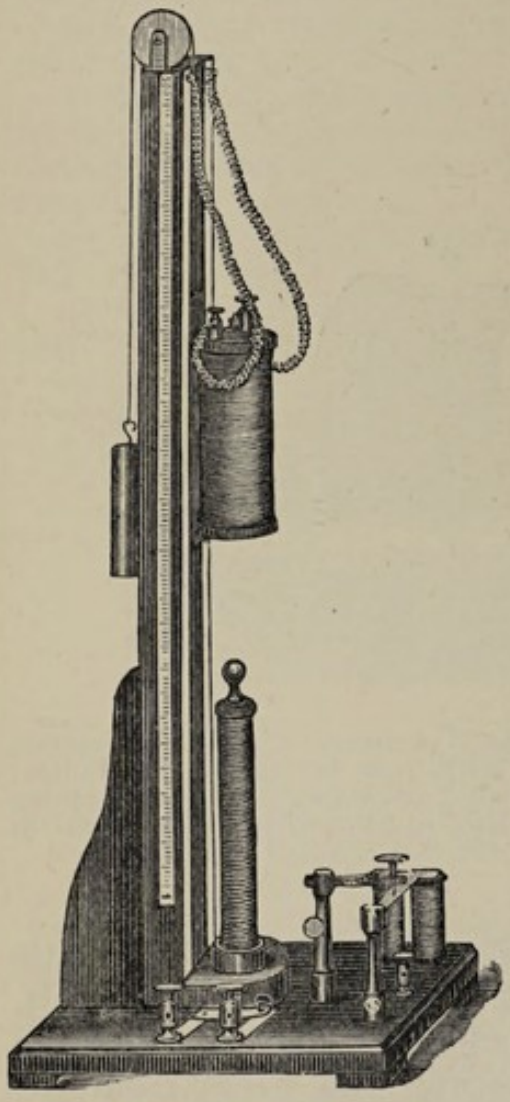


Fig. 105.—Inductorium with secondary coil moving in a vertical slot.

4. **Graduated induction apparatus.**—In the ordinary apparatus the distance between the S and P coils is indicated by a millimetre scale attached to the instrument. When the S coil is moved along equal distances, there is not a corresponding increase or decrease in the induced current; on the contrary, the strength of the induced currents undergoes a very unequal change. Fick and Kronecker use a graduated coil; on one side of the slot is a millimetre scale, and the other is divided into units.

5. **Bowditch's rotating secondary spiral.**—The S coil is withdrawn from the P to the unit mark 30 on the scale. The S coil rotates on a vertical axis, so that it can be placed at varying angles with the P. In proportion as it is rotated from its conaxial position the current is diminished. Test this by removing the S coil from the slot and placing it at variable angles to the P.

6. **Ewald's sledge coil.**—This coil is, with the exception of the interrupting arrangement, similar to the du Bois coil; the iron core (fig. 106, K) is arranged movable, and the S coil slides over the P and can be adjusted in any position by means of a rack and pinion arrangement. The interrupter consists of an upright electro-magnet, over the poles of which swings a small steel bar-magnet; this magnet forms the lower end of a pendulum which swings with very little friction, and is counterbalanced on its upper end by a small weight. The electro-magnet, when traversed by the current, becomes magnetised in such a way that its poles are the same as those of the little bar-magnet above it, thus repelling the latter, the swing of which is limited by the stop spring, B.

The magnetic circuit now being broken, the pendulum swings back until it again touches the contact D, when it is repelled again, and so on. According to the position which is given to the spring by means of the milled head A, the amplitude and speed of the interrupter swings can be varied between the limits of 1 and 200 per second.

Z, Z, are the battery terminals; P and S the terminals for primary and secondary current (fig. 106).

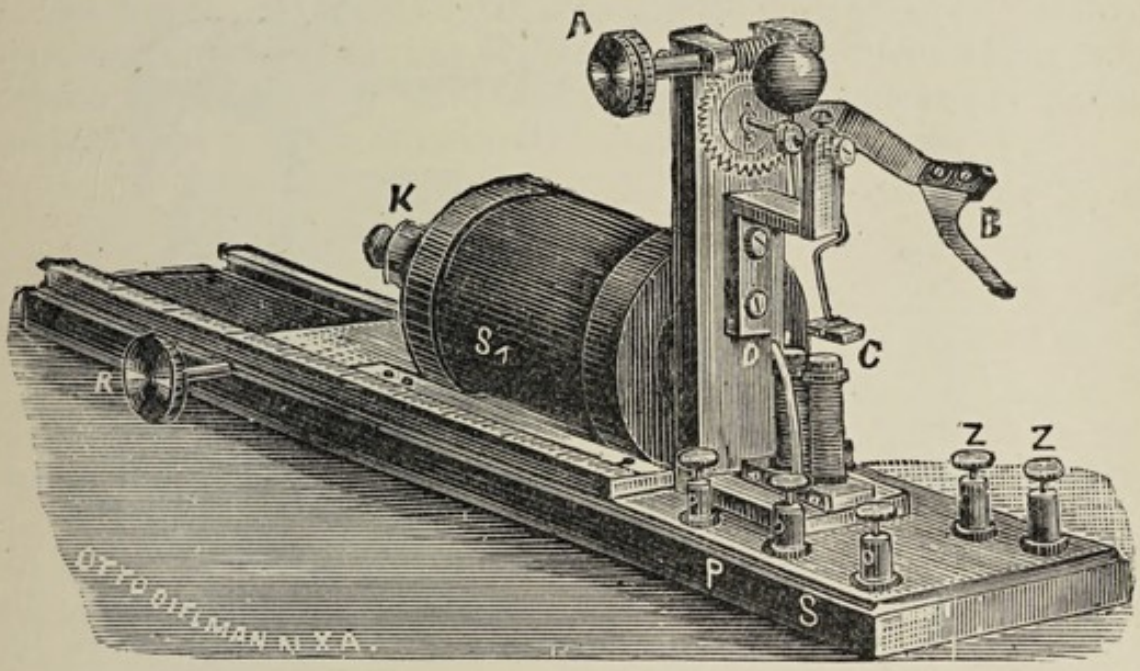


Fig. 106.—Ewald's sledge inductorium. *S'*, Secondary coil moved by milled head, *R*; *K*, Core of primary coil; *A*, Milled head to alter position of stop, *B*; *C*, Magnet; *Z*, *Z*, Battery terminals; *P* and *S*, Those for primary and secondary current. (It is made by A. Hurst & Co., 66 Fenchurch Street, London.)

Albrecht (Tübingen) makes an Ewald coil (inductorium)—like the original Ewald form—in which the secondary coil moves on a rack and pinion arrangement and on metallic wheels along a metal track. The secondary electrode connections are thus made stationary at the end of the track, and the wires are thus independent of movement of secondary coil.

7. **Shielded electrodes.**—For some purposes, *e.g.*, stimulation of the vagus, these electrodes are used—*i.e.*, the platinum terminals are exposed only on one side, the other being sunk in a piece of vulcanite. A pair of shielded electrodes is easily made by fixing the ends of two fine wires—arranged parallel to each other and about one-eighth of an inch apart—in a thin layer of gutta-percha cement. A little of the cement is scraped off to expose a small piece of both wires.

8. **Du Bois-Reymond electrodes** (fig. 107).—The two wires end in triangular pieces of platinum, P, which rest on a glass plate. The whole is supported on a stand, V, and can be moved in any direction by the universal joint, B.

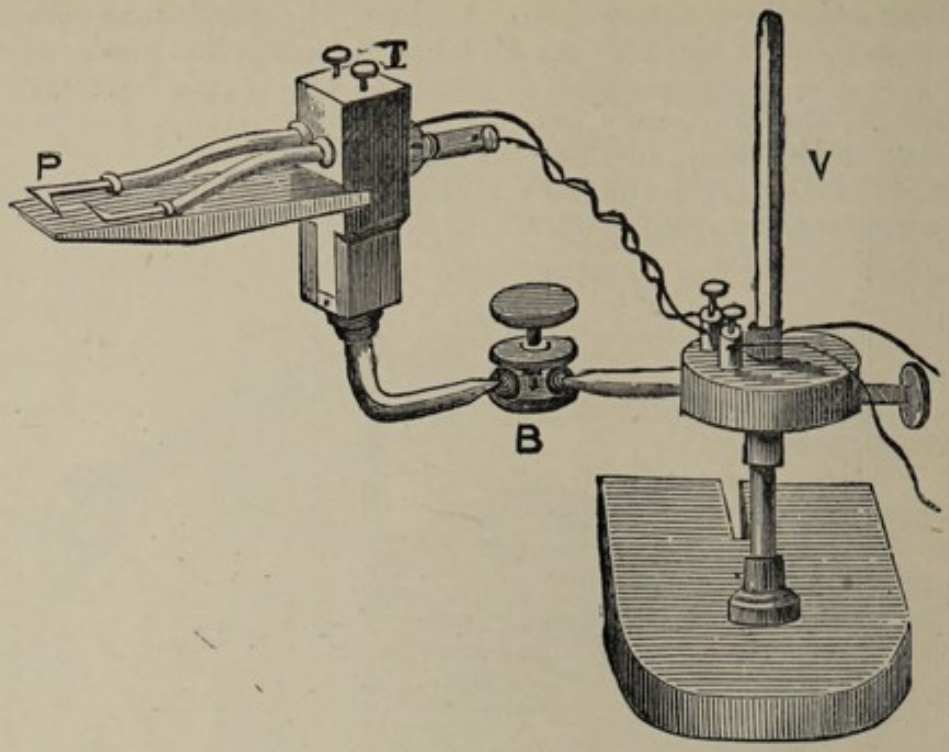


Fig. 107.—Du Bois-Reymond's platinum electrodes. The nerve is placed over the two pieces of platinum, *P*, which rest on glass; *B*, Universal joint; *V*, Support.

LESSON XXVIII.

SINGLE INDUCTION SHOCKS — INTERRUPTED CURRENT — BREAK EXTRA-CURRENT OF FARADAY — HELMHOLTZ'S MODIFICATION.

1. Single induction shocks.—Apparatus.—Leclanché cell, induction coil, wires, two du Bois keys (or one du Bois and spring or mercury key), and electrodes.

(*a*) Make connections as in fig. 108. The key in the primary circuit—preferably a mercury key—is used to make or break the primary current. Keep the key open. To the binding screws of the S coil attach two wires, and connect them to the short-circuiting du Bois key, and to the latter the electrodes. First set up connections in P coil, then those of S coil, noting specially the du Bois key as the short-circuiting key in this circuit—*i.e.*, contact key in P and short-circuiting key in S circuit.

(*b*) **Effect of single induction shocks on tongue.** (*Break shock is stronger than make shock.*)—Open the short-circuiting key, push the S coil pretty near to the P, and place the points of the electrodes on the tip of the tongue, or hold them between the moist forefinger and thumb. Close the key in the P circuit, *i.e.*, make the circuit, and instantly at the moment of making a shock or prick—the closing or **make induction shock**—is induced in the S coil, and is felt on the tongue or moist finger. All the time the key is closed the galvanic current is circulating in the primary coil, but it is only when the primary current is made or broken that a shock is induced in the secondary coil.

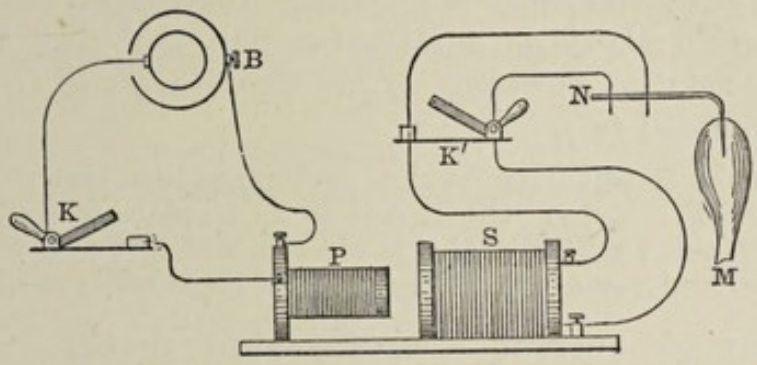


Fig. 108.—Scheme for single induction shocks. B, Battery; K, K′, Keys; P, Primary, and S, Secondary coil of the induction machine; N, Nerve; M, Muscle.

(*c*) Break the P current by raising the key, and instantly a shock—the opening or **break induction shock**—is felt.

(*d*) The *break is stronger than the make shock.* Push the S coil a long distance from the P, and, while the electrodes are on the tongue, make and break the P circuit. Gradually move the S near the P coil until the *minimal* shock is felt. Read off the position of S coil. The break shock is felt first, and on pushing the S nearer the P coil both shocks are felt, but the break is stronger than the make shock. Read off position of S when the *minimal make* shock is felt. It is considerably nearer P. Note that:—

(i.) The break shock is the stronger.

(ii.) On nearing the S to the P coil, a shock is felt at make also—*i.e.*, when the P circuit is made.

(iii.) If the P circuit be kept closed—*i.e.*, made—no shock is felt.

(iv.) The shocks increase in intensity the nearer the S coil is to the P.

N.B.—Tabulate the results showing the distance of the S coil from the P when testing the relative effects of M. and B. shocks.

Single M. and B. Induction Shocks (1 Daniell).

Distance of Secondary Coil from Primary in cm.	Effect on Tongue. M.	B.
19	0	0
18	0	Slight shock.
17	0	Stronger shock.
...	...	...
...	...	...
...	...	...
...	...	...
9	Slight shock.	Maximum shock.
8	Stronger shock.	,, ,,
7	Maximum shock.	,, ,,

Other parts of the apparatus being unchanged:—*The strength of the induction shock as a stimulus can therefore be varied by changing the relative positions of the secondary coil (S) in relation to the primary coil (P).*

(*e*) Remove the S coil from its slot, and place it in line with and about 15 cm. from the P. Rotate the S coil so as to place it at variable angles with the P. Make and break the P circuit, and test how the strength of the induced current varies with the extent of rotation of the S coil.

2. Interrupted current—*i.e.*, repeated shocks—with Neef's hammer—(Alternating currents)—Faradisation.

(*a*) Connect the battery wires (fig. 109) to P′ (+) and P″ (−). Introduce a du Bois key in the primary circuit as in the make

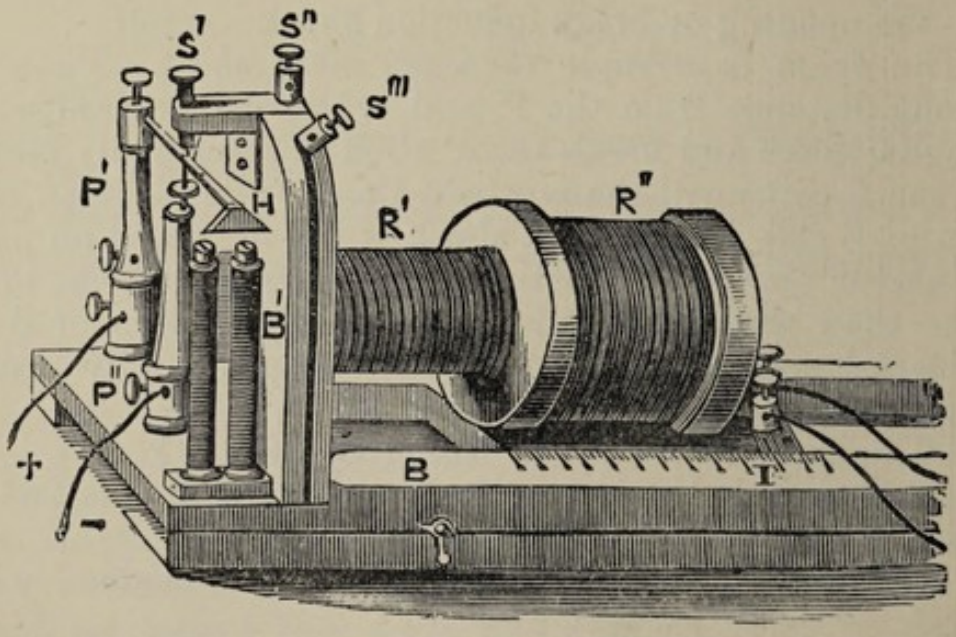


Fig. 109.—Induction coil arranged for interrupted or repeated shocks, with Neef's hammer in the primary circuit.

and break arrangement. The automatic vibrating spring, or Neef s hammer, is now included in the P circuit. Set the spring

vibrating. Close the key in the P circuit. The spring, H, is attracted by the temporary magnet, B′, thus breaking the contact between the spring, H, and the screw, S′, and causing a break shock in the S coil. B′ is instantly demagnetised, the spring recoils and makes connection with S′, and causes a make shock. Thus a series of make and break induction shocks following each other with great rapidity is obtained, but the make and break shocks are in alternately opposite directions.

(*b*) **Effect on tongue.**—With Neef's hammer vibrating, apply the electrodes to the tongue. Note effect, and how it varies on altering distance between S and P coils.

(*c*) Note how strength of induced shocks varies with the angular deviation of the S coil, the distance between the two coils being kept constant.

3. Break extra-current of Faraday.—When a galvanic current traversing the P coil of an induction machine is made or broken, each turn of the wire exerts an inductive influence on the others. When the current is *made*, the direction of the extra-current is *against* that of the battery current, but at *break* it is in the same direction as the battery current. **Apparatus.**—Cell, two du Bois keys, five wires, P coil of induction coil, electrodes (or nerve-muscle preparation).

(*a*) Arrange apparatus as in fig. 110, removing the S coil. Notice that both keys and the P coil of the induction machine are in circuit, the keys being so arranged that either the primary coil, P, or the electrodes attached to key, K′, can be short circuited. The secondary coil is not used.

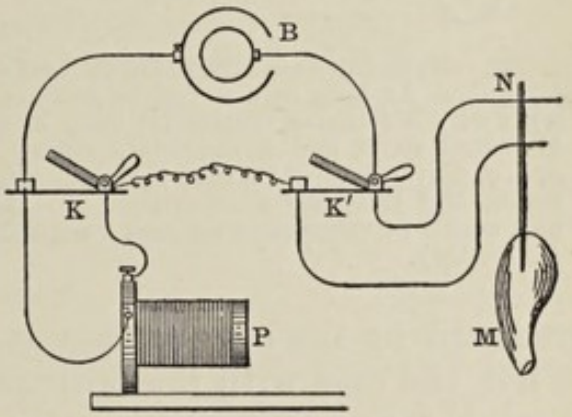


Fig. 110.—Scheme of break extra-current. B, Battery; K and K′, Keys; P, Primary coil; N, Nerve; M, Muscle.

(*b*) Test (*α*) either by electrodes applied to the tongue, or (*β*) by means of a nerve-muscle preparation (*β* to be done after the student has learned how to make a nerve-muscle preparation).

(*c*) Close the key K, thus short-circuiting the coil. Open and close key K′. There is very little effect.

(*d*) Open K, the current passes continuously through the P coil. Open key K′; a marked sensation is felt, due to the break extra-current.

[The consideration of the make extra-current is omitted.]

4. Helmholtz's modification.—The break shock is stronger than the make, so that if repeated—*i.e.*, interrupted shocks be sent

through a tissue for some time—polarisation effects are set up. To equalise them Helmholtz devised the following modification:—

(*a*) Connect the battery wires to the two pillars (fig. 109), P′ and P″, or to *a* and *e* (fig. 111). In fig. 111 connect a wire—"Helmholtz's side wire"—from *a* to *f*, thus bridging or "short-circuiting" the interrupter. Raise the screw (*f*) clear of the spring (*c*), but turn the screw (*d*) until it touches the spring, when the latter is in contact with the electro-magnet (*g*) close the key, the spring vibrates. By this means the make and break shocks are nearly equalised. Test this on the tongue. Both shocks, however, are weaker, so that it is necessary to use a stronger battery. The P circuit is always closed during the vibration of the spring.

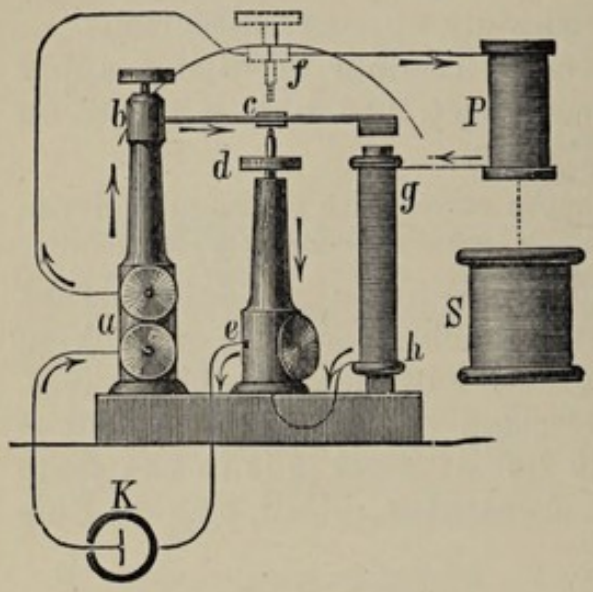


Fig. 111.—Helmholtz's modification of Neef's hammer. As long as *c* is not in contact with *d*, *g h* remains magnetic; thus *c* is attracted to *d*, and a secondary circuit, *a*, *b*, *c*, *d*, *e*, is formed; *c* then springs back again, and thus the process goes on. A new wire is introduced to connect *a* with *f*. *K*, Battery.

It is always advantageous, when using faradic shocks for physiological purposes, to use make and break shocks of nearly equal intensity, *i.e.*, use Helmholtz's side wire. Why? Because any "polarisation" produced by the one current is neutralised by the other. This is not the case with the ordinary arrangement, where the break shock is stronger than the make, whereby there is a progressive summation of the polarisation effects of the break shocks.

N.B.—The Helmholtz modification should, as a rule, be used when stimulating nerve or muscle for the reasons given above.

5. Equalise single make and break induction shocks.—As we have seen, the extra-current is the cause of the greater intensity of the break shock. If, however, the intensity of the extra-current be the same at make and break, this inequality will disappear.

(*a*) Connect the terminals of a cell with the upper binding screws of an induction coil (fig. 112), and to the same coil terminals connect two other wires with a make and break key (K) in their circuit ("deriving circuit," D, D). Thus the P current is never broken.

(*b*) Arrange S coil with short-circuiting key and electrodes.

(*c*) On closing the key in the deriving circuit the current in the P coil is diminished, and on opening it the P current is increased. Induced currents of opposite directions are thereby produced, which, though weaker than the make induction shock, are approximately equal to each other.

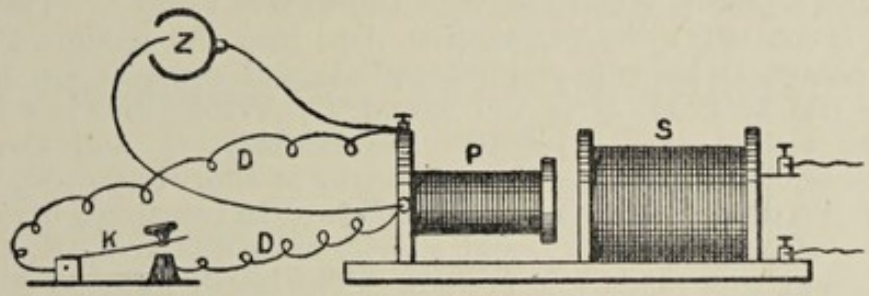


Fig. 112.—Arrangement to approximately equalise M. and B. shocks. *P*, Primary; *S*, Secondary coil; *K*, Key in deriving circuit, *D*, *D*.

6. To cut out M or B shock (1).—Arrange as in fig. 108—*i.e.*, with K as a M and B or "gap" key in the P circuit, and K′ as a short-circuiting or "bridge key" in the S circuit.

To cut out B shock.—Open K′, close K, when a M shock is obtained; close K′, and open K, ready to begin again.

To cut out M shock.—Close K′, then close K, then open successively K′ and K, when a break shock passes through the electrodes.

An automatic arrangement may be used, when a succession of either M or B shocks is required.

7. Or use the "**rotating key**" of Brodie (fig. 113). It consists of a horizontal axis supported on two ebonite uprights fixed to an ebonite base. This axis consists of two metal rods, A B and C D, united together by an

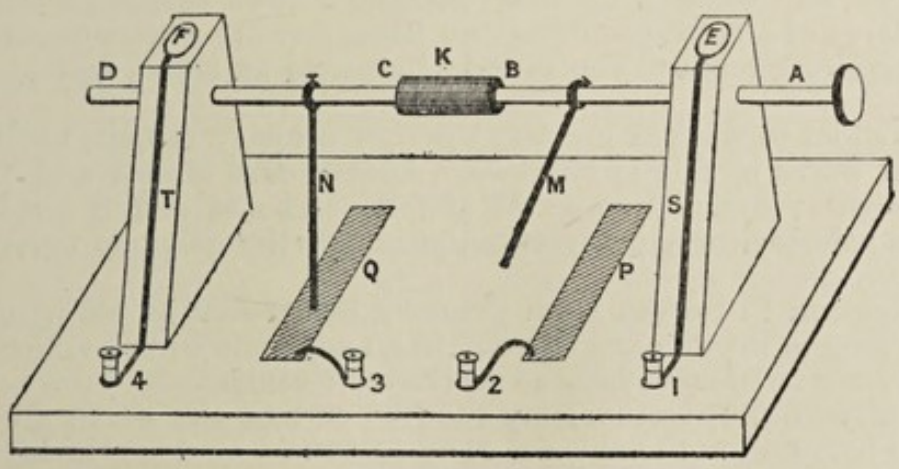


Fig. 113.—Brodie's "rotating key" to cut out the M or B shock.

insulating piece of ebonite, K. A B passes through a cup, E, cut in the upright and filled with mercury. The other rod, C, D, is similarly con-

nected to the second upright. Two stout wires, S, T, lead from the two mercury cups, E, F, to two binding screws, 1 and 4 respectively. Attached to the two rods are two metal arms, M and N, which can be rotated round the rods and clamped in any position. These dip into two mercury troughs, P and Q, which are respectively attached by stout wire to two binding screws, 2 and 3.

The action for which the key was devised is as follows :—

The primary circuit is connected with the two screws 3 and 4; the secondary and a pair of electrodes with the screws 1 and 2. Then, as the axis, A, D, is rotated, the arm, M, first dips into the trough, P, and the secondary circuit is thereby short-circuited, and remains so during the whole time the arm, M, is in the mercury. While this is still in the mercury the second arm, N, enters the mercury, Q, and the primary circuit is thus closed, but, as the secondary is short-circuited, the make induced current does not reach the electrodes. On rotating a little further, the arm, M, leaves the mercury, and shortly after the arm, N, leaves the mercury, Q, and the current is broken. The break induced current can now pass through the electrodes since the secondary circuit is not now short-circuited.

By reversing the rotation only make shocks can pass through the electrodes, the break shocks being short-circuited.

The key may also be used in other ways. By placing the two arms, M and N, parallel to one another, the key may be used to close two circuits simultaneously—*e.g.*, a primary current, and a current working a signal.

Further, by altering the angular distance between M and N, and having the axis driven at a constant rate, the key may be used for sending in two successive stimuli at different intervals of time.

8. Extra-current at break of primary current.—Remove secondary coil, use only primary, and connect a cell with the binding screws, as for single M. and B. shocks interposing a simple key. Attach the electrode wires to the same binding screws. Close the primary circuit. Place electrodes on the tongue, and open the key. A shock will be felt due to the self-induced currents in the primary coil. Remove secondary coils.

9. Extra-currents at M and B of primary current.—Connect a cell through a simple key to the terminals of a rheochord. Connect the slider—placed hard up against the positive pole—to the primary coil arranged for single shocks, and connect up these binding screws through a simple key with the nerve of a N.M. preparation. Close key in primary circuit; make and break nerve circuit. The muscle contracts at make, and possibly at break.

Remove slider to weaken current through primary circuit, until neither M nor B of nerve circuit is effective. Close nerve circuit and M and B primary circuit; contraction at M and B. The M and B self-inducted currents in primary cause contraction, although the galvanic current itself is ineffective.

[**10. Induction in nerves from primary coil.**—Just as one wire with a current flowing in it induces a current in an adjacent wire, so currents are induced in a nerve brought near to a primary circuit.

Prepare a N.M. preparation; lay the free end of the nerve on another part of the nerve or on the muscle, so as to form a circle. On laying this preparation on paraffin paper, and bringing the whole near the primary coil, contraction occurs at M and B of the primary circuit.]

LESSON XXIX.

NERVE-MUSCLE PREPARATION—DISSECTION—NORMAL SALINE.

1. Pith a frog.—Wrap the body, fore and hind legs, in a towel, leaving the head projecting. Stun the animal by giving it a sudden blow on the head. Grasp the towel with the little, ring, and middle fingers and thumb of the left hand, leaving the index-finger free. With the index-finger bend down the frog's head over the radial surface of the second finger until the skin over the back of the head is put on the stretch. With the nail of the right index-finger feel for a depression where the occiput joins the atlas. With a sharp, narrow knife held in the right hand, divide skin, membrane, and bulb transversely. Avoid wounding the vertebral arteries, which run laterally. Destroy brain—decerebration—with a seeker. Only the brain may be destroyed, or the spinal cord as well. To prevent oozing of blood, a piece of a wooden match or piece of cotton wool may be thrust into the brain cavity. If it is desired, destroy also the spinal cord with the seeker or a wire. The knife used must not have too broad a blade, else two large blood-vessels will be injured. The operation should be performed without losing any blood.

2. Anatomy of nerve-muscle preparation—Dorsal dissection.—On a dead frog study the arrangement of the muscles, as shown in fig. 114. Place the frog on its belly—legs extended—on a frog-plate. Slit up the skin—from trunk to foot—covering the back of the leg. Pin the skin aside, and view the exposed muscles from behind. On the outside of the thigh, the *triceps femoris* (*tr*), composed of the *rectus anterior* (*ra*), the *vastus externus* (*ve*), and the *vastus internus*, not seen from behind. On the median side, the *semi-membranosus* (*sm*), and between the two the small narrow *biceps* (*b*). The biceps is readily observed, at the lateral margin of the large semi-membranosus, by its shining tendon in the middle of the lower half of the thigh. Notice, also, the *coccygeo-iliacus* (*ci*), the *gluteus* (*gl*), the *pyri-*

formis (*p*), and the *rectus internus minor* (*ri*). In the leg, the *gastrocnemius* (*g*), with its tendo Achillis, the *tibialis anticus* (*ta*), and the *peroneus* (*pe*). Slit up the skin over the back, and note the fine white dorsal cutaneous nerves which traverse the extensive dorsal lymph sac.

(*a*) Remove the skin from the leg of a frog; with a blunt needle, seeker, finder, or a pointed glass rod, gently tear through

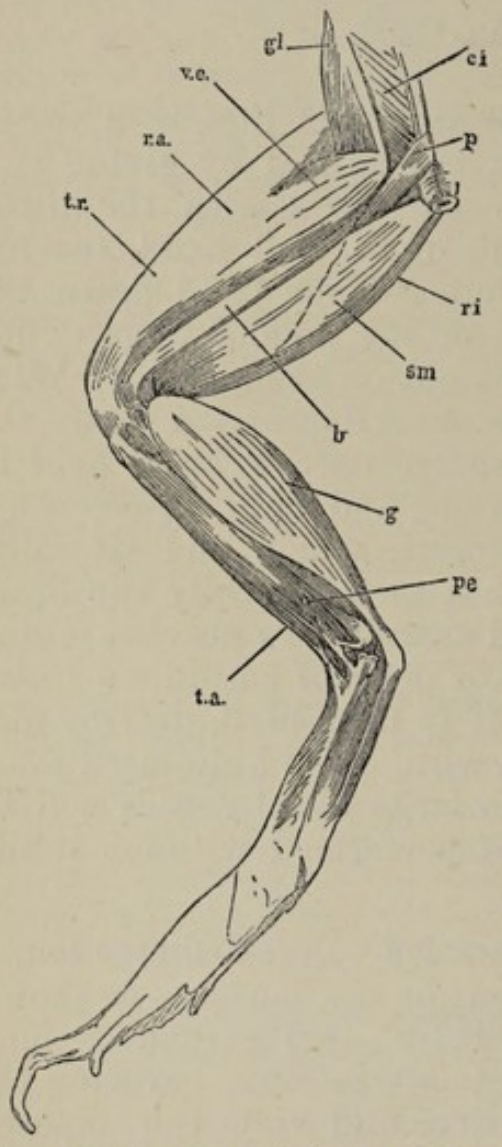


Fig. 114.—Muscles of left leg of frog from behind. *ci*, Coccygeo-iliacus; *gl*, Gluteus; *p*, Pyriformis; *ra*, Rectus anterior; *ve*, Vastus externus; *tr*, Triceps; *ri*, Rect. int. minor; *sm*, Semi-membranosus; *b*, Biceps; *g*, Gastrocnemius; *ta*, Tibialis anticus; *pe*, Peroneus.

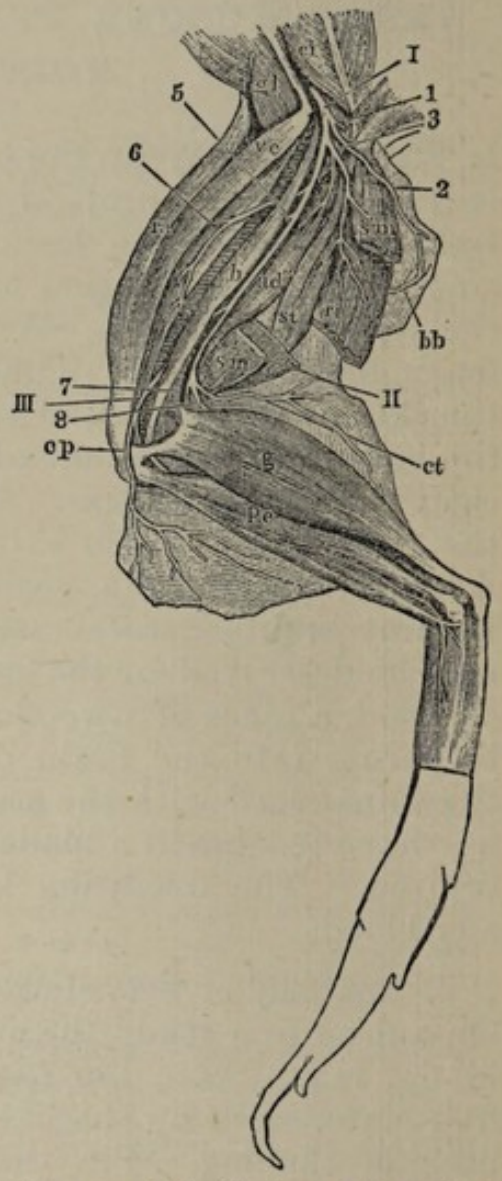


Fig. 115.—Distribution of the sciatic nerve (I.) of the frog (see also fig. 114). *st*, Semitendinosus; *ad'''*, Adductor magnus; (II.) its tibial, and (III.) peroneal divisions.

the fascia covering the thigh muscles. Separate the semi-membranosus from the narrow biceps, and in the interval between

them beneath the biceps observe the *sciatic nerve* and the *femoral vessels*, the sciatic artery crossing the nerve from without inwards. The dark coloured vein runs with the artery in the lower part of the thigh, but above it diverges to join the renal portal vein. Carefully isolate both, beginning at the knee, where the nerve divides into two branches—the tibial and peroneal—and work upwards (fig. 115). In clearing the nerve, two small lateral branches have to be cut. The corresponding muscles contract owing to the mechanical stimulation of their nerves.

The tibial or inner branch passes over the knee-joint towards the middle plane, and enters the under surface of the gastrocnemius; the peroneal branch passes between the lateral tendinous origin of the gastrocnemius and the tendon of the biceps, and then under the latter.

(*b*) Follow the nerve upwards to the vertebral column, and observe that it is necessary to divide the pyriformis (*p*), and also the ilio-coccygeal muscle; lift the urostyle, cut it away, keeping the scissor points close to the bone, when three spinal nerves—the 7th, 8th, and 9th—which form the sciatic nerve, come into view. Keep them moist by covering with filter paper soaked in normal saline. On its way from the sacral plexus to the thigh, it gives off cutaneous and muscular branches for the pelvis and thigh.

3. Hyoglossus preparation (Lesson XXXV.).

4. Sartorius (Lesson XXXI.).

5. Double semi-membranosus and gracilis (*Fick's method*).—I am indebted to the late Professor Fick, of Würzburg (†1901), and Professor Schenck, now of Marburg, for showing me this method.

(*a*) In a pithed frog divide the fascia at the outer margins of the semi-membranosus and gracilis, until the insertion of these two muscles at the knee is reached, then divide the bone just under the knee-joint, so that the osseous insertion of both muscles is retained. Divide the femur just above the knee-joint, and separate all the muscles inserted into it, save the two muscles one is isolating. Separate the two muscles up to the symphysis. Leave the two muscles in connection with the symphysis, divide the other muscles, disarticulate the femur at the acetabulum. In preparing the muscles in this way the semi-tendinosus is usually left. It is easy to separate it by dividing its insertion into the femur, and then its two heads at the pelvis.

(*b*) Make a similar dissection on the opposite side. Bore a hole with an awl through both acetabula. Through this a hook can be placed. We have two muscles with nearly straight fibres which can be placed "side by side," thus giving a short muscle with large sectional area, or they can be placed "one behind the other," a piece of bone, the symphysis intervening—*i.e.*, equivalent to a long muscle with half the sectional area.

6. Indifferent fluids—Normal saline.—Dissolve 6 to 7·5 grms. of dried sodium chloride in 1000 c.c. of tap water. This is the best fluid to use to moisten the tissues of the frog, when a large quantity is required. A solu-

tion containing 0·6 to 0·75 p.c. of NaCl is supposed to be "isotonic" with the tissues of the frog—*i.e.*, it neither takes from nor gives water to the tissues. It is, however, not so "indifferent" as its name would imply (Lesson XXX.). For mammals, normal saline 0·9 p.c. is used. The tissues should be moistened rather than soaked in the fluid. Mendel suggests the term "isotonic saline" instead of "normal saline"; a 0·6 p.c NaCl is an indifferent fluid only for the frog. The principle is an important one—viz., the use of isotonic fluids. For nerve the aqueous humour of the frog's eye is the best. It can readily be obtained by perforating the cornea with a fine glass pipette.

[The standard work on the frog is *Die Anatomie des Frosches*, by A. Ecker; translated by G. Haslam, 1889. New German edition (3rd) by R. Wiedersheim and E. Gaupp, 1896, 1899, 1901.]

LESSON XXX.

NERVE-MUSCLE PREPARATION — MECHANICAL, CHEMICAL, THERMAL STIMULI.

1. **Nerve-muscle preparation.**—Frog, seeker, scalpel, a small and a large pair of scissors, forceps, towel, and a frog-plate.

(A) (*a*) Destroy the brain and spinal cord of a frog, and place it on its belly on a frog-plate. With scissors make an incision through the skin along the back of one thigh—say the left—from the knee to the lower end of the coccyx, and prolong the incision along the back a little to the left of the urostyle. Reflect the skin, and expose the muscles shown in fig. 114.

(*b*) Gently separate the semi-membranosus and biceps with the "seeker," so as to bring into view the sciatic nerve and femoral vessels. Some use a glass rod drawn to a thin, slightly-bent point, instead of a "seeker." Still working with the seeker and beginning near the knee, clear the sciatic nerve, but do not scratch or stretch the nerve, or touch it with forceps. Divide the pyriformis and ilio-coccygeus, and trace the nerve up to the vertebral column.

(*c*) Divide the spinal column above the seventh lumbar vertebra; seize the tip of the urostyle with forceps, raise it, and with strong scissors free it from its connections as far as the last lumbar vertebra, and then divide the urostyle itself. Divide the left iliac bone above and below, and remove it with its attached muscles. The lumbar plexus now comes into view. Bisect lengthways the three lower vertebræ, and use the

quadrilateral piece of bone to manipulate the nerve. With forceps lift the fragment of bone, and with it the sciatic nerve; trace the latter downwards to the knee, dividing any branches with fine scissors. Moisten with normal saline.

(*d*) Divide the skin over the gastrocnemius, and expose this muscle. Cut the tendo Achillis below its fibro-cartilage, lift the tendon with forceps and detach the gastrocnemius from its connections as far up as the lower end of the femur. Cut across the knee-joint, and remove the tibia and fibula with their attached muscles. Taking care to preserve the sciatic nerve from injury, clear the muscles away from the lower end of the femur, and then divide the femur about its middle. This preparation (fig. 116) consists of the gastrocnemius, and the whole length of the sciatic nerve, to which is attached a fragment of bone, by which the preparation can be manipulated without injuring the nerve. *N.B.* (1)—Leave a small piece of the vertebral column attached to the nerve. (2) Cut the tendon as long as possible, and separate the muscle from below upwards. (3) Leave $\frac{1}{4}$ inch of femur attached to muscle. (4) The nerve must not be held or pinched with forceps, nor should it be pulled on. (5) Keep it and muscle moist with normal saline. It must not be allowed to dry. Cover it with blotting-paper, moistened with normal saline. (6) The outer surface of the skin must not be allowed to touch the muscle or nerve.

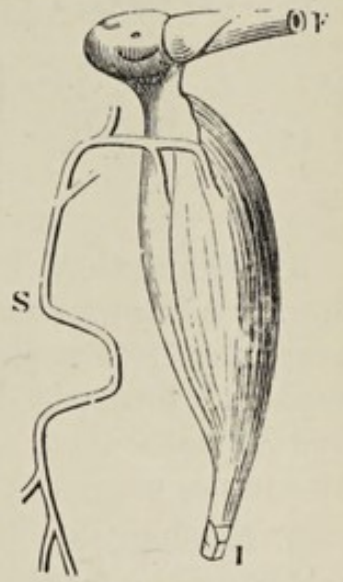


Fig. 116.—Nerve-muscle preparation. *S*, Sciatic nerve—the fragment of the spinal column is not shown; *F*, Femur; and *I*, Tendo Achillis.

(B) (*a*) **Rapid method.**—Destroy a frog's brain and spinal cord. With the left hand seize the hind limbs and hold the frog with its belly downwards. With one blade of a sharp-pointed pair of scissors transfix the body behind the shoulder-blades, and divide the spinal column. The head now hangs down, and by its weight it pulls the ventral from the dorsal parts.

(*b*) With the scissors divide the wall of the abdomen on both sides parallel to the vertebral column, and remove the abdominal viscera. With the left hand seize the upper end of the divided spinal column, and with the right the skin covering it, and pull. The lower end of the trunk and the lower limbs are denuded of skin.

(*c*) Take the thigh muscles between the thumb and forefinger of the left hand, and with the point of one blade of a pair of scissors tear through the fascia between the biceps and semi-membranosus to expose the sciatic nerve, and then proceed as directed in 1.

2. The most obvious function of the skeletal-muscular tissue is that it reacts only in response to stimuli. Stimuli may be applied direct to the muscle itself—**direct stimulation**; or to the motor nerve of the muscle—**indirect stimulation.**

Stimuli may be classified as follows :—

(1) **Mechanical,** *e.g.*, cutting, pinching, or tapping a nerve or muscle.

(2) **Chemical,** *e.g.*, by dipping the tissue in a saturated solution of common salt or glycerin.

(3) **Thermal,** *e.g.*, applying a heated wire.

(4) **Electrical**—
- (*a*) *Continuous current.*
- (*b*) *Single induction shocks.*
- (*c*) *Interrupted current or repeated shocks.*
- (*d*) *Equalised repeated shocks.*
- (*e*) [*Condenser shocks.*]

3. **Stimulation of muscle and nerve.**—It is convenient to modify somewhat the physiological limb, in order to render the muscular contraction more visible. **Apparatus.**—Seeker, scalpel, scissors, forceps, straw flag, pins, muscle-forceps, camel-hair brush, saturated solution of common salt in a glass thimble, ammonia, copper wire, spirit lamp or gas-flame.

4. **Mechanical stimulation.**—(*a*) Prepare a N.M. preparation, isolating the sciatic nerve, but modify the subsequent details as follows :—

(*b*) After the nerve is cleared as far as the spine, cut away the muscles from the femur, and divide the latter about its middle. Divide the sciatic nerve as high up as possible. Pin a straw flag to the toes by means of two pins. Fix the femur in a clamp or pair of muscle-forceps, supported on a stand (fig. 117), taking care that the gastrocnemius is upwards. The nerve hangs down, and must be manipulated with a camel-hair brush dipped in normal saline, or by means of a glass seeker.

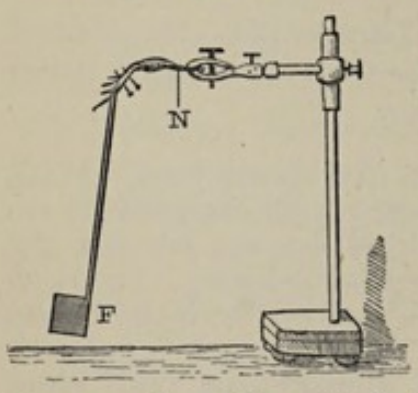


Fig. 117.—Straw flag attached to a frog's leg fixed in a clamp. *N*, Nerve; *F*, Flag.

(*c*) Pinch the free end of the nerve sharply with forceps; the muscles contract. No change is visible in the nerve, but the straw flag is suddenly raised and depressed. Cut off the dead part of the nerve with scissors; contraction also occurs.

(*d*) Prick the muscle with a needle, it contracts; or strike it smartly with the handle of a scalpel.

It is sufficient to expose one sciatic nerve *in situ*, and observe the movements of the foot and leg.

For the "muscle-telegraph" of du Bois-Reymond, see *Appendix*.

(*e*) A tetanomotor is sometimes employed, but the part stimulated is apt to be injured by the stimuli. Heidenhain (1856) devised a tetanomotor for this purpose. It consisted of a Wagner or Neef's hammer, with one end prolonged and carrying a small ivory hammer, which beat the nerve placed under it. The same result is obtained by attaching a piece of lead to the vibrating style of a "time marker" (Lesson XXXV. Recently v. Uexküll has devised apparatus for this purpose (*Zeits. f. Biol.*, Bd. xxxi.). Ewald and Schäfer have used the stimulus obtained by drops of mercury falling on the nerve from a reservoir (*Journ. of Phys.*, xxvi.).

(*f*) **Mechanical stimulation by removal of pressure.**—Place the nerve of a nerve-muscle preparation on a moist glass plate, press the nerve slowly and steadily with a curved 1 mm. thick glass hook. If pressure be applied steadily and uniformly the nerve is not excited, but on suddenly removing the pressure the muscle contracts (*v. Uexküll*).

N.B.—Pressure may be applied slowly and steadily, and the nerve destroyed functionally without causing a contraction of its muscle.

5. Thermal stimulation.—To the same preparation apply, either to muscle or nerve, a stout copper wire heated to a dull heat; a contraction results in either case. Cut off the dead part of the nerve.

6. Chemical stimulation.

(*a*) Place **saturated solution of common salt** in a glass thimble, or on a slide, and allow the free end of the nerve to dip into it. The nerve floats on the surface of the fluid, but the salt diffuses into the nerve. After a few moments the joints of the toes twitch, and by-and-by the whole limb is thrown into irregular, flickering spasms, which terminate in a more or less continuous contraction, constituting *salt tetanus*, but the contractions are not completely fused. Cut off the part of the nerve affected by the salt; the spasms cease. Some apply finely-powdered salt to the nerve, others glycerine.

(*b*) Using a similar preparation, cover the leg with the skin of the frog, or wrap it in blotting-paper saturated with normal saline. Expose the fresh-cut end of the nerve to the vapour of **strong ammonia.** The ammonia must not act directly on muscle, hence the glass vessel must be placed above the nerve, and the nerve raised to the ammonia. There is no contraction of the muscle, but the ammonia kills the nerve.

Instead of doing this, the whole leg may be laid on a card with a hole in it just sufficient to allow the sciatic nerve to pass through it, the whole being covered with blotting-paper moistened with normal saline. The card is placed over a test-tube containing a drop of ammonia; the nerve hanging in the vapour of the latter is speedily killed, but there is no contraction of the muscle. Apply ammonia to the muscle; it contracts.

(*c*) Dissect out a sartorius; hold it over ammonia, it curls up ("contracture"). (Lesson XXXI., 6.)

Although ammonia applied directly to a motor nerve does not cause contraction of the corresponding muscle, yet when it is applied to the central end of the divided vagus of a rabbit it causes marked reflex movements of the respiratory muscles.

(*d*) How does muscle respond to indirect stimulation by glycerine or alcohol?

[(*e*) Place a sartorius in "normal saline" for 20 minutes. If then its muscle curve be recorded, in response to a single maximum induction shock, it will give not a simple twitch, but a tetaniform contraction. Therefore, so-called "normal saline" is not without its influence on muscle tissue.

(*f*) Presence of calcium.—If the above muscle be now placed in normal saline (0·6 p.c.), to which 10 p.c. of a saturated solution of calcium sulphate is added, the tetaniform contraction will disappear, and the response will be a single twitch. Calcium salts play an important part in the maintenance of the excitability of several tissues. It is partly for this reason that tap water and not distilled water is used for making normal saline.

(*g*) Distilled water.—Observe effect produced in a sartorius due to withdrawal of certain substances by osmosis. It is a powerful poison (*Porter*).]

(*h*) Chemical stimulation and rhythmical response.—Dissolve in 1000 c.c. water, NaCl 5 grms., Na_2HPO_4 2 grms., and Na_2CO_3 0·4 grm. (Biedermann's fluid), and suspend in it a sartorius. Usually the muscle contracts rhythmically. Here the stimulus is a constant one, the response rhythmical.

7. Drying.—If the nerve be allowed to hang free in the air for some time, it gradually dies, and the muscles twitch irregularly (movements of toes). If the excitability of the nerve be tested with induction shocks it will be found to be increased at first, but it disappears rapidly as the nerve dries. Moisten the nerve with normal saline and the twitching *may* cease. Glycerine may act as a stimulus by absorbing water.

LESSON XXXI.

SINGLE AND INTERRUPTED INDUCTION SHOCKS —TETANUS—CONSTANT CURRENT.

1. Electrical stimulation.—Single induction shocks—Apparatus.—Cell, coil, two du Bois keys (or one spring or mercury key and one du Bois key, the last to be used as a short-circuiting key), five wires, electrodes.

(*a*) Arrange a cell and coil for single induction shocks (fig. 118) with a spring contact-key or Hg-key in the P circuit. Electrodes are fixed to the du Bois short-circuiting key, K′, in the S circuit, and over them place the nerve.

(*b*) Expose the sciatic nerve in a pithed frog, place it on electrodes—preferably a pair fixed in ebonite, and so shielded that only one surface of their platinum terminals is exposed under it; or use ordinary electrodes. Pull the S coil, S, far away—say 25 cm.—from the P coil, raise the short-circuiting key, K′, make and break the primary circuit by means of the key, K. At first there may be no contraction, but on approxi-

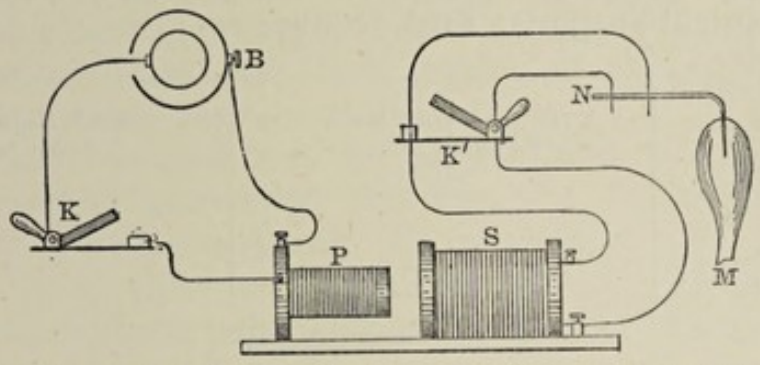


Fig. 118.—Scheme for single induction shocks. *B*, Battery; *K*, *K′*, Keys; *P*, Primary, and *S*, Secondary coil of induction; *N*, Nerve; *M*, Muscle.

mating the S to the P coil a **single muscular twitch** or **contraction** will be obtained, first with the break shock, and on approaching the S nearer to the P coil, also with the make. Find the distance at which the *minimal* break result is obtained, as shown by the contraction of the muscles, first shown in the twitching of the toes. Then push up till contraction is also obtained at make. Weaker currents are required than those used to give a sensation from stimulating a sensory surface like the tongue. The one is called a **make** and the other a **break** contraction. Enter in a note-book the results obtained. *N.B.*—In all cases the student should keep an account of the experiment, and especially of all numerical data connected therewith, *e.g.* :—

Single M and B shocks—Du Bois coil, Daniell.

Distance of P from S coil = in cm.	Response at Make (M).	Response at Break (B).
45	0	0
44	0	Min. twitch.
43	0	Slight ,,
42	0	Stronger ,,
41	0	,, ,,
20	0	Max. ,,
19	Slight twitch.	,, ,,
18	Max. ,,	,, ,,

[A very good exercise is to stimulate any exposed muscles of a frog. Stimulate them directly as a whole, or stimulate certain fibres to observe the action of these fibres in producing movements in the parts to which they are attached—*e.g.*, exposed pectoralis in flexing and adducting rapidly the fore limb.]

Compare ordinary with Helmholtz arrangement, and tabulate the results as follows, to show the distance of the S coil at which mechanical response first occurs :—

	Ordinary du Bois-Reymond Coil.	With Helmholtz's Modification.
Nerve make, .		
,, break, .		

The same may be done by applying the electrodes directly to the gastrocnemius muscle—*i.e.*, *direct* stimulation, that through the nerve being *indirect* stimulation.

	Ordinary du Bois-Reymond Coil.	With Helmholtz's Modification.
Muscle make, .		
,, break, .		

2. Interrupted current or repeated shocks.

(*a*) Arrange the induction machine so as to cause Neef's hammer to vibrate (Lesson XXVIII., 2). Slide the S coil to 30 to 35 cm., and stimulate the nerve or push-up coil, and note when response is first obtained. The distance will be greater than for single shocks, because the make and break of the P current are regular and rapid, and also because, although single shocks may be inadequate, they become adequate when repeated. On applying a strong current, at once the muscle is thrown into a state of rigid spasm or continuous contraction, called **tetanus,** this condition lasting as long as the nerve or muscle is stimulated, or until exhaustion occurs.

(*b*) **Stimulate different nerves.**—Stimulate the seventh, eighth, or ninth nerves, near the spinal column, or the peroneal or tibial nerves, with different strengths of current, and observe the results on the flexors and extensors of the limb.

3. Constant current—Apparatus.—Cells, du Bois key (or, preferably, a contact key), four wires, electrodes, forceps, and N.M. preparation; or simply expose the sciatic nerve *in situ*.

(*a*) If two or more cells be used, always connect them in series—*i.e.*, the + pole of one cell with the – pole of the next. Connect two wires, as in fig. 119, to the free + and – poles of the battery B, and introduce a du Bois key, K′, to short-circuit the battery circuit. Fix two shielded electrodes in the other binding-screws of the du Bois key, and having prepared a nerve-muscle preparation, lay the divided sciatic nerve, N, across them, as shown in fig. 119. A simple key to make or break the current is preferable to the short-circuiting key, as the latter allows polarisation currents to pass when it is closed.

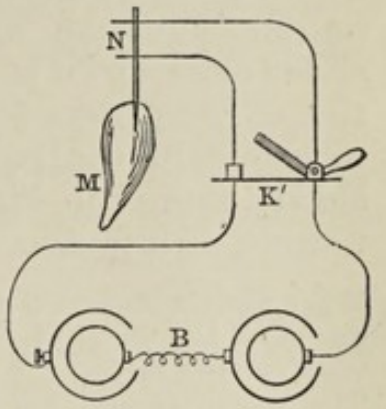


Fig. 119.—Scheme of constant current. *B*, Battery; *K′*, Short-circuiting key; *N*, Nerve; *M*, Muscle.

(*b*) Make and break the current, and a **single muscular contraction** or **twitch** is obtained, either at make or break, or both. If the key be raised to allow the current to flow continuously through the nerve, no contraction occurs, provided there be no variation in the intensity of the current. Contraction, however, does take place under certain conditions during the flow of a galvanic current, either through nerve or muscle. The electrodes may also be applied to the muscle directly.

(*c*) Rapidly make and break the current by opening and closing the key; a more or less perfect *tetanus* is produced. If imperfectly fused it is called *clonus*.

(*d*) To test the effect of a constant current on muscle alone, then the terminations of the motor nerves in the muscle must have been paralysed previously by curare. The electrodes must be applied directly to the muscle.

The above results would seem to indicate that a muscle is stimulated only by sudden changes in the intensity of the current, a view propounded by du Bois-Reymond (Lesson XXXII.). There are, however, important exceptions, more especially in sluggish contractile tissue—*e.g.*, smooth muscle.

(*e*) **Sudden change in intensity of a constant current.**—Set up a cell connecting the poles through a closed du Bois key, A. Connect a second cell, the copper with one binding-screw of A. To the other binding-screw attach the wire of an electrode connected with a muscle. Between the zinc of the second cell and the other electrode applied to the muscle introduce a simple

make and break du Bois key, B. Close B = contraction. Open A, which *suddenly* increases the strength of the current = contraction or twitch.

(*f*) Gradual change.—Arrange two cells in series, and connect the outer zinc through a simple M and B key to one (O) end of a metre rheochord, and the outer copper to the other end. Connect O end of the rheochord with an electrode resting on the muscle, and the other electrode with the slider. Bring the slider close up to O—*i.e.*—pole, so that only a small fraction of the current passes through the tissue. Close the key; the muscle will contract. With the key closed move the slider slowly along the wire; an increasing amount passes through the muscle, still it does not contract.

(*g*) *Constant current and simple rheochord.*—With simple rheochord (fig. 103) test the effect of varying strength and direction—using a commutator with crossbars—on the contraction when the current is applied to a nerve. The nerve should be laid over N.P. electrodes.

(*h*) The effect of a constant current on smooth muscle and those under certain conditions on muscle and nerve, are referred to later (Lesson LII.).

4. Muscle on mercury.—Lay the muscle of a nerve-muscle preparation on the surface of mercury. Stimulate the nerve, the muscle contracts, but does not elongate; it shows little tendency to elongate unless it be weighted.

5. Dead muscle and nerve.—Immerse a nerve-muscle preparation for a few minutes in water at 40° C. Both are killed, and none of the above stimuli cause contraction.

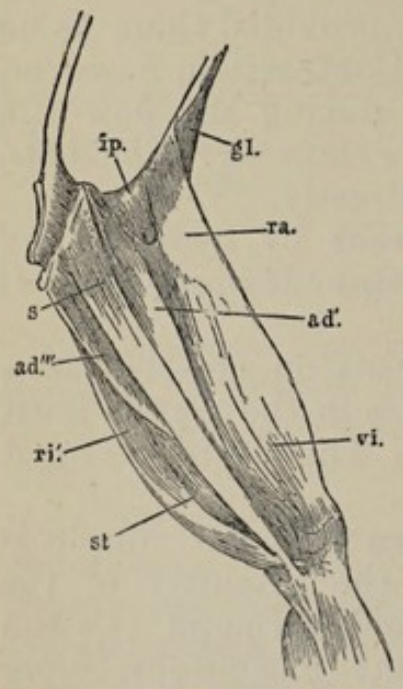


Fig. 120.—Muscles of left leg of frog seen from the front. *ip*, Ileo-psoas; *s*, Sartorius; *ad'*, Adductor longus; *vi*, Vastus internus (See figs. 114 and 115.)

6. Sartorius (Dissection of).—A muscle with parallel fibres.

Lay a pithed frog on its back, and remove the sartorius. The thin narrow sartorius (fig. 120), stretching from the ilium to the tibia, is best seen if it be moistened with blood, which differentiates its edges. Raise its tibial tendon, and round it tie a fine silk thread. Gradually raise the muscle by means of the thread, and with fine scissors cut it free from its fascial connections right up to the ilium. Cut it out with the ilium attached. Its nerve enters on its under surface about the middle of the muscle. Stretch it on a slip of glass or hang it up by its iliac bony attachment in a clamp (Lesson XXXIII., 4).

7. Unipolar stimulation (Induction shocks have great power of overcoming resistance). **Apparatus.**—Cell, coil, du Bois keys, (muscle-chamber), wires, electrodes.

A. (*a*) Place a frog on a dry cork plate, or glass, or block of paraffin, and expose its sciatic nerve. Arrange a coil for faradisation with the electrodes short-circuited, and placed under the sciatic nerve clear of all adjoining muscles. Open the short-circuit key and find a strength of current (S coil at 25 to 30 cm.) which on faradisation gives feeble tetanus.

(*b*) Disconnect one electrode wire from the preparation, so that only one terminal is in connection with the nerve. There is no contraction when the secondary key is open. Insulate the preparation by placing it on a block of paraffin or dry beaker.

(*c*) Find the distance of the secondary coil (8 to 10 cm.) at which no response is obtained with unipolar stimulation, but a response is obtained when the preparation is touched with finger. Why is there a response? Because by touching the preparation one suddenly diminishes the resistance to the passage of the induction currents to earth.

B. (*a*) Set up a cell and coil with electrodes for single shocks. Disconnect one of the electrodes of the S coil, the other one being under the sciatic nerve or the nerve of a N.M. preparation insulated on a glass plate. If the frog is on a frog-plate, put the frog-plate on a dry beaker to insulate it. No contraction occurs at make or break.

(*b*) Connect the disconnected electrode to a gas-pipe, and so to the earth. Contraction takes place at make or break. *N.B.*—Unipolar stimulation is due to defective insulation, and it is in order to avoid unipolar stimulation that a du Bois key is used to short-circuit the secondary circuit, as an ordinary break-key always allows especially strong induction currents to pass to the preparation.

C. (*a*) Connect the battery to the P coil either for single shocks or tetanus, introducing a du Bois key in the circuit. Connect one wire with the S coil, and attach it to one of the binding screws on the platform of the muscle-chamber, to which the nerve electrodes are attached. See that the battery and induction machine are perfectly insulated by supporting them on blocks of paraffin.

(*b*) Prepare a N.M. preparation, and arrange it in the muscle-chamber in the usual way, laying the nerve over one electrode. One of the electrodes will therefore be connected with the secondary circuit.

(*c*) Make and break the primary circuit; there is no contraction.

(*d*) Destroy the insulation of the preparation by touching the muscle, or what does better, allow the brass support of the muscle to touch a piece of

moist blotting-paper on the inner surface of the glass shade of the chamber. Every time the brass binding of the shade is touched, or the brass support itself, the muscle contracts. Touch the S coil and contraction results.

D. Arrange an induction coil for tetanising shocks. Prepare four N.M. preparations and lay them on a clean glass plate, arranged so that the nerve of the first rests on the single electrode from the S coil. Let the second nerve rest on the first muscle, the third on the second, and the fourth nerve on the tendon of the third muscle. Gradually approximate the secondary to primary coil. Note that No. 1 muscle contracts first as the tension rises, and as the distance between the coils is lessened the second muscle also begins to contract, and finally all four will contract.

Here the unipolar action is greater nearer the secondary coil—*i.e.*, when the conducting surface is small. This exemplifies the danger of unipolar stimulation in tissues near the secondary coil when the currents are strong.

8. Relative excitability of muscle and nerve.—Using an N.M. preparation, find the minimal shock or tetanising current to cause a muscular response when the nerve is stimulated. Apply the same current to the muscle—direct stimulation. It will be inadequate; no response. Slide up S coil until response is obtained—indirect stimulation. Tabulate results.

Nerve is more excitable to this form of stimulation than muscle, but one must not conclude from this experiment that the muscle itself is directly excitable, because the nerve-terminals in the muscle are not excluded. They can be eliminated by curare. (Lesson XXXIII.)

9. Nerve more excitable than muscle.—Arrange a coil for single B shocks. Prepare two N.M. preparations A and B. Lay the end of the nerve of B on the muscle A, and arrange electrodes to pass a current directly through A.

Stimulate A directly with single break shocks of gradually increasing intensity. B will contract before A, although it is only stimulated by the current which passes through the part of its nerve lying in A.

10. Summation of inadequate single stimuli.—Find on a N.M. preparation a strength of single break induction shock—*i.e.*, by removing secondary from primary coil—that is just not quite sufficient or adequate to cause a muscular response. Wait a minute; at intervals of five seconds repeat the break, inadequate stimuli. After a time the muscle will respond. Why? Each shock causes an excitation, and leaves an after-vibration which lasts longer than the shock itself, and thus a certain amount of stimulation is possible.

11. Changes in excitability in a dying nerve.—Place a nerve with vertebral column attached on waxed paper. Moisten with normal saline, cover with a shade to form a moist chamber. Test excitability by single minimal induction shocks at the middle of the nerve, judging the excitability in terms of the muscular response. Test near and far from muscle. Usually the excitability is not equal. Cut the nerve near the spinal column. After a time the cut end is more excitable than the end near muscle, but later on it becomes less.

12. **Stimulation by condenser.**—Connect (1) a cell with the two pools (5 and 6, fig. 99) of a Pohl's commutator *without* cross bars; (2) the middle pair of pools (1 and 2) to the terminals of a condenser; and (3) the remaining two pools (3 and 4) to a nerve of a N.M. preparation.

When the cradle of the commutator is turned in the direction of the battery the condenser is "charged." When it is turned over the battery circuit is cut off, and the condenser charge is "discharged" through the nerve, the muscle responding with a single twitch.

LESSON XXXII.

RHEONOME—DIRECT, INDIRECT STIMULATION—MUSCLE SOUND—DYNAMOMETERS.

1. **Rheonome and law of excitation.**—This instrument (fig. 121) is useful for showing du Bois-Reymond's so-called (Lesson XXXI.) law, that it is not the absolute intensity of a galvanic current flowing through a nerve which excites it, but the *rapidity* of the variations in the intensity which excite a motor nerve. The apparatus consists of a square ebonite base, with a grooved circular channel in it, and two binding screws, with zinc attached, and bent over so as to dip into the groove, which is filled with a saturated solution of zinc sulphate. A vertical arm, with binding screws attached to two bent strips of zinc, moves on a vertical support. Stimuli so obtained have been called "time stimuli" by v. Kries to distinguish them from the make and break shocks, which are called "momentary stimuli." It is a kind of revolving rheochord.

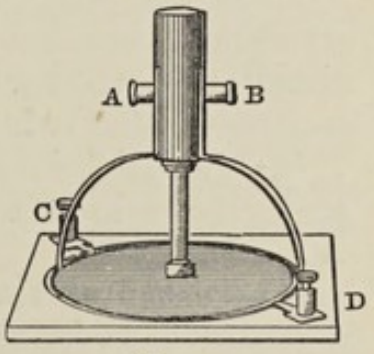


Fig. 121.
Fleischl's rheonome.

(*a*) Connect two or three cells coupled together for intensity—*i.e.*, copper to zinc—with the fixed binding screws C and D, introducing a du Bois key in one wire. Attach the electrodes, introducing a du Bois key to short-circuit them, to the binding screws A and B. Fill the groove with a saturated solution of $ZnSO_4$. Amalgamate the zinc tips which dip into the $ZnSO_4$.

(*b*) Arrange the nerve of a N.M. preparation over the electrodes, or simply expose the sciatic nerve of a frog *in situ*.

Pass a constant current through the nerve, observing the usual effects—viz., contraction at make or break, or both, but none when the current is passing. Then suddenly rotate the handle with its two zinc arms; this is equivalent to a sudden variation of the intensity of the current—the current, of course, continuing to pass all the time. The muscle suddenly contracts.

When the two ends of the zinc arc stand as in the fig.—*i.e.*, opposite C and D, then on closing the current, most of the current goes through the zinc arc to the preparation, and only a small part through the zinc sulphate solution from C to D. When the arm ends are near C and D, and the battery circuit closed, there are three paths open to the current—one in each half of the circular trough, and the third by the zinc arms and electrodes. The muscle contracts according to the direction and intensity of the current, either on closing or opening the key, or at both. Turn the handle so that the zinc arc is vertical to a line joining C and D—*i.e.*, exactly at right angles to its former position. There is no current through the arm, as its ends are in contact with points of equal potential, so that there is no response either on closing or opening.

If, while the zinc arc is in this position, the circuit be closed, and the zinc arc suddenly rotated into the position of the line C, D, the muscle contracts, provided in the first experiment a closing—*i.e.*, make, contraction was obtained. If it be rotated slowly then there is no response. Thus one can allow the current to glide or slide into the nerve ("*einschleichen*") without causing excitation.

Summary.—The response of a muscle or nerve, or indeed excitable tissues as a whole, to electrical stimulation is due, not to the simple flow or intensity of a current through the tissues, but rather to the more or less *sudden* change in the strength of the current. Sudden opening or closing of a galvanic current, or its sudden increase or decrease, may act as an efficient stimulus, but the *gradual* increase or decrease of the current causes no response.

2. **Direct and indirect stimulation of muscle.**—When the stimulus is applied directly to the **muscle** itself, we have **direct stimulation**; but when it is applied to the **nerve**, and the muscle contracts, this is **indirect stimulation** of muscle.

(i.) *Induced current.*—(*a*) Arrange a N.M. preparation, and coil induction for single or repeated shocks. (Lesson XXXI., 1).

(*b*) Test first the strength of current—as measured by the distance between the S and P coils—which causes the muscle to contract when the stimulus is applied to the nerve—*i.e.*, for **indirect stimulation.**

(*c*) With the S still at the same distance from the P, try if a contraction is obtained on stimulating the muscle **directly.** It does not contract; but make the current stronger, and it does so. It will be found that by direct stimulation the interval between the strength of current to obtain minimal and maximal

contractions is greater than by indirect stimulation. The excitability of muscle to direct stimulation is best done after the nerve-terminations have been paralysed by curare. (Lesson XXXIII.)

(ii.) *Constant current.*—Connect the electrodes with two cells, with a Hg-key in circuit. Place the electrodes under the nerve. Contraction occurs at make only, and at break only if the preparation is very excitable, but there is usually no contraction when the current is passing through the nerve.

3. **Muscle sound produced during voluntary muscular contraction.**—(*a*) Insert the tips of the index-fingers into the auditory meatuses, forcibly contracting the biceps muscles. A low rumbling sound is heard. It is transmitted by the tissues of the arm to the cranial bones, and thence to the internal ones.

(*b*) When all is still at night, firmly close the jaws, and, especially if the ears be stopped, the sound is heard. It is a note of low pitch, 36 vibrations per second (*Wollaston*).

(*c*) Place one wrist against the ear, alternately clench and relax the fist, and listen; or, with a stethoscope, auscultate the flexor surface of the fore-arm during the same movements, or place the stethoscope over the contracting masseter.

[It was regarded as the first over-tone of the note of 18 vibrations per second produced in the muscle itself. Had this been so, the vibrations in a muscle during voluntary muscular contractions—"voluntary tetanus"—would be 18 per second. It appears to be the natural resonance-tone of the ear, which becomes intensified by a mixture of notes and noises. Most voluntary contractions show 8 to 10 vibrations per second.]

(*d*) Set up a coil, introducing reeds vibrating at various rates—20, 30, 50, 100 per second—in P coil. Connect the S coil by long wires to a key in another room. Stimulate the fore-arm muscles, and listen for a note of a pitch corresponding to the rate of stimulation.

4. **Telephone experiment.**—(*a*) Arrange the nerve of a N.M. preparation over a pair of electrodes. Connect the latter with a short-circuiting key. To the key attach the two wires from a telephone.

(*b*) Open the short-circuiting key; shout into the telephone, and observe that on doing so the muscle contracts vigorously.

5. **Rupturing strain of muscle and tendon.**—(*a*) Dissect out the femur and gastrocnemius with the tendo Achillis of a frog. Fix the femur in a strong clamp on a stand. To the tendo Achillis tie a short stout thread, and hang a scale-pan on to it.

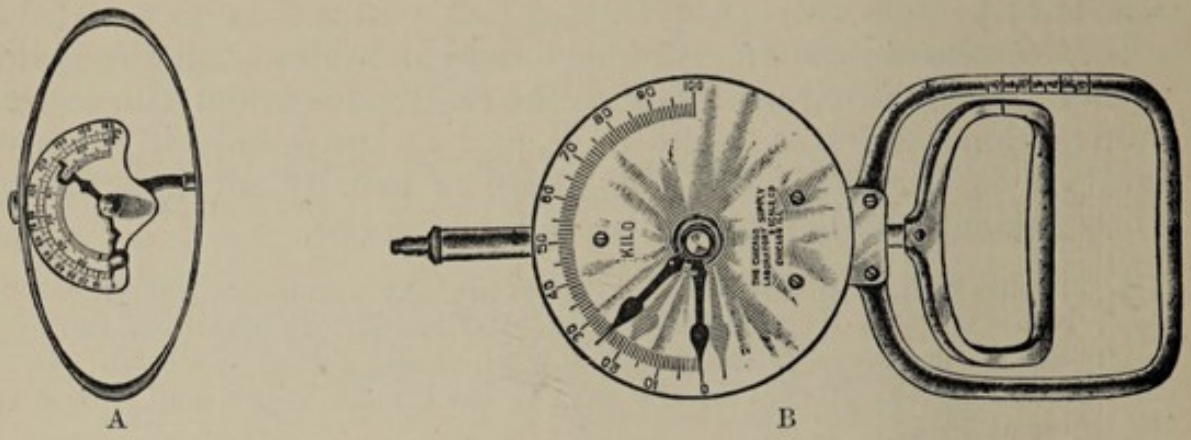
(*b*) Place weights in the scale-pan, and note the weight required to rupture the tendon or muscle. Usually the muscle is broken first. The weight may be 1 kilo., more or less, according to the size of the frog.

(*c*) Compare the rupturing strain of a frog's gastrocnemius which has been dead for 48 hours. A much less weight is required.

6. **Dynamometers** (see also "Fatigue") (*a*) **Hand.**—Test the force exerted, first by the right hand and then by the left, by means of Salter's or Colin's dynamometer (fig. 122).

(*b*) **Arm.**—Using one of Salter's dynamometers, test the strength of the arm when exerted in pulling, as an archer does when drawing a bow.

7. **Specific gravity of muscle.**—This is determined in the same way as that of blood (Lesson V., 4) by placing part of a fresh muscle free from adhering air bubbles in a solution of NaCl of known S.G. The



A B

Fig. 122.—A, Colin's dynamometer. B, Salter's dynamometer as made by Chicago Laboratory Supply and Scale Company.

variations are between 1048 to 1074. Weber gives 1058 as about the mean. The red muscles, as a rule, have a lower S.G. than the pale. The heart has a less S.G. than other visceral muscles (J. Carvallo and G. Weiss, *Journ. de Phys. et de Path. gen.*, 1899).

LESSON XXXIII.

INDEPENDENT DIRECT MUSCULAR EXCITABILITY—CURARE.

1. Independent inherent muscular excitability.—Curare paralyses the intra-muscular terminations of motor nerves. **Apparatus.**—Cells, coil, two keys, three ordinary and two fine wires, hand electrodes, waxed paper, scissors, fine-pointed forceps, fine aneurism-needle, or fine sewing-needle fixed in a handle, with the eye free to serve as an aneurism-needle, thin tape, fine threads, pithing-needle, 1 p.c. filtered watery solution of curare, hypodermic syringe or glass pipette. [For much interesting information regarding curare and Bernard's experiments, see Cl. Bernard's *Leçons sur les effects des substances toxiques*, 238, *et seq.*, 1856, Paris.]

(*a*) Arrange the battery and coil for an interrupted current with a key in the P circuit, and a du Bois key to short-circuit the S, as in Lesson XXXI., 2.

(*b*) Destroy the brain of a frog, and plug the skull cavity (to prevent bleeding) with a match or cotton wool. With a hypodermic syringe or a fine glass pipette inject into the ventral or dorsal lymph-sac two drops of a 1 p.c. watery solution of curare. [The curare of commerce is only partly soluble in water, but its active constituent curarin ($C_{10}H_{15}N$) is soluble in tap water. Rub up 1 grm. curare in 100 c.c. water and filter.] The poison is rapidly absorbed. At first the frog draws up its legs when pinched. It soon ceases to do so, and it lies in any position in which it is put. The legs are not drawn up on being pinched, and the animal lies flaccid and paralysed—toneless and without cutaneous reflexes—and it exhibits no respiratory movements.

(*c*) Expose the heart, and observe that it is still beating.

(*d*) Expose one sciatic nerve.

(i.) Stimulate it with interrupted shocks = *no contraction.*

(ii.) Stimulate the corresponding *muscles* = *contraction.*

Therefore curare has paralysed some part of the motor nerves [*or connection between N and M*], *but not the muscles.*

In curare poisoning the nerve-trunk itself is not inexcitable, but the nerve-endings in the skeletal muscles are so affected as to prevent the excitatory state of the nerve being propagated to the muscle. The following experiment proves this :—

2. Curare acts at periphery of motor nerve.

(*a*) Arrange coil as in the previous experiment.

(*b*) Destroy the brain of a frog. Expose the sciatic nerve and the accompanying artery and vein on one side—*e.g.*, the *right*—taking great care not to injure the blood-vessels (fig. 123). Isolate the sciatic nerve, and then ligature all the other structures of the thigh as low down as possible to prevent blood from passing into the limb. None of the poison passes by a collateral circulation into the parts below the ligature. Or ligature the middle of one thigh with tape to obstruct the circulation, but not crush the nerve. This is the protected, or unpoisoned, limb.

(*c*) Inject a few drops of a solution of curare into the ventral lymph-sac. The poison will be carried to every part of the body except the right leg below the ligature. The animal is rapidly paralysed (20 to 30 mins.), but if the non-poisoned leg (right) is pinched, it is drawn up, while the poisoned leg (left) is not—*i.e.*, there is a reflex movement of the non-poisoned limb, so that the afferent (sensory) nerves, spinal centre and motor nerves are still unaffected.

(*d*) Wait until the animal is thoroughly curarised—*i.e.*, when

all reflexes cease—and then expose *both sciatic nerves* as far up as the vertebral column and as far down as the knee. Place the upper ends of the nerves on waxed paper.

(i.) Fix the knees with pins, so that the leg movements can be seen (fig. 123). Stimulate the *left* sciatic nerve. There is no contraction. Therefore the poison has acted either on nerve or muscle.

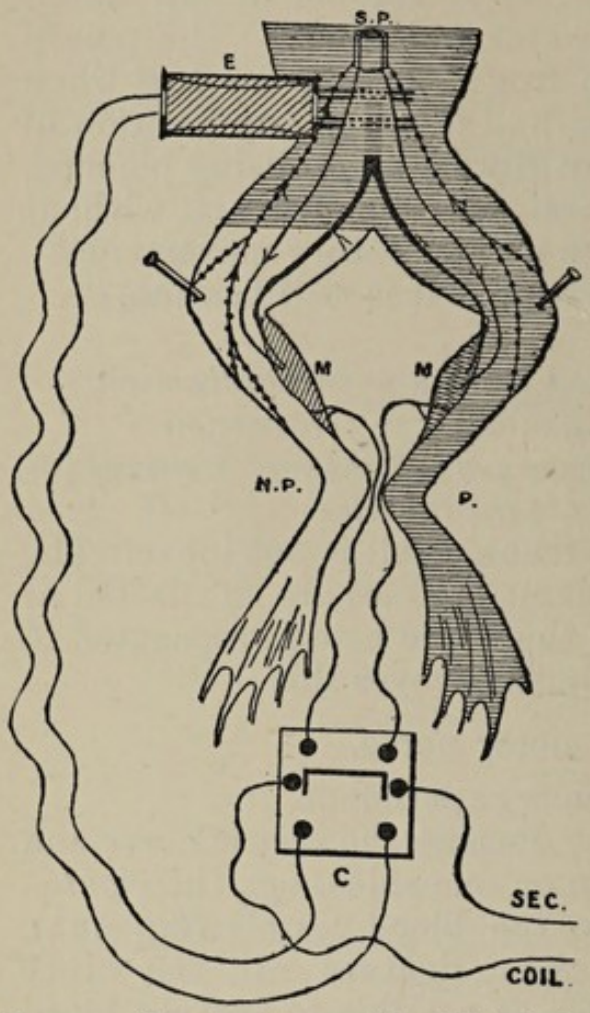


Fig. 123.—Curarised frog with right sciatic artery ligatured. *S.P.*, Spinal cord; *N.P.*, Non-poisoned limb (right); *P.*, Poisoned (left); *E.*, Electrodes; *C.*, Commutator; *M.*, Gastrocnemius muscles; *Sec. Coil*, Wires to S coil.

(ii.) Stimulate the *left* gastrocnemius muscle; it contracts. Therefore the poison has acted on some part of the nervous path, but not on the muscle.

(iii.) Stimulate the *right* sciatic *above the ligature;* the right leg contracts. The part of the nerve above the ligature was supplied with poisoned blood, so that the nerve-trunk itself is not paralysed, as may be proved by stimulating any part of the *right* sciatic as far down as its entrance into the gastrocnemius. Stimulating any part of the right nerve causes contraction. Therefore neither nerve-trunk nor muscle is affected. The nerve-impulse is blocked somewhere, in all probability by paralysis of the terminations of the motor nerves within the muscle.

(*e*) Apply several drops of a strong solution of curare to the *right* gastrocnemius, and after a time stimulate its nerve; there is no contraction, but on stimulating the muscle itself contraction takes place.

3. Or curarise a frog after ligaturing the blood-vessels of one limb. Expose the whole length of both sciatic nerves from the spine to the gastrocnemius muscles. Pin out the legs on a cork plate. Slip hand electrodes, E—guarded below with sheet india-rubber—under the nerves near the spine, and connect with a Pohl's commutator, C, as in fig. 123. Bring other two wires

direct to the gastrocnemii from the commutator; connect up the commutator with the secondary coil of an induction machine served with two Leclanché cells and arranged for interrupted shocks. The current can then be sent through either pair of wires—*i.e.*, through both nerves or both muscles.

(1) Stimulate both nerves with minimal interrupted shocks. The muscle on N.P.—*i.e.*, the protected side—contracts, that on P—poisoned—does not. The nerve of N.P. is not paralysed, for it is stimulated in the poisoned area.

(2) Reverse commutator and stimulate both muscles, but use a stronger current. Both respond. They are not paralysed.

(3) Stimulate the muscles with minimal current, and note that M. on the non-poisoned side responds first (*Rosenthal's modification*). Push up the S coil, and the muscle on the poisoned side also responds. Why? *Nerve is more excitable than muscle* to induction shocks. In N.P. the end terminals are excited by a feeble current and cause contraction in the N.P. side. In the P. side these terminals are poisoned, and thus it happens that the strength of the current has to be so increased as to discharge a response by what is practically direct stimulation of *nerveless* muscle. Curare thus paralyses the motor nerve-terminals—the weakest link perhaps between the nerve and its muscle.

The **direct excitability of muscle** is further proved by other experiments, which we owe to W. Kühne († 1900).

(1) Sartorius experiment (p. 239).
(2) Kühne's curare experiment (p. 242).
(3) Gracilis experiment (Lesson L.).

4. Kuhne's sartorius experiment (*Glycerine stimulates nerve, but not muscle*).

(*a*) Isolate the sartorius (fig. 120 and p. 230); suspend it by a thread tied round its tibial attachments—*i.e.*, with its iliac end downwards. The upper third of the iliac end is free from nerves, or, to be more exact, Kühne showed that at both ends, for about $\frac{1}{8}$ of the entire length of the muscles, there are no nerves (*Arch. f. Anat. u. Phys.*, 1895. There are in the same volume other papers by Kühne on direct excitability of muscle).

(*b*) Allow the iliac end to dip into a drop of glycerine placed on a greasy surface. The muscle gives no response. Why? Because it is devoid of nerve-fibres. Then, with sharp scissors, cut across the muscle about 4 mm. higher up and dip the fresh section into glycerine. Soon the muscle twitches. Why? As glycerine stimulates nerve and not muscle, there is no response

until the glycerine is either directly applied to nerve-fibres, or diffuses to affect them.

Kühne used this experiment to demonstrate the independent or direct excitability of muscle and nerve.

That glycerine stimulates nerve is shown in Lesson XXX. If the end of the nerve dipped in glycerine be cut off, the twitchings cease.

A substance may stimulate muscle and not nerve—e.g., *ammonia.* (Lesson XXX.).

The foregoing and some subsequent experiments clearly prove that—

(*a*) Muscle is excitable without the intervention of nerves or nerve-endings—*i.e.*, direct or independent excitability.

(*b*) The excitability of muscle is independent of the excitability of its nerve.

Some drugs may paralyse nerves and not muscles. Some agents may act as stimuli to muscle, but not to nerve; whilst others stimulate nerve, but not muscle.

There is much other evidence bearing on these important points, but it is omitted here.

5. Relative excitability of muscle and nerve.

(*a*) Prepare a frog as for the curare experiment—*i.e.*, protect one leg by a ligature, then inject curare into a lymph-sac. After the curare has acted, expose both sciatic nerves and both gastrocnemius muscles.

(*b*) Note the approximation of the S coil to the P required to obtain a mechanical response or contraction to—

(i.) Single make induction shocks,
(ii.) Single break induction shocks,
(iii.) Faradisation,

when the electrodes are applied to the sciatic nerve of the ligatured limb—*i.e.*, the protected limb. Tabulate results.

(*c*) Apply the electrodes directly to the gastrocnemius muscle of the opposite side—*i.e.*, the poisoned limb, which is practically nerveless, as curare paralyses the terminations of the motor nerves. It will be found that a stronger shock is required to cause the muscle to contract than is necessary through the intervention of the nerve—*i.e.*, muscle is less excitable than nerve.

Direct stimulation of nerveless muscle.		Distance of Primary from Secondary coil in cm.		Stimulation of nerve of ligatured limb.	
M.	B.			M.	B.
o	o	22	30	o	C
o	C	21	29	o	C
o	C	20	28	o	C
C	C	19	27	C	C

Faradisation.

Nerveless muscle.	Distance of P from S.	Ligatured limb.
o	35	C
o	34	C
o	33	C
C	32	C

Additional Exercises.

6. **Curare** (*Rosenthal's modification*).

(*a*) Prepare a frog as in the previous experiment, ligaturing the left leg —all except the sciatic nerve—and inject curare. After complete paralysis occurs, dissect out both legs with the nerves attached. Attach straw flags, NP and P, of different colours to the toes of both legs by pins, and fix both femora in muscle-forceps, F, with the gastrocnemii uppermost (fig. 124). Place the nerves, N, on the platinum points of du Bois-Reymond's electrodes (fig. 107).

(*b*) Arrange the coil for tetanising shocks (fig. 124), connecting the terminals of the secondary coil with a Pohl's commutator *without cross-bars*, H. Two other wires pass from the commutator to the electrodes, N, while two *thin* wires pass from the other two binding screws, C, and their other ends are pushed through the gastrocnemii muscles. The commutator enables the tetanising currents to be passed either through both nerves or both muscles. It is more convenient to have a key in the S circuit.

(i.) Set Neef's hammer going, and pass the current through *both nerves;* only the non-poisoned leg, NP, contracts.

(ii.) Reverse rocker, pass current through *both muscles; both contract.*

(iii.) *Rosenthal's modification.*—Push the S coil far away from the primary, and pass the current through *both muscles.* At first, if the coils be sufficiently far apart, neither muscle contracts. Gradually push up the S coil, and notice that the *non-poisoned limb contracts* first. Why? Because the terminations of the nerves are directly excited, and they are more excitable than the muscle. Continuing to push up the S coil, both muscles ultimately contract.

7. **Curare**—*Bernard's method.*—Prepare two N.M. preparations, and dip the nerve of one (A) and the muscle of the other (B) into a solution of curare in two watch-glasses. On stimulating the nerve of A, its muscle contracts; on stimulating the nerve of B, its muscle does not contract, but

the muscle contracts when it is stimulated directly. In A, although the poison is applied directly to the nerve-trunk, the nerve is not paralysed.

8. Kühne's curare experiment.—(*a*) To the margin of a meat-plate fix two copper slips, to serve as attachments for the electrodes, and between them place a strip of filter-paper moistened with normal saline.

(*b*) Cut the excised sartorius transversely into five pieces of nearly equal length. Place them in their original order on filter-paper, numbering them 1 to 5. Pass a feeble tetanising current through the muscle. The central parts—*i.e.*, 2, 3, and 4—contract, while 1 and 5 do not. On making the current stronger the terminal parts also contract. Why? Because there are no nerves at the ends of the sartorius, and in the first instance the muscular fibres are really excited by stimulation of the intramuscular termination of the nerves, while the end parts were stimulated directly.

(*c*) If a curarised sartorius be used in the same way, all the parts contract at once, because all its motor nerves are paralysed.

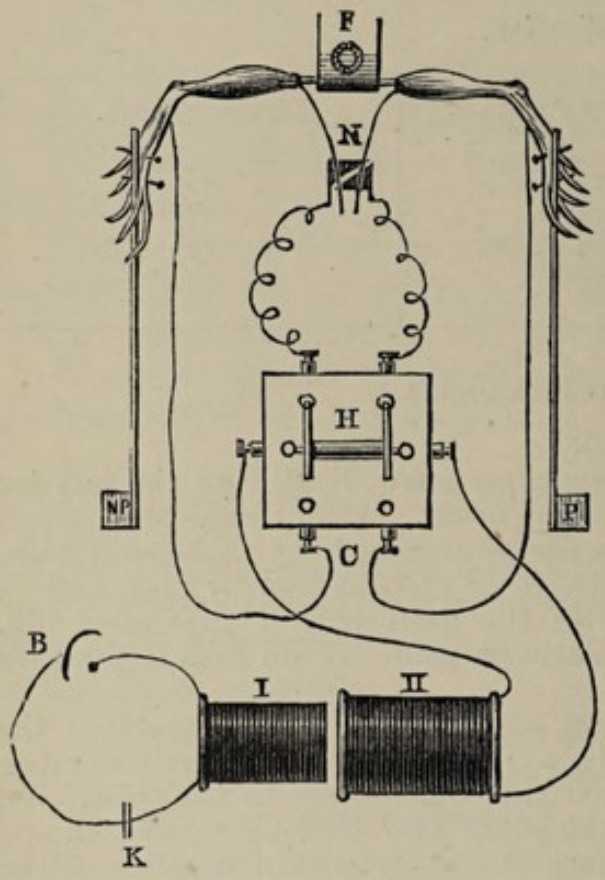


Fig. 124.—Scheme of curare experiment. *B*, Battery; *I*, P; *II*, S coil; *N*, Nerves; *F*, Clamp; *N.P.*, Non-poisoned leg; *P*, Poisoned leg; *C*, Commutator; *K*, Key. Short-circuiting key in circuit omitted in diagram.

9. Curarised sartorius and constant current.

Dissect out a curarised sartorius in its whole extent—*i.e.*, a nerveless muscle with parallel fibres. Lay it on waxed paper or a paraffin block, and arrange under it a pair of electrodes so that the ends of the muscle rest one on each electrode. Connect the electrodes with a Pohl's commutator with crossbars, and the latter to two Daniell cells with a key in one wire.

(1) Stimulate with the constant current until the muscle is nearly exhausted. Observe that only the end of the muscle connected with the zinc pole—*i.e.*, kathode—contracts when the current is made. At the kathode the excitability rises from normal owing to the appearance of the kathelectrotonic state.

(2) A contraction may start from the anode when the current is broken—*i.e.*, there is a rise of excitability from the anelectrotonic (lower) state to normal.

This experiment not only shows the direct excitability of muscle, but also that when a part of a muscle passes suddenly from a lower to a higher state of excitability an impulse is generated at that point.

10. Curare does not affect nerve-centres or centripetal nerves.—Apply to the skin of a pithed frog (cord intact) a drop of strychnine solution (0·1 p.c.). This increases the reflex excitability. After a time protect one leg with a ligature and curarise. Pinch the motionless unprotected or

poisoned limb, the non-poisoned or protected limb will give a reflex response. *Curare* does not appreciably affect *centrifugal or centripetal nerve-fibres or spinal nerve-centres. Its action is on the junction between nerve and muscle*—i.e., *on the motor end-plates.*

LESSON XXXIV.

GRAPHIC METHOD—MOIST CHAMBER—SIMPLE CONTRACTION—TWITCH.

A RECORDING surface is required, and for most purposes a revolving cylinder covered with glazed paper and smoked over a turpentine or gas flame is the most convenient. Most of the movements we have to study are so rapid that they cannot be properly investigated by the unaided eye. By the graphic method we obtain a permanent record of each movement, which can be studied at one's leisure, the general principle being that the tissue whose movements we wish to study records its movements usually through the intervention of a lever, and thus—correspondingly magnified—upon a moving surface. The change of form is one of the indications of the transformation of energy in the tissue. A muscle in action evolves also chemical and thermal energy. In order to determine the *time* occupied by any given movement, we require to record the vibrations of a tuning-fork of known rate of vibration.

1. Arrangement of work-table.—The recording drum, which revolves from right to left, is placed on the table to the left of the student. On the extreme right of the table is the battery and between the two the induction coil, with a M and B key in the P circuit between the battery and the coil, and a du Bois key—arranged to short-circuit the S circuit—between the coil and the drum. The P circuit is then coupled up (spirally wound wires), and the same with the S circuit; the wires of the latter or electrode wires being attached to some kind of a myograph with a moist chamber to protect the N.M. preparation. Each student is provided with a frog-plate, a vessel for *débris*, wires, millimetre scale, normal saline, and the necessary apparatus.

It is important to remember that the more complex the machinery the greater the liability of errors due to the apparatus.

2. **Recording surface.**—Use a revolving cylinder or other moving surface covered with smoked glazed paper. The velocity of the moving surface is usually determined by recording simultaneously the vibrations of a tuning-fork of known rate of vibration, or an electro-magnetic time-marker, or by a vibrat-

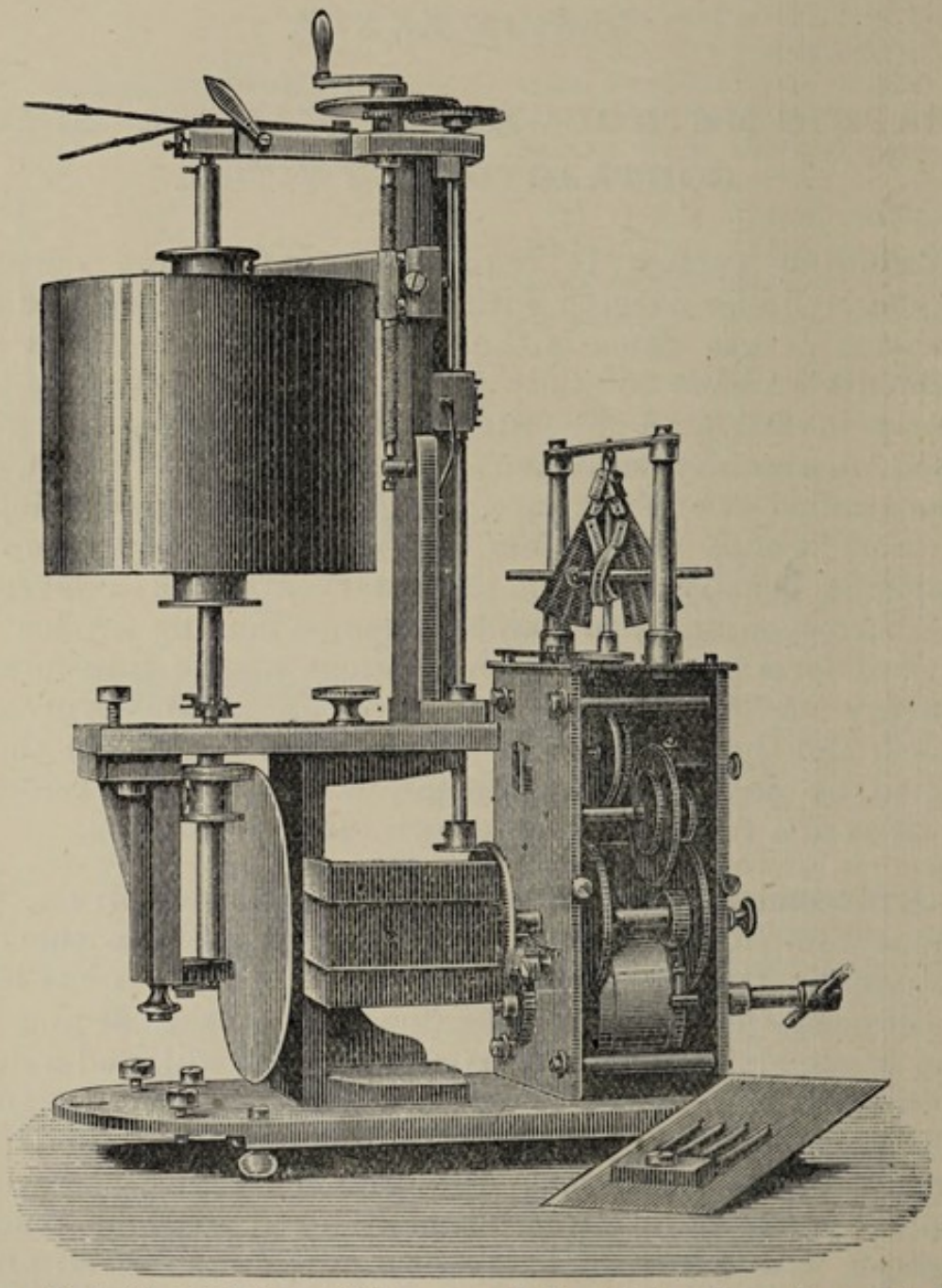


Fig. 125.—Ludwig's revolving cylinder, moved by clockwork, and regulated by a Foucault's regulator on the top of the box. A disc, moved by clockwork, presses upon a wheel, which can be raised or lowered by a screw, thus altering the position of the wheel on the disc so as to cause the cylinder to rotate at different rates. The cylinder itself can be raised by turning a handle. Made by Petzold.

ing reed. It does not matter particularly what form of recording drum is used, provided it moves smoothly and evenly, and is capable of being made to move at different speeds as required. Sometimes this is done by placing the drum on different axles, moving at different velocities. In Ludwig's form (fig. 125) a small wheel moves on a large brass disc. The drum is 13 cm. high, and has a circumference of 50 cm. It is driven by friction rollers, and can be run either vertically or horizontally. According to the position of the small wheel or the large friction wheel, and by adjusting the regulator, one can vary the rate of revolutions within very wide limits. An arrangement of shafting, moved, say, by a water-motor, or turbine, or electric-motor, from which several drums can be driven by cords, is very convenient. The Thirlemere motor may also be used for actuating a number of recording cylinders. Each pair of students is provided with recording apparatus, which is placed at the left end of the table, so that the other apparatus can be arranged to the right of it. The vertical cylinder or drum (6 ins. diameter) can be adjusted on its upright spindle, from which it can be removed to cover it with the paper, which is to be smoked.

3. **Covering the drum and smoking the paper.**—The paper is glazed on one surface, and is cut to the necessary size to suit the drum. The drum can be removed, and is then covered with a strip of paper, the latter being laid on evenly to avoid folds, glazed side outermost. One edge of the paper is gummed, and overlaps the other edge. Wrap it round the drum, and leave it for a few minutes until the gum dries. The paper should be cut wider than the height of the drum. This protects the drum from the effects of the smoke. The paper has then to be smoked in a draught chamber, by holding the drum and keeping it moving uniformly over a fan-tailed or bat's wing gas-burner, or paraffin lamp (6-inch wick). Take care that the soot from the flame is deposited evenly and lightly, and see that it is not burned into the paper. At the overlap on the paper rub off a vertical layer of soot, leaving a white streak. The drum is then placed in position. (See *Appendix.*)

To obtain a very fine film of soot, Hürthle has invented a "smoke-spray." The soot from the flame of a turpentine lamp is blown by means of an elastic ball-bellows against the paper. We find that a large gas flame is the most convenient.

4. **General rules for graphic experiments.**

(1) Arrange the apparatus completely, cover the drum with paper, and smoke it, before beginning the dissection.

(2) Test all the connections stage by stage as they are made. In recording, let all curves begin just after the overlap.

(3) With a smooth point inscribe on the tracing your name, the date, and what it shows; then varnish it. In removing the tracing from the drum, hold the drum vertically in the left hand, with the left thumb pressing near the overlap, and cut through the paper near the overlap edge.

(4) Determine the magnification of the movement as recorded by the lever.

5. Myographs.—Various forms are in use, but most of them consist of a light lever which is raised by the contracting muscle, and so arranged as to record its movement on a smoked surface of paper or glass. Such curves are called "**isotonic**" by Fick. The movements of the muscle are thereby magnified and rendered visible to the eye. Or the lever may record its movements on a moving surface. Taking advantage of the fact that a muscle when it contracts becomes both shorter and thicker, myographs have been constructed on three principles :—

(α) Shortening of muscle attached to a lever.
(β) Thickening of muscle on which the lever rests.
(γ) Changes in tension.

Suppose a muscle to be so fixed that during activity it can contract but little we can record changes of tension by the so-called "**isometric**" method introduced by Fick. (Lesson XXXVI.).

The recording surface on which the lever style writes may be—

(1) Stationary (*Pflüger's*).
(2) Revolving (*Helmholtz's*).
(3) Swinging pendulum (*Fick's*).
(4) Moved by a spring, either vertically or horizontally (*du Bois-Reymond*).

6. Muscle-lever (change in length of muscle).—It is customary to use such a simple muscle-lever as is shown in fig. 126, with the weight attached directly under the point of attachment of the muscle to the lever. This has its disadvantages, as it is set into vibration by the rapid rise of the lever. Fick has shown that by using a light straw lever, the muscle itself being made tense not by a weight applied directly under the point of attachment of the muscle to the lever, but by attaching the weight over a small pulley fixed to the steel axis to which the lever is attached, the weight is raised but little, and even with a rapid

contraction does not move quickly. A crank-myograph is very serviceable.

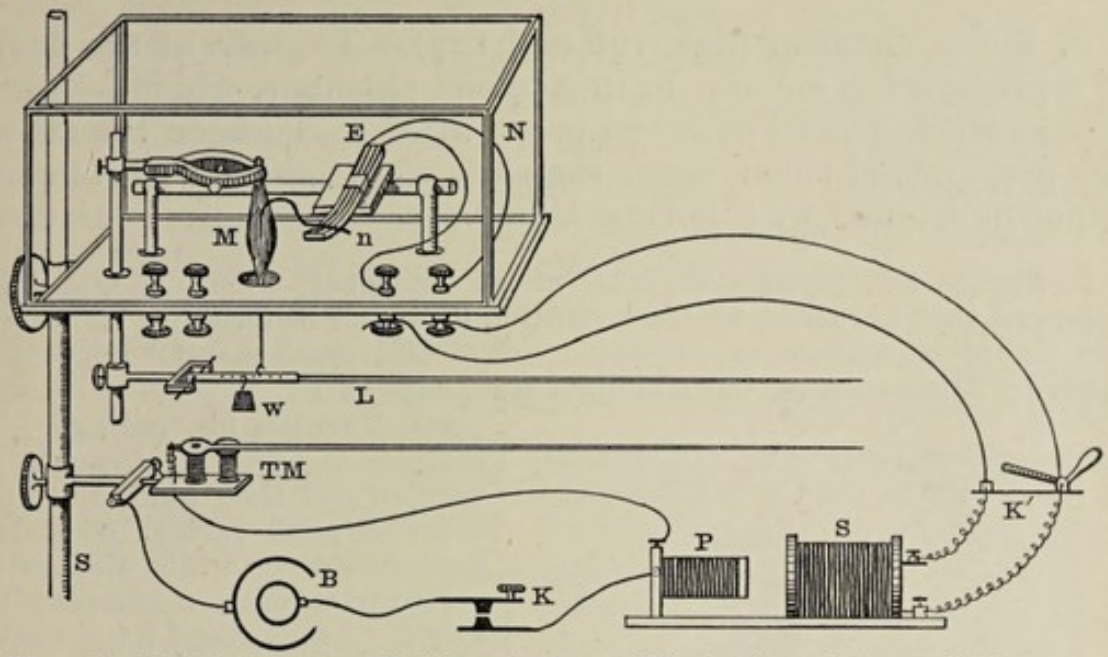


Fig. 126.—Moist chamber. *N*, Glass shade; *E*, Electrodes; *L*, Lever; *W*, Weight [illegible] *TM*, Time-marker; other letters as in previous figures.

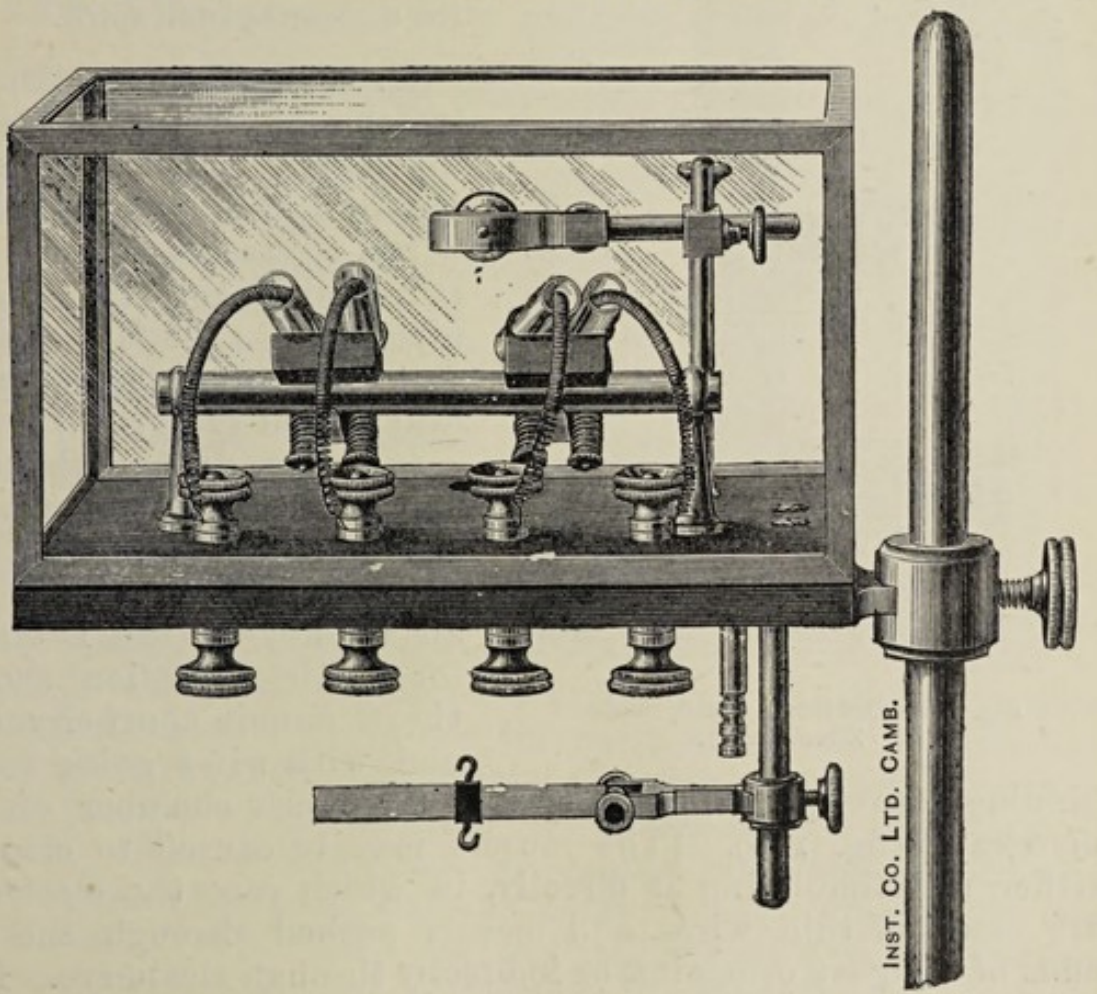


Fig. 127.—Moist chamber for muscle, with two pairs of electrodes, muscle-forceps, and recording lever. To the latter is fixed a straw.

7. **Writing points.**—A triangular piece of vegetable parchment or thin sheet aluminium fixed to the end of the recording straw lever does very well.

8. **Moist chamber** (figs. 126 and 127).—To prevent the drying of a preparation enclose it in a moist chamber, which is merely a glass shade placed over the preparation. To keep the air and the preparation moist, cover the sides of the shade with blotting-paper moistened with normal saline.

9. **Stand for myograph and moist chamber.**—This should be so arranged that the point of the recording lever can be switched off or on to the recording surface as required. A "Universal stand" (fig. 128) is made by Zimmermann, but a simpler form suffices.

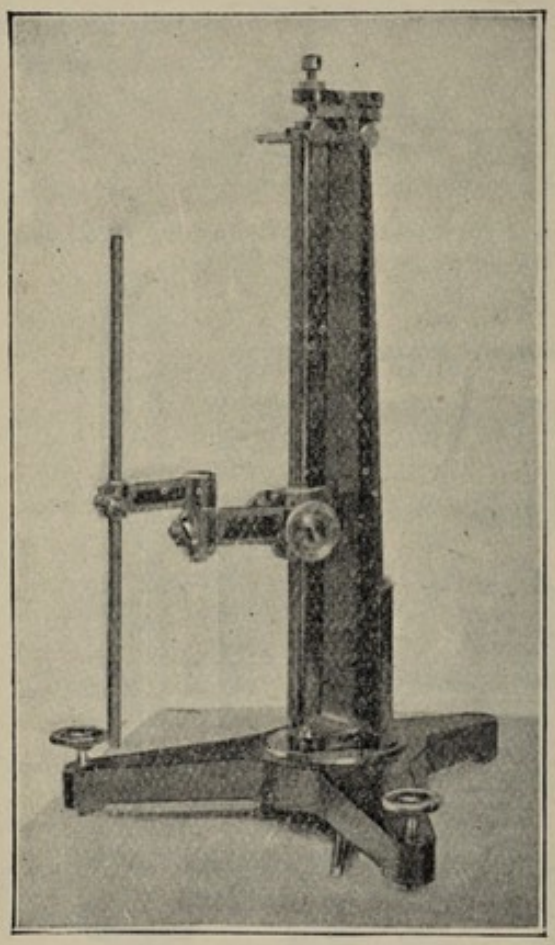


Fig. 128.—Universal stand, made by Zimmermann.

10. **Varnish for tracings.**—The tracing is drawn through the varnish and then hung up to dry.

(*a*) A good varnish consists of gum mastic or white shellac dissolved to saturation in methylated spirit. Let it stand for a week or two.

(*b*) Dissolve 15 grms. of rosin in 100 c.c. methylated spirit.

11. **Single contraction or twitch—Apparatus.**—Recording drum, Leclanché cells, Hg-key, coil, du Bois key, wires, electrodes, moist chamber and lever (or crank-myograph), blotting-paper, stout ligatures, hook, pins, lead weight (20 grms.).

(*a*) Cover drum with glazed paper, smoke it, and arrange it to move slowly.

(*b*) Arrange the cells and an Hg-key in the P circuit for single induction shocks, the S circuit short-circuited, and with wires going to the binding screws on the platform of the moist chamber on the myograph (fig. 126). [The muscle may be caused to contract either by stimulating it **directly**, in which case the electrodes are made of thin wires, and merely pushed through the two ends of the gastrocnemius, or **indirectly** through the nerve. It is convenient to use the latter method (Lesson XXXII.).]

(*c*) Make a N.M. preparation, leaving the lower end of the femur attached to the gastrocnemius, and cut away the tibia and fibula. With the point of a sharp pair of small scissors make a hole in the tendo Achillis, and insert in it an S-shaped hook, made by bending a pin. Fix the femur in the muscle clamp of the moist chamber, and by means of a stout thread or fine wire attach the hook in the tendo Achillis to the writing-lever. See that the thread or wire goes clear through the hole in the stage. Place the nerve on the electrodes, and cover the preparation with the glass shade lined on three sides with moist blotting-paper. Load the lever with 20 grms. either directly, or place the weight scale-pan near where the muscle is attached to it. The lever itself must be horizontal. The writing-style may be made of very thin aluminium or parchment paper, fastened to the lever with sealing-wax or telegraph composition. As arranged, the P circuit is made and broken by hand.

According as the recording surface is stationary or moving when the muscle contracts and raises the lever, either an upward line or a curve will be made upon the paper. In the latter case the form of the curve will vary with the velocity of the drum.

A. Simple twitch—Drum stationary (Isotonic contraction).—By this arrangement one registers only the lift or height of the contraction, and its relation to the strength of the stimulus; yielding **minimal** and **maximal** contractions. A light (isotonic) lever is chosen, such as will amplify the movement six to eight times, while the weight to be lifted is such that the tension of the muscle is about 8 to 10 grms.

(*a*) Push the S coil away from the P, open the key in the secondary circuit, and make and break the primary circuit. There may be no contraction at either M or B. Close the secondary circuit key.

(*b*) Open the short-circuiting key, gradually push up the S coil, and break the primary circuit by means of the key in it. Observe when the first feeble single contraction or **twitch** is obtained = **minimal twitch** or **contraction.** When the muscle just contracts, this gives the **threshold value**—*i.e.*, a minimal twitch or response from a minimal stimulus. Make the primary circuit, there is no contraction. The *break shock is stronger than the make.* Record under each contraction whether it is a make (M) or break (B) shock, and the distance in centimetres of the S from the P coil. The minimal twitch may first be obtained when the S coil is 35 to 40 cm. from the P. Move the drum a short distance with the hand; the lever inscribes a base line or abscissa.

(*c*) Push up the S coil 0·5 cm. at a time. Test the effect of the make and break shocks, after each test moving the drum with the hand, and recording the result as to M or B, and the distance in centimetres of the S from the P coil. After a time a M twitch appears, and on pushing up the S coil the M contraction becomes as high as the B (fig. 129).

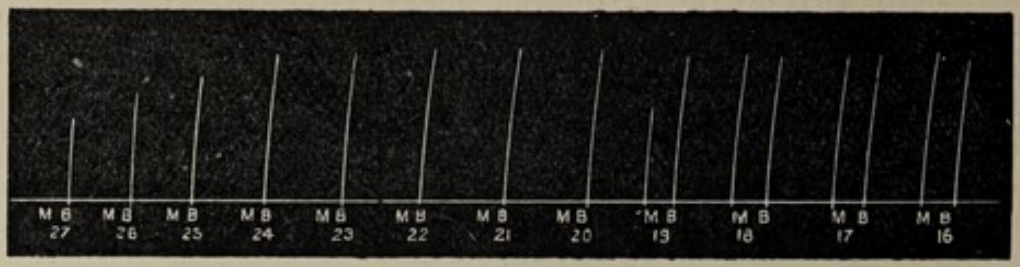


Fig. 129.—Contractions obtained with single make (M) and break (B) induction shocks. The numbers indicate the distance of the S from the P coil. The drum is stationary during each twitch, and is then moved a little distance by hand.

(*d*) Increase the stimulus by bringing the S nearer the P coil, and notice that the contractions do not become higher = maximal twitch or contraction. Keep the M and B twitches obtained with each strength of current close together. Their relative heights can then be readily compared (fig. 129).

N.B.—It is obvious that there is a certain relationship between the *strength* of the *stimulus* and the corresponding *twitch* or *contraction*. The twitch in response to each B stimulus increases with the strength of the stimulus, at first rapidly, then slowly up to a certain point; but increased strength of stimulus produces no further increase in the twitch—*i.e.*, both twitch and stimulus are maximal.

The "threshold value" of the minimum stimulus will be the same whether 10 or 15 grms. are used to load the muscle—*i.e.*, *threshold value is independent of load.*

B. Twitch with cylinder revolving (fast speed).—Arrange the experiment as in A, but allow the cylinder to revolve about 50 centimetres per second. Mark the paper-overlap by removing some of the soot with your finger. The curve must not be inscribed at or near the overlap.

(*a*) Select a strength of stimulus (B shock only) which is known to cause a contraction, and while the cylinder is revolving cause the muscle to contract by opening the key in the P circuit.

(*b*) Study the curve, a so-called "isotonic" curve (fig. 138).

C. Vary velocity of cylinder, and observe how the form of the curve varies with the variation in velocity of the cylinder (fig. 130). Use only the break shock, and record the contractions either (i.) all on one abscissa, or (ii.) record each contraction on a different abscissa, recording a time-curve under each. (Lesson XXXV.)

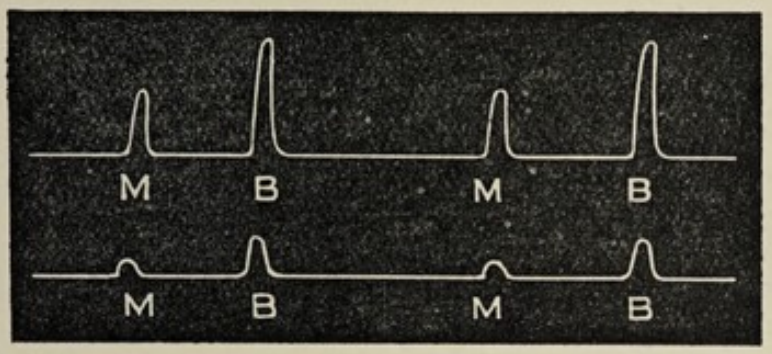


Fig. 130.—Frog's gastrocnemius stimulated by single M and B shocks, the distance between the P and S coil being the same for both shocks. In the lower figure the muscle was fatigued. Slow rate.

12. Relation of "lift" to strength of stimulus.—Suppose one uses only break shocks, and, beginning with the first effective stimulus ("**minimal contraction**") and gradually increasing the strength of the stimulus, one obtains a gradual increase in the height of the "lift" until a certain maximum of lift ("**maximal contraction**") is reached, above which, even though the stimulus

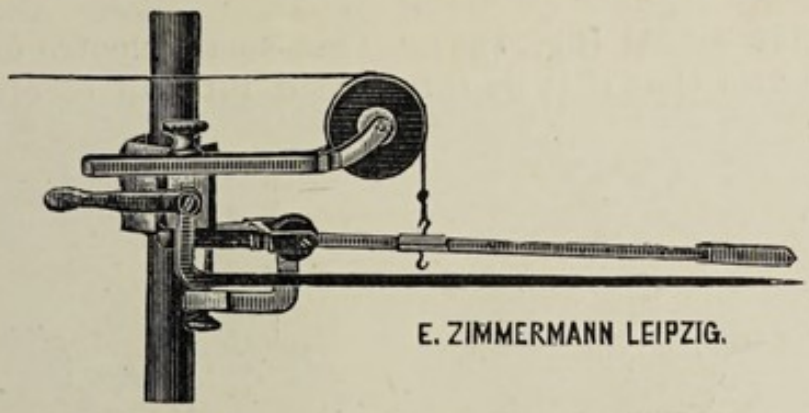


Fig. 131.—Simple form of recording lever with independent abscissa recorder.

be increased, there is no further shortening of the muscle. If a muscle be stimulated directly—*i.e.*, the electrodes applied to the muscle direct—the difference between the first effective stimulus (minimal) and the first effective maximal stimulus is considerably greater than by indirect stimulation—*i.e.*, where the stimulus is applied through the nerve.

LESSON XXXV.

CHRONOGRAPHS AND CHRONOGRAMS—CRANK-MYOGRAPH—AUTOMATIC BREAK—MUSCLE CURVE—HYOGLOSSUS.

1. **Chronographs and chronograms.**—The rate of movement of the recording surface, if rapid, is ascertained by some form of time-marker or chronograph. As the times to be measured in experiments on muscle and nerve are very small—*e.g.*, $\frac{1}{100}$ to $\frac{1}{1000}$ second—some speciala pparatus is required. The simplest is a tuning-fork—vibrating, say, 100 D. V. per second—one arm of which carries a writing-style, and makes the vibrations on a smoked surface. It is convenient to keep the tuning-fork vibrating by means of electricity, introducing a recording signal in the circuit, the whole constituting an *electrical chronograph.* It consists of a battery, a vibrating tuning-fork or reed, and a signal or marker.

B. **Desprez signal** (fig. 132).—This small electro-magnet has so little inertia that if it be introduced into an electric circuit,

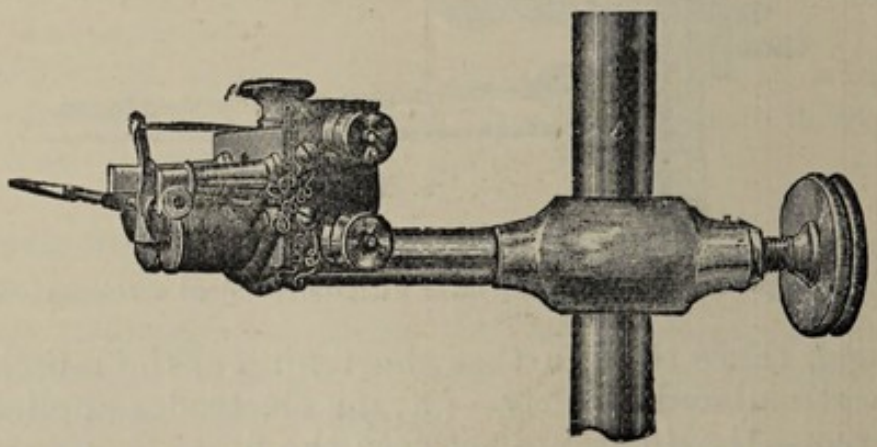


Fig. 132.—Desprez electric signal or chronograph, as made by the Cambridge Scientific Instrument Company.

its armature, which is provided with a very light writing point, vibrates simultaneously with the vibrations of an electric

tuning-fork introduced into the same circuit. The drum must move more rapidly, the more rapid the vibrations of the tuning-fork used.

Arrange a Desprez or other signal in circuit with a tuning-fork of known rate of vibration—*e.g.*, 100 D.V.—and actuated by two dry cells or Grenet battery (fig. 133). The handiest

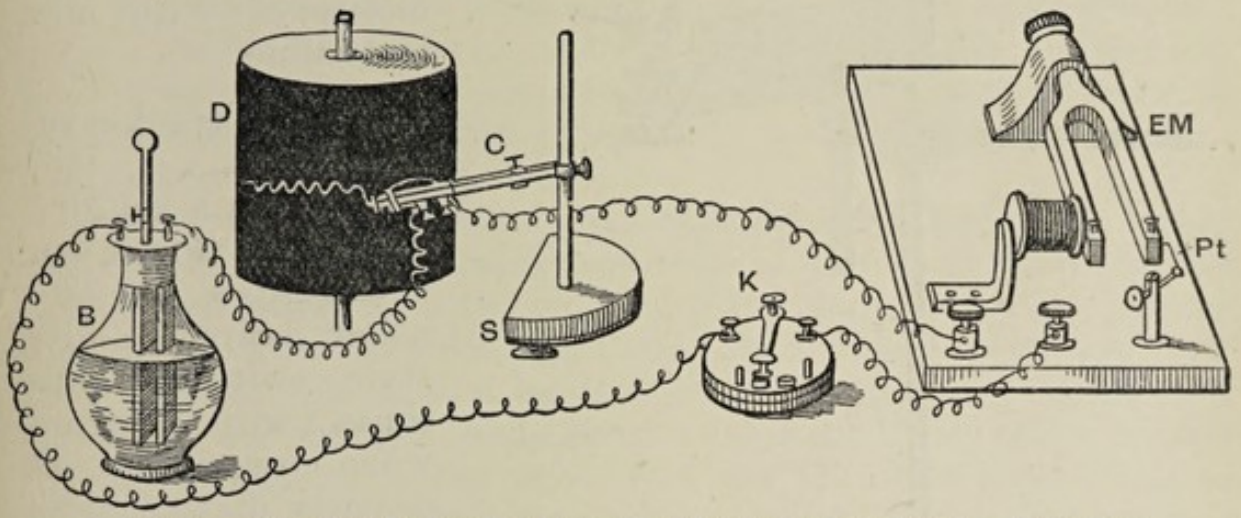


Fig. 133.—Signal and vibrating tuning-fork in an electric circuit. *D*, Drum; *C*, Signal; *EM*, Electric tuning-fork; *Pt*, Platinum contact.

form of fork is made with a dry platinum contact, Pt. Set the tuning-fork vibrating. Record the result on a quick-moving cylinder.

2. **Take chronograms** (fig. 134).—(1) With tuning-fork with writing-style which has been made to vibrate 100 D.V. per second by giving it a small blow with an india-rubber stopper

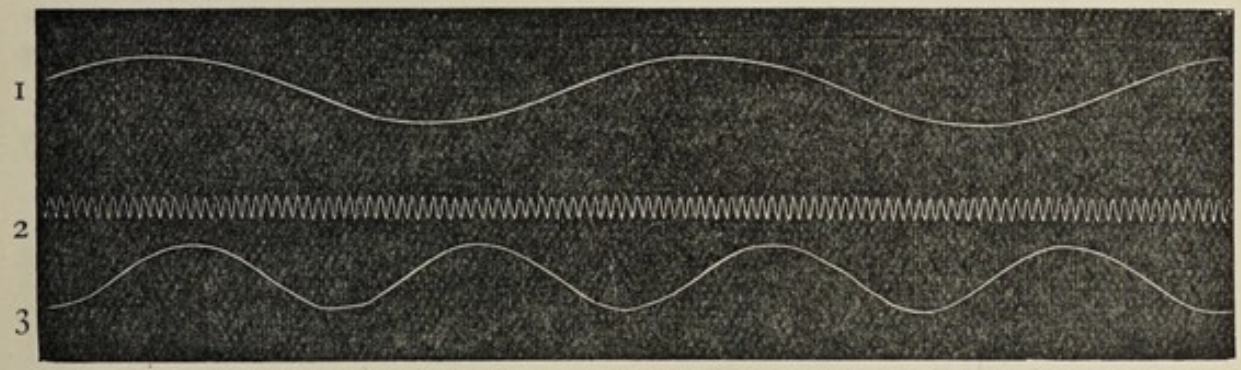


Fig. 134.—Chronograms. 1 and 3 Spring chronograph, 0·5 second, 0·25 second. 2, 100 D.V. per second. Same speed of drum.

fixed on a wooden handle. Do this with slow-moving drum and quick-moving drum, and calculate the speed of the drum from the time curve.

(2) Record $\frac{1}{4}$ and $\frac{1}{2}$ seconds with the spring chronograph (fig. 145) near the 100 D.V. per second curve, and compare the time.

(3) Record with an electrical chronograph of 50 or 100 D.V. per second.

Muscle-curve.—Instead of the lever shown in fig. 126, the crank-myograph is used (figs. 135, 136). The muscle is kept moist by covering it with blotting-paper moistened with normal saline.

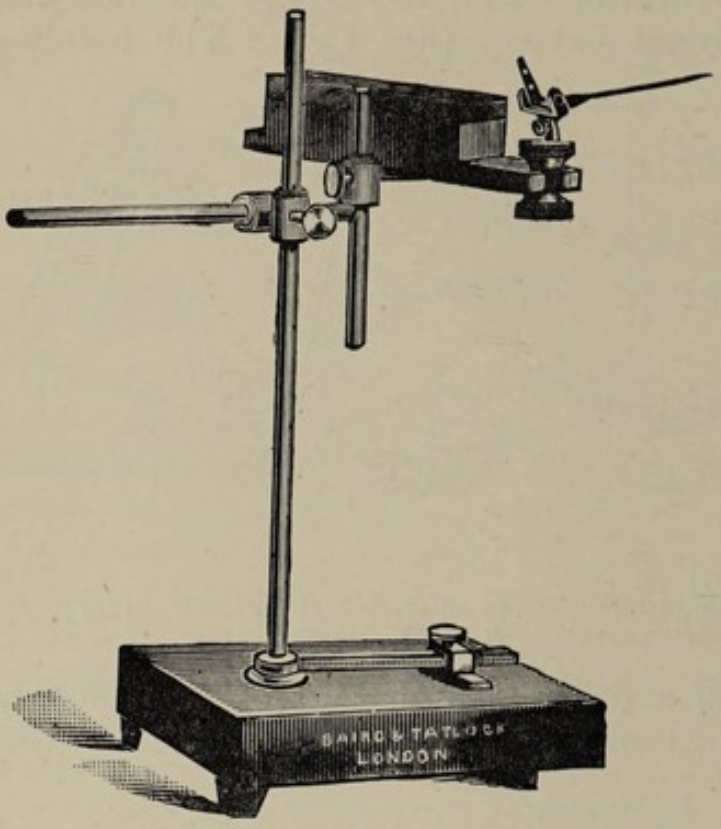


Fig. 135.—Crank-myograph with T-pieces and fixed support moving on a vertical axis—*i.e.*, a tangent stand.

3. **The crank-myograph** (figs. 135, 136) is fixed on a suitable support, so that it can be adjusted to any height desired, and its style switched into contact with the drum when required. It consists of two arms—long and short—fixed at right angles to each other. The muscle lies horizontally, so that the movement of the recording point is at right angles to the recording surface. It is convenient to fix the myograph with T-pieces, as in the fig. 135, as then the writing point can be made to move in the plane of the recording surface.

Measure the length of the short and long arms respectively

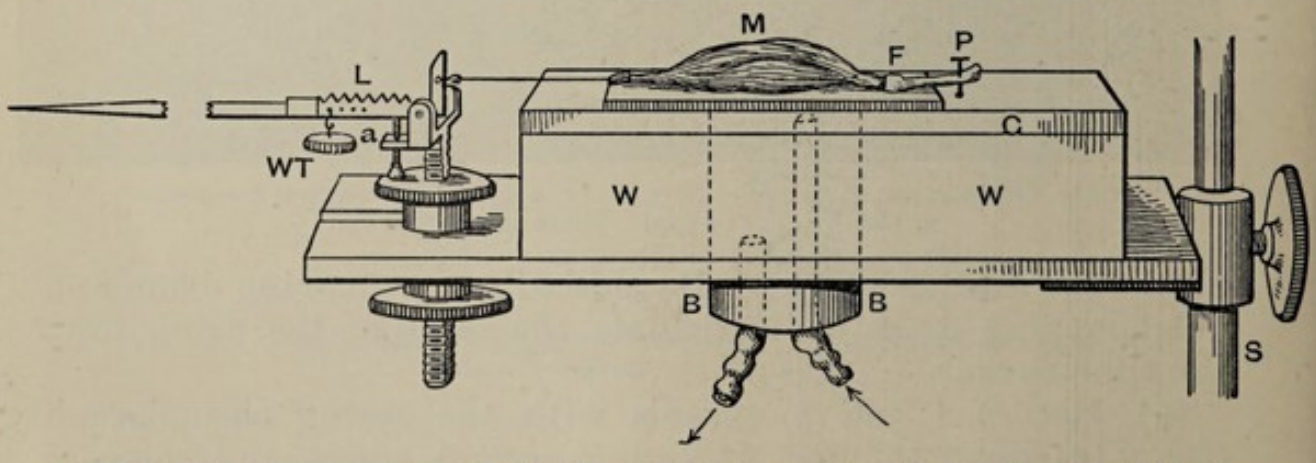


Fig. 136.—Crank-myograph. *W*, *W*, Block of wood: *M*, Muscle; *F*, Femur; *P*, Pin to fix *F*; *L*, Lever; *WT*, Weight; *a*, Screw for after-load; *C*, Cork; *B*, *B*, Brass box.

from the fulcrum, so as to ascertain the *amount of magnification* of the muscle curve.

After-load.—In the crank-myograph, under the lever, is a screw on which the horizontal arm of the crank rests (fig. 136, *a*), so that the muscle is loaded only during its contraction. Thus, a muscle may be "loaded" or "after-loaded"; in the former case the muscle is loaded with a weight, both when it is at rest and when contracting, but in an "after-loaded" muscle the muscle raises the weight only during contraction, and is not stretched by it when at rest. The experiment is arranged in the same way as in Lesson XXXIV., 8.

(*a*) Prepare the gastrocnemius with the lower end of the femur attached. Pin the femur securely to the cork plate of the myograph covered with blotting-paper moistened by normal saline. Instead of a pin, the little clip "tie-fasteners" are very convenient. Tie a stout ligature round the tendo Achillis, by a hook fix the ligature to the short arm of the lever, add a weight of 10 to 12 grms. to the lever, and see that the lever itself is horizontal. Thrust two fine wires—which act as electrodes—from the du Bois key in the S circuit through the upper and lower end of the muscle.

(*b*) Arrange style of lever so that it writes on the drum, and repeat the experiments of the previous Lesson.

(*c*) Use different weights—5, 20, 50 grms.—and observe how the form and other features of the curve vary on increasing the weight attached to the lever.

4. Automatic break—*i.e.*, method of excitation.—It is convenient to use a single *break* induction shock—*i.e.*, the S coil is at such a distance from the P that only the break shock is effective. One may, of course, break the primary circuit by the hand, as in the previous experiments, but this is not convenient. It is better to have an "automatic break" (fig. 137) done by the drum itself as it revolves, the drum being introduced into the P circuit. Two binding screws are placed on the stand, but one is insulated. The axis of the drum carries a horizontal (adjustable) arm or "striker" carrying a platinum wire which touches a wire fixed on a support on the insulated binding screw. Thus, every time the drum revolves a shock is induced, and always at the *same* moment, so that successive shocks can be recorded on the same abscissa, and the moment of stimulation can be found at once.

5. Simple muscle-curve (Twitch) crank-myograph and automatic break.—Recording drum moving at a fast rate (about

50 cm. per second); crank-myograph; chronograph vibrating 100 times per second; coil; keys.

(*a*) Arrange apparatus as in fig. 137, with drum, D, in P circuit. When the striker, S, fixed to the vertical spindle, touches the upright the circuit is made and then broken. Select a *break* shock—*i.e.*, when the make is not yet effective. The support, IS, is insulated from the drum support.

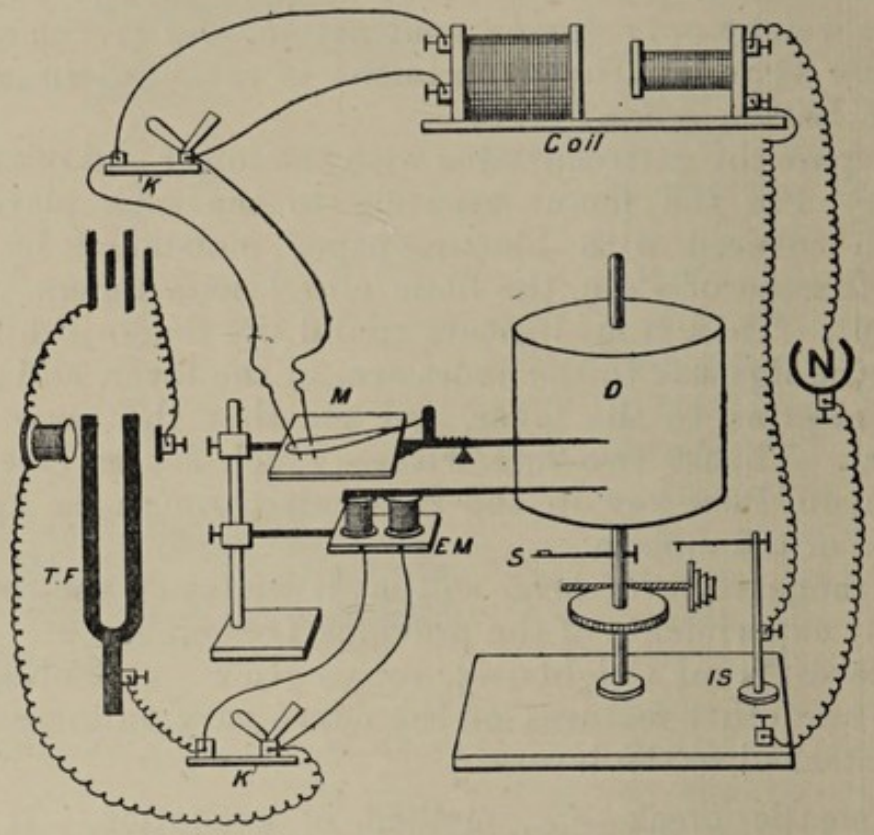


Fig. 137.—Arrangement for analysis of muscle-curve by means of crank-myograph (*M*) with "Automatic break" in P circuit. *S*, Striker on axis of *D* cylinder; *PC*, Primary, and *SC*, Secondary circuits; *TC*, Time-circuit with *EM*, Electro-magnet; *IS*, Insulated support in *PC*.

(*b*) Short-circuit the S current, arrange a N.M. preparation on a crank-myograph, M, placing the nerve on the electrodes, and arrange the lever to record on the drum. Mark the overlap on the paper, and arrange the time of automatic breaking so that the curve will fall about the middle of the paper.

(*c*) *Adjust the writing points.*—Arrange the lever of a chronograph (vibrating 100 times per second, and actuated by a dry cell in circuit with a tuning-fork, T.C. time-circuit) so that its writing point records exactly under the muscle lever point.

(*d*) *Mark overlap and take a record.*—Adjust the position of the break key in order to have the tracing near the middle of the paper and not near where it is gummed. Open the short-circuiting key, set the chronograph vibrating, and the cylinder

in motion during one revolution. When the striker, S, comes in contact with support, IS, a break induction shock is obtained, and the muscle records a simple muscle-curve. Close the short-circuiting key.

(*e*) Record the moment of stimulation by bringing S in contact with the style on IS, depress and raise the lever until it cuts the time tracing at *a* in Fig. 139. The distance between this point and the beginning or rise of the curve indicates the **latent period.** In a similar manner make vertical arcs—the drum being stationary—opposite (1) the point at which the tracing rises from the abscissa, *b*, (2) highest point of the curve, *c*, (3) the point where it again meets the abscissa, *d*; the after-oscillations are seen in all tracings, and are diminished by using as light levers as possible.

Make base lines and ordinates—muscle-lever and time-lever—on the cylinder to mark the relative positions of the two writing points, or cause one to write exactly over the other.

(*f*) Study the "**muscle-curve**" (fig. 138), noting particularly the latent period, the ascent and descent. The latent period may be represented by a distance of 4 or 5 millimetres, but this

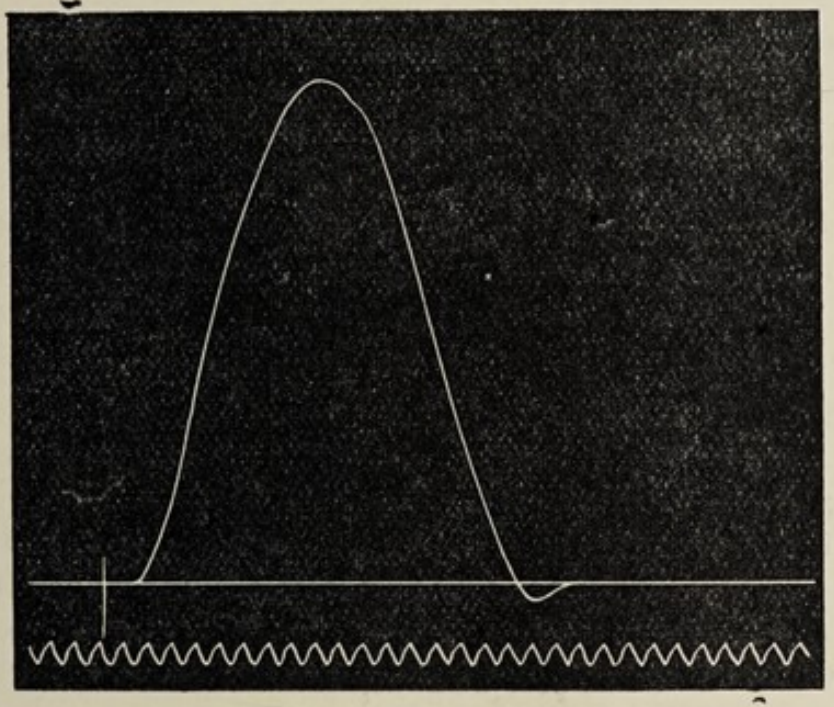


Fig. 138.—Muscle-curve of frog's gastrocnemius. The lower line indicates time and each double vibration (*D.V.*) = $\frac{1}{100}$ second.

delay does not represent the actual latent period, which is really much shorter. The long latent period is really largely due to the apparatus, and therefore instrumental. Estimate, by means of the tuning-fork vibrations, the duration of each of the phases.

6. *Measure* (fig. 139) as accurately as possible the *duration* of—

(i.) The latent period, *a-b*.
(ii.) The period of contraction, *b-c*.
(iii.) The period of relaxation, *c-d*.

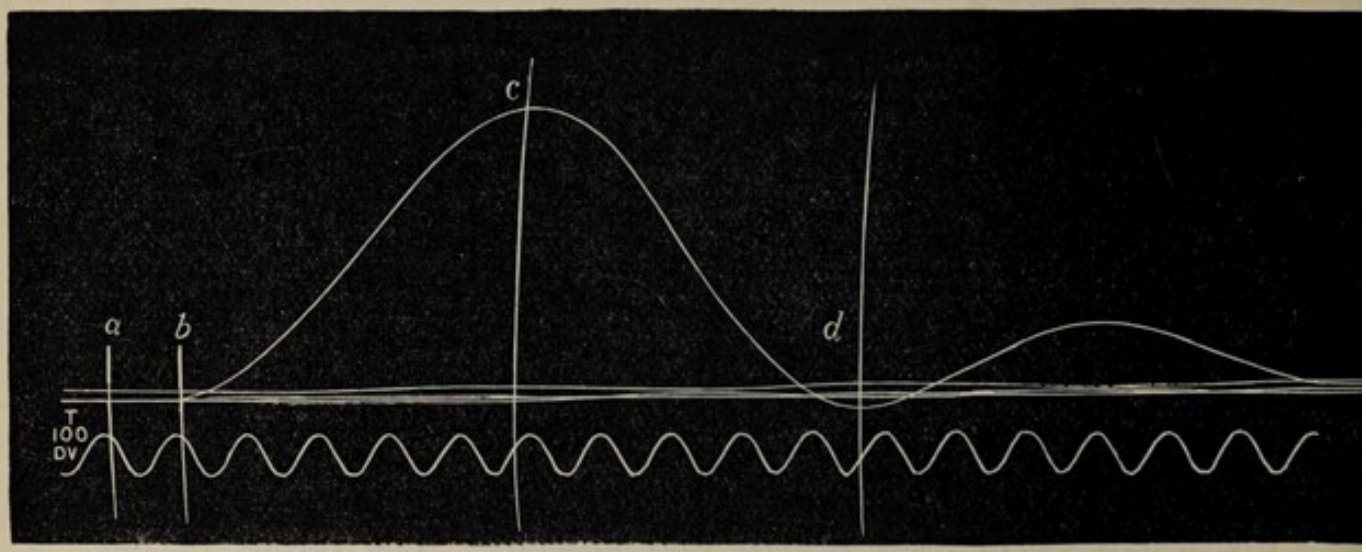


Fig. 139.—Muscle curve of hyoglossus. Time tracing 100 D.V. per second. *a-b*, L.P. *b-c*, ascent; *c-d*, descent.

(i.) **Latent period** from point of stimulation, *a*, to commencement of contraction, *b*, there is no change of length. Suppose the chronograph tracing from *a-b* to show 1 D.V., the period occupied would be ·01 second.

[This estimate is too high, and the excess over the true latent period, about 0·003 to 0·008, for a frog's muscle is partly due to the non-simultaneous contraction of all parts of a muscle, and largely to inertia and instrumental delay.]

(ii.) **Period of contraction**, *b-c*, from *b* to highest point of curve *c*. At first it is usually slightly convex to the abscissa—*i.e.*, rate of contraction increases gradually—at first slowly, it then increases rapidly, and soon reaches a maximum of rapidity; then it becomes slower and concave towards the abscissa, and gradually ceases to shorten. Suppose the time be equal to 8 D.V., then the time occupied was $\frac{8}{100}$ of a second—*i.e.*, 0·08 second.

(iii.) **Period of relaxation**, *c-d*, from the highest point *c* to *d*, but it may be difficult to be sure exactly where *d* takes place, as the lever often gives several secondary rebounds. The curve shows practically similar changes to those on the ascent, but in the reversed order. Suppose the number of D.V. to be 9, then the time is $\frac{9}{100}$ = 0·09 second.

Total time of twitch, 0·01 + 0·08 + 0·09 = 0·18 second.

7. Magnification by lever.—Measure height of curve at *c* from the abscissa. This indicates the *extent of shortening*—*e.g.*, 74 mm. The *magnification* has previously been ascertained by comparing the length of the two arms of the lever. Suppose lengths of lever were respectively 10·3 mm. and 257·5 mm., then the magnification would be $\frac{257{\cdot}5}{10{\cdot}3} = 25$. The amount the muscle contracted was $\frac{75}{25} = 3$ mm.

The length of the muscle was 23 mm., consequently the muscle contracted $\frac{75}{25} \times \frac{1}{23}$.

8. Work done during twitch—*i.e.*, product of load or weight lifted × height through which it is raised, but the magnification has to be taken into account. The actual load lifted was 5 grms.

$$\text{Work} = \frac{\text{Height}}{\text{Magnification}} \times \text{Wt.} = \frac{75}{25} \times 5 = 15 \text{ grm. mms.}$$

This work was done in 0·08 second, and the mean rate of work was $\frac{15}{0{\cdot}08} = 175$ grm. mms. per second.

The lever then falls again, and in so doing the load performs almost the same amount of work on the muscle as the muscle upon the load.

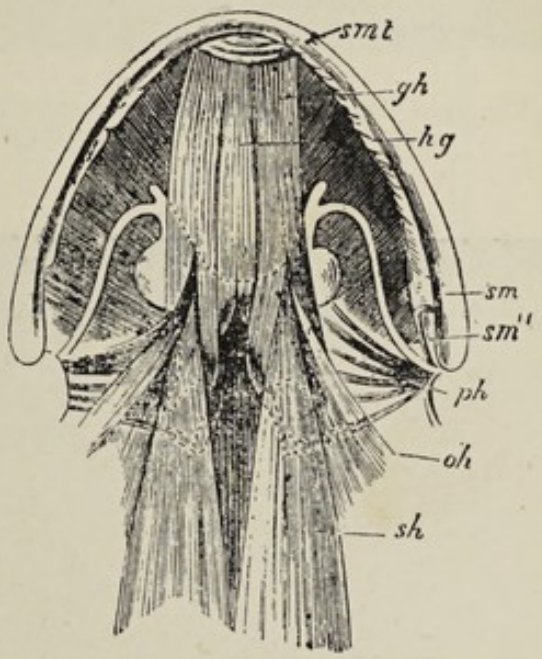


Fig. 140.—Hyoglossus of frog, *hg*; *sh*, *oh*, *ph*, *gh*, sterno-, omo-, petro-, and genio-hyoid muscles.

9. Hyoglossus of frog.—This may be called a classical muscle, as it was used by E. H. Weber. Comparatively recently its advantages have been recalled. It arises from the anterior edge of body of the hyoid cartilage, and runs forward under the mucous membrane of the lower jaw to the tip of the tongue with its fellow of the opposite side. It is supplied by the hypoglossal nerve (fig. 140, *hg*).

(*a*) Remove whole of lower jaw by carving incisions from its joint towards the shoulder girdle, and then join the two by a transverse incision. Place it on a crank-myograph, mucous surface upwards. As the tongue is attached in front, lift it by its tip (behind) and tie a thread round the latter. Pull the tongue straight and attach it to a myograph. To give a fixed point of support, fix the hyoid with a pin. Another way is to remove the upper jaw by a transverse cut, leaving the lower

jaw. The muscle is composed of parallel fibres, and is not exposed to air, as it is covered by its own mucous membrane. It may be stimulated directly with two pins thrust through a cork, or indirectly through its nerve the hypoglossal.

(*b*) Take a tracing, using a light lever, fine electrode wires, and an automatic break in the primary circuit. Add a time

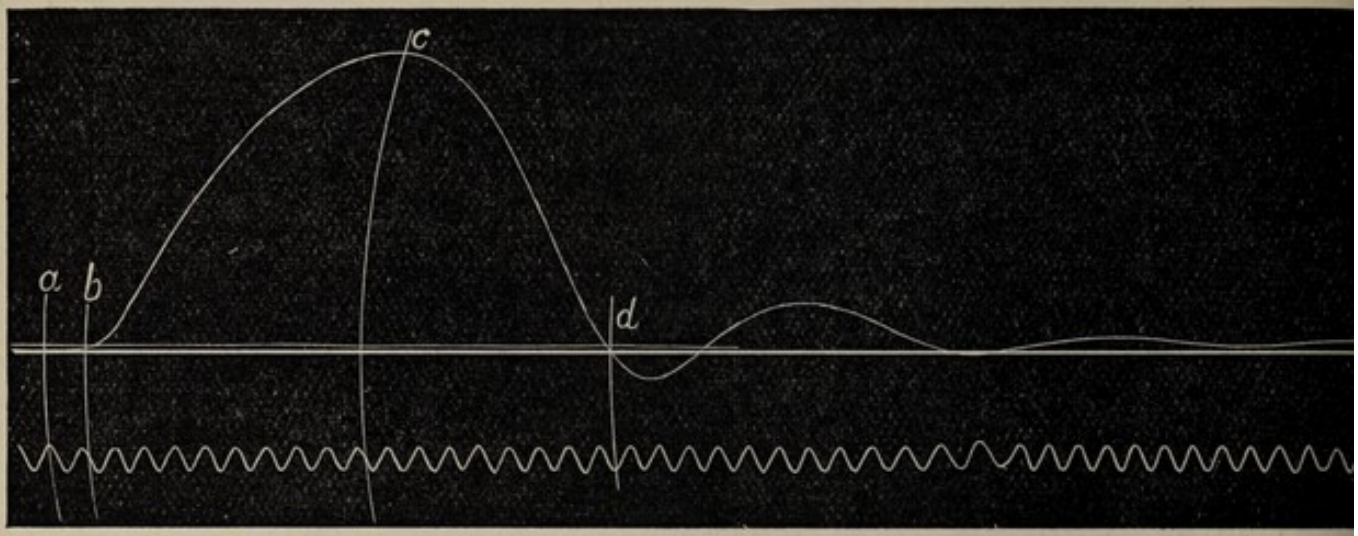


Fig. 141.—Muscle-curve of gastrocnemius of frog recorded with hear suspension lever, magnification 10, but reduced by photography. Time, 100 D.V per second.

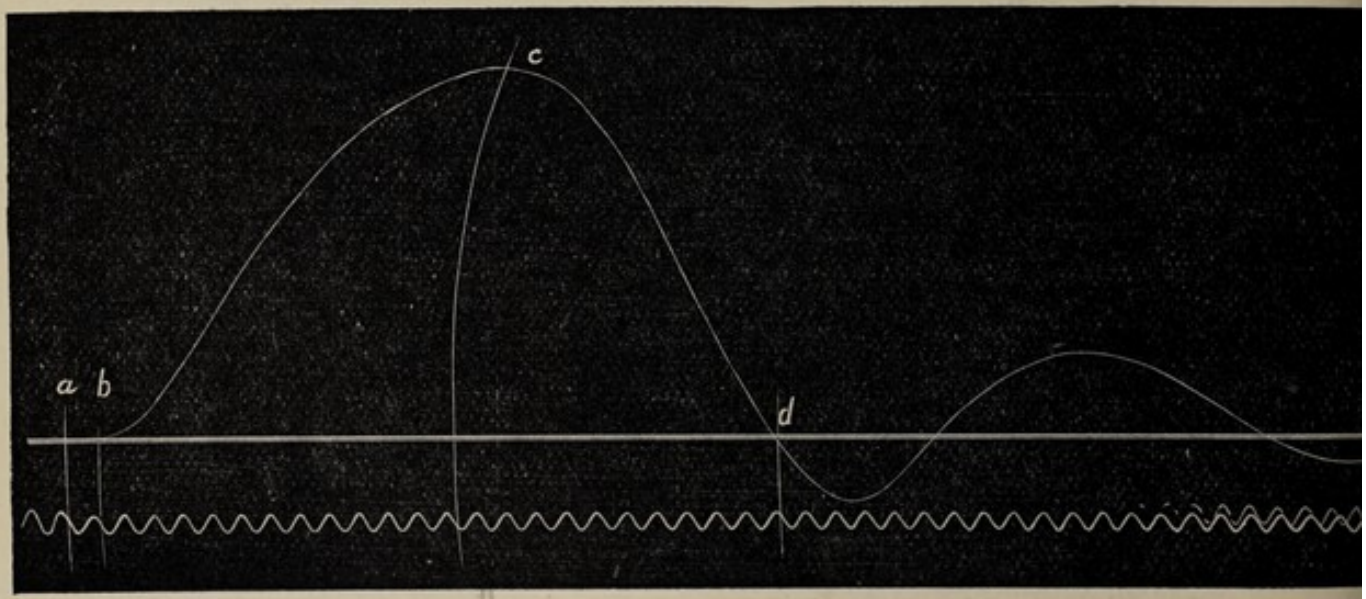


Fig. 142.—As above, but of hyoglossus of same frog.

tracing, and mark the movement of stimulation—the beginning and end of the contraction—by vertical arcs. It is easy to record with a "suspension heart-lever." Compare the time relations of the hyoglossus and gastrocnemius curves (figs. 141, 142).

10. Correction for lever.—Obviously the curves obtained are not the true form, as the point of the lever records an arc of a circle and not a

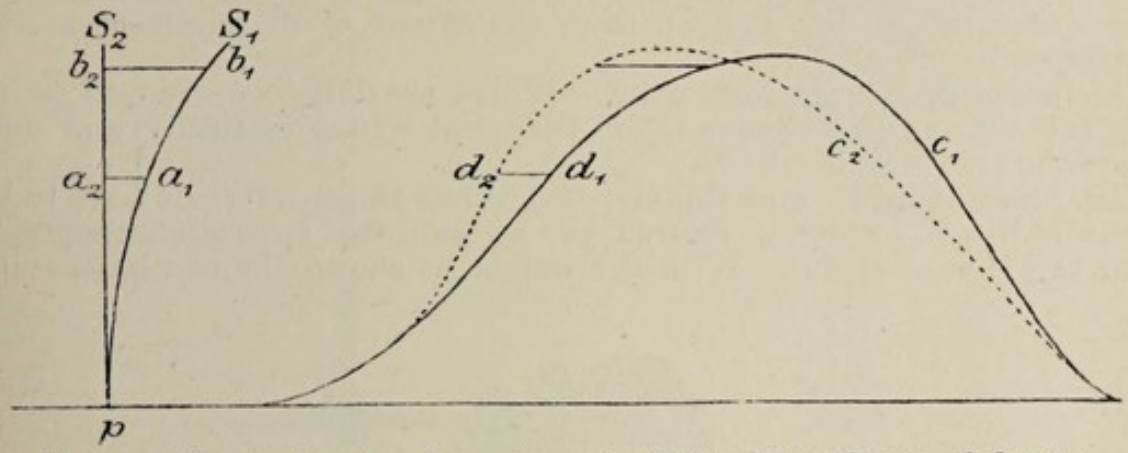


Fig. 143.—Showing how true form of curve is obtained from the recorded curve.

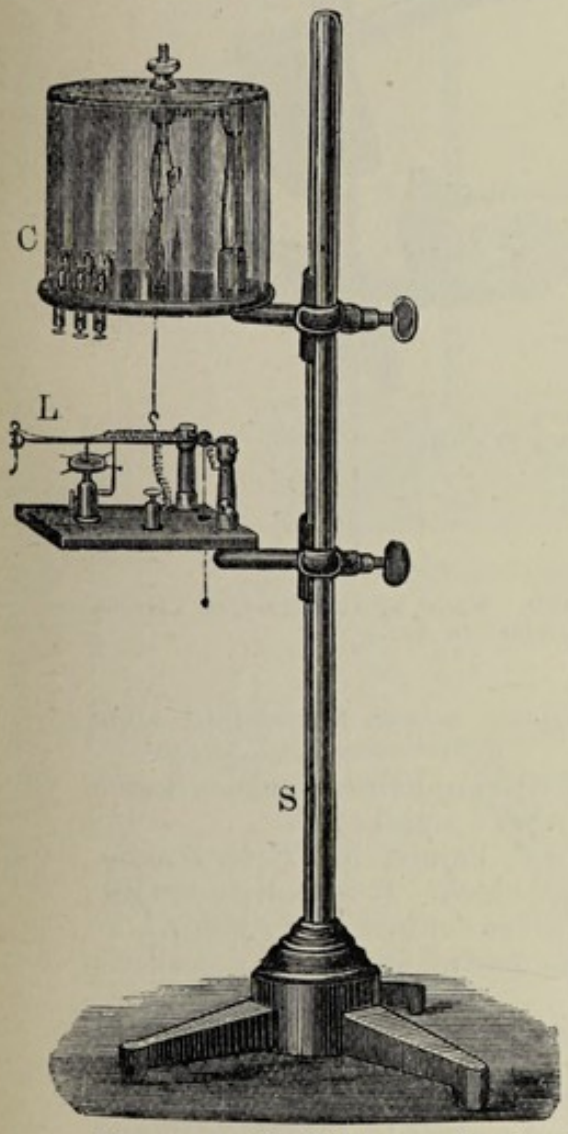


Fig. 144*a*.—*C*, Moist muscle chamber; *L*, Tigerstedt's recording lever.

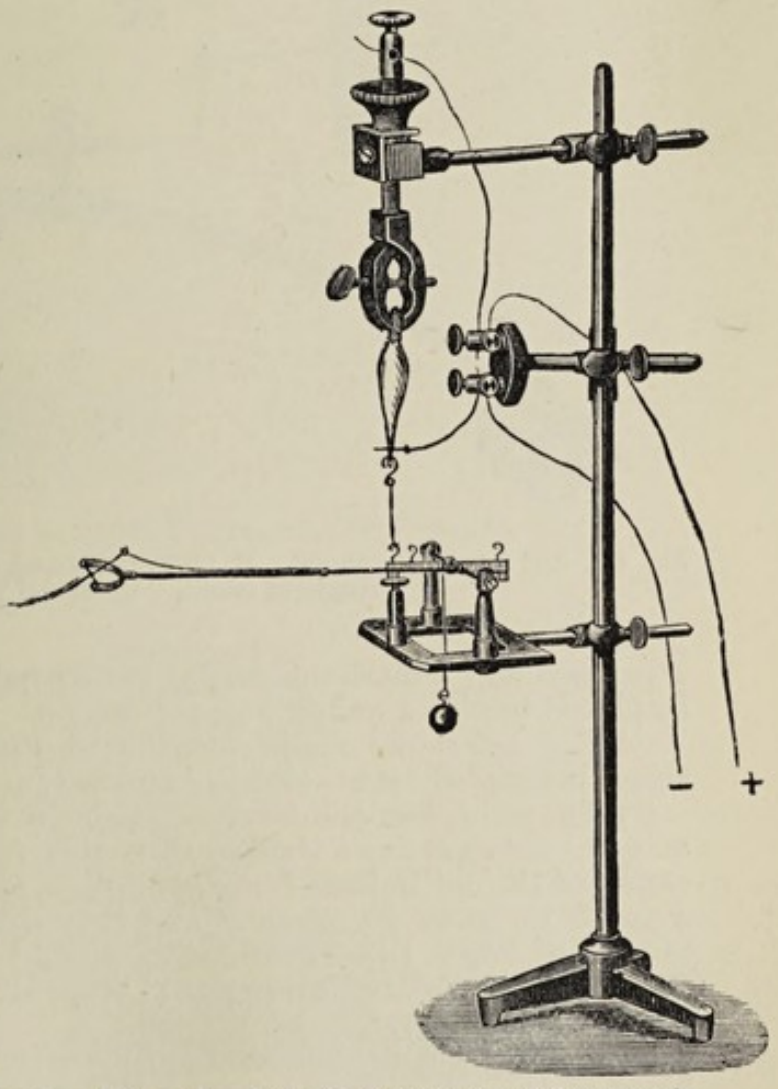


Fig. 144*b*.—Ludwig's arrangement for recording a muscular contraction (direct stimulation) with a Tigerstedt's lever, which writes at right angles to the recording surface.

vertical line. Fig. 143 shows how the true form of the curve can be obtained. The dotted line shows its true form.

To the left of the recorded curve record an arc and a vertical ordinate; the distances, $d_2 - d_1$, $a_2 - a_1$, show the extent of displacement at the corresponding levels.

11. Recording lever and point.—Where the lever is arranged as in fig. 144—*i.e.*, in Tigerstedt's form—the point writes vertically, and thus gives the true form of curve.

12. Chronographs and signals.—(*a*) When longer intervals have to be measured—*e.g.*, 2 or 4 records per second—the **spring chronograph** (fig. 145) is most useful. With the weight as shown the oscillations are

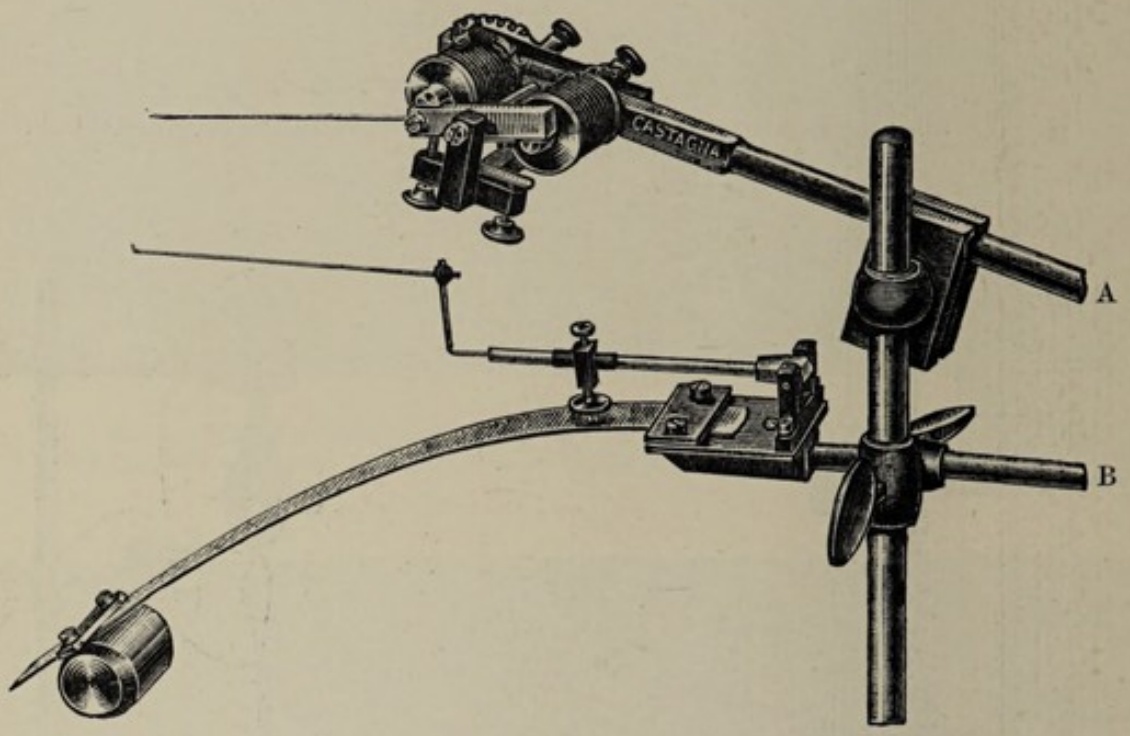


Fig. 145.—A, Recording signal; B, Spring chronograph. Made by Castagna, of Vienna. Observe useful "Universal holder" in A.

2 per second; a mark on the flat spring indicates where the weight must be placed to give 4 oscillations per second.

(*b*) Fig. 146 shows a convenient method of writing half-seconds, where a signal is coupled up in electrical connection with a small clock.

(*c*) The *pendulum contact clock*, made by C. F. Palmer for T. G. Brodie, may be used either as a time-marker or exciting clock. It is really a modification of the old Baltzar-Ludwig clock. It gives contact every second, or 2 to 10 to 30 or 60 seconds, and is a most useful apparatus; cost £4 (*Journ. of Phys.*, Dec., 1900).

(*d*) The form of recording signal originally made by Pfeil is an excellent instrument (fig. 147).

(*e*) Some use a flat metallic spring or reed (arranged as in fig. 148) in place of the tuning-fork to give a succession of shocks by vibrating in and out of a pool of HG in the circuit.

(*f*) Instead of using a signal, the tuning-fork kept vibrating with a couple of dry batteries may be made to record its movements directly, as

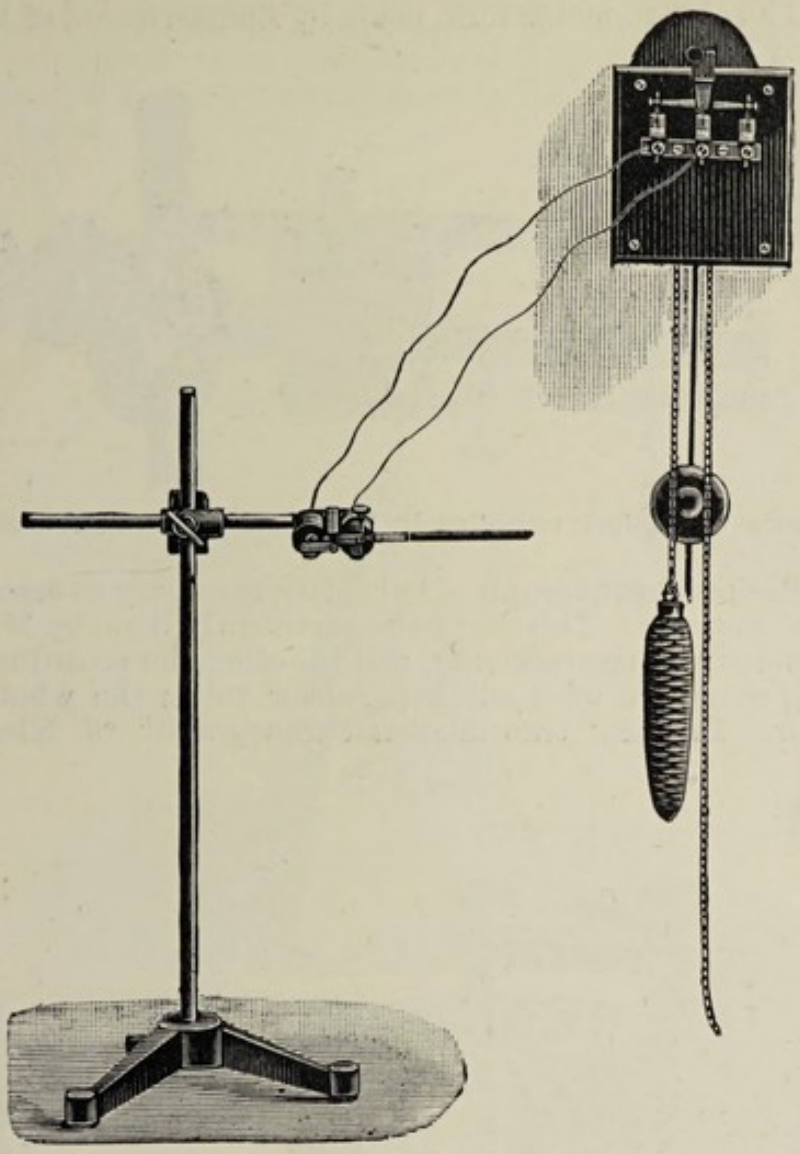


Fig. 146.—Clock and signal to record half-seconds (W. Petzold).

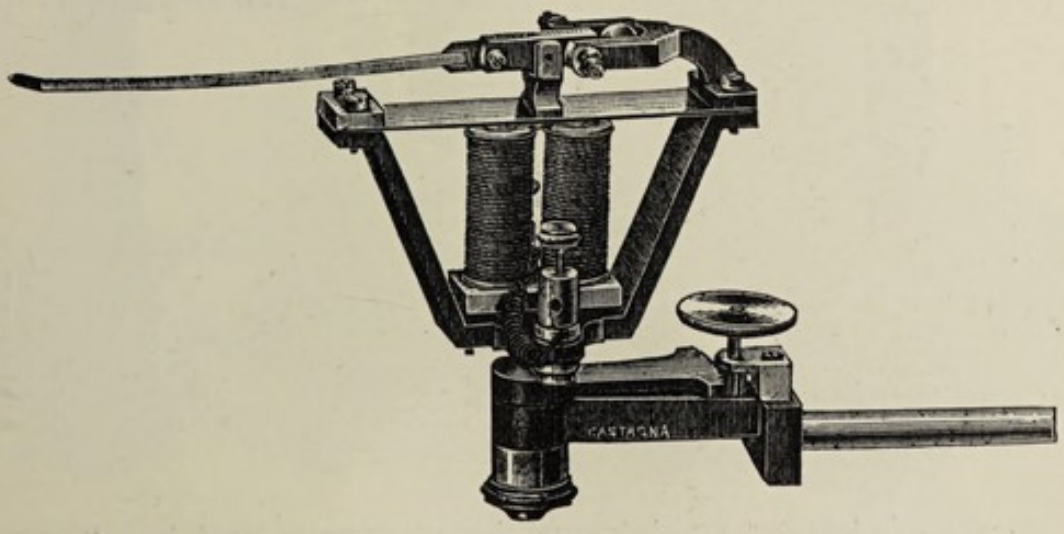


Fig. 147.—Pfeil's (Kronecker) recording signal. Made by Castagna, of Vienna.

in fig. 148. The electro-motor fork, made by Zimmermann, of Leipzig, can be fixed on any stand.

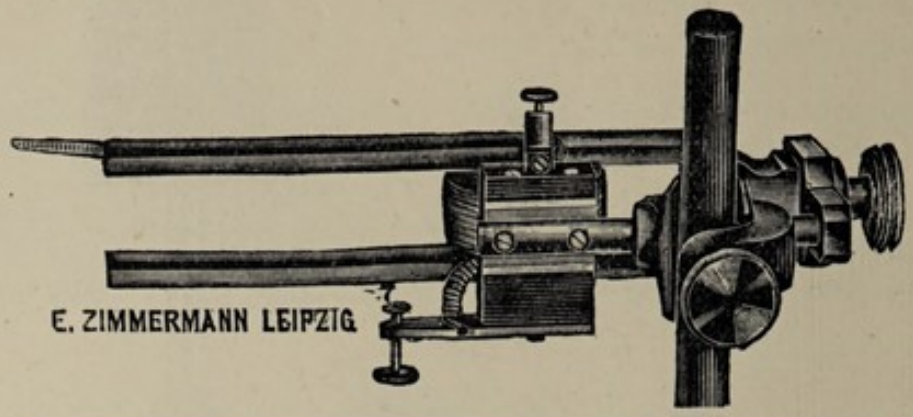


Fig. 148.—Electrical tuning-fork, recording directly. Made by Zimmermann, of Leipzig.

(*g*) **Transmission chronograph.**—It is often necessary to transmit movements from a distance. This is most conveniently done by Marey's tambours (fig. 149); one is the receiving, and the other the recording tambour, the two being connected with an india-rubber tube; the whole system is filled with air. In the "transmission chronograph" of Klemensiewicz

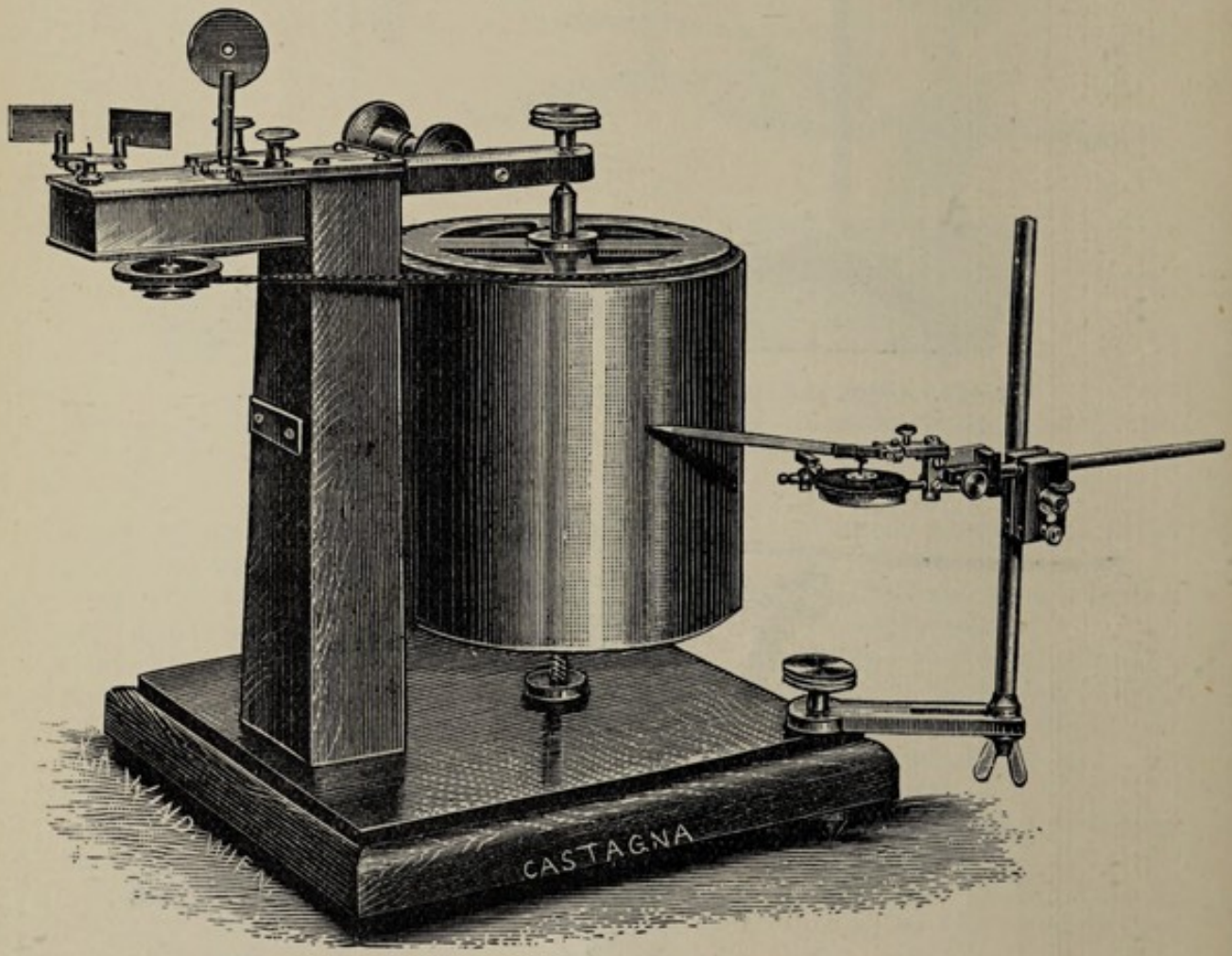


Fig. 149.—Marey's tambour for air-transmission experiments, fixed in a "Universal holder" and recording on a clockwork cylinder.

(fig. 149*a*), the pendulum of a metronome strikes right and left against two air capsules, and these are connected by a common tube to the recording Marey's tambour, which records the oscillations of the column of air in the tubes.

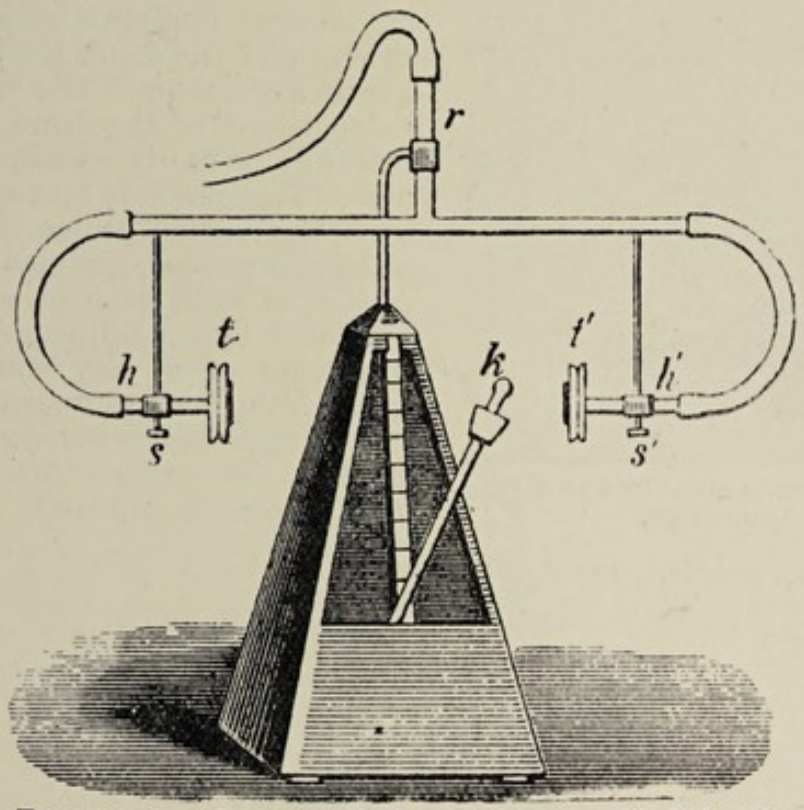


Fig. 149*a*.—Transmission chronograph, Klemensiewicz. Made by Zimmermann.

(*h*) An air-transmission chronograph modified in connection with a metronome is made by Verdin. (See "Circulation.")

(*i*) Sometimes it is convenient to record on a drum moving on a horizontal axis (fig. 150).

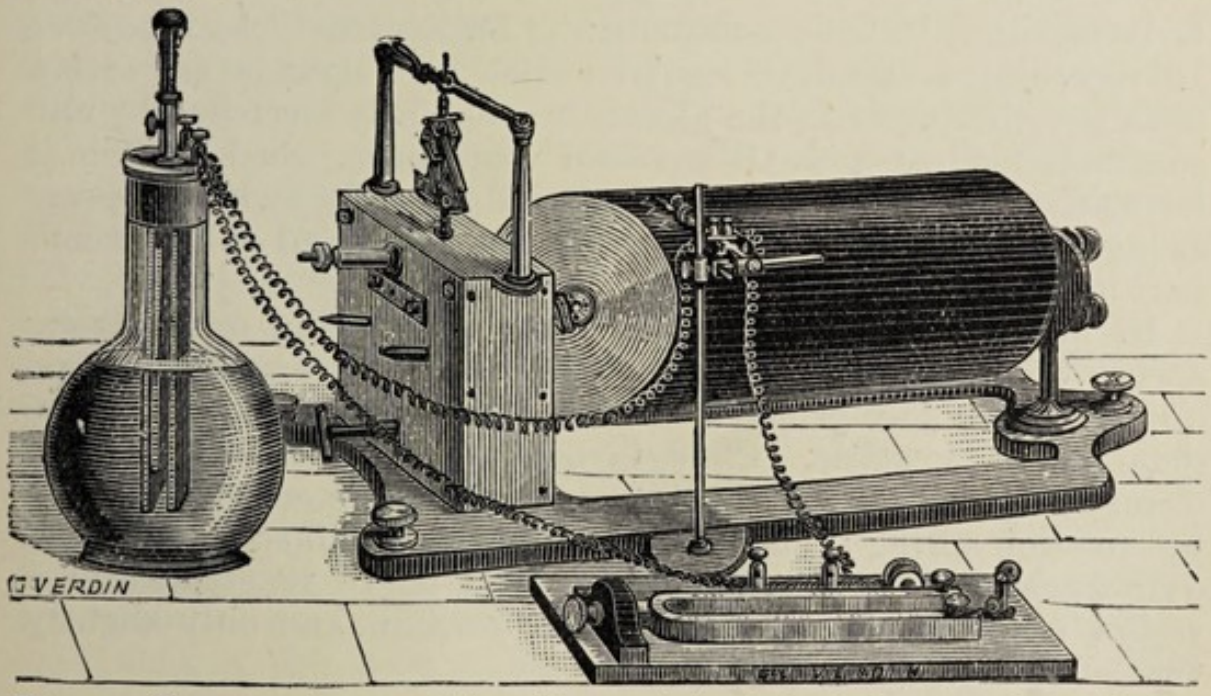


Fig. 150.—Chronograph arranged to write on a horizontal cylinder, as made by Verdin.

13. Drums.—Many forms are used, and a great variety are figured in the later lessons. A useful, inexpensive drum moved by clockwork, that made by Pye of Cambridge, is very convenient (fig. 151). It has a side support for levers. It gives a great variety of speeds, and the drum is standard size, 6 inches diameter. The clockwork is independent of the drum, which can therefore be easily removed for smoking. The clock is arranged to work from the centre pinion or from the mainspring arbor, giving a fast or slow motion at will, each of which can be regulated by an adjustable fly, thus admitting of a very wide range of speed from both sources. The drum can be raised or lowered, without removing the writing levers, when a fresh tracing is required.

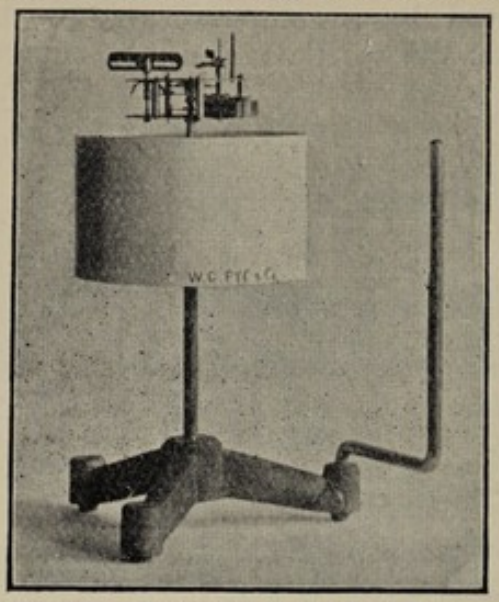


Fig. 151.—Drum made by Pye & Co., Cambridge.

(For Birch's pattern, see *Appendix*.)

LESSON XXXVI.

ISOTONIC AND ISOMETRIC CONTRACTIONS—WORK—HEAT-RIGOR.

1. Isometric *v.* isotonic contraction or twitch (A. Fick, †1901).—In recording a simple muscular twitch by a light lever (with a light weight attached), the muscle is constantly stretched by and contracts against a small constant resistance. Such a curve is **isotonic.** Most of the energy set free during the twitch appears as molar or mechanical energy of the lever, and only a small part as tension.

If, however, the muscle contracts by pulling on a strong spring of great resistance—such a spring, for example, as requires about 500 grms. to bend it slightly—then the curve obtained is **isometric.** Changes of *tension* are recorded, while change of length is practically prevented. The energy is stored up in the spring tension. The curves obtained by clinical dynamometers are of this class. The curve is practically a record of the change of energy into tension, and only slightly that of change of form.

For isometric curves Fick attached a muscle to the short arm of a lever, the other arm being prevented from moving much by the resistance of a strong spring, as shown in fig. 152. In this way one obtains a curve which shows little change of form, but indicates the increase and decrease of *tension* during the contraction, the length of the muscle remaining nearly constant ; and for this reason Fick called it "isometric." Of course, an absolutely isometric curve cannot be recorded. If numerical results are desired the spring must be graduated.

2. Record tension of muscle—Isometric curve (Fick).—When the two ends of a muscle are so fixed that during activity they cannot approximate towards each other, then the muscle does not change its length, but only its tension.

One can record the change in tension by means of Fick's "**tension-recorder**" (fig. 152). One end of the muscle is fixed ;

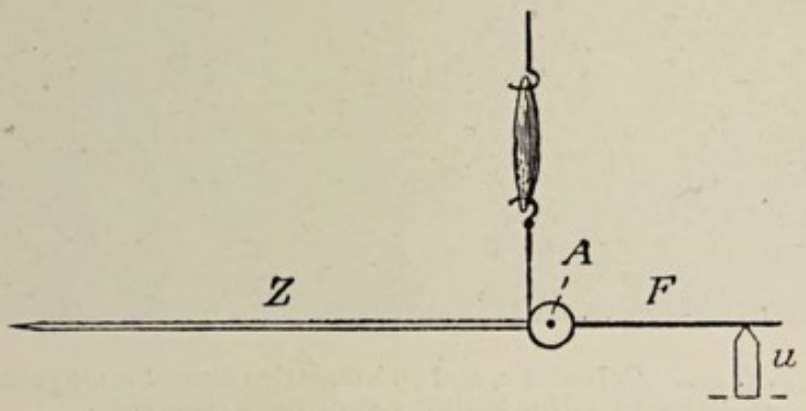


Fig. 152.—Scheme of Fick's tension-recorder. *A*, Axis movement ; *F*, Strong spring resting on support, *u* ; *Z*, Writing-lever. Principle of isometric method.

the other is attached by means of an inextensible thread which passes round a small pulley fixed on a steel axis, A. This axis carries (1) a long, light recording lever, Z, and (2) a horizontally-placed steel spring, F, whose free end rests on a support, *u*. When the muscle contracts, the spring, F, is pressed against the support, *u*. In consequence of the opposing tension of the spring the axis can only be turned slightly, but this movement is greatly amplified by the lever.

Schönlein has devised a myograph (*Pflüger's Archiv.*, Bd. 52, p. 112), which is so arranged that one can record either isotonic contractions or isometric contractions. The isometric curves so obtained have been called "**tonograms.**"

If one compares an isotonic and isometric curve from the same muscle (fig. 153), there is a latent period—a period of increasing tension and one of decreasing tension. The apex of the isometric curve lies nearer the beginning of the contraction than that of the isotonic curve—*i.e.*, the length remaining the

same, the isometric curve reaches the maximum of its tension sooner than it (the tension being the same) reaches the maximum of its shortening. Moreover, the isometric curve is flat-

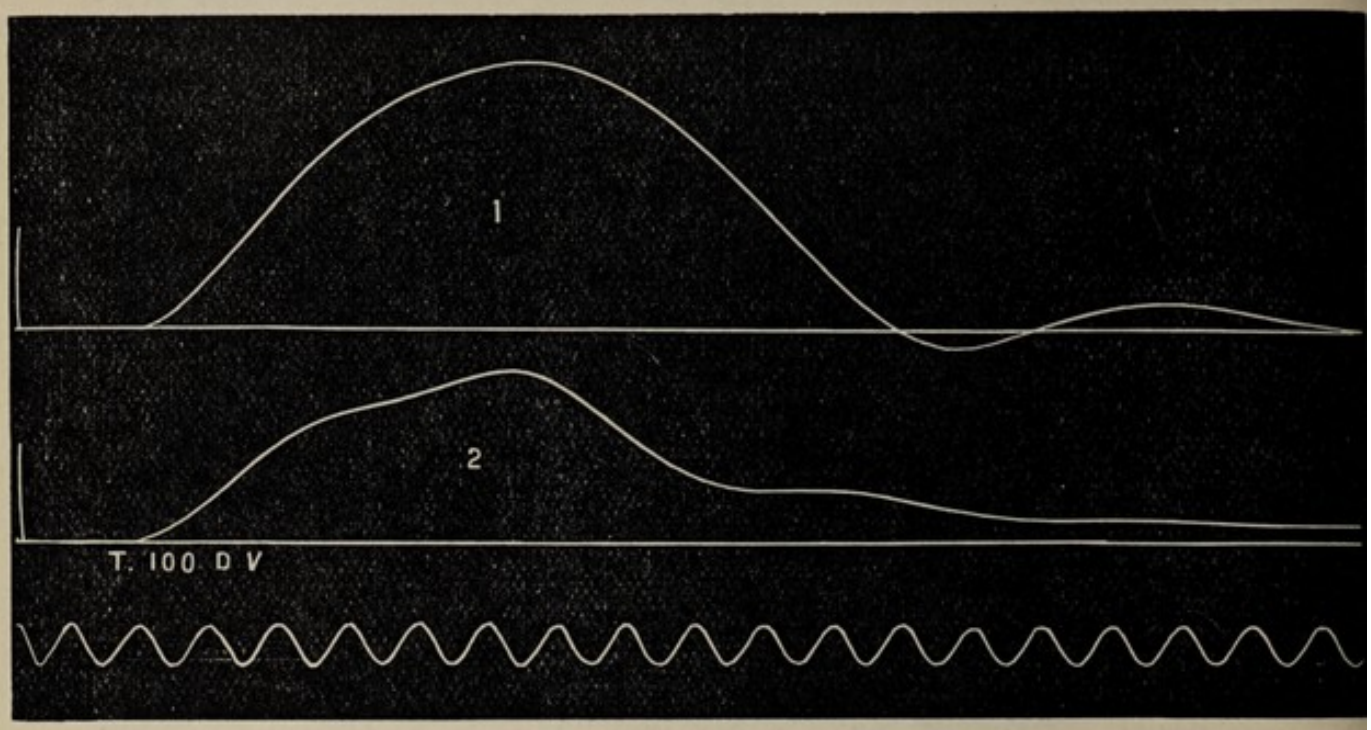


Fig. 153.—T. D.V. 100. (1) Isotonic, and (2) isometric curve of same gastrocnemius; in 2 contracting against considerable resistance.

topped, so that it remains for some time in contraction (fig. 154). If curves be taken with varying tensions, tabulate and measure out the results obtained.

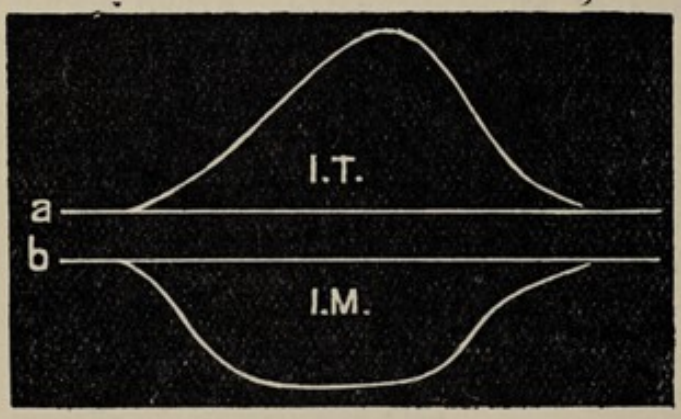


Fig. 154.—*a*, Diagram of isotonic; *b*, Isometric muscle-curves.

3. Myotonometer (fig. 155) of Burdon-Sanderson is used as (1) a myograph to obtain "isometric" myograms, and (2) to measure the tension and stress. The femur is fixed on the clamp, and the tendon is attached to a strong spring, with writing-style above. For observations on the tensions of tetanised muscle the spring is so stiff that when the muscle is tetanised its length is scarcely altered. The small movement is visible when magnified by a lever. "By ascertaining the weight which, when hung on the spring, will produce a movement equal to that produced by the traction of the muscle, the force of traction (tension) of the muscle can be estimated." (Fig. 156.)

4. Work by a simple twitch or contraction.—Arrange a gastrocnemius to record on a stationary cylinder, but record only the "lift," as in Lesson XXXIV., moving the cylinder with the hand as required. On the lever under the muscle attachment place a scale-pan, and in this place weights of known value. With each twitch the muscle lifts the weight, and thus does a certain amount of work which is easily calculated.

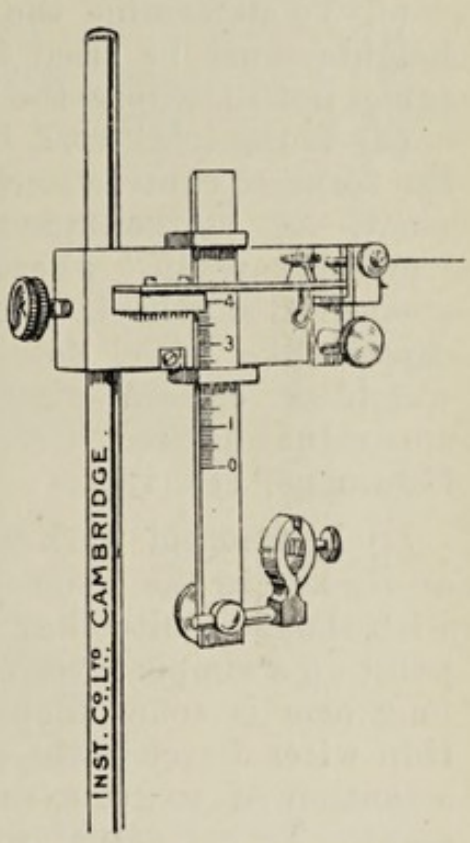


Fig. 155.—Myotonometer of Burdon-Sanderson.

(*a*) Measure the height of the tracing from the base line or abscissa. This is conveniently done by a paper millimetre scale fixed to a microscopic slide. The work done, W, is equal to the weight, *w*, lifted multiplied by the height, *h*, to which it is lifted—

$$W = wh.$$

But, of course, a long lever being used, the tracing is much higher than the actual shortening of the muscle.

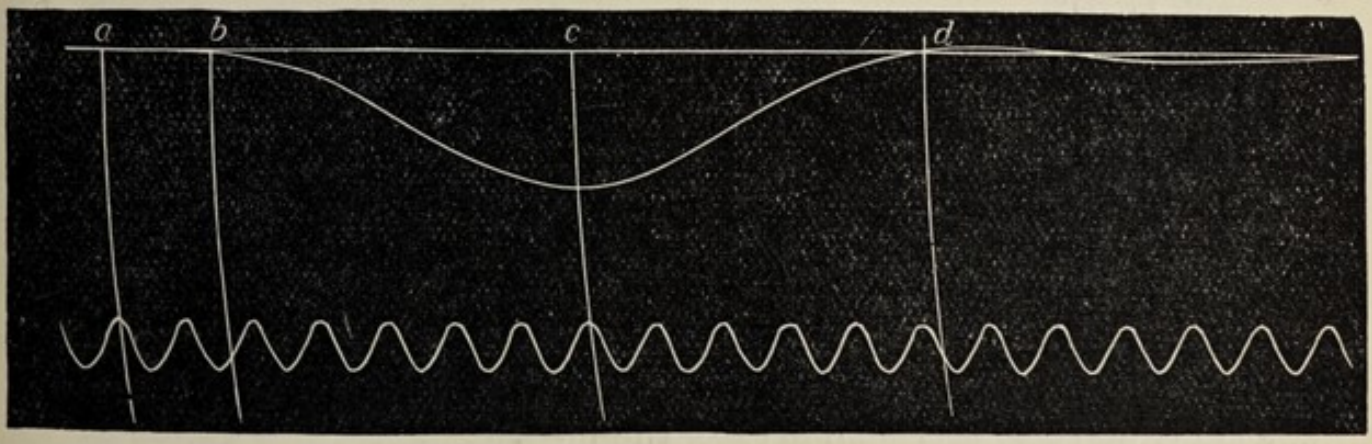


Fig. 156.—Tension-curve taken with instrument shown in fig. 155.

(*b*) To determine the exact amount of the lift, one must know the length of the lever and the ratio between its arms and the magnification of the curve. Suppose the one to be ten times as

long as the other, then the total work in gram-millimetres must be divided by 10.

(*c*) To determine the **maximum** of work obtainable, various heights must be tried to get the largest product, care being taken not to fatigue the muscle.

(*d*) If the **total work** be required, record only the heights of the series of contractions. Fick uses a special capstan arrangement, or "**Arbeitssammler**" "work-adder," "muscle-crank," "muscle-capstan," whereby a muscle lifts a known weight by means of a wheel. The apparatus is so arranged that the capstan-like wheel does not slip back again, but winds up the weight a certain distance at each twitch. Description of apparatus omitted here. (Figure in Cambridge Sc. Inst. Co. Catalogue, 117, 1899.)

(*e*) **Relation of work to load.**—Arrange the coil with a contact or Hg-key in the primary circuit to give only break shocks, and with short-circuited key in the S current. Attach a muscle at a point on a simple lever, so that the ratio between the short and long arm is some simple multiple—*e.g.*, 5. As electrodes are thin wires direct to the end of the muscle, add a weight to give a tension of 30 grms. nearer the axis than the muscle attachment. To get actual weight multiply 30 by ratio of distance of axis from fixation point of muscle to distance of axis from weight attachment. Take a maximum twitch. Proceed as above, adding a tension of 30 grms. each time. A series of vertical ordinates is obtained. Determine the work done at each twitch. It will be found that the work increases rapidly as the load increases, and reaches its maximum with a load of 150 to 160 grms. After this it decreases.

During tetanus no weight is raised; it is held suspended, and of course no visible mechanical work is done, but that there is a great expenditure of energy is shown by the rise of temperature of the muscle and other chemical phenomena.

(*f*) The "**absolute force**"—*i.e.*, the weight a muscle is just able to lift—can also be determined. This is usually expressed in grms. per sq. cm. of sectional area, as it depends directly on the transverse sectional area of the muscle.

5. **Curve of heat-rigor.**—(*a*) Arrange a frog's gastrocnemius to record by means of a crank-myograph on a slow drum, weighting it with 30 to 50 grms. Inscribe the change of form of the muscle produced by pouring water at 70° C. on the muscle.

(*b*) Or use Ludwig's apparatus (fig. 157) with a sartorius muscle. When a frog's muscle is gradually heated from 12° to 90°, three distinct contractions are observed—the initial contraction (about 33°) being most marked,

the first secondary at 50° to 56°, and the second one about 70° C. Excitability is lost about 38·5° C. The heart shows only a slight initial contraction and a second single well-marked secondary. Distinctive curves are also given by smooth muscle.—"Heat-rigor in Cold-blooded Animals," by H. M. Vernon (*Journ. of Phys.*, xxiv., 239, 1899).

(*c*) Suspend the hind legs (with skin) in water at 50°. Observe how the parts of the limb are extended at all the joints—as coagulation of the proteids—and heat-rigor takes place. Note change in muscles — opaque, white, tough.

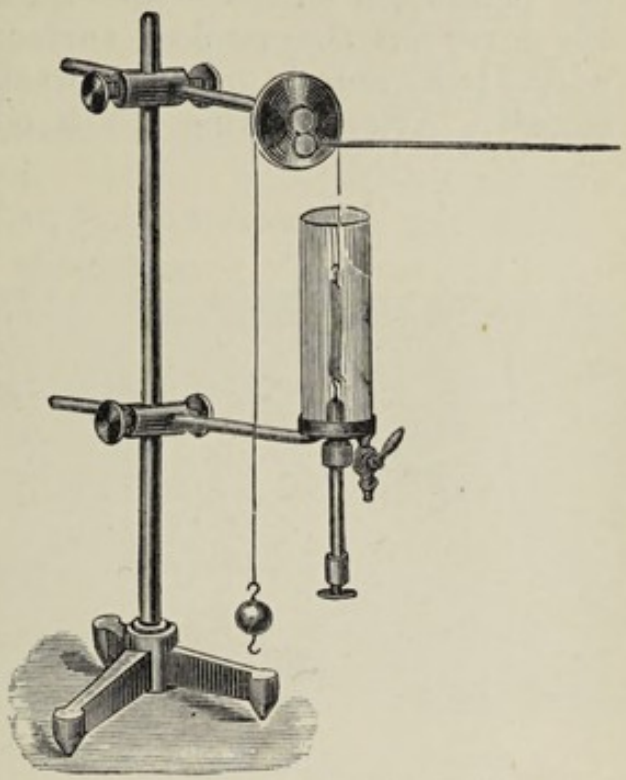


Fig. 157.—Ludwig's apparatus for heat-rigor curve of sartorius, made by Petzold.

6. **Chordogram.** — Engelmann (Croonian Lecture, R. S., 1895) has shown that when a short length (5 cm.) of an E violin string, previously swollen in water, is fixed so as to record any alteration in its length, on suddenly heating the string the lever rises, and on cooling the lever falls and a curve is recorded just like a contraction curve of muscle. Or a string may be made to swell by dipping in hot water and then soaking in concentrated glycerine. This can then be heated in air and the movements recorded.

LESSON XXXVII.

PENDULUM-MYOGRAPH—SPRING-MYOGRAPH.

1. Pendulum-myograph muscle-curve.

(*a*) Cover the oblong glass plate with glazed paper, smoke its surface, and fix it to the pendulum. The plate must be so adjusted that the pendulum, on being set free from the "detent" (fig. 158, C), shall be held by the "catch," C. Test this.

(*b*) Arrange the P circuit for single break shocks (fig. 158), interposing the knock-over key, K′. Short-circuit the S coil.

(*c*) Fix the femur of a N.M. preparation in a clamp on a suitable stand, attach the tendo Achillis to the writing-lever, S, and place the nerve over the electrodes in a moist chamber, or

use a crank-myograph. Load the lever with 20 grms., and direct its point to the side to which the pendulum swings. Fix the pendulum with the detent, and adjust the writing-style of the lever on the smoked surface. Connect the electrodes (or wires) from muscle or nerve to the short-circuiting key in the S circuit. After opening the S circuit, with the hand break the P circuit to make certain that the muscle responds at break.

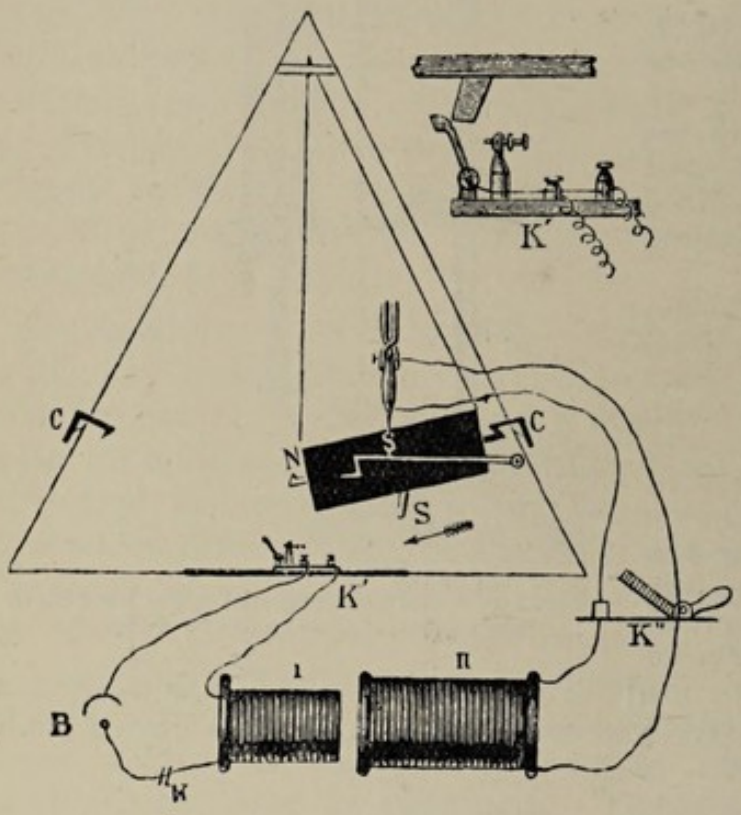


Fig. 158.—Scheme of arrangement of pendulum. *B*, Battery; *I*, Primary, *II*, Secondary coil; *S*, Tooth; *K'*, Key; *C*, *C*, Catches; *K'* in the corner, scheme of *K'*; *K*, Key in primary circuit; *K''*, Short-circuiting key in secondary circuit.

(*d*) Close the trigger-key, K', in the P circuit, and open the key in the S circuit. Allow the pendulum to swing; as it does so it knocks over the key in the P circuit and breaks the current, thus inducing a break shock in the S circuit, whereby the muscle is stimulated and records its contraction or muscle-curve on the smoked surface.

(*e*) **Abscissa** — *i.e.*, the base line. Rotate the stand supporting the muscle to remove the writing point of the lever from the recording surface. Bring the pendulum back to the detent, adjust the writing-style, close the trigger-key, and keep the secondary circuit short-circuited. Allow the pendulum to swing. This records the base line. Note that it is *curved*, and is an arc of a circle with a radius equal to the length of the pendulum.

(*f*) **Latent period.**—Bring the pendulum to the detent, short-circuit the S circuit, and withdraw the writing-style as in (*e*). Close the trigger-key; with a finger of the left hand keep it closed, allow the lever to touch the glass plate in its original position, and with the right hand bring the knife-edge of the pendulum in contact with the trigger-key, so as just to open it. An arc of a circle is inscribed on the plate, and indicates the moment of stimulation.

(*g*) **Time-curve.**—Remove the muscle-lever, place the pendulum in the detent, close the trigger-key, and adjust the writing-style of a tuning-fork, vibrating, say, 120 or 250 D.V. per second, in the position formerly occupied by that of the muscle-lever. Set the fork vibrating, either electrically or by striking it. Allow the pendulum to swing, and record the time-curve under the muscle-curve (figs. 159, 160 D.V.). All the conditions must be exactly the same as when the muscle-curve was taken.

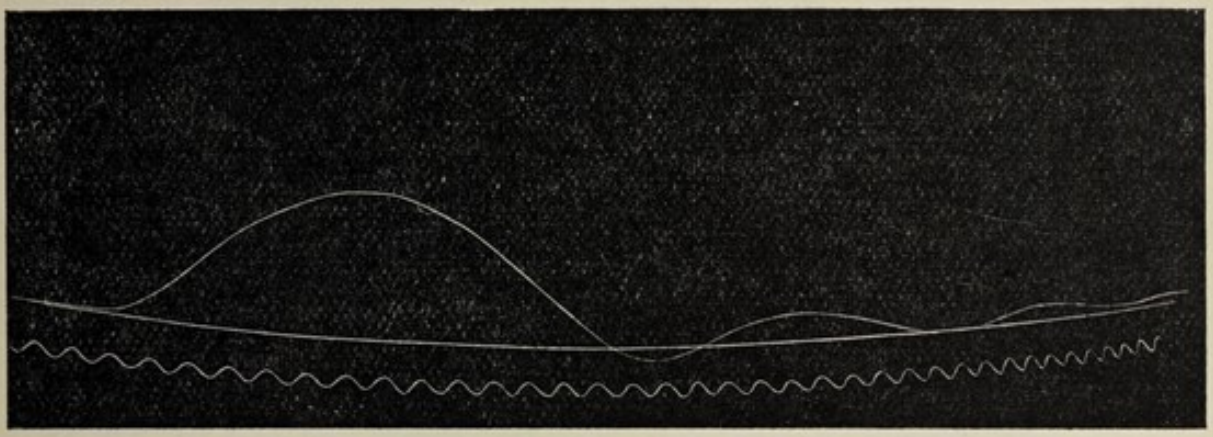


Fig. 159.—Pendulum-myograph curve-time, 100 D.V. per second (reduced).

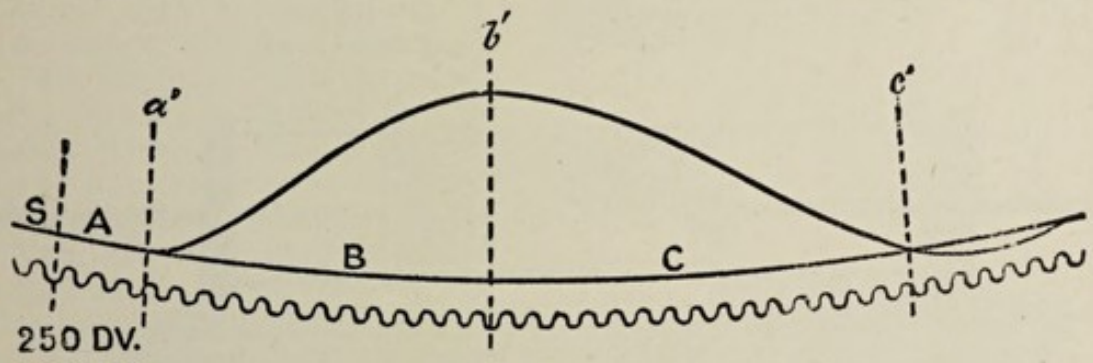


Fig. 160.– Scheme of pendulum-myograph curve. *S*, Point of stimulation; *A*, Latent period; *B*, Period of shortening, and *C*, of relaxation.

(*h*) Varnish the curve, and measure its phases. *N.B.*—Note that the zero abscissa is an arc of a circle with a radius equal to the length of the pendulum. Bring ordinates vertical, *a'*, *b'*, *c'*, to the abscissa, and make time measurements of the "latent period" (figs. 159, 160, A), shortening (B), relaxation (C), and contraction remainder. Record magnification.

In a system with the abscissa represented by an arc of a circle, the ordinates are radii of the corresponding circle. Such a system is called a "polar co-ordinated system."

2. Spring-myograph (fig. 161).—The arrangements are the same as for the pendulum-myograph, the trigger-key of the myograph being placed in the P circuit.

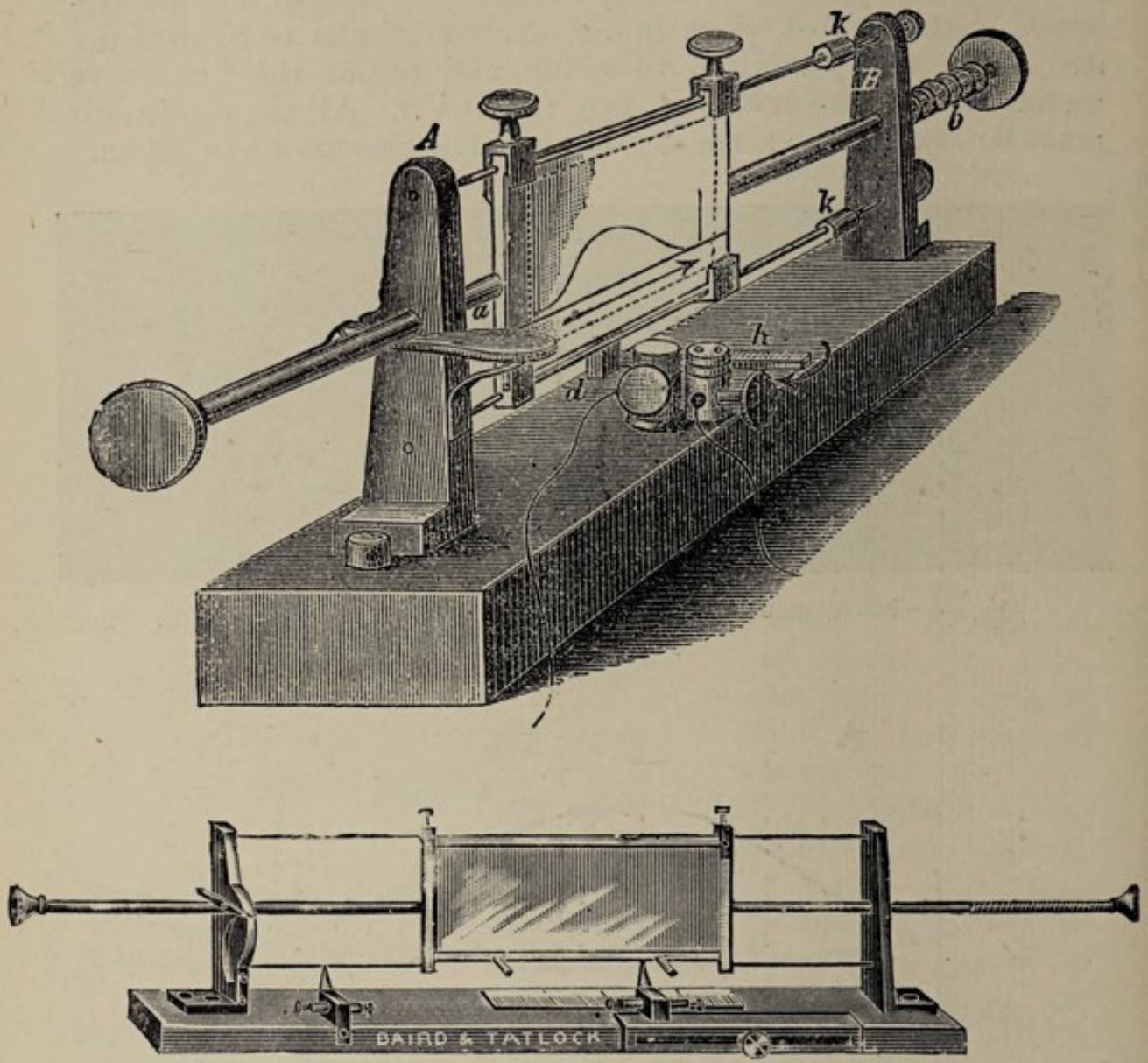


Fig. 161.—*Upper figure*, Spring-myograph, seen obliquely. *Lower figure*, Simple form of spring-myograph, seen from front (du Bois-Reymond).

(*a*) Cover the glass with glazed paper, smoke it, and fix it in the frame. Push the plate to one side, and fix it with the catch. Close the trigger-key, *h*.

(*b*) Arrange a N.M. preparation to write on the glass plate. Open the secondary circuit.

(*c*) Press on the thumb-plate, *a*, thus liberating the spring, when the glass plate shoots to the other side, the tooth, *d*, on its under surface breaking the primary circuit, and the muscle-curve is recorded.

(*d*) Short-circuit the S circuit, push back the plate, and fix it with the catch; close the trigger-key, and shoot the plate again to record the abscissa.

(*e*) Time-curve.—Push the plate back again, and fix it; close the trigger-key, in order that the conditions may be exactly the same as before; set a tuning-fork in vibration (120 D.V. per second), and adjust its writing-style under the abscissa. Shoot the plate again, and record a time-curve.

3. Study the improved form of this instrument in which the glass plate is set free, and the tuning-fork vibrations are recorded simultaneously when a handle is pressed. It has a simple mechanism for adjusting the writing-styles for the muscle and abscissa (*du Bois-Reymond*).

4. Analysis of twitch (Revolving drum).

(*a*) Arrange the drum to move at a fast speed (50 cm. per second).

(*b*) Arrange coil for single B shocks, the S circuit short-circuited, and arranged to stimulate a nerve attached to a muscle placed in a moist chamber, or on a crank-myograph, as directed for the foregoing experiments. In the P circuit introduce, besides the spring-key, an electro-magnet with a marking lever (figs. 126, 162, *e*), and cause its point to write exactly under the muscle-lever. Arrange, with its point exactly under the other two, a Desprez chronograph or signal, in circuit with a tuning-fork of known rate of vibration, actuated by a dry battery. The three recording levers are all fixed on the same stand, which should preferably be a tangent one—*i.e.*, the rod bearing the recording styles can by means of a handle be made to rotate so as to bring the writing-styles in contact with the recording surface.

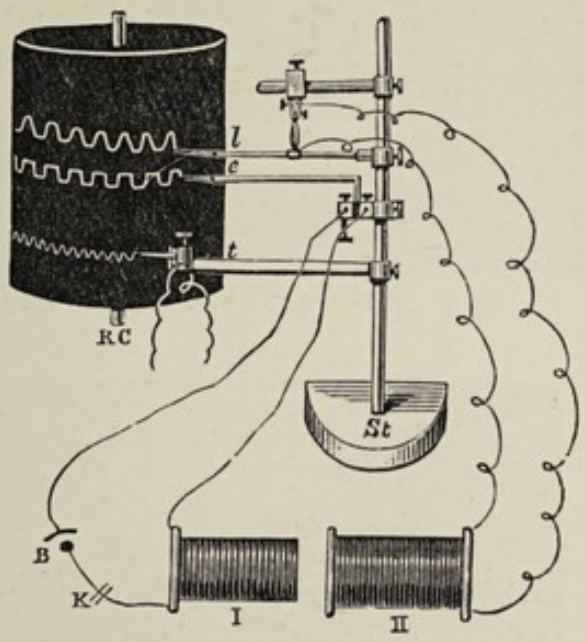


Fig. 162.—Arrangement for estimating time-relations of a simple twitch. *B*, Battery; *K*, Key in primary circuit; *I*, Primary, *II*, Secondary coil, with *K'*, Short-circuiting key; *l*, Muscle-lever; *e*, Electro-magnet in primary circuit; *t*, Electric signal; *St*, Support; *RC*, Drum.

Open the S circuit, break the P; the muscle contracts, and at the same time the style of the electro-magnet is attracted and records the moment of stimulation (fig. 126).

The analysis may also be done by means of the "automatic break" arrangement attached to the revolving drum. (Lesson XXXV.)

5. Vibrating reed as a chronograph.—For measuring small intervals of time this is very convenient. The arrangement was first adopted by Grunmach, acting on the suggestion of Kronecker. A steel tongue, vibrating a hundred times per second, covers an oblong aperture placed at the lower part of a gradually-narrowing brass tube, closed at the narrow end. To the tongue is attached a stylette, which records the movements

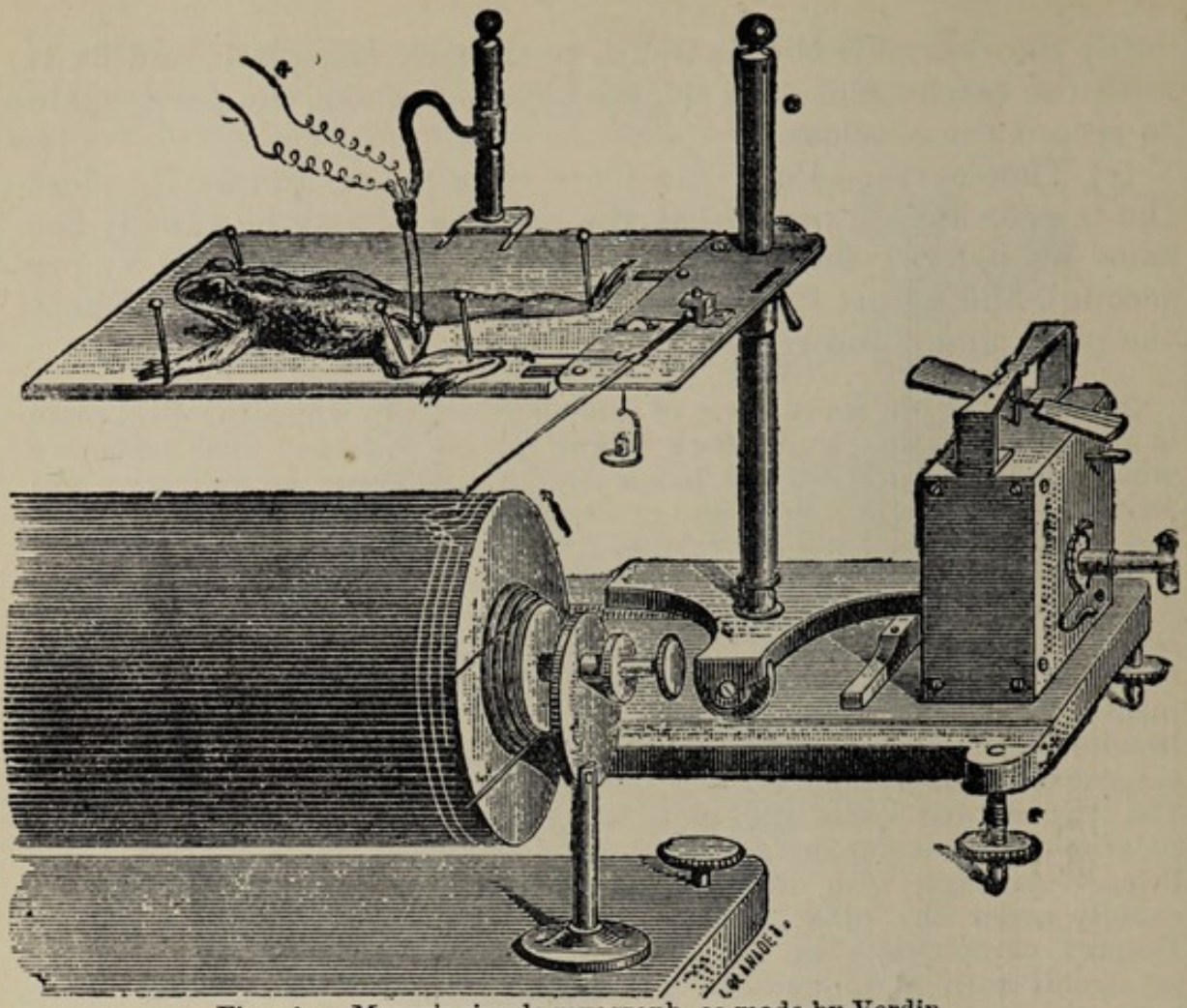


Fig. 163.—Marey's simple myograph, as made by Verdin.

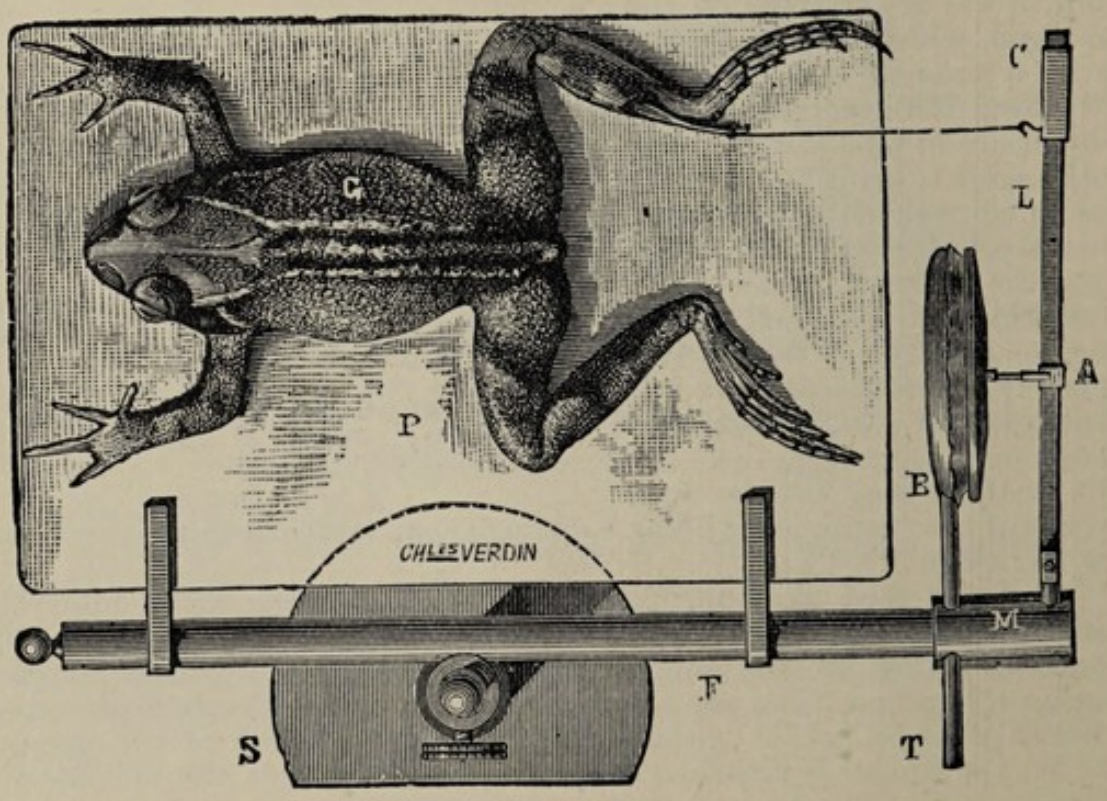


Fig. 164.—Marey's transmission myograph.

of the former. To the open end of the brass tube of the instrument is attached a brass ball or resonator, and to the latter a caoutchouc tube.

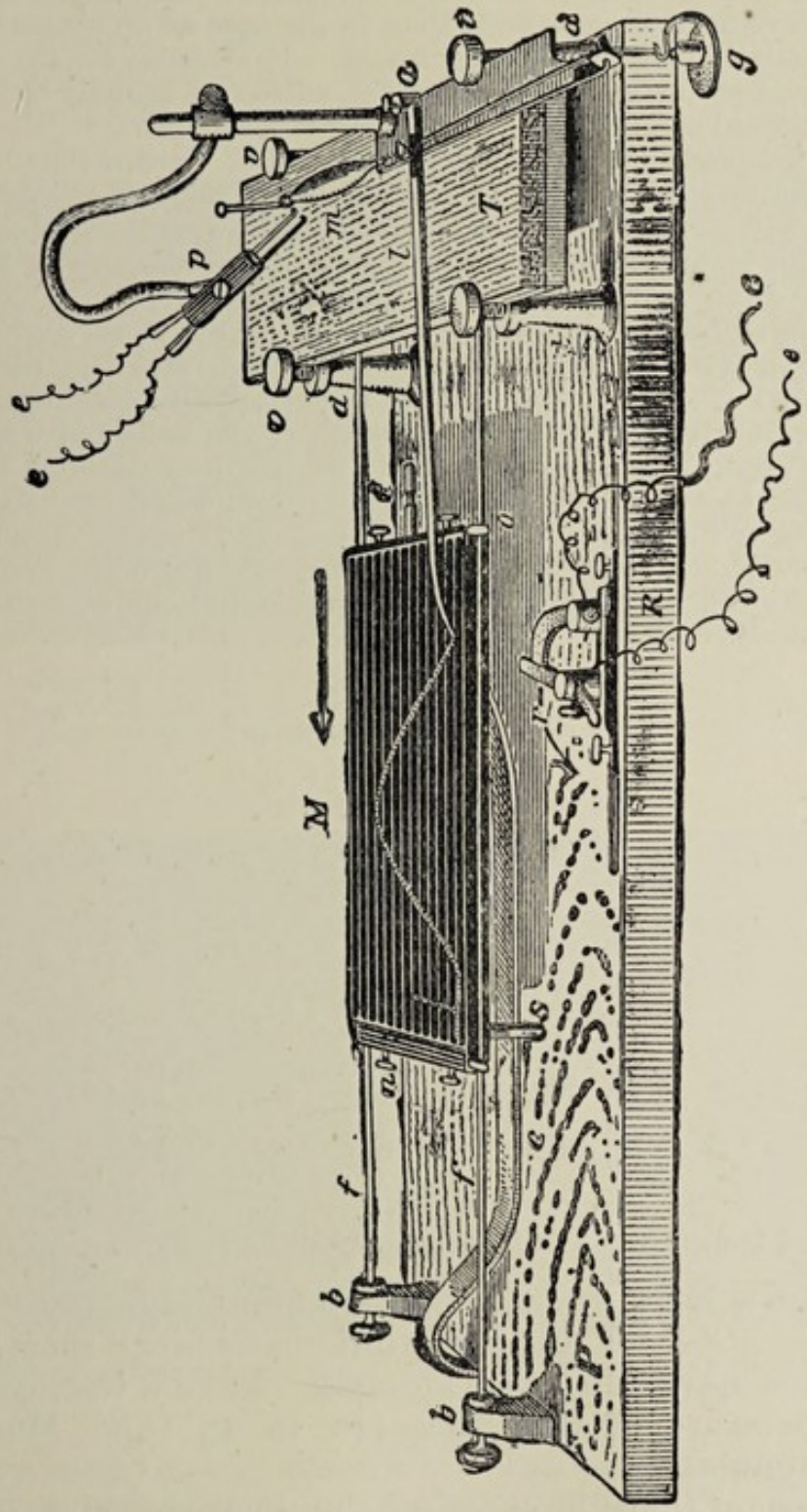


Fig. 165.—Horizontal myograph of Fredericq. *M*, Glass plate, moving on the guides *f*, *f*; *l*, Lever; *m*, Muscle; *p*, *e*, *e*, Electrodes; *T*, Cork plate; *a*, Counterpoise to lever; *R*, Key in primary circuit.

When air is sucked through the apparatus the reed and stylette are set vibrating. They may be kept vibrating by means of an aspirator placed in connection with a water-tap.

6. **Marey's simple myograph** (fig. 163).—A pithed frog is pinned on a cork plate; the tendon of the gastrocnemius is dissected out and attached to a writing-lever, which is weighted with a counterpoise; the sciatic nerve is exposed and stimulated in the ordinary way. The cylinder moves on a horizontal axis. The muscle can be stimulated while it is still *in situ*, and is under more normal conditions than in the case of an excised muscle —*e.g.*, the circulation of the blood is intact. It is useful for the study of the action of poisons on muscle and for the action of stimuli on a muscle under nearly normal conditions.

7. **Marey's transmission myograph**, made by C. Verdin (fig. 164), is a most convenient apparatus when it is desired to transmit the record of a contraction to a distance. The muscle pulls on the arm lever of a Marey's tambour, and the latter is connected by means of an india-rubber tube to a similar recording tambour in relation to a drum (fig. 149).

8. **Spring-myograph of Fredericq** (fig. 165).—This is arranged as the spring-myograph, but the glass plate is horizontal, and is pulled along rapidly by a band of caoutchouc. A key in the primary circuit is opened by means of a pin attached to the frame carrying the glass plate when the plate is discharged. In an improved form of the instrument, a steel rod made to vibrate at the moment the plate is discharged records a time-curve beside the muscle-curve.

9. **Demonstration myographs.**—The "railway" form of Waller and that of M'Kendrick, and the water-pressure myograph of Schäfer are excellent. (See *Appendix.*)

LESSON XXXVIII.

INFLUENCE OF TEMPERATURE, LOAD, AND VERATRIN ON MUSCULAR CONTRACTION.

1. Influence of temperature on muscular contraction.

(*a*) Arrange a N.M. preparation on a crank-myograph—after-loaded—(Lesson XXXV.), using an automatic break shock drum. All the curves have the same abscissa. Take a tracing at the normal temperature of the room (10 to 15° C.). Mark the moment of stimulation.

(*b*) Place ice on the skin covering the gastrocnemius, or pour iced salt solution on the exposed muscle, and then take a tracing on the same abscissa. The contraction is longer and lower, the latent period is also longer, and relaxation is greatly increased.

(*c*) Pour on the muscle warm salt solution, and take another tracing. Note temperature. Do not overheat the muscle, or heat-rigor results (fig. 166). Note that cold increases the L.P. and prolongs the whole contraction—both the rise and the fall —but especially the latter, and there is often a contraction remainder. The "lift" may not be greatly altered. The curve is not unlike that of a fatigued muscle.

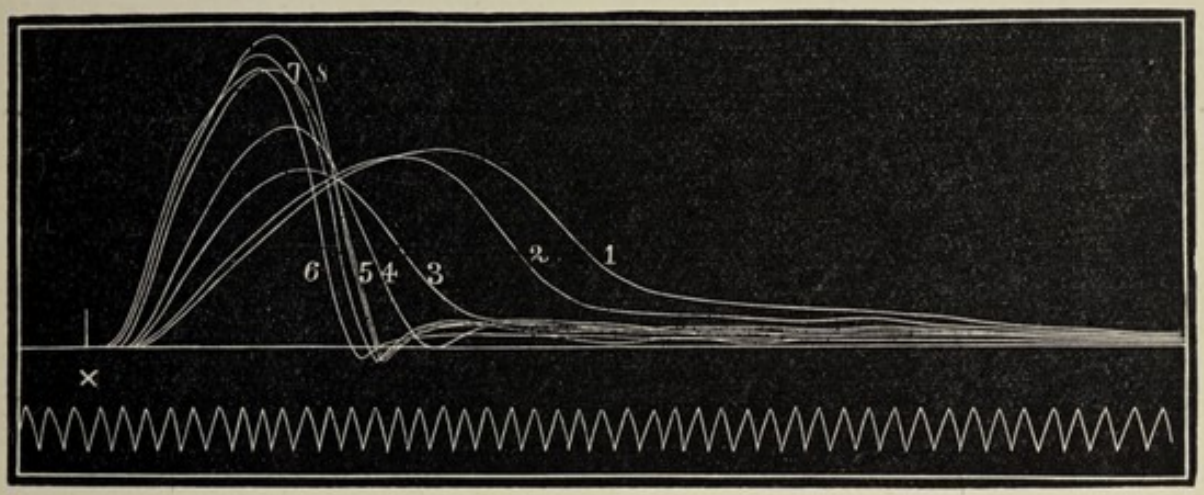


Fig. 166.—Showing how a muscle-curve varies with temperature. 1 at 5° C.; 2, 10°; 3, 15°; 4, 20°; 5, 25°; 6, 30°; 7, 35°; and 8, 40° C. The lowest tracing indicates time, 100 D.V. per second; ×, moment of stimulation by automatic break.

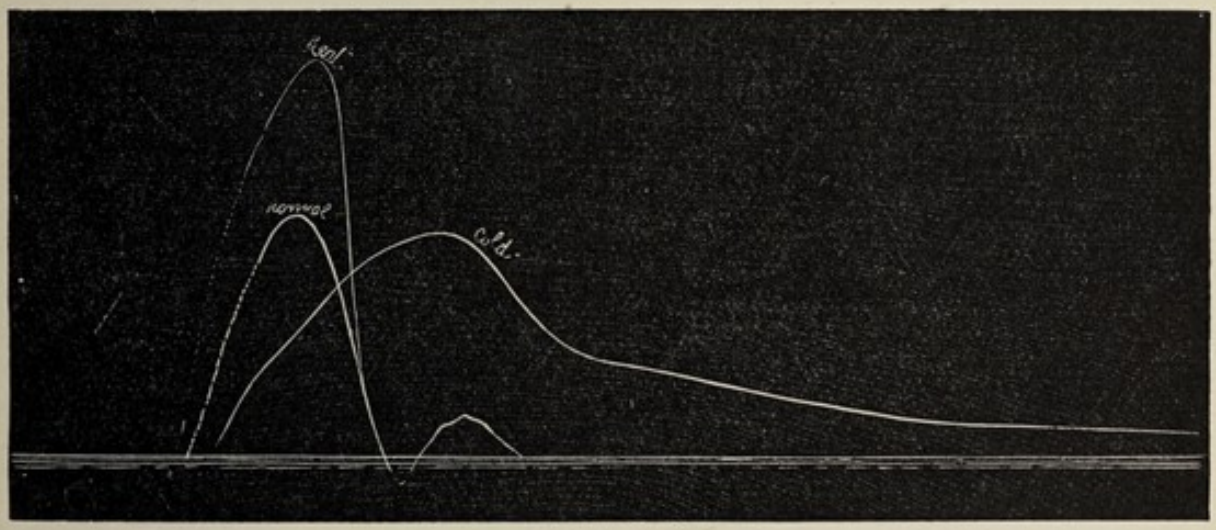


Fig. 167.—Muscle-curve of gastrocnemius, showing normal, effect of heat, and of cold.

Other methods.—(*d*) Place the muscle on a circular brass box fitted into the wooden plate of the crank-myograph. The box, B, has an inflow and outflow tube, through which water of the desired temperature can be passed (fig. 136).

(*e*) Or fix a muscle in an apparatus resembling that of Wild (fig. 196), normal saline of the appropriate temperature being added.

2. **Hyoglossus.**—Record as in 1, or use heart suspension apparatus. Test effect of heat and cold (fig. 168).

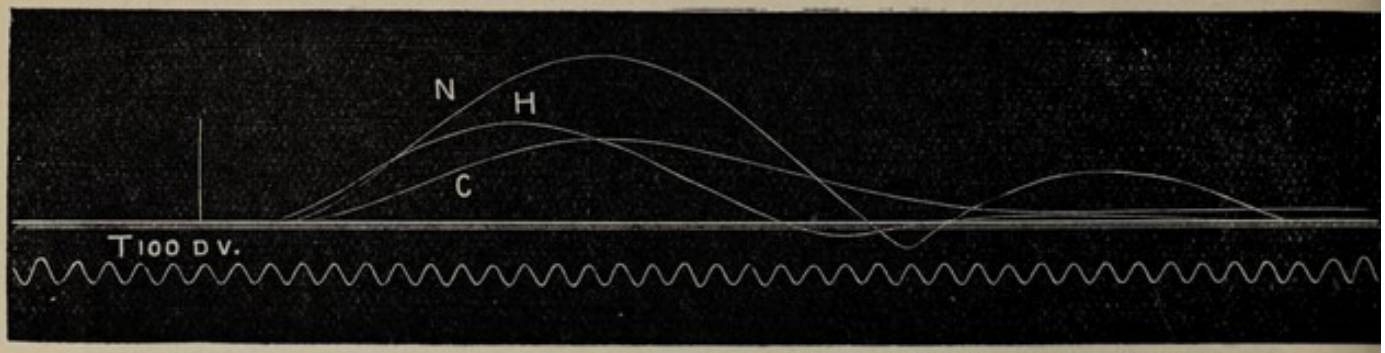


Fig. 168.—Curve of hyoglossus. *N*, Normal; *H*, Heat; *C*, Cold. Normal saline applied directly (*Stirling*).

MEASURE OUT THE CURVES AND TABULATE THE RESULTS.

Temperature.	Latent period.	Contraction time.	R elaxation time.	"Lift" of weight in mm.	Work.
C.					

3. **Muscle is paralysed at a lower temperature than nerve.**—Arrange two N.M. preparations in normal saline, as in fig. 169. B is entirely immersed, and only the nerve of A. Heat the fluid (37° to 50° C.). Stimulate both nerves. A contracts, but not B (Cl. Bernard).

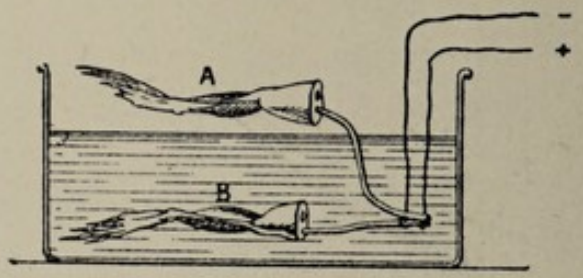


Fig. 169.—Apparatus to show effect of heat on nerve and muscle.

4. **Load on muscle-curve.**

(*a*) Arrange the pendulum-myograph as in Lesson XXXVII., using either a muscle-lever or a crank-myograph. Or, arrange a crank-myograph (after-loaded) to write on a cylinder ("automatic break"). If there is a double crest the make shock is active as well, and must be eliminated. Take all the curves on the base line.

(*b*) Record a muscle-curve with the lever only.

(*c*) Then load the lever successively with different weights (5, 20, 50, 70 . . . 100 grms.) If the crank be used, hang the

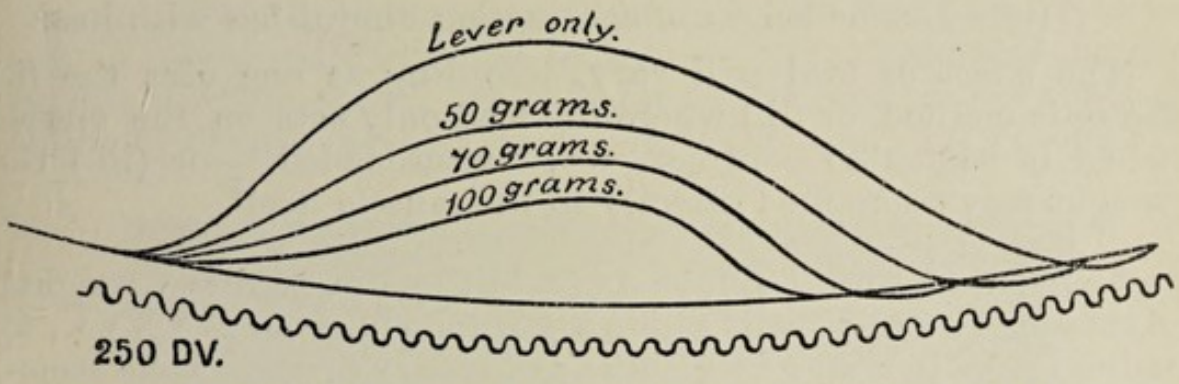


Fig. 170.—Pendulum-myograph curves, showing the influence of the load on the form of the curve.

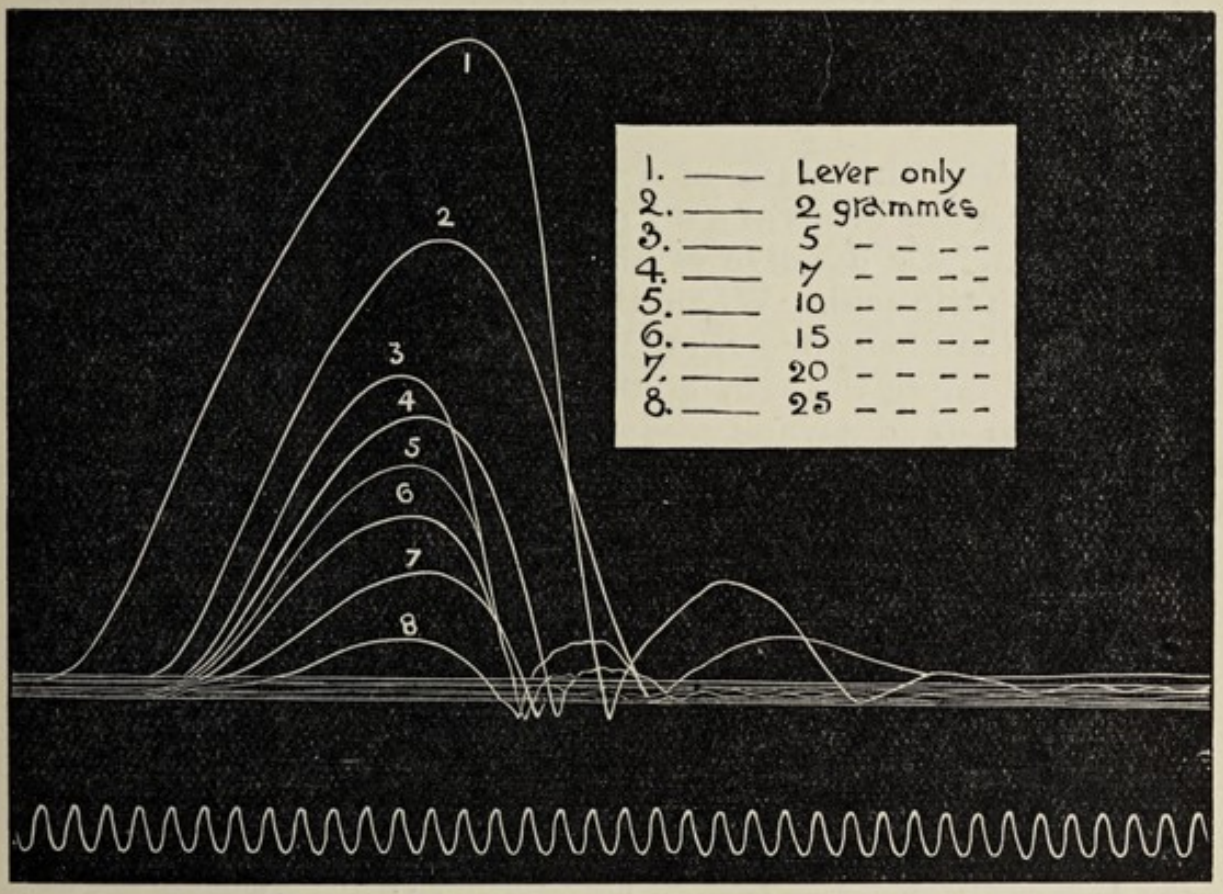


Fig. 171.—Effect of load. Revolving drum (*Stirling*).

weight near the axle by means of a string. The weights will extend the muscle, but in every case the writing-point must be brought back to its original position (fig. 171). In each case record a curve and observe how the curve varies (fig. 170).

(*d*) Record abscissa and time-curve. Study result. Note—

(i.) That L.P. increases with load.
(ii.) Period of contraction increases.
(iii.) Period of relaxation at first is diminished.
(iv.) Lift or height of contraction diminishes with load.

The effect of load will vary, according as one uses the (i.) isotonic method, or (ii.) where the load only acts on the muscle when it begins to contract—*i.e.*, "after-loaded"—or (iii.) the weight may be applied directly to the muscle itself.

5. Load on work.—Successive additions of separate weights. Arrange the experiment as for work done (Lesson XXXVI., 4), using break twitches only and a stationary drum. Take record of successive contractions—recording, of course, only the "lift." Move the drum by hand 0·5 cm. after each contraction. Add successive weights with constant increment of 10 grms. until the muscle ceases to respond. Measure the tracing and calculate the effect of load on work, as in Lessons XXXV. and XXXVI.

6. Veratrin on contraction.

(*a*) Destroy the brain of a frog, and inject into the ventral lymph-sac a few drops of a 0·1 p.c. solution of sulphate or acetate of veratrin. Or dissolve veratrin in normal saline with aid of weak sulphuric acid. When the frog is under the influence of the drug (half-hour or more), discharge a reflex act by mechanically stimulating the skin of the leg. The limbs are extended, and remain so for several seconds, due to the prolonged contraction of the extensors overcoming the flexors and thus causing extension of the legs. Or, apply the drug by Stirling's sublingual method if the hyoglossus is used. (Lesson LXV., 5).

(*b*) Arrange the coil for single break shocks. Make and break the P circuit by the hand with a simple key. Short-circuit the secondary—*i.e.*, recording and stimulating arrangements as for a simple twitch. Do not stimulate the muscle often, as the veratrin effect diminishes with activity of the muscle.

(*c*) Make a N.M. preparation and fix it on a crank-myograph. On dividing the spinal cord notice the prolonged extension of the legs. Or the muscle may be stimulated directly.

Record on a slow drum (1 to 2 cm. per sec.) and take a tracing. Note that the muscle contracts quickly enough, but the twitch is high compared with that of a non-poisoned muscle, while the muscle relaxes excessively slowly. The relaxation phase may last several seconds—*i.e.*, a kind of "contracture." Record

half-seconds or seconds under the tracing. The excessive prolongation of the twitch is the remarkable feature. It may last 50 to 60 seconds instead of the normal $\frac{1}{10}$ of a second. Barium salts have a similar effect. The tracing may show an uneven curve, due to irregular spasms of the muscular fibres, or a rapid initial contraction, as in fig. 172, may precede the characteristic veratrin curve. Is this due to the presence of different muscular fibres—red and white—in the muscle? (*Grützner.*)

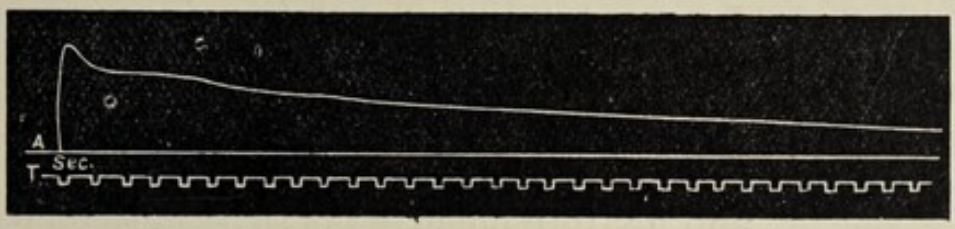


Fig. 172.—Muscle-curve from a "veratrinised muscle," recorded on a slow-moving drum. *A*, Abscissa; *T*, Time in seconds.

(*d*) Take a tracing with a quick drum, and such a curve as fig. 173 will be obtained, where the drum goes round several times before the relaxation is complete.

(*e*) If the "veratrinised" muscle contracts several times, the effect passes off—only a simple twitch being obtained—but is re-established after rest. A high temperature also causes it to disappear temporarily.

(*f*) The direct action of veratrin on muscular tissue may also be studied by the apparatus described in Lesson XLIII., and by

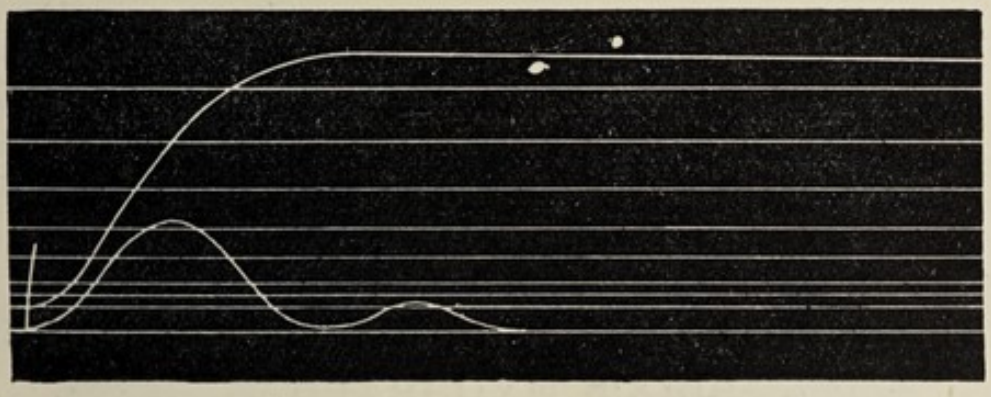


Fig. 173.—Veratrin curve (upper). Normal muscle-twitch (lower). Quick-moving drum.

this method it is easy to compare the form of the curve before and after the action of the poison (fig. 173). The drum makes many revolutions before the lever comes to the abscissa again.

(*g*) Investigate the effect of heat and cold in modifying the curves obtained. Under heat the veratrin influence passes off.

(*h*) To contrast its effects, ligature one thigh as for the curare experiment (Lesson XXXIII.), and take tracings of both muscles.

(*i*) Glycerin injected into the lymph-sac of a frog causes great prolongation of the relaxation phase of a simple twitch. "Veratrin-like action of Glycerin," Langendorf (1891) and Lyle (*Journ. of Phys.*, 1901).

7. **Hyoglossus and veratrin.**—Fix the muscle to a crank-myograph, or better a heart-suspension apparatus. Inject a few drops of very weak solution of veratrin in normal saline (1 in 100,000) into the sublingual lymph-sac by Stirling's method. This affects the muscle directly and promptly. Record the first contraction, and the sixth or tenth, or some later one. The veratrin effect passes off, the curve becomes shorter, and not infrequently the characteristic "double apex" may be obtained. If several contractions are taken, the last may be almost normal. If a very weak solution be used, the experiment may be repeated over again. If very weak solutions of veratrin are used (1 in 100,000 or 500,000 of normal saline) and the muscle exposed to its action for a long time, the height and duration and form of the response to a single stimulus is exactly the same as that obtained with rapidly-interrupted stimuli (F. Buchanan, *Journ. of Phys.*, xxv., 137, 1899.) For the action of strychnin on the red and pale muscles of the rabbit, see J. Carvallo and G. Weiss, *Journ. de Phys. et de Path. gén.*, 1899. Excellent figures are given of the anatomical arrangement of these muscles in the hind limbs.

LESSON XXXIX.

ELASTICITY AND EXTENSIBILITY OF MUSCLE—BLIX'S MYOGRAPH.

1. **Extensibility and elasticity of muscle.**

(*a*) Prepare a frog's gastrocnemius with the femur attached, fix the femur in a strong clamp, attach the tendon to a muscle-lever with a scale-pan of known weight, or a hook to carry weights. See that the lever writes horizontally on a drum. It is better to do the experiment with the sartorius (or with the

semi-membranosus and gracilis, Lesson XXIX.), as they have parallel fibres. Turn the drum and record a short abscissa, then return to the starting point.

(*b*) Add to the lever, successively, different weights (10, 20, 30, 40 . . . 100 grms.). After each addition let the muscle extend fully, move the drum (1 cm.), then add the next weight, and move the drum, noting that with each weight the muscle extends less, and not in proportion to the weight added. Remove the weights successively, moving the drum as before. The muscle shortens, retracts by elastic reaction, but it does so very slowly and incompletely if heavy weights have been added. The effects of extensibility and elasticity may be combined thus. On adding 10 grms., the lever descends; remove the weight and the lever ascends. Move the drum by hand 1 cm., and add 20 grms. to the scale-pan. This time the vertical line drawn is longer, indicating greater extension of a muscle by a greater weight, but nevertheless the muscle-lever will rise to its original height on removing the weight. Repeat this with other weights. With the heavier weights see that everything is securely clamped. If the apices of all the lines obtained be joined, they form a *hyperbola*. The muscle, therefore, has not a large amount of

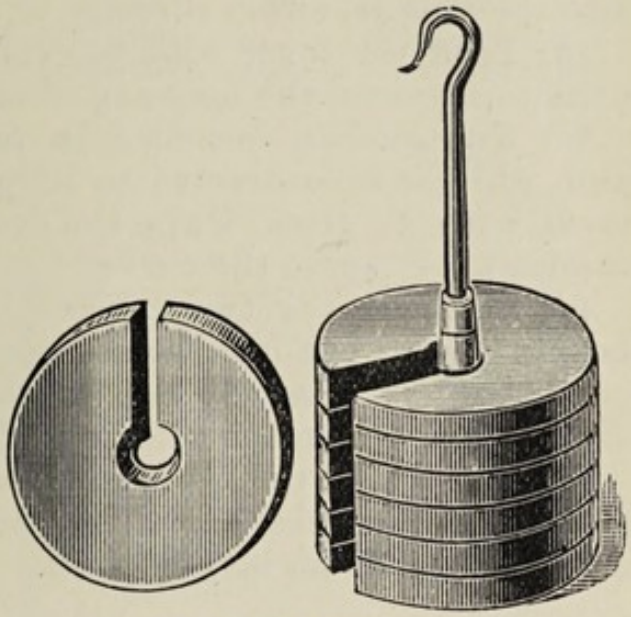


Fig. 174.—Weights to load muscle.

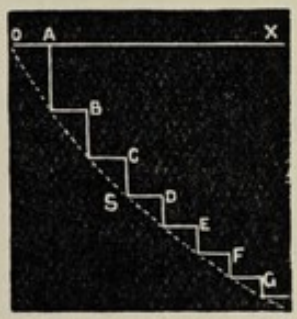


Fig. 175*a*.—Curve of elasticity of a frog's muscle.

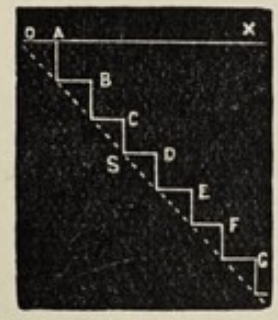


Fig. 175*b*.—Curve of elasticity of india-rubber.

elasticity—*i.e.*, it is easily extended by light weights, and on removal of the weight it regains its original length, so that its elasticity is said to be perfect. The *hyperbola* obtained shows

further that the increase in length is not directly proportional to the weight, but diminishes as the weights increase (fig. 174).

(*c*) Repeat the same experiment with a strip of *india-rubber*. In this case equal increments of weight give an equal elongation, so that a line joining the apices of the vertical lines drawn after each weight is a *straight line* (fig. 175).

2. Extensibility of muscle increases during contraction, elasticity diminishes.

(*a*) Fix the gastrocnemius—or preferably semi-membranosus and gracilis—in a strong clamp, connecting it to a lever to record on a drum, and adjust an interrupted current to stimulate the muscle, either directly or indirectly.

(*b*) Load the lever with 50 grms. to record on a slow-moving drum. Remove the load and observe the curve obtained.

(*c*) *Extensibility increased in tetanus.*—Tetanise the muscle, and, while it is contracted to its greatest extent, again load the lever with 50 grms. while the drum is in motion, and remove the load. Observe the curve.

(*d*) Compare the two curves. The second curve, of course, begins higher, but its absolute descent is greater than the first curve, and it does not rise to the horizontal zero again—*i.e.*, there is an "extension remainder."

(*e*) Begin with the drum stationary, using single break shocks or tetanising shocks, and then record with the drum in motion. A stationary drum may be used.

3. Blix's myograph.—The following summary is based on the description given by Prof. F. Schenck (*Marburg*).

The muscle-clamp and the part to which the steel lever is attached form a rectangular piece, S S, which glides in a slot formed by the guides, R R and R_1 R_1. The slider, S S, carries at *a* the axis of the lever *a b*, and also a lateral piece, A, placed at right angles for the attachment of the muscle, and one end of which is fixed to the lever at *b*. The weight is represented by P, which, by means of the collar, *r*, presses on the lever. This collar, *r*, moves to and fro—not from side to side—between two pairs of fixed studs, *t t* and t_1 t_1 (fig. 176).

Suppose the slider to be pushed so far to the left that the axis, *a*, just lies opposite to the collar, *r*—a point which is adjusted on the apparatus—then the tension of the muscle is nil. On moving the slider with the hand towards the right, so that the weight, P, acts on points of the lever more and more removed from *a*, then the tension of the muscle increases steadily, when the writing point, *p*, records the curve of extension, *p*, on a horizontally placed and stationary wooden board or glass plate covered with smoked glazed paper. In using the apparatus, board, slot, and slider are placed horizontally, the weight, P, is not applied directly to *f*, but to the

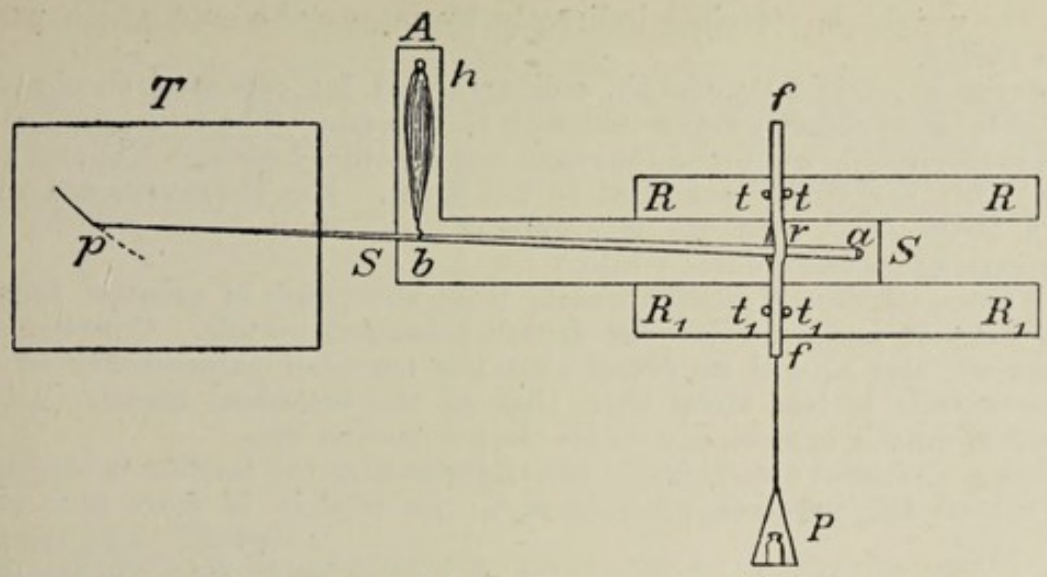


Fig. 176.—Scheme of Blix's myograph for taking extension curves of muscle with the load increasing or decreasing. *S S*, Slider; *R R* and R_1 R_1, Guides for slider; *a b*, Lever; *A*, Support for muscle; *P*, Weight; *r*, Collar; *t t* and t_1 t_1, Guides for collar carrying weight; *p*, Recording point.

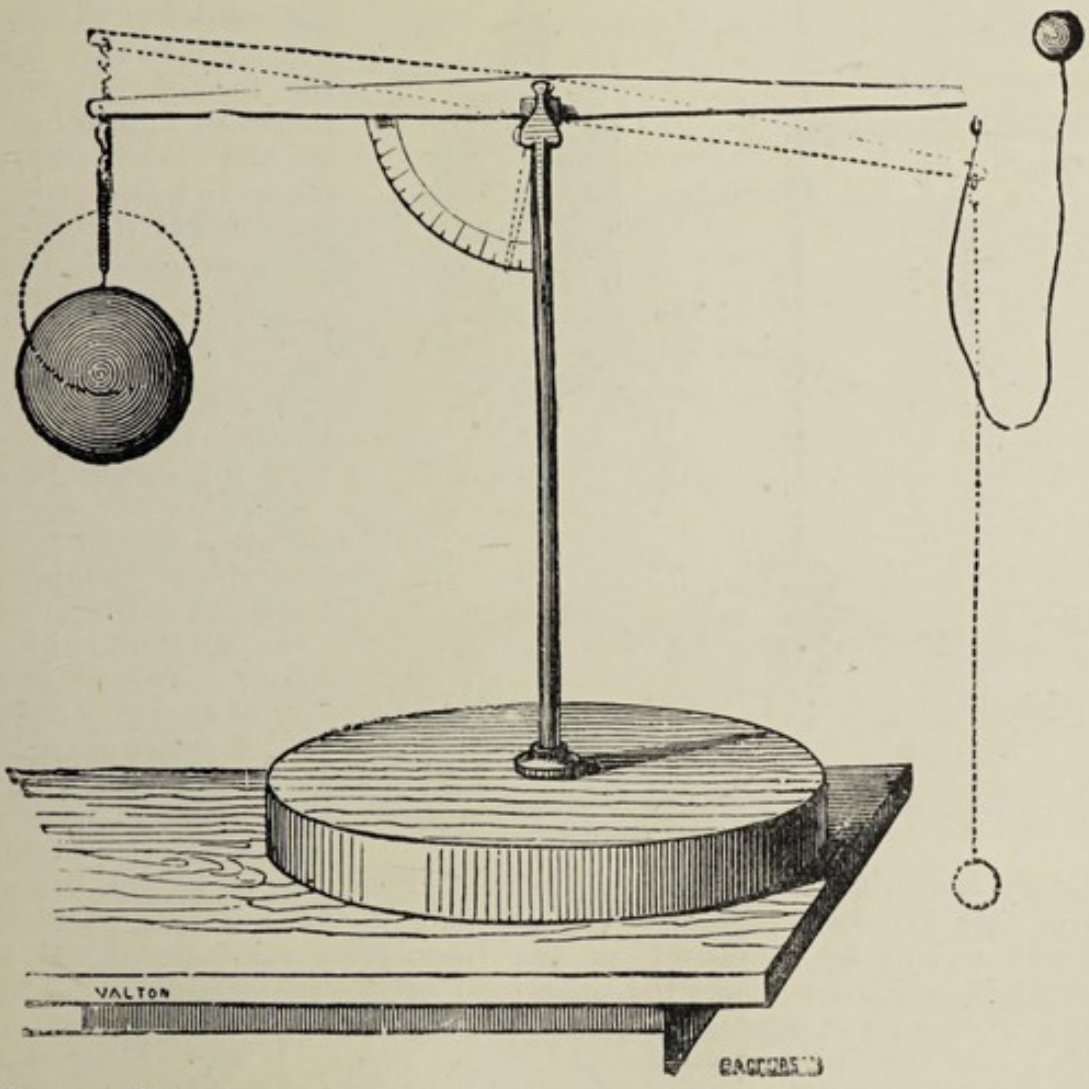


Fig. 177.—Marey's apparatus to show action of a weight acting through an elastic medium There is an improved form of the apparatus made by Verdin.

latter the weight is attached indirectly by means of a cord which passes over a pulley.

Apparatus.—Blix's myograph, coil arranged for repeated shocks, the electrodes being directly connected with the muscle. The best preparation to use is the double semi-membranosus and gracilis (Lesson XXIX.) placed side by side and firmly attached to the lever. For these muscles taken from a large *Rana esculenta* a weight of 2 kilos. is used, and for the corresponding gastrocnemius 1 kilo.

(*a*) Take a curve of a *passive* muscle from the point of greatest tension to nil tension, and a similar one from a tetanised muscle. Compare the two curves, and it will be found that the curve of extensibility of the passive muscle is less steep than that of the tetanised muscle—*i.e.*, a contracted muscle is more extensible than a passive one.

(*b*) On a tetanised muscle move the slider so that the tension is increased from nil to the greatest possible—*i.e.*, the muscle is more and more "loaded"—and then reverse this, so that from the greatest tension there is gradually "unloading." The two curves so obtained do not coincide; the latter lies considerably below the former. It would therefore appear, as far as the contraction is concerned, that it is not a matter of indifference whether the muscle is being gradually "loaded" or "unloaded."

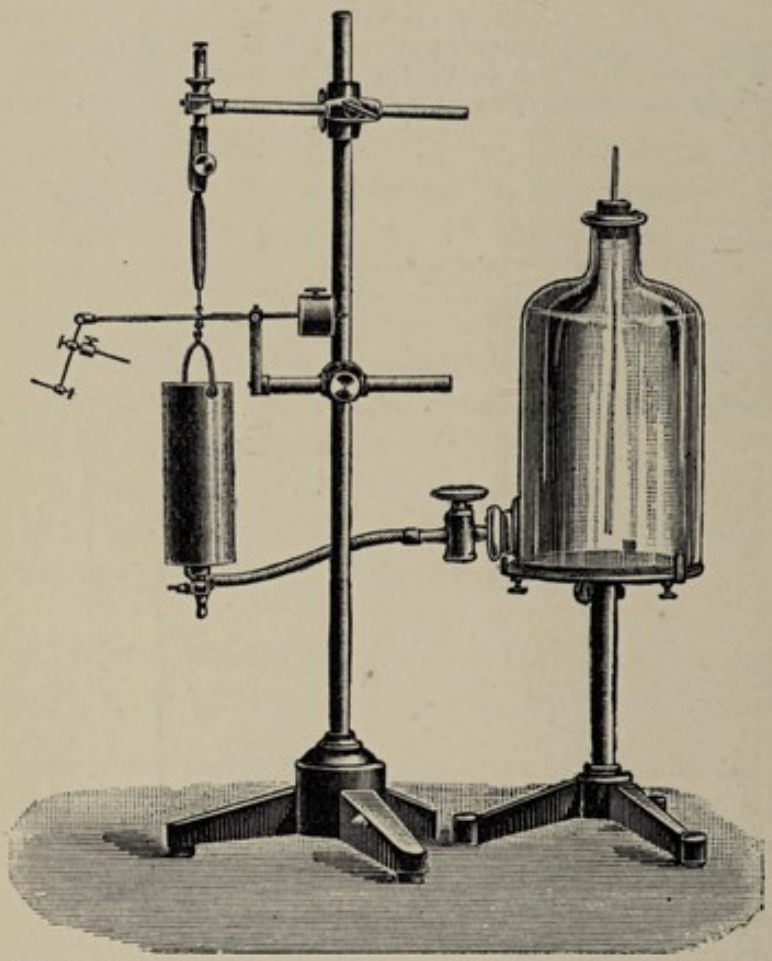


Fig. 177*a*.—Apparatus of Nicolaides for extensibility of muscle (*Petzold*).

A simpler "tension-writer" is described by Blix for recording isometric and isotonic curves synchronically (*Skandin. Arch. f. Phys.*, 1892, iii., 295).

4. Elasticity of an artery.—Test the elasticity of a strip of aorta.

5. Marey's apparatus for elasticity (fig. 177) consists of what is practically a modified ordinary beam balance with a wheel and ratchet to allow the beam to move only in one direction. To one end of the beam is suspended a given weight by means of, at one time, (1) a rigid metal rod, and then by (2) an elastic band; the other end of the beam is attached by a long cord to a given lighter weight, which is allowed to fall from a known height, and thus to act on one end of the lever. If the weight at the opposite end of the beam be suspended by a rigid rod, the falling weight produces a sound—a shock—but it fails to move the balance beam. If the

weight to be raised is suspended by an elastic cord, then the energy of the falling body is no longer lost in shock and sound, but the weight is raised. The same amount of energy transmitted through an elastic medium results in the production of mechanical work. [See also Nicolaides, *Arch. f. Phys.*, 1896, for another simple method of studying extensibility of muscle (fig. 177*a*)].

LESSON XL.

TWO SUCCESSIVE SHOCKS—TETANUS—COLD—METRONOME.

1. Two successive shocks.—The P current may be broken by means of a revolving drum—*i.e.*, using break automatic shocks (fig. 137). Two strikers are arranged on the same support (IS), and their angular deviation adjusted to give any required interval between the two successive shocks.

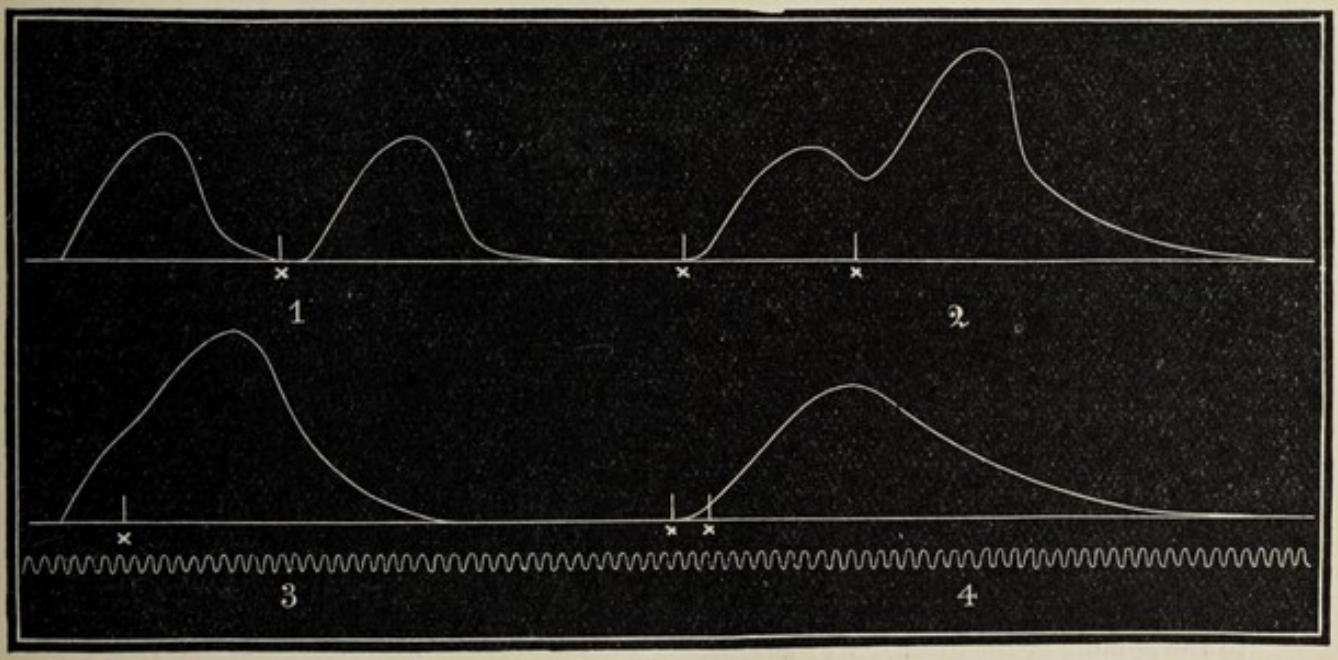


Fig. 178.—Effects of two successive shocks on a muscle. 1, Second stimulus applied at ×; 2, Second stimulus applied at the second ×; 3, Second stimulus applied at ×; 4, Second stimulus applied at the second ×. Notice "apex-time" sooner in 1 than 2.

Fig. 178 shows the effect of summation or superposition of one contraction on another, and how the result varies with the phase of the contraction at which the second shock is applied.

Make four successive experiments, using *break* shocks.

(i.) Arrange the two closures for stimulation so that they are a full muscle-curve apart. The second is usually slightly higher than the first (fig. 178, 1).

(ii.) Arrange closures to fall on a different part of the cylinder, but on the same abscissa, so that the second stimulus comes in on the relaxation of the foregoing contraction. As the second contraction occurs before the first one has ended, it starts from a higher level (fig. 178, 2).

(iii.) If the second stimulus is so arranged as to be thrown in on the ascent of the first curve, and before the apex is reached, the second curve is superposed on the first, and the height of the compound is greater than the original muscle-curve (fig. 178, 3).

(iv.) Apply the second stimulus during the latent period of the first contraction. There is practically no alteration in the height of the curve (fig. 178, 3).

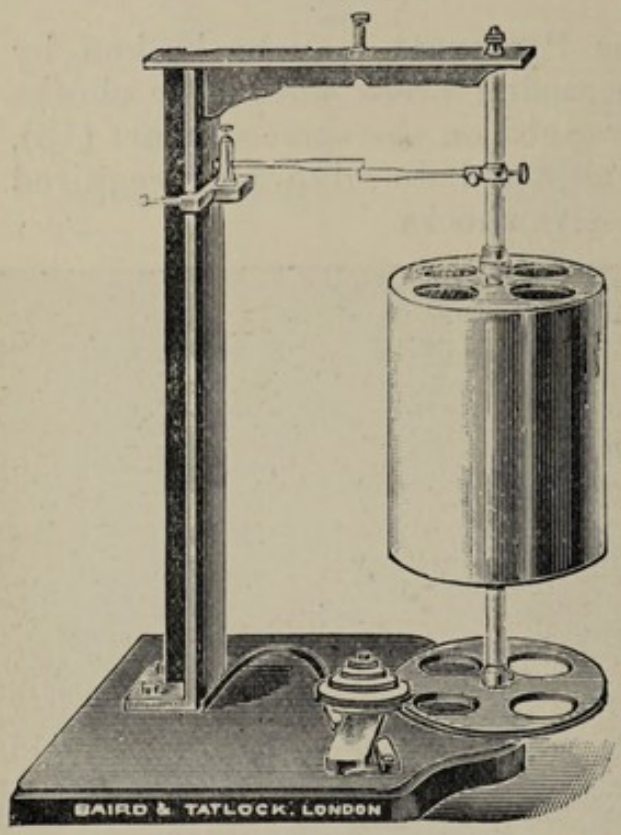


Fig. 179.—Drum (Sherrington) showing a "striker" for automatic break-shocks attached to the spindle of the drum. Key at side.

It is found that

(1) The greatest rise—lift—of the double contraction occurs when the second stimulus falls in the latter part of the ascent of the curve—*i.e.*, contraction.

(2) The "apex-time" ("Gipfel-zeit") or time of highest elevation of the second curve occurs sooner in the double contraction than when the second contraction is discharged on the descent.

Summation—(*a*) *Of contractions.*—When two successive stimuli are rapidly applied to a skeletal muscle, the second twitch is, as it were, piled on the first, and a greater "lift" height is obtained. If several rapidly-recurring shocks be applied, there is a summation of many twitches, and physiological tetanus is produced. As ordinarily recorded, a maximal twitch is not so high as a tetanus (fig. 183). This is due to the fact that the elongation or relaxation phase begins before the muscle overcomes the inertia of the

mass to be moved, and thus contracts to its greatest extent. v. Kries and v. Frey have shown that if the muscle be supported —*e.g.*, by means of the after-load—the muscle contracts more and more until, with shortening, it is equal to that obtained in tetanus. In a slow, prolonged contraction—*e.g.*, in a veratrinised muscle—there is no difference between the height of a simple response and one due to tetanic stimuli. (Lesson XXXVIII.) We might therefore expect that a slow contracting muscle would not show summation, as is the case in the heart.

(*b*) Some muscles, however, give a *summation of stimuli—i.e.*, although each stimulus is itself ineffective or inadequate, repetition of such stimuli increases the excitability of the tissue, so that in the end ineffective stimuli become effective. (Lesson XXXI., 10). Summation of stimuli is a marked feature in nervous processes.

2. **Genesis of tetanus** (*Summation of muscular contractions or mechanical responses*).—A tetanising current may be obtained by Neef's hammer, or by means of a vibrating rod. **Apparatus.**—Leclanché's cells, five wires, flat vibrating spring (35 cm. long and

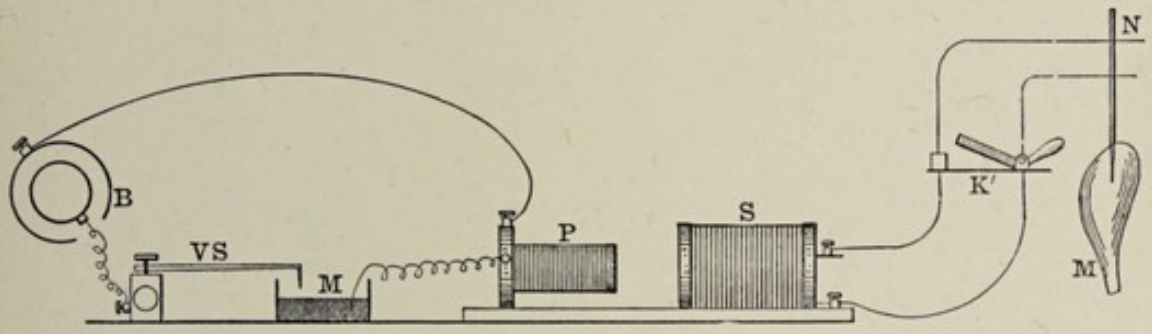


Fig. 180.—Scheme of arrangement for tetanus. *V S*, Vibrating spring; *M*, Cup for mercury. Other letters as before.

2 cm. broad), cup of mercury in a wooden stand, coil, du Bois key, drum (rate 5 cm. per sec.)—*i.e.*, the cylinder moves once round in 10 seconds—crank-myograph.

(*a*) Arrange the experiment as in fig. 180; the coil for single break-shocks, short-circuiting the S circuit. Place in the P circuit the flat metallic spring, acting as a Hg-key. One end of the spring has a needle fixed at right angles to it, which dips into a cup of mercury. The needle hangs just above the mercury cup when the spring is at rest, but dips in and out of the mercury when it vibrates. The clamped end of the spring is connected with the battery, while the mercury cup is connected with the induction coil. Cover the mercury with alcohol and water (1 : 3) to prevent oxidation, and keep the resistance more

uniform. Select a shock which gives response only at *break*, thus eliminating the make shock.

(*b*) Arrange a N.M. preparation as in fig. 136 to record on a slow drum, using a short writing-lever.

(*c*) Fix the flat spring firmly in the clamp, with 10 inches projecting. Allow the drum to revolve, set the spring vibrating, and while it is doing so open the key in the S circuit, and before the spring ceases to vibrate short-circuit again. With 5, 8, and 10 stimuli per sec. there is a series of independent separate break contractions; as the frequency of stimulation rises there is less and less relaxation of the muscle, and fusion of the individual contractions is seen.

(*d*) Shorten the vibrating spring and repeat the experiment, making the tracing follow the previous one.

(*e*) Make several tracings on the same abscissa, following each other at regular intervals, shortening the spring until the tracing no longer shows any undulations—*i.e.*, until it passes from "incomplete" (= clonus) into "complete tetanus" (fig. 181).

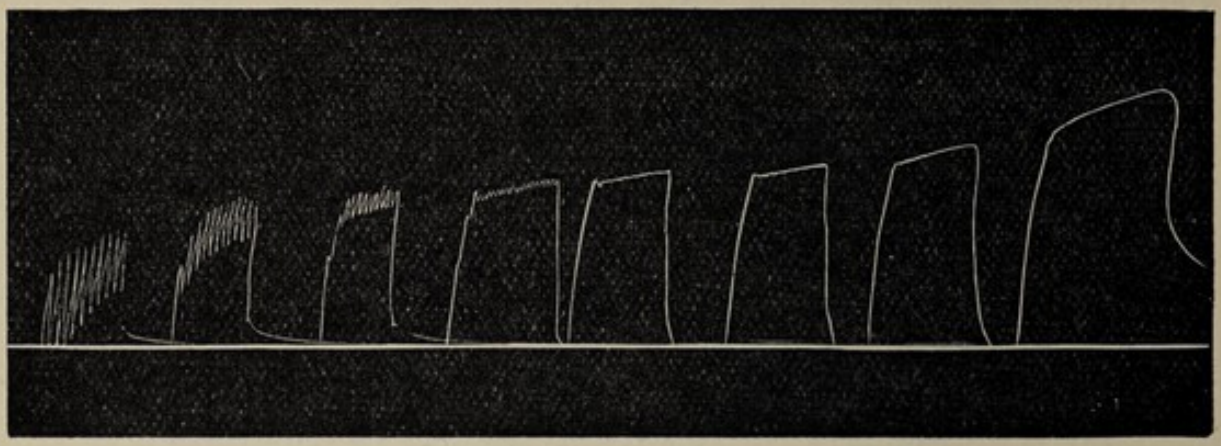


Fig. 181.—Curves of incomplete (clonus) and complete tetanus (*Stirling*).

(*f*) Take a tetanus-curve by introducing Neef's hammer (Helmholtz's side wire) instead of the vibrating flat spring.

(*g*) It is interesting to take a (i.) simple twitch and (ii.) a complete and prolonged tetanus-curve on the same abscissa. Observe the much greater shortening during tetanus (fig. 183).

(*h*) Study the tracings. The first tracings are indented, showing separate contractions, but gradually there is more and more fusion of the teeth, until a curve unbroken by depressions is obtained. In the curve of complete tetanus the ascent is at first steep, then slightly more gradual, speedily reaching a maximum, when the lever practically records a horizontal line parallel to the abscissa. When the current is shut off the descent is very steep at first, and towards the end very slow.

The result is that the stimuli begin to follow one another so rapidly that the contractions are no longer separate, but fuse and become superposed—so that the second is higher than the first, the third than the second, &c. With about 25 to 27 shocks per second there is complete fusion, and a smooth even curve is recorded, until a maximum of contraction is reached—*complete tetanus*. Where the fusion is not complete there is *incomplete tetanus* and *clonus*. The result is easier obtained with a N.M. preparation than with a muscle alone.

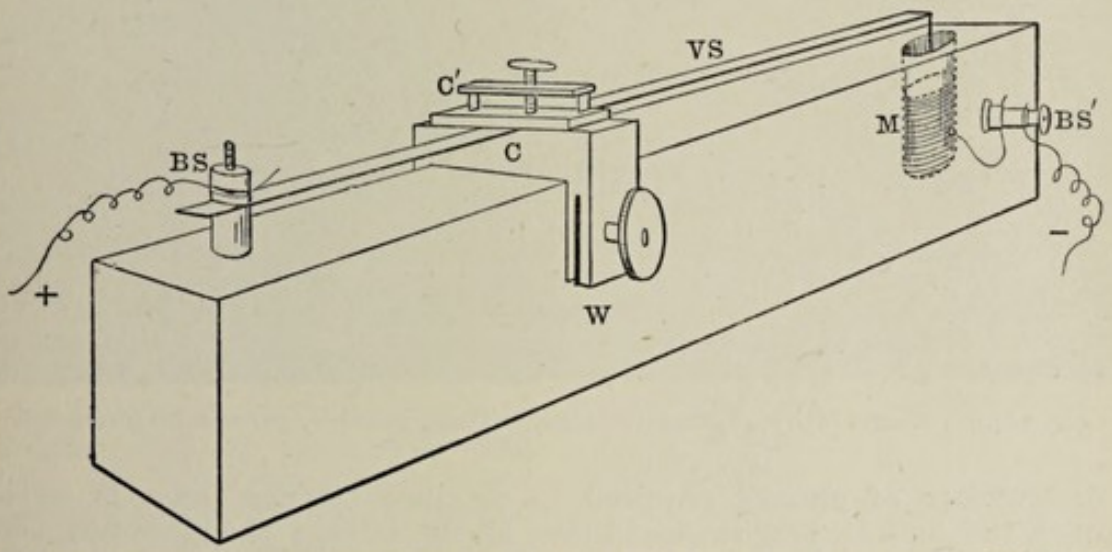


Fig. 182.—Tetanus interrupter. *W*, Wood block; *VS*, Vibrating spring; *BS*, *BS'*, Binding screws; *C*, Movable clamp; *C'*, Clamp to fix spring; *M*, Cup of mercury.

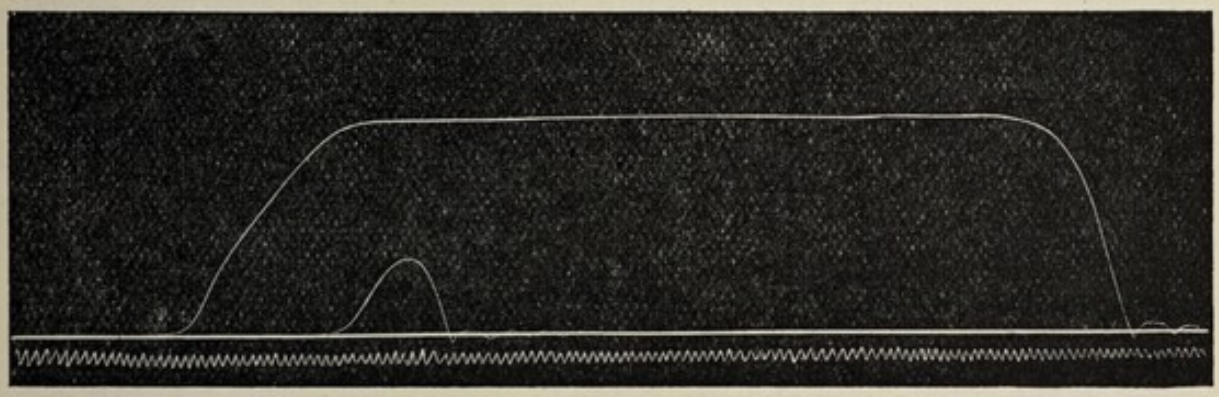


Fig. 183.—Simple twitch and tetanus (greatly reduced. Curve taken on same base line (*Stirling*).

3. **Tetanus of hyoglossus.**—Take a tetanus curve. Observe long period of extension after the sudden relaxation (fig. 184).

4. **Effect of (1) cold, (2) fatigue on fusion of successive muscular contractions.**

(*a*) Arrange a N.M. preparation to record on a slow drum. Cool the preparation, and by means of a graduated interrupter determine the number of single break shocks required to produce tetanus—*i.e.*, complete fusion of successive muscular contractions.

(*b*) Arrange a similar experiment, but partly fatigue the preparation by faradisation before applying the rhythmical break-shocks. A fatigued muscle is more readily tetanised than a fresh one.

5. **Increasing strength, but frequency the same.**—Arrange a certain rate of strength and frequency of stimulation. Observe effect, and then push up S coil, thus increasing strength, but leaving frequency of stimulation unaltered. Observe more rapid rise of curve.

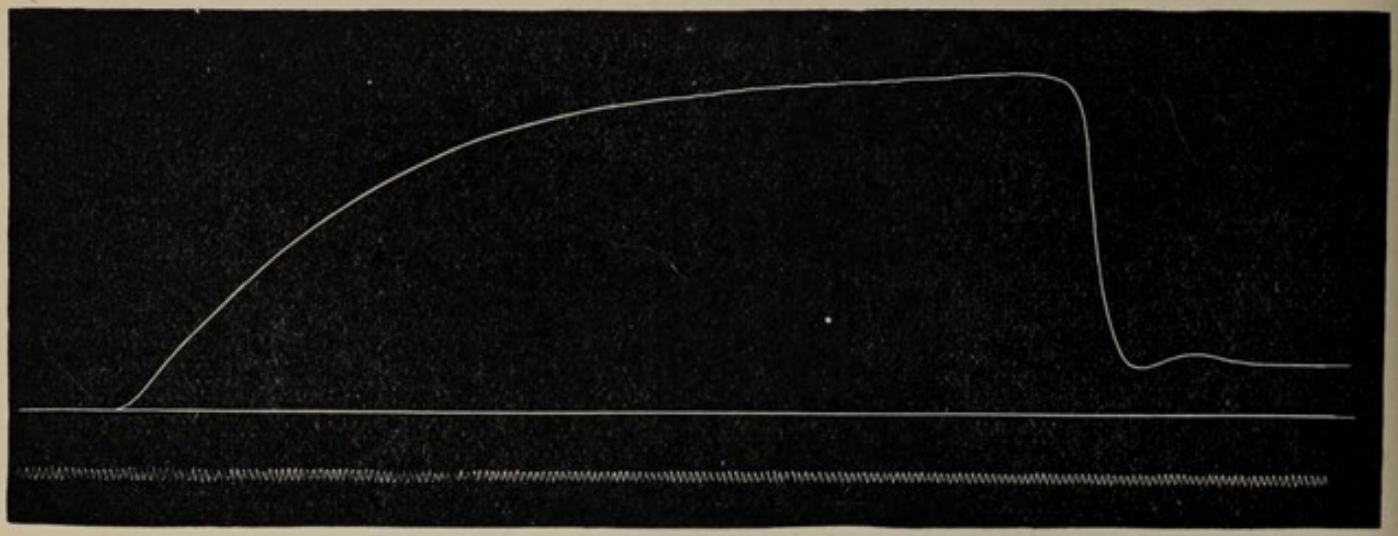


Fig. 184.—Tetanus of hyoglossus (reduced). Time, 100 D.V. per second (*Stirling*).

6. **Number of shocks** required to produce tetanus depends on the animal, the muscle, and the condition of the latter; the more fatigued a muscle is the slower it contracts, and, therefore, the more readily does fusion of contractions take place. A fresh frog's gastrocnemius requires about 27 to 30 shocks per second to produce complete tetanus. The following table shows approximately the number of shocks per second required to produce tetanus. If the muscle be fatigued, then more or less complete fusion takes place with a smaller number of shocks per second.

	Shocks per sec.	
Tortoise,	2	(*Marey*).
Frog (hyoglossus),	10-15	
,, (gastrocnemius),	27-30	
Lobster (claw),	20	(*Richet*).
,, (tail),	40	(*Richet*).

	Shocks per sec.	
Rabbit (red muscle),	4-10	(*Kronecker* and *Stirling*).
,, (white ,,),		
Bird,	100	(*Richet*).
Insects,	300-400	(*Marey*).

7. **Heat and cold on tetanus.**—Take a tracing with 10 or 15 shocks per second, and then test the effect of different temperatures on the curve. Pour on the muscle normal saline at the required temperature. Cold helps the fusion, while heat makes the tetanus less complete.

8. If Ewald's coil be used (fig. 106) any number of shocks from 1 to 200 per second can be obtained.

9. **Shortening during twitch and tetanus.**—Record height of contractions of a muscle *with lever only*, no load. (*a*) Single maximum B induction shock. (*b*) Stimulate with tetanising shocks for brief period. The lift is almost the same in both for an *unloaded* muscle. (*c*) Repeated with load of 20 grms. The shortening in tetanus is now much greater than that due to a maximum single shock. (*d*) Load with 10 grms., using the after-loading screw, which supports the weight until the contraction begins. Record a twitch on a stationary drum (maximal induction

stimulus), turn drum 1 mm., raise lever 1 mm. by after-load screw, record from new abscissa, and repeat the process, screwing up the after-load until the muscle cannot shorten any further, so as to raise the lever. It would appear that the total shortening of the successively after-loaded muscle is as great as that of a loaded muscle during tetanus (*v. Frey*).

10. Interruption by a metronome.—Instead of the vibrating rod or Neef's hammer, introduce into the primary circuit a metronome (fig. 185), provided with a wire which dips into a mercury cup introduced into the P circuit. Vary the rate of vibration of the metronome, and observe the effect on the muscle-curve. It is easy to allow the free end of the rod to beat against the membrane of a receiving tambour connected with a recording tambour; thus the beats can easily be recorded.

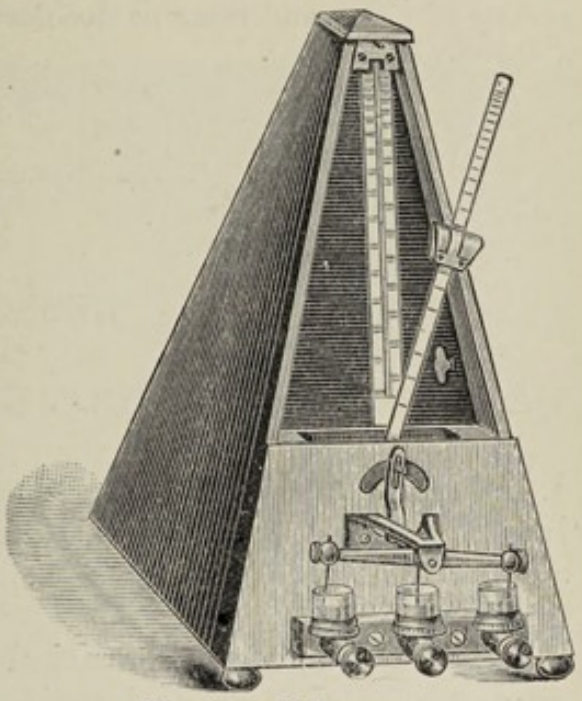


Fig. 185.—Metronome.

11. Instead of using the vibrating spring, a convenient form is shown in fig. 182. The spring is kept vibrating by an electro-magnet actuated by two dry cells.

12. Magnetic interrupting tuning-fork.—Instead of a vibrating spring, the P current may be interrupted by means of a tuning-fork of known rate of vibration, and kept in motion by means of an electro-magnet. The instrument (fig. 186) is introduced into the primary circuit, and every time the style on one of the arms of the tuning-fork dips into and comes out of the mercury placed in a small cup, the primary current is made and broken. One

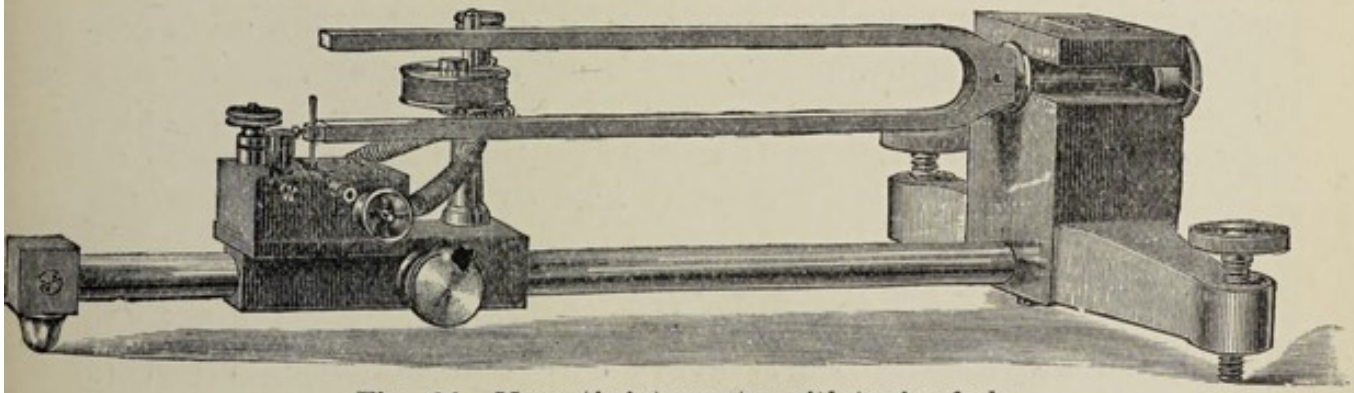


Fig. 186.—Magnetic interrupter with tuning-fork.

of the most important points in connection with the use of this instrument is to keep the surface of the mercury clean and bright, so that the successive shocks shall be of nearly equal intensity. In Kronecker's apparatus for this purpose the vibrating flat spring is so adjusted that stimuli from 1 to 50 or 60 per second can be obtained.

13. Acidity of tetanised muscle (Lesson XIV.).—Inject during the course of 24 hours into the ventral lymph sac of a pithed frog two or three

Pravaz syringe fulls of a 5 p.c. watery solution of acid fuchsin. Passive muscles show scarcely any reddish tinge, because they are alkaline. Kill the animal, tetanise a leg for 10 to 15 minutes by stimulating its sciatic nerve. The muscles soon become red, proving the formation of an acid. One cannot, however, determine by this means whether the acidity is due to CO_2 or lactic acid (see also Lesson XIV., 1).

A weak neutral solution of acid fuchsin is decolorised by an alkali. The colour is restored even by CO_2. This solution is decolorised by heat—escape of CO_2—and remains decolorised in the cold (Dreser, *Centralblatt f. Physiol.*, i., p. 195).

LESSON XLI.

FATIGUE AND RECOVERY OF EXCISED MUSCLE.

1. **Fatigue and recovery of excised muscle.**—(*a*) Arrange an induction coil for break-shocks, but interrupt the P circuit automatically. Arrange so that curve is not near the overlap (fig. 137).

(*b*) Fix a N.M. preparation on a crank-myograph—or use a curarised muscle and stimulate it directly—with a lever and a weight of 40 to 50 grms., lay the nerve over the electrodes from the short-circuited S coil, and let the lever record on the drum. A break-shock is obtained each time the drum revolves. The myograph should be supported on a tangent stand. If a tangent support be used for the muscle-lever, although the muscle contracts at each revolution of the cylinder, one may record every tenth or fifteenth contraction (fig. 187). This is necessary, as a muscle may give one or two hundred contractions—depending on load, &c.—before it is exhausted, and so many tracings would blur the record.

(*c*) Observe that the height of the curves fall, while their duration is longer—the great slowing of the relaxation being a marked feature. In nearly every case the fatigue-curves from muscle show a "staircase" character (fig. 188), the second curve being higher than the first one, and the third than the second.

(*d*) *Recovery.*—If the muscle be allowed to rest for a few minutes and then stimulated, it contracts on recovery.

(*e*) Similar experiments may be made with an isometric lever.

2. **Fatigue-curve of excised muscle.**—(*a*) Use a slow-revolving drum on which to record, so slow that the ascent and descent of the lever form

merely one line. Let the P current be broken at regular intervals with an automatic key, using break shock. In this way a curve such as fig. 188 is obtained.

(*b*) Note the "staircase" character of the curve—*i.e.*, the second contraction is higher than the first, the third than the second, and so on for a certain number of contractions. Afterwards the height of the contraction (lift) falls steadily, so that a line uniting the apices of all the contractions forms a straight line (approximately).

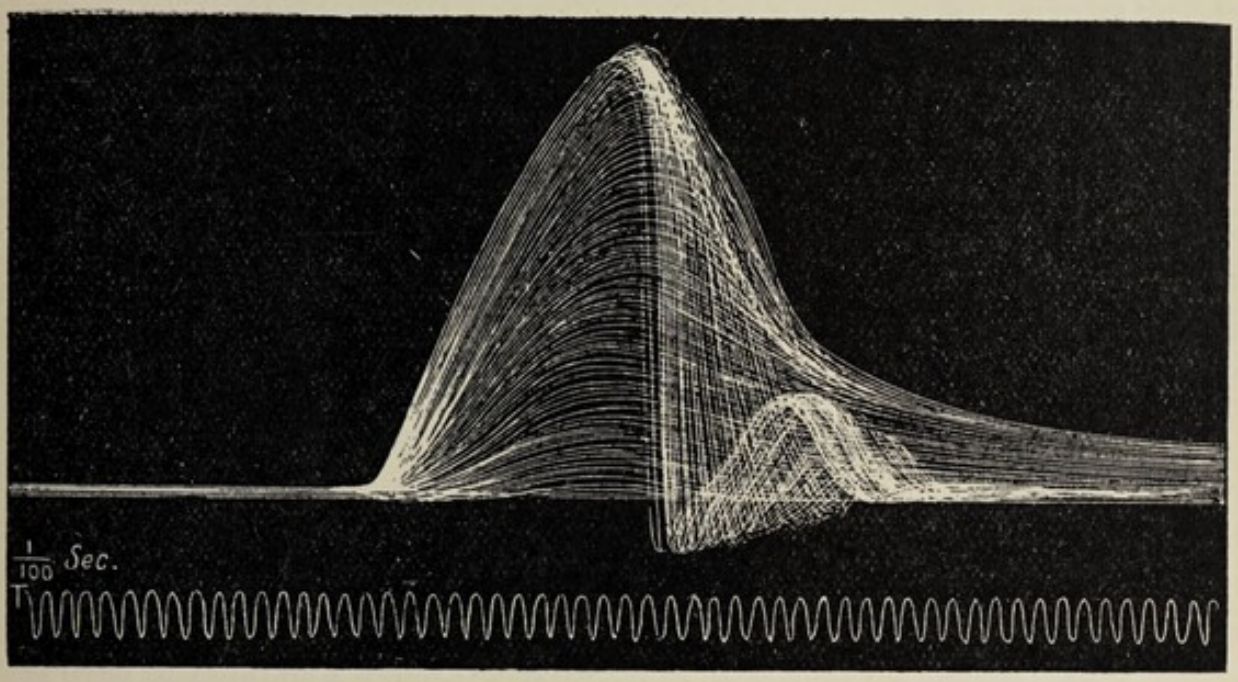


Fig. 187.—Tetanus-curve produced with break-shocks. Stimulation every second by automatic break key in P circuit. *T*, Time-curve, 100 D.V. per second.

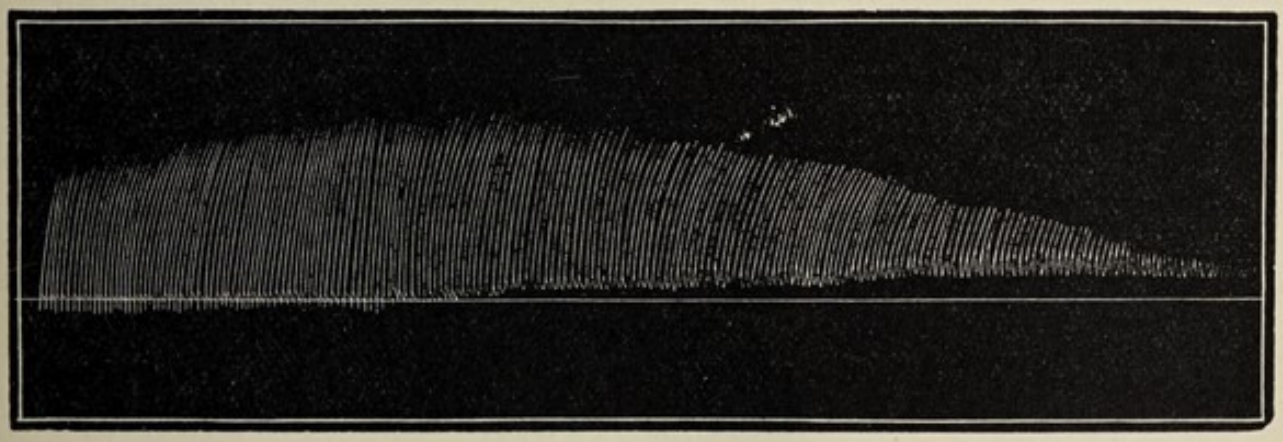


Fig. 188.—Fatigue-curve of an excised frog's muscle recorded on a slow-moving drum.

(*c*) In a fatigue-curve, where only the "lift" is recorded, note that the rise of the lever increases with the number of stimuli—the strength of the stimulus remaining constant, so that one gets the phenomenon of the "Treppe" or "staircase." After a time it falls steadily until the excitability is (temporarily) extinguished (fig. 188). Note also that in the phase

of relaxation the lever does not reach the abscissa—*i.e.*, relaxation takes place slowly, as if one had to deal with a so-called "contracture." If the march of events be arrested, and time given for repose, then, on stimulating, the lift increases, but the effect lasts only for a short time.

(*d*) *Silhouette curve.*—If a muscle be stimulated, say, 5 times per second, weight 10 to 20 grms., and made to record on a *very slow* drum, it is obvious that instead of distinct vertical lines indicating the lift, the soot will be completely removed, and a "silhouette," white on black, will show the development of fatigue and contracture.

3. Fatigue by tetanising shocks.—Arrange the apparatus with automatic break as for recording the latent period (fig. 137). Record a single twitch. Remove writing lever from drum and tetanise the muscle for 10 seconds. Another twitch. Tetanise again for 10 seconds, and after every period of tetanus record a twitch. Do this 8 to 10 times. Observe the increased L.P., the curves are successively more extended, the relaxation less and less, the contractions lower and lower, and both rise and fall are slower, the P relaxation being more affected than the contraction.

4. Stimulation of nerve, then of muscle.—Arrange a N.M. preparation to record only the height (lift) of its contractions (fig. 188). Connect the preparation with a coil, putting in a commutator without cross-bars, so that the current can be sent either through nerve or muscle.

(*a*) Stimulate the nerve at short regular intervals (single or tetanising shocks) until the muscle ceases to respond.

(*b*) Turn the cradle and apply similar shocks direct to the muscle, it responds, although it did not do so to indirect stimulation.

(*c*) Continue to stimulate the muscle until it is exhausted—direct fatigue—*i.e.*, until it no longer responds by change of form. Allow it to rest; it recovers.

5. Polar fatigue.—Connect N.P. electrodes through a simple key with a battery, and arrange a coil for single induction shocks. Fatigue an excised sartorius by alternate M and B shocks of the galvanic current, leaving the current made for a short interval. On making, finally there will be no response to the galvanic current. Stimulate with induction shocks when the current is passing. The muscle will respond, save when the kathodal area is stimulated. The fatigue is localised in this area.

LESSON XLII.

INDEFATIGABILITY OF NERVE—SEAT OF EXHAUSTION.

1. Can nerve be fatigued?—A muscle manifests fatigue—*i.e.*, its store of material and energy are gradually used up, so that it shows a diminished capacity to respond to stimulation. Does a nerve manifest such phenomena? From the fact that the only known sign apparently obtainable during the activity of a

nerve is the "negative variation of the nerve-current," one is led to suppose that probably nerve-fibres partake but little, if at all, in the phenomena of fatigue. Nerve is practically inexhaustible.

Suppose one stimulated a nerve of a N.M. preparation with tetanic or maximal induction shocks until the muscle ceased to respond to indirect stimulation. This would afford no proof that the muscle is fatigued. (Lesson XLI., 4.) Why? Stimulate the muscle directly, and it responds. Therefore the seat of fatigue in this case is not primarily in the muscle, but must be sought for either in the nerve itself or at the end-plates where the nerve comes into relation with the muscular substance.

2. Seat of exhaustion—is it in muscle, nerve, or end-plates? (*J. Bernstein*, 1877).

A. *Not primarily in muscle.*—(*a*) Arrange for repeated shocks. Connect the S coil with a Pohl's commutator without cross-bars.

(*b*) Attach a N.M. preparation to a crank-myograph, and place its nerve on electrodes attached to a commutator without cross-bars. Pass two fine wires through the muscle and attach them to the other two binding screws of the commutator.

(*c*) Tetanise the nerve until tetanus ceases. Reverse commutator, stimulate muscle. It contracts. Therefore, *the seat of fatigue is not in the muscle.*

B. *Not in the nerve* (*nerve is practically inexhaustible*).—(*a*) Arrange a N.M. preparation coil for repeated shocks as before. Place the nerve on the electrodes from the S coil.

(*b*) Arrange two Leclanché cells connected to N.P. electrodes, and short-circuited for a constant current—the "polarising current" (Lesson XLVIII.)—and place the N.P. electrodes next the muscle, so that the - pole is next the muscle—*i.e.*, with the polarising current descending. The "polarising current" so lowers the excitability of the nerve as to "block" the passage of a nerve impulse through this part of the nerve. The tetanising electrodes are placed near the upper cut end of the nerve.

(*c*) See that the muscle responds when the stimulating current acts on the nerve, then throw in the polarising current, when at once the muscle ceases to respond, because the nerve impulse is blocked. Stimulate the nerve for an hour or longer. We know that if there had been no "block" the muscle would long ere this have ceased to respond to indirect stimulation.

(*d*) Close the key of the polarising circuit—*i.e.*, remove the block. The muscle responds at once. Therefore the loss of

excitability or *seat of exhaustion is not in the nerve.* Where is it? It must lie primarily somewhere between the nerve and muscle—*i.e.*, *in the end-plates*, or where nerve joins muscle. Moreover, Bowditch has shown that the sciatic nerve of a curarised cat may be stimulated for hours, there being no muscular response, but as the effects of curare, which is known to paralyse the nerve-terminals in striped muscle, pass off, the muscles of the foot respond.

C. The results of A and B may be combined thus :—

(*a*) Dissect out two N.M. preparations (A, B), clamp both femurs in one clamp, and attach straw flags to legs (fig. 124). Lay both nerves over du Bois electrodes.

(*b*) Attach the electrode wires to two of the binding screws of a commutator without cross-bars.

(*c*) To the nerve of B, beyond the du Bois electrodes and the muscle, apply a "polarising current" with its – pole next the muscle.

(*d*) Pass an interrupted current through *both nerves;* A becomes tetanic, while B remains quiescent ; the impulse cannot pass because of the "block" produced by the "polarising current."

(*e*) Continue to stimulate the nerves until A ceases to respond. Break the polarising circuit—*i.e.*, remove the block on B ; B becomes tetanic.

As both nerves have been equally stimulated, both are equally fatigued or non-fatigued. As B becomes tetanic, the seat of the fatigue is not in the nerve-trunk.

As in A, the seat of fatigue was not in the muscle, and as B shows that nerve-fibres practically do not manifest the signs of fatigue, it would seem that its seat must be somewhere between muscle and nerve—in all probability in the *end-plates.*

D. Ether.—The nerve impulse in one nerve may be blocked by the local application of ether to that nerve.

Nerve fibres, therefore, offer a marked contrast to the other parts of the nervous system. The lines of junction of nerves with other parts seem to be more readily fatigued. Nerve cells, or the fields of conjunction in the central nervous system, seem to be markedly susceptible to fatigue.

LESSON XLIII.

MYOGRAPHIC EXPERIMENTS ON MAN—ERGOGRAPH AND DYNAMOGRAPH.

1. Mosso's Ergograph for fatigue and work.—By means of this instrument (fig. 189) the student can study the process of fatigue

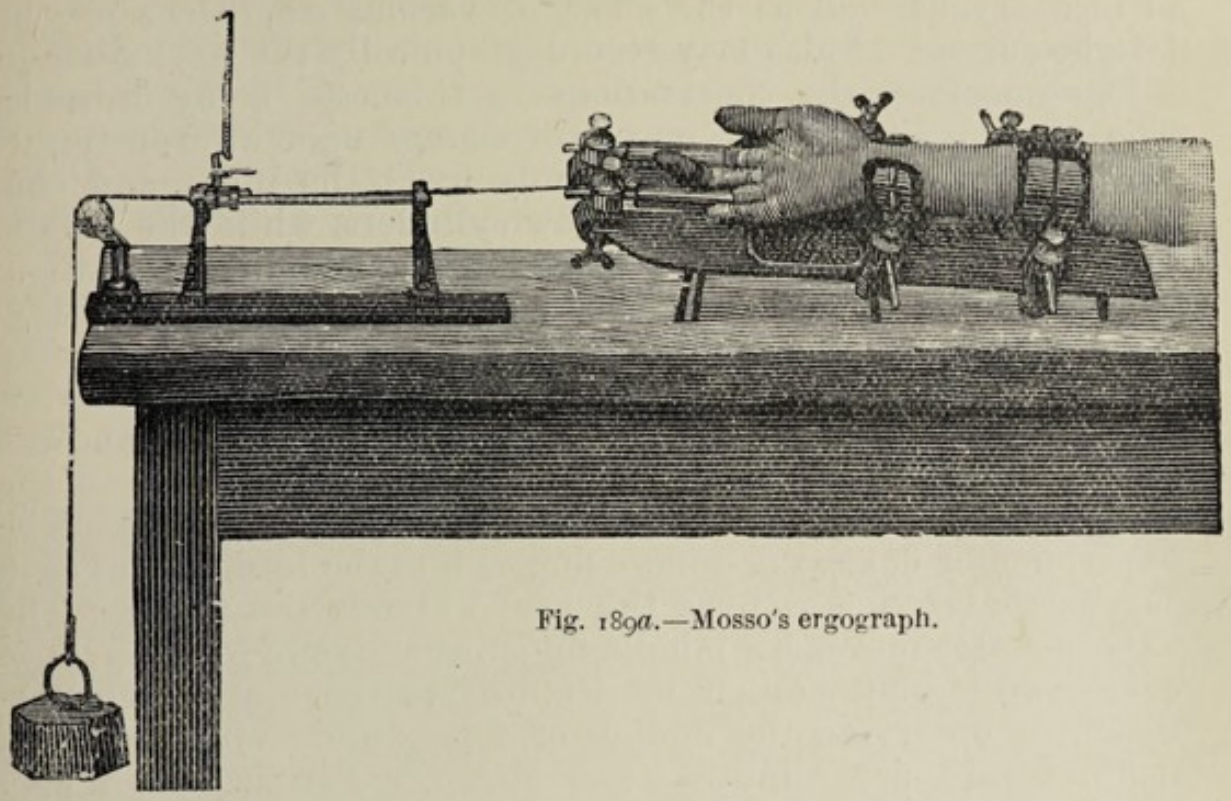


Fig. 189*a*.—Mosso's ergograph.

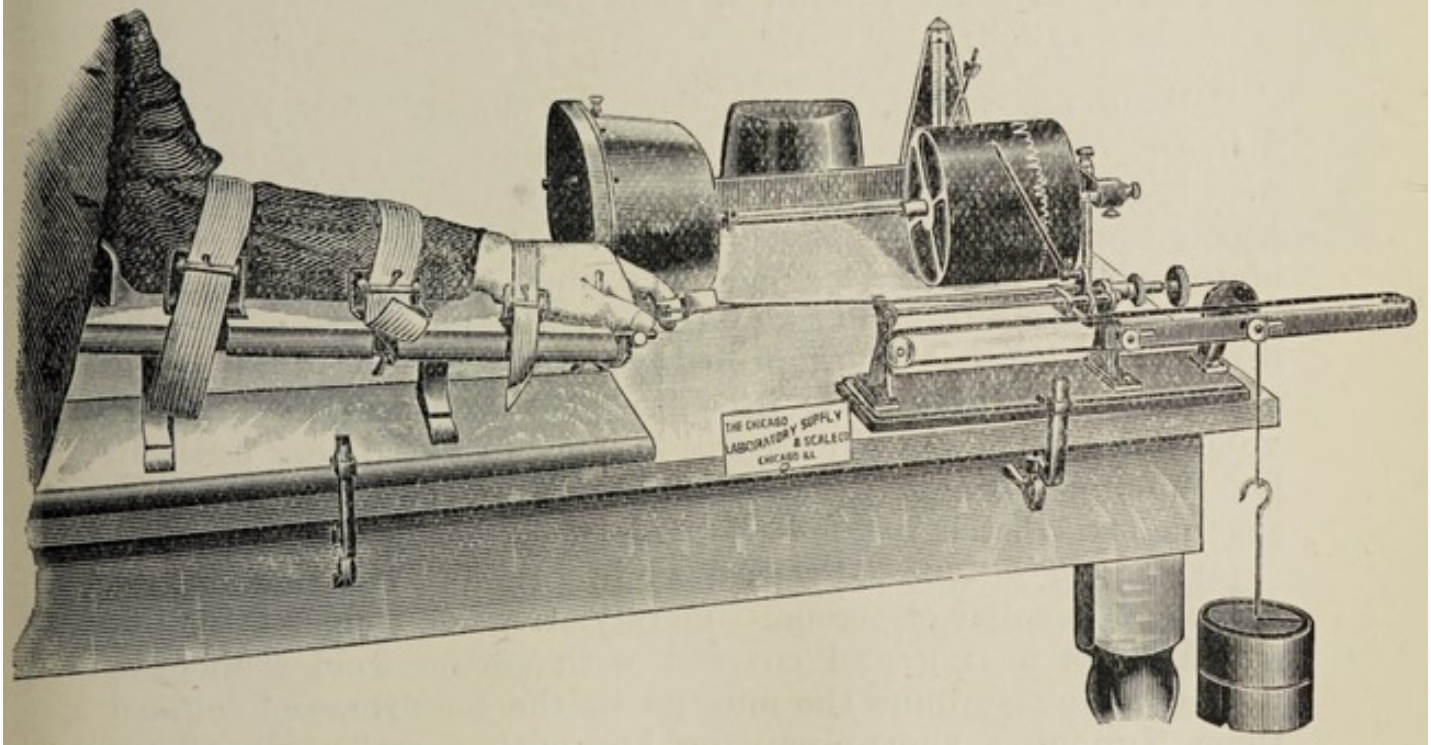


Fig. 189*b*.—Shows complete arrangement.

on himself, the conditions that predispose to it, and the process of recovery, as well as the effect of various conditions on the fatigue-curve. It also may record graphically the work done in kilogrammetres, the contractions in this case being isotonic. The forearm is fixed by means of clamps upon an iron framework, while the hand also is firmly fixed, the index and ring fingers being placed in brass hollow cylinders, while the middle finger is free. The forearm is placed in a half-supinated position. To the middle finger is attached a cord, passing to the writing-style, and to the latter is attached a weight, which can be varied. The style writes upon a recording drum moving horizontally. The forearm is fixed in the apparatus, and the middle finger attached to the writing apparatus, and to the latter is added a load of known weight—*e.g.*, 2 to 3 kilos. The experimenter flexes the middle finger, lifts the load, and as soon as the contraction is over the load extends the finger. The experimenter moves his middle finger at a given rate—say, once every two seconds—either by listening to the beat of a metronome, or observing the motion of a pendulum vibrating at a definite rate per minute. (A. Mosso, "Fatigue of human muscle," *du Bois-Reymond's Archiv.*, 1890, and *Die Ermüdung*, Leipzig, 1892; Warren P. Lombard, "Some of the influences which affect the power of voluntary muscular contraction," *Journal of Physiology*, xiii., 1, 1892; Z. Treves, *Archiv. ital. de Biol.*, xxx., 1898).

Fatigue for voluntary muscular contraction.—(*a*) *Voluntary fatigue.* With the arm arranged and fixed, as in fig. 189, connect it to the recording lever, weighted with 3 or 4 kilos., go on lifting the weight with the middle finger every 2″ at the sound of the click of the metronome, and then relax the finger. In this way a series of voluntary records will be inscribed on the drum (fig. 190). They probably will show a "staircase" character, but gradually, in spite of the operator's greatest voluntary efforts, the curves will become less and less until, in fact, he no longer can lift the weight. A state of "fatigue" has set in. To what is it due?

(*b*) *Seat of fatigue.*—Proceed as in (*a*), but before doing so arrange two collar electrodes—merely strips of copper bent into the form of a ring and covered with cotton wool soaked in normal saline—around the muscles of the forearm, and connect them through a short-circuiting key with a coil arranged for tetanising shocks—the coil supplied with 4 or 5 dry cells coupled in series in the primary circuit. As soon as the flexor

muscles cease with the greatest voluntary effort to raise the weight, open the short-circuiting key, and stimulate the muscles directly; they contract and the weight is raised; go on repeating the stimulation by opening and closing the secondary circuit at regular intervals, while the primary circuit is kept close, and another series of contractions will be obtained. Therefore, the seat of fatigue is *not in the muscles.*

(*c*) That it is not in the nerves or nerve-endings in the case of voluntary fatigue can be shown by doing as in (*a*)—*i.e.*, causing fatigue of the finger, and then stimulating the median nerve at the bend of the elbow by sponge electrodes. As the seat of fatigue in voluntary muscular movements is not in

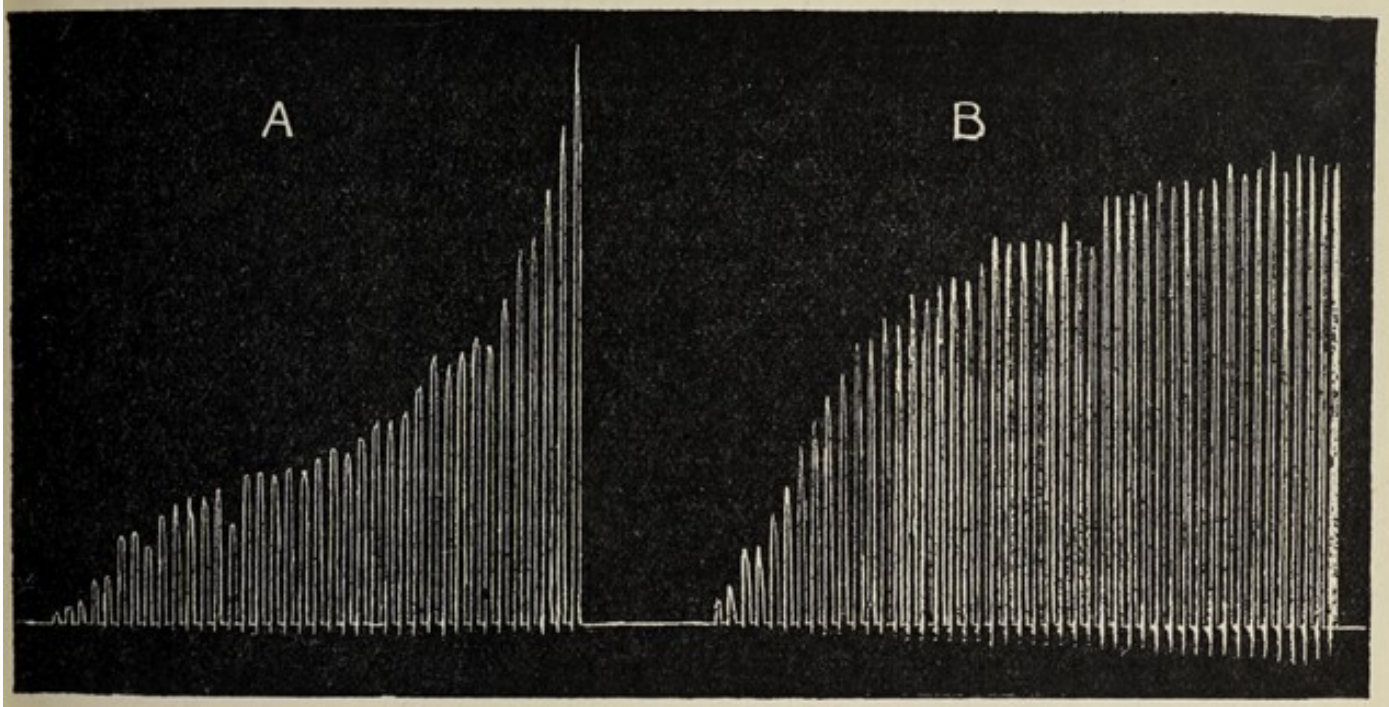


Fig. 190.—Ergographic curves from two different individuals, A and B. Read from right to left. Voluntary fatigue.

muscle, nerve-endings, or peripheral nerve fibres, it must be in the cord or higher centres. We have no reason to believe that the nerve fibres of the central nervous system—in cord or brain—differ from peripheral fibres. We may assume that the seat of the fatigue is to be sought either in the central nerve cells or at the nerve network "synapses" in relation to them. Is this a protective arrangement?

(*d*) Of course, fatigue may be induced by keeping the weight lifted. Try this, and observe form of curve.

(*e*) Vary the load, the interval of stimulation. Try the effect of short pauses, mental fatigue, general fatigue, tobacco, &c.

(*f*) Record on same drum ergographic curves, showing successively (1) voluntary fatigue; (2) fatigue produced by stimulation of the nerve; and (3) stimulation of muscle. In the last case, after (1) and (2), the muscle soon fails.

(*g*) *Recovery.*—If the muscle be completely exhausted, as in (*f*), it takes a long time to recover; not so if the fatigue be voluntary, as in (*a*). The central factor is sooner restored than the peripheral.

(*h*) *Work done.*—This is easily calculated from the weight and height of the lift.

A line joining the apices of the several contractions gives the "curve of fatigue" for each individual. It is in some individuals nearly a straight line falling steadily from the first to the last contraction. In others there is a slight concavity or convexity upwards.

Lombard's modification of the ergograph is so made that there are two arm rests for right and left arms respectively, and the apparatus is so arranged as to record on a drum moving on a vertical axis (fig. 47 in Petzold's Catalogue).

On making maximal voluntary muscular contractions with the "weight-ergograph"—*i.e.*, isotonic contractions—say, every two seconds, to study the "laws of fatigue," it is found that the extent of movement at first is great, but gradually decreases, until after 50 to 100 contractions the weight is no longer raised, and no mechanical work is accomplished. It is assumed that the total work which a muscle can do before it is fatigued (exhausted) is the product of the weight and the sum total of the extent of the separate contractions. This is not quite so, for the muscle, although unable to lift a given weight, may be able to lift a lighter one. It is for this reason that various "spring-ergographs" or ergometers have been introduced (Franz, *Amer. Journ. of Phys.*, iv., 1900).

2. Myographic experiments on man.

Fick has devised a simple apparatus for investigating the isometric curves of the *Abductor indicis* or *interosseus dorsalis primus* of the hand. This muscle arises by two heads from the adjacent surfaces of the metacarpal bones of the thumb and index-finger, and is inserted into the dorsal aponeurosis of the latter.

Apparatus.—In a prismatic piece of wood, H, firmly fixed to a base, a hole is cut down to the level, K, through which one can conveniently place one's hand (fig. 191); the ulnar surface of the hand rests on the rounded lower end of the hole, while the thumb rests against the lateral wall of the hole; in this way the hand is sufficiently fixed. Over the index-finger is placed a collar made of strong iron wire, and through this collar project the three other fingers, which hang free, the collar itself lying over the joint between the second and third phalanges. To the collar is attached a strip of iron with a notch in it, by means of which it is attached to the

axis of the lever, which is one so arranged as to give isometric contractions as in fig. 189.

When one attempts to raise the index-finger, the muscle records an isometric curve. As the collar can at most move only 1 mm., and as the muscle itself acts on a lever about five times shorter than the distance of the point of attachment of the collar from the axis of rotation of the index-finger, the muscle can at most contract $\frac{1}{5}$ mm. The muscle records on a revolving surface. (From the description of Shenck. See Fick, *Pflüger's Archiv.*, Bd. 41, p. 176).

With this apparatus one can study (1) force of contraction; (2) fatigue and recovery; (3) electrical stimulation of muscle; (4) mechanical response elicited by electrical (tetanic) and the normal physiological stimulus, and learn that during a voluntary contraction there is a greater contraction—*i.e.*, a greater liberation—of energy than during the strongest contraction elicited by electrical stimulation.

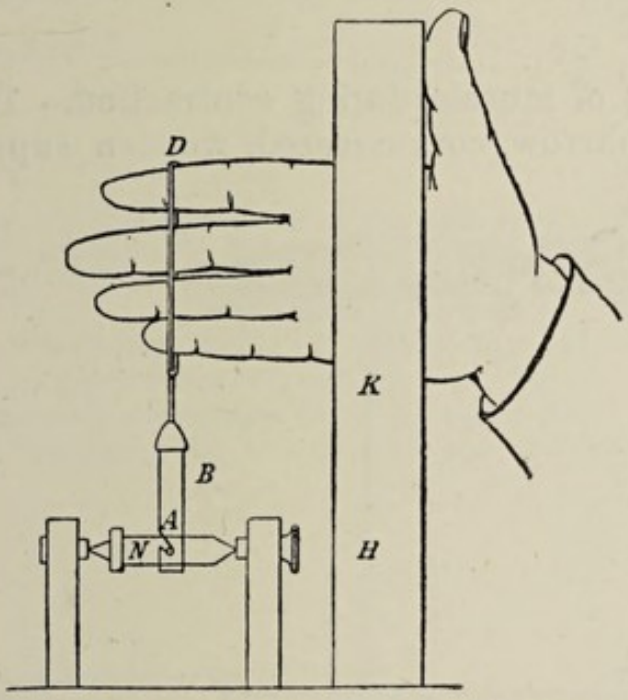


Fig. 191.—Fick's apparatus for studying tension of abductor indicis. *H*, Wooden rod with hole, *K*, for hand; *D*, Iron-wire collar, acting through *B* on an axle, *N*, to which a lever is attached. Seen from the end.

3. **Dynamographs.**—(*a*) *Voluntary muscular efforts.*—Waller has devised a simple form. To the vertical arm of a dynamometer of Salter (p. 235), a strong steel spring with a long 60 cm. recording arm is attached, the record being made on a very slow drum—*e.g.*, a cylinder placed vertically on the hour-spindle of an American clock. The dynamograph is so arranged that it can be clamped to a table. The observer, by grasping the handle of the instrument, makes a series of maximal efforts, say, 30 per minute—*i.e.*, each lasting two seconds—then he takes one minute's rest, and repeats the experiment.

One can measure the muscular strength and how it declines with each contraction or series of contractions, together with its recovery during rest. We have a series of *isometric* contractions.

(*b*) A simple form of dynamometer is described by Scripture ("The new psychology," fig. 4, p. 74, 1897).

LESSON XLIV.

MUSCLE THICKENING—MUSCLE WAVE.

When a muscle contracts it shortens, but its volume remains the same. What is lost in length is gained in thickness, so that during the twitch of a muscle not only does it shorten, but it also becomes thicker. The amount and extent of this thickening can be recorded.

1. **Thickening of muscle during contraction.**—Place a gastrocnemius on a narrow, cork-covered, wooden support (fig. 192).

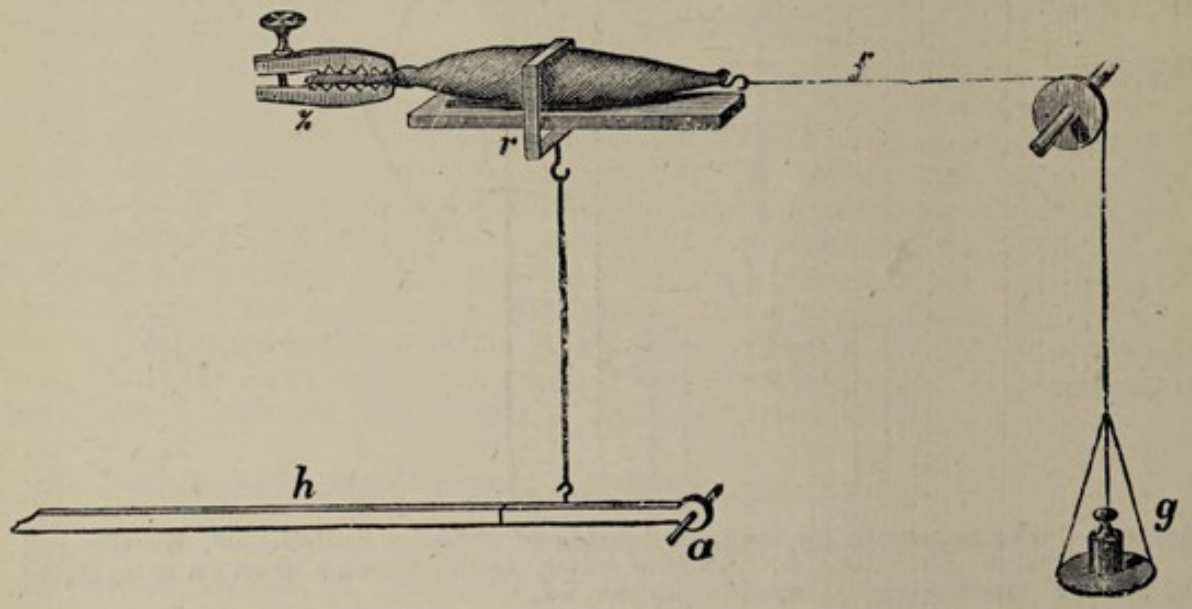


Fig. 192.—Von Bezold's apparatus to record thickening of muscle. *z*, Muscle clamp; *r*, Bridle over muscle; *g*, Weight over pulley; *h*, Recording lever; *a*, Fulcrum (*Langendorff*).

Fix the femur end of the muscle by two pins which have wires soldered to them to serve as electrodes, and attach the tendon by a thread to a band of india-rubber, the other end of which is so fixed by a pin, as gently to stretch the muscle. Over the belly of the muscle rests a flattened plate or bridle of aluminium, ending in a loop, which is attached to a recording lever placed below. The apparatus used is practically a modification of that in fig. 193.

(*a*) Take a tracing—simple twitch (automatic break).

(*b*) If desired, from the same preparation record a tracing with a crank lever to show curve of shortening. Record time.

(*c*) Compare the two curves.

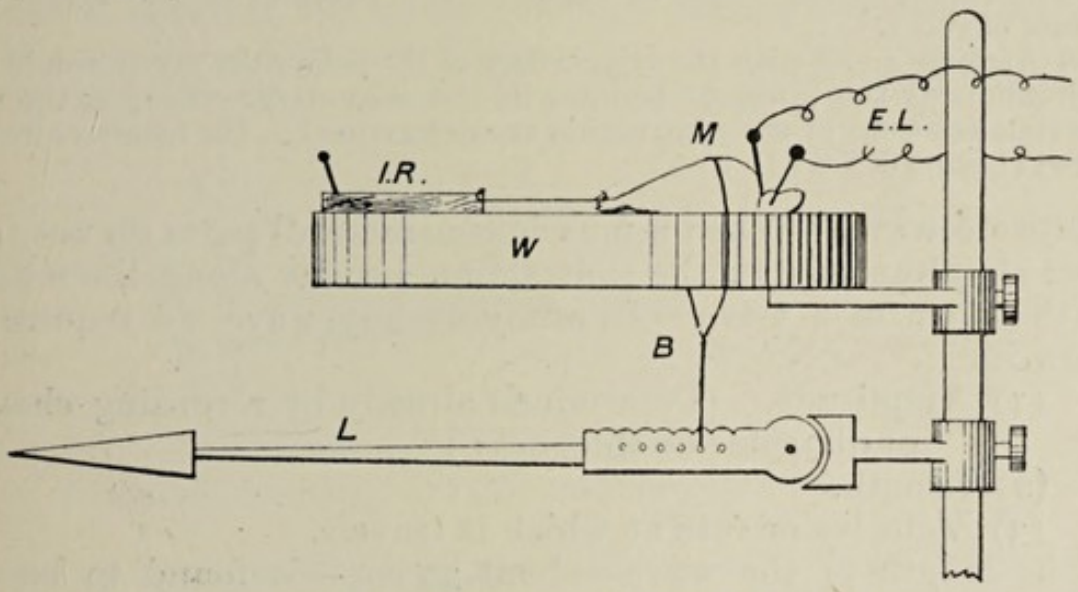


Fig. 193.—Apparatus for recording increase of thickness of muscle during contraction. *M*, Muscle; *EL*, Electrodes; *IR*, India-rubber band; *W*, Wooden support; *B*, Bridle over muscle; *L*, Recording lever.

2. Thickening of a muscle during contraction.—(*a*) Arrange a Marey's tambour to write on a pendulum-myograph (fig. 194).

(*b*) Fix Marey's *pince myographique* (fig. 194*a*) so as to compress the adductor muscles between the thumb and the metacarpal bone of the

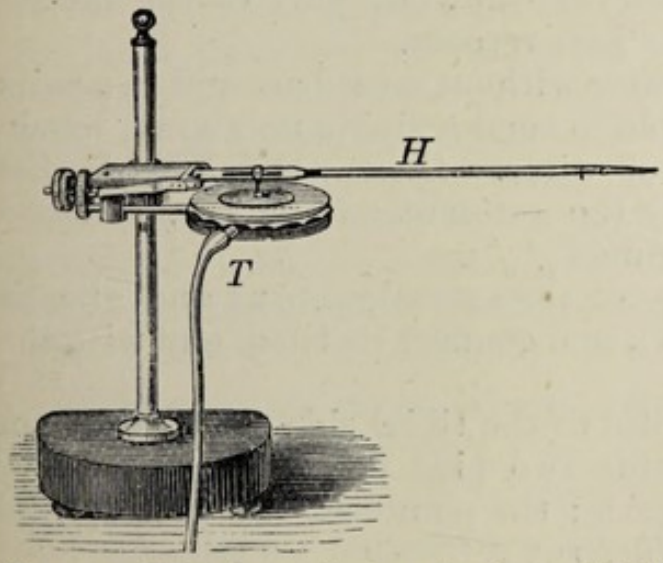


Fig. 194.—Marey's registering tambour. Metallic capsule, *T*, covered with thin india-rubber, and bearing an aluminium disc, which acts on the writing lever, *H*.

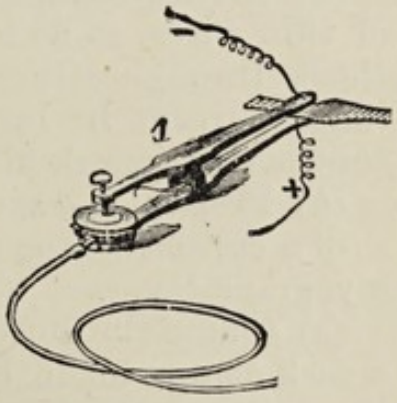


Fig. 194*a*.—Marey's *pince myographique* (*Verdin*).

index-finger, keeping the two arms together with an elastic band. Or use a pair of toy bellows, to the arms of which plate-like electrodes are fitted and connected with binding screws. Keep the handles of the bellows

pressed upon the adductor muscles by means of an elastic band. Connect the receiving tambour of the pince or the nozzle of the bellows with the recording tambour, introducing a valve or T-tube with a screw clamp into the connecting elastic tube, to regulate the pressure of air within the system of tubes.

(*c*) Arrange a coil with the trigger-key of the pendulum-myograph in the P circuit, and the pince or bellows in the secondary. Take a tracing. The time relations of the contraction are determined in the manner already stated (Lesson XXXVII.).

Muscle wave.—When a muscle contracts all parts do not contract simultaneously; the contraction travels along the muscle in the form of a wave. In studying any wave we require to know its

(1) Amplitude. (Determined already by recording change of diameter or thickness.)

(2) Length.

(3) Velocity or rate at which it travels.

The length of the wave—about 30 cm.—is found to be far greater than the length of the individual fibres of the muscle.

3. Velocity of muscle-wave.—This is best measured by Biedermann's modification of v. Bezold's method. A muscle with parallel fibres—sartorius—is fixed a little to one side of the middle line in a cork clamp so that the *direct* transference of the change of muscle form, but not the excitation process in the muscle, is prevented from passing—*i.e.*, one part of the muscle is stimulated while the other part records.

(*a*) Arrange coil, commutator without cross-bars and two pairs of thin wires, so as to be able to send a single maximum break-shock through either pair of wires. (Lesson XXXIII.) Let the P current be broken by the automatic drum key. Record contraction (crank-M) and time ($\frac{1}{100}''$).

(*b*) Prepare the sartorius—or semi-membranosus and gracilis—of a *curarised* frog (p. 221), and connect its tibial end with the myograph-lever.

(*c*) Clamp the muscle a little to the tibial side of its middle in a cork clamp, made by pushing two pins parallel to each other through two thin pieces of cork; the points of the pins fix the preparation on the support (fig. 195).

(*d*) Thrust two pin electrodes through the muscle close to the clamp and two near its free end, and connect them with the commutator, so that the muscle can be stimulated either near the clamp or far away from it. Stimulate the muscle first near the clamp and record the contraction, reverse commutator—excite it away from the clamp and record. Two curves, one

rising later than the other. The distance between the two indicates the time taken by the wave of contraction to pass over the distance from the far to the near electrodes. Measure the distance between the electrodes and calculate its velocity. It varies from 1 to 2 metres per sec.

(*e*) Test the effect of cold normal saline in slowing its rate.

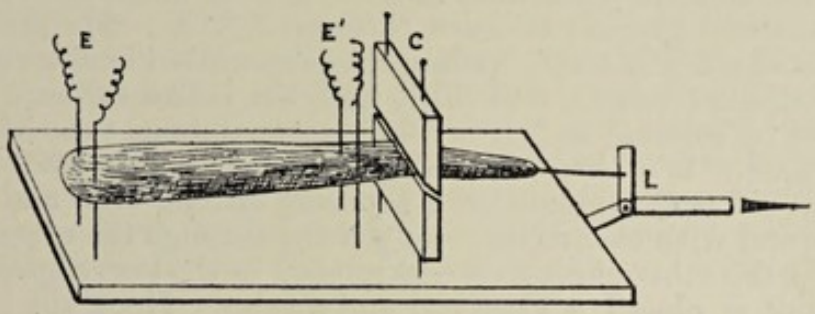


Fig. 195.—Muscle wave. *E*, *E'*, Pin electrodes; *C*, Cork clamp; *L*, Lever.

4. (*a*) Arrange two long straw levers on a frog-plate so that the two free ends of the levers record exactly over each other on a drum. Record time ($\frac{1}{100}''$).

(*b*) Remove the double semi-membranosus and gracilis (p. 221) of the thigh from a *curarised* frog, together with their bony attachments, and place them under the levers—the levers lying across them, and as far apart as possible. Let the muscles rest on paraffined paper. Fix the muscles through their bony attachments by means of pins. Through one end of the muscles near the lever push two pins attached to wires to act as electrodes. Some prefer the two sartorii muscles, fastened together, the one lying on the other and fixed by means of pins.

(*c*) Stimulate with a maximal break induction shock and note that two curves on different abscissæ are obtained, the one a little later than the other. The distance between the two indicates the time taken by the contraction to pass from the one lever to the other. Test the effect of cold normal saline.

(*d*) **Measure the curves.**—Suppose the distance between the two levers resting on the muscle to be 15 mm. Each lever, of course, records the increase of thickness at its point of application, but lever 2 rises later than 1. Measure the latent periods of the two curves. Suppose them to be 0·004 sec. and 0·009 sec. respectively; difference = 0·005 sec.—*i.e.*, the muscle wave travelled 15 mm. in this time. Therefore its *rate* was 3·3 metres per sec.

$$15 \text{ mm.} : 1'' :: 0{\cdot}005'' : N = 3{\cdot}3.$$

The *length* of the wave is represented by velocity × duration. From the time tracing ascertain the time occupied from the rise of lever (1) until lever (2) reaches the abscissa. Suppose it to be 0·08 sec. Hence velocity (3·3 metres) × duration (0·08 sec.) = 26·4 cm., length of muscle wave.

5. Volume of contracting muscle.—Although the muscle changes its form, there is no change of volume. There are several simple methods of showing this.

(*a*) Use a small glass vessel like a pycnometer for estimating the S.G., with two platinum contacts fused into its lower end to serve as electrodes. The vessel is provided with a well-ground stopper prolonged into a capillary tube—*i.e.*, with a very narrow lumen.

Remove the skin from the hind limbs of a frog, or use the double semimembranosus and gracilis muscles (Lesson XXIX., 5), prepared under boiled but cold 0·6 p.c. NaCl. Place the preparation in the vessel between the two platinum contacts, and fill it with the boiled saline. The stopper is then filled by sucking it full of fluid, a caoutchouc tube being attached for the purpose. It is then inserted into the vessel. See that there are no air-bubbles. Or use a volume tube, tapering to one end, and provided at its opposite end with electrodes—one passing through the upper caoutchouc stopper, and the other through a cork placed in the lower opening. In the upper stopper is placed a glass rod and a tube. Hook the hind limbs to the upper electrode, drawn out to a narrow capillary. Overfill the tube with boiled normal saline, and replace the stopper in the tube, excluding all air-bubbles. Adjust the height of the fluid in the capillary tube by raising or depressing the glass rod in the stopper until the water niveau is distinctly seen. On passing a single break induction shock through the preparation and causing it to contract, no change in the level of the water is observed.

6. Wild's apparatus consists of a glass cylinder made by inverting the neck-end of a two-ounce phial. The neck is fitted with a cork, the upper end is open (fig. 196, B). A wire connected with a key (K′) short-circuiting

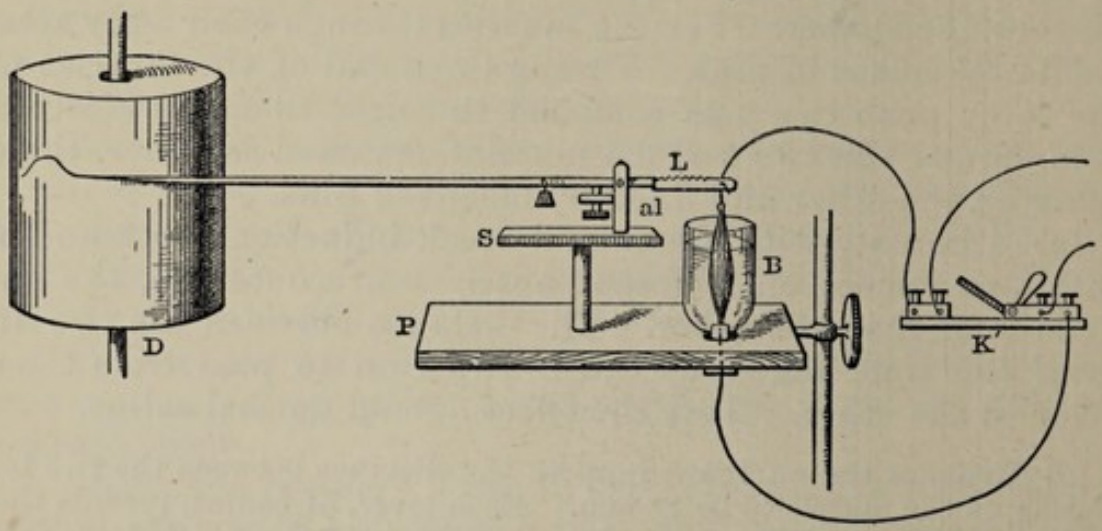


Fig. 196.—Wild's apparatus for studying the action of drugs on muscle. *D*, Drum; *P*, Platform; *S*, Stand; *al*, After-load; *L*, Lever; *B*, Bottle with muscle; *K′*, Key.

the secondary coil of an induction machine perforates the cork. Arranged above is a light lever (L), provided with an after-load (*al*), and moving on an axis, the short arm projecting over the mouth of the jar. The whole arrangement is fixed on a platform (P), with an adjustable stand (S) bearing the fulcrum of the lever and the after-load. The cork must be renewed with each new drug used.

(*a*) Dissect out the gastrocnemius, divide the femur with the gastrocnemius attached just above the origin of the latter, and the tibia below the

knee-joint. Pass a metal hook through the knee-joint or its ligaments, and attach it to the hook on the short arm of the lever. Fix tendo Achillis to a hook connected with the wire passing through the cork in the neck of the glass cylinder.

(*b*) Nearly fill the vessel with normal saline. Stimulate directly with a break-shock, using a Hg-key in the P circuit, and take a tracing.

(*c*) Remove the normal saline with a pipette, and replace it with a solution of the drug whose action you wish to study—*e.g.*, veratrin 1 in 5000, or barium chloride 1 in 1,000. Study the veratrin tracing (fig. 173).

7. Interference-phenomenon in N.M. preparation.—Arrange a N.M. preparation in a moist chamber, and weight the recording lever with 20 grms. Place the central end of the nerve over platinum electrodes, and allow a portion of the nerve nearer the muscle to hang in the form of a loop in contact with strong glycerin. The muscle becomes tetanic. Throw in an interrupted current, the tetanus is diminished. Is this interference-phenomenon an inhibitory one? (Kaiser, *Zeitsch. f. Biol.*, 1891, p. 417.)

LESSON XLV.

GALVANOMETER—N.P. ELECTRODES—SHUNT—CURRENTS IN MUSCLE, NERVE, HEART.

For studying the electromotive phenomena of muscle and nerve a sensitive galvanometer is required.

1. Thomson's (Kelvin's) high-resistance astatic galvanometer.

In this instrument the pair of suspended magnets are very nearly astatic, and each is surrounded by many turns of fine insulated wire, thus giving a high resistance of 10,000 to 20,000 ohms. As the resistance of both nerve and muscle, as well as the N.P. electrodes, is very high, this is of no moment. This explains why the galvanometer is defined as "high resistance" and "astatic;" "reflecting," because a slightly concave mirror, by means of which a beam of light can be reflected on a scale, is attached to the upper magnet.

(*a*) Place the galvanometer (fig. 197) on a stand unaffected by vibrations—*e.g.*, on a slate slab fixed in the wall, or a solid stone pillar fixed in the earth, taking care that no iron is near.

(*b*) Let the galvanometer mirror face *west*—*i.e.*, with the plane of the coils in the magnetic meridian—the magnetic meridian being ascertained by means of a magnetic needle. As the

galvanometer is a differential one, make it a single one by connecting the two central binding screws on the ebonite base with a copper wire.

(*c*) By means of the three screws level the galvanometer.

(*d*) Take off the glass cover and steadily raise the small milled head on the top of the upper coils, which frees the mirror, and allows it to swing free. Replace the glass shade.

(*e*) Place the scale (fig. 198) also in the magnetic meridian and 1 metre from the mirror, taking care that it is at the proper height. Instead of a slit in the scale, it is better to fix in it a thin wire, and by means of a lens of short focal distance to bring the image of the wire to a focus in the middle of the illuminated disc of light reflected from the mirror upon the scale.

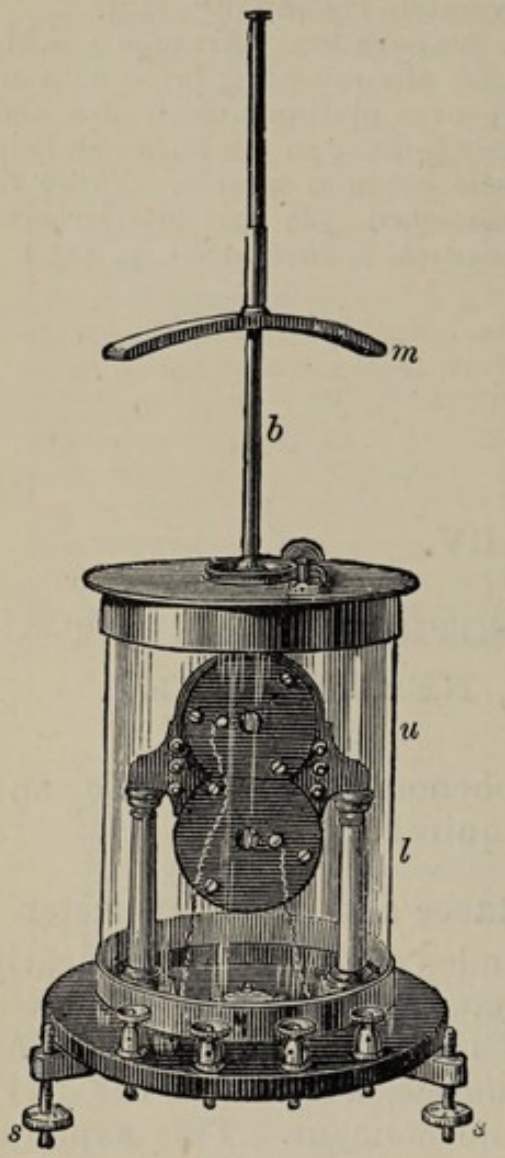


Fig. 197.—Thomson's (Kelvin's) reflecting galvanometer. *u*, Upper; *l*, Lower coil; *s*, *s*, Levelling screws; *m*, Magnet on brass support, *b*.

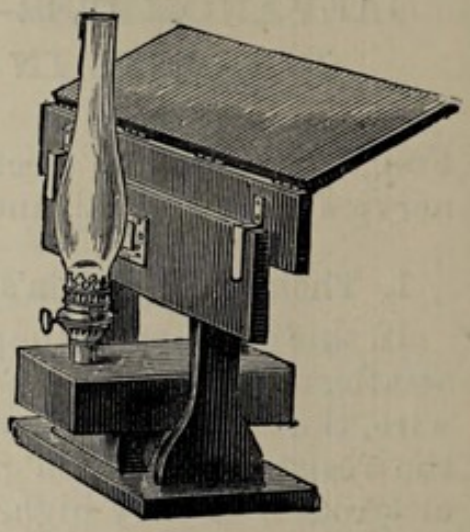


Fig. 198.—Lamp and scale for Thomson's galvanometer.

(*f*) Light the paraffin lamp, place the edge of the flame towards the slit, darken the room, and see that the centre of the scale, its zero, the slit in the scale, the flame of the lamp, and the centre of the mirror are all in the same vertical plane, so that a good light is thrown on the mirror and a distinct image on the scale. A better arrangement is an incandescent electric

lamp enclosed in a blackened asbestos box, with a vertical slit in the dial. The light from this is collected by a projecting lens upon the mirror.

(*g*) Make the needle all but astatic by means of the bar magnet, *m*. The needle is *most sensitive when it swings slowly*.

(*h*) Test the sensitiveness of the galvanometer by applying the tips of two moist fingers to the two outer binding screws of the instrument, when at once the beam of light passes off the scale.

2. **Non-polarisable (N.P.) electrodes.**—One may use the old form of du Bois-Reymond, the simple tube electrodes, or the "**brush electrodes**" of v. Fleischl (fig. 203).

The contact of metal electrodes with an electrolyte leads to the generation of polarisation currents, hence metallic electrodes should not be used for studying the effect of galvanic currents on nerve or muscle, or for electromotive phenomena.

A. (*a*) Use perfectly clean glass tubes (3 cm. × 5 mm.), tapering somewhat near one end. Plug the tapered end with china clay, made by mixing kaolin into a paste with normal saline. Push the clay into the lower third of the tube, using a fresh-cut piece of wood or thin glass rod to do so, allowing part of the clay to project beyond the tapered end (fig. 199, *t*, *t*).

(*b*) With a clean pipette half fill the tube with a *saturated* neutral solution of $ZnSO_4$. Make *two* such electrodes.

(*c*) In each tube place an amalgamated zinc wire with a copper wire soldered to its upper end, Z, Z, fix the electrodes in suitable holders in a moist chamber, and attach the wires of the zincs to the binding screws of the moist chamber. The zinc should not touch the clay.

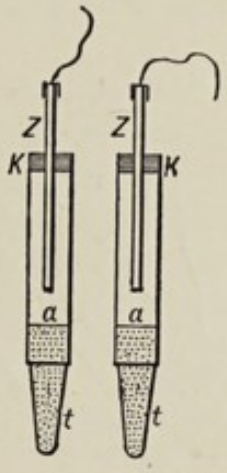


Fig. 199.—Non-polarisable electrodes. *Z*, Zincs; *K*, Cork; *a*, Zinc sulphate solution; *t*, *t*, Clay points.

B. Some prefer a U-shaped glass tube held in a suitable holder attached to a vulcanite rod in the moist chamber (Sanderson's pattern). The tube contains a saturated solution of $ZnSO_4$. In one limb of the tube is placed a rod of amalgamated zinc, and in the other a straight tube with a slight flange at its upper end filled with kaolin moistened with normal saline, the kaolin projecting as a cap above the level of the U-shaped tube. The muscle is placed on the two corresponding kaolin caps.

3. The shunt is an arrangement by which a greater or less proportion of a current can be sent through a galvanometer (fig. 200). The brass bars on the upper surface are marked with the numbers $\frac{1}{9}$, $\frac{1}{99}$, $\frac{1}{999}$, indicating the ratio between their resistance and that of the galvanometer, so that when the plug is inserted in the several positions $\frac{1}{10}$, $\frac{1}{100}$, or $\frac{1}{1000}$ of the whole current can pass through the galvanometer. The short-circuiting key, C, enables all the current to be sent through the battery circuit and none through the galvanometer.

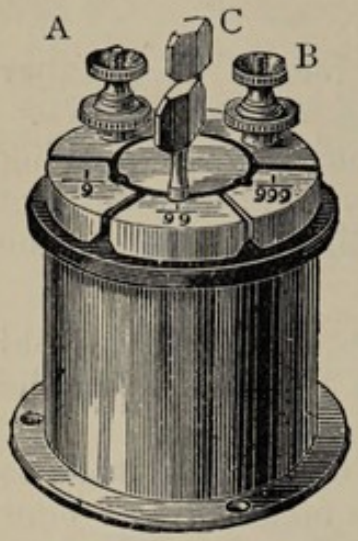


Fig. 200.—Shunt.

4. Muscle demarcation-current (current of injury).

(*a*) Arrange apparatus (fig. 201). Place shunt between the N.P. electrodes and galvanometer. Connect two wires from the electrodes to the binding screws, A, B, of the shunt, and from

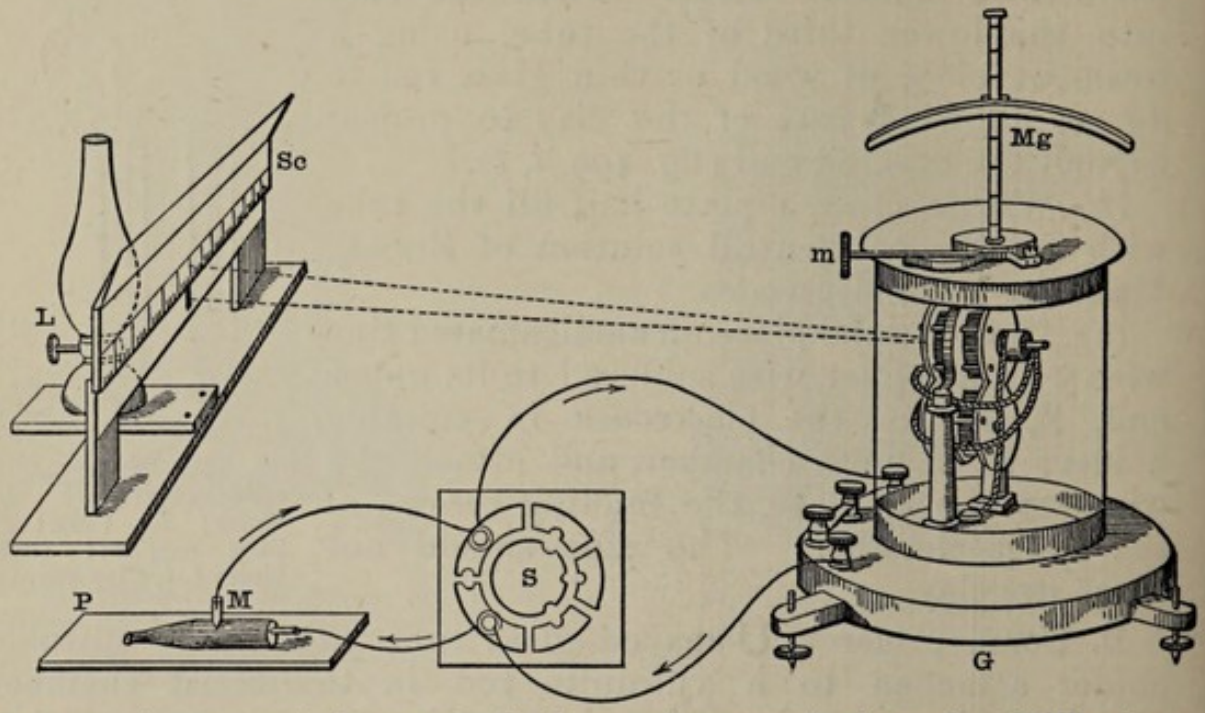


Fig. 201.—Arrangement of apparatus for demarcation-current of muscle. *M*, Muscle on a glass plate, *P*; *S*, Shunt; *G*, Galvanometer; *Mg*, Its magnet moved by the milled head, *m*; *L* and *Sc*, Lamp and scale.

the same binding screws attach two wires to the galvanometer. Insert a plug, C, between A and B, thus short-circuiting the muscle-current. When working with muscle, keep a plug in the gap opposite $\frac{1}{9}$ on the shunt. Arrange lamp and scale to give

good image of the mirror on the scale zero—adjusting, if necessary, by means of the magnet moved by the milled head on the top of the glass shade (fig. 201, *m*).

(*b*) Test the electrodes, either by bringing them together or by joining them with cotton thread covered with china-clay paste. After removing all the plugs from the shunt, there ought to be no deflection of the spot of light. If there is none, there is no polarity, and the electrodes are perfect. If there is much deflection, make new electrodes.

(*c*) *Ascertain direction of current in galvanometer.*—Make a small Smee's battery with a 2-ounce bottle. Place in a w.m. bottle dilute sulphuric acid (1 : 20), as also a zinc (-) and a copper wire (+), with wires soldered to them. Connect them with the galvanometer. Arrange the shunt so that $\frac{1}{100}$ or $\frac{1}{1000}$ part of the galvanic current thus generated goes through the galvanometer. Note the deflection and its direction. Arrange the N.P. electrodes in the same way, and observe which is the negative and which the positive pole corresponding to the zinc and copper of the battery. If the north binding screw of the galvanometer be positive, the spot of light moves to the left, and *vice versâ*.

(*d*) *Prepare muscle.* — Prepare either sartorius or semimembranosus, which consist of parallel fibres, but avoid touching the muscle with the acid skin. Lay it on a glass plate or block of paraffin in a moist chamber. Keep one plug in the shunt at C to short-circuit the electrodes, and the other plug at $\frac{1}{9}$. Make a fresh transverse section at one end of the muscle, and adjust the point of one electrode exactly over the centre (equator) of the longitudinal surface of the muscle. Apply the other electrode exactly to the centre of the freshly-divided injured transverse surface (fig. 201).

(*e*) *Current of injury.*—Remove the short-circuiting plug, C, from the shunt, keep one plug at $\frac{1}{9}$, so that $\frac{1}{10}$ of the total current from the muscle goes through the galvanometer. Note the direction and extent of the deflection. By noting the direction, and from the observation already made (*c*) one knows that the longitudinal surface of the muscle is +, and the transverse section −. Replace the plug-key, C, and allow the needle to come to rest at zero. The deflection was caused by the current of injury, and it flows outside the muscle from the equator or middle of the muscle towards the cut end. It is also called the *demarcation* current, because the difference of potential appears at the demarcation between the dying and the injured muscle. The injured part of a muscle is negative to the uninjured part, and the current in

the galvanometer is from the longitudinal (+) surface to the injured negative transverse surface.

(*f*) Bring the N.P. electrode on the longitudinal surface nearer to the end of the muscle, and note diminution of deflection of needle. Replace plug C.

(*g*) Vary the position of the electrodes and note the variation in deflection. If equidistant from the equator, there is no deflection. It is greatest with one electrode on the equator and the other on the centre of the transverse section of a muscle with parallel fibres. The deflection—*i.e.*, the electro-motive force—diminishes as the electrodes are moved from the equator or the centre of the transverse section. In certain positions no deflection is obtained.

5. Negative variation of muscle-current.

(*a*) Use the same muscle preparation or the gastrocnemius with the sciatic nerve. Divide the muscle transversely, and lay the artificial transverse section on one electrode, and the longitudinal surface on the other. Observe the extent of the deflection.

(*b*) Adjust at some distance from the galvanometer a coil for repeated shocks.

(*c*) Take the demarcation-current, observe the deflection, and allow the spot of light to take up its new position on the scale. Tetanise the muscle through its nerve, the spot of light travels towards zero. This is the "**negative variation of the muscle-current.**" If the gastrocnemius be used, stimulate the sciatic nerve. Care must be taken that the muscle does not shift its position on the electrodes.

According to Hermann's theory, it is brought about as follows:—An injured part of a muscle (or nerve) is negative to an uninjured part—"negativity of injury," and similarly an active part of a muscle is negative to an inactive part—"negativity of activity." The demarcation-current or injury-current passing in the galvanometer from the longitudinal + to the transverse – surface is diminished, because when the muscle contracts there is a current set up—**action-current**—in the opposite direction, which diminishes the total current acting on the galvanometer.

6. Demarcation-current of nerve.—(*a*) Make the galvanometer as sensitive as possible by adjusting at a suitable height the north pole of the magnet over the north pole of the upper needle.

(*b*) Prepare N.P. electrodes for a nerve. In this case the electrodes are hook-shaped, and one is adjusted over the other. The upper hooked electrode has a groove on its concavity communicating with the interior of the tube (fig. 202). Place only one plug in the shunt between A and B.

(*c*) Dissect out a long stretch of the sciatic nerve, make a fresh transverse section at both ends, hang it over the upper N.P. electrode, N, and resting with its two cut ends on the lower electrode, C, thus doubling the strength of the current (fig. 202).

(*d*) Remove the plug from C in the shunt and pass the whole of the demarcation nerve-current through the galvanometer, noting the deflection.

(*e*) Instead of adjusting the nerve as in (*c*), it may be so placed on the ordinary tube N.P. electrodes that the cut end rests on one electrode and the longitudinal surface on the other, thus leaving part of the nerve free. Observe the deflection in this way.

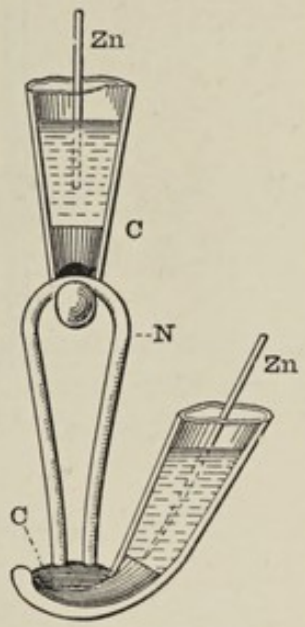


Fig. 202.—Nerve N.P. electrodes. *N*, Nerve; *C*, Clay of electrodes; *Zn*, Zincs.

7. Action current of nerve.—(*a*) Observe the amount of deflection as in 6 (*e*). Stimulate with an interrupted current the free end of the nerve. The spot of light travels towards zero. This was formerly called the "negative variation" of the nerve-current.

8. Electromotive phenomena of heart.—Arrange the apparatus the same as in Lesson XLV., 4 (*a*).—(*a*) Make a Stannius preparation of the heart, using only the first ligature (Lesson LV., 1) to arrest the heart's action. Lead off with brush N.P. electrodes from base and apex of the quiescent uninjured heart; there is no deflection.

(*b*) Pinch the apex so as to injure it, it becomes negative; a difference of potential is at once set up, and the spot of light oscillates with each beat of the heart.

(*c*) Excise a heart so as to get a spontaneously beating ventricle; lead off from the base and apex of the latter; observe the so-called "negative variation" with each contraction.

(*d*) See also Lesson XLVII., 7, for secondary contraction excited by the beating heart.

9. Capillary electrometer for heart.—(*a*) Lead off a muscle to the two binding screws of a capillary electrometer. The fine thread of mercury must be observed with a microscope. (Lesson XLVI.)

By means of the capillary electrometer Waller has shown the diphasic variation of the heart-current in man and dog.

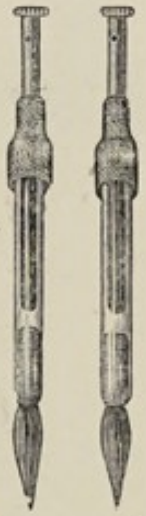


Fig. 203.—Brush electrodes of v. Fleischl.

10. Brush electrodes of Fleischl (fig. 203) consist of glass tubes (4 cm. × 5 mm.). In one end is fitted a perfectly clean camel-hair pencil, and into the other dips a well-amalgamated rod of zinc with a binding screw at its free end. Place some clay in the lower part of the tube, and then fill it with a saturated solution of $ZnSO_4$. No metallic instruments to be used. A piece of india-rubber tubing fits as a cap over the upper end of the glass tube. The brushes are moistened with a mixture of kaolin and normal saline.

11. D'Arsonval's N.P. electrodes (fig. 204) consist of a silver wire coated with fused silver chloride. The threads projecting from the tapered points

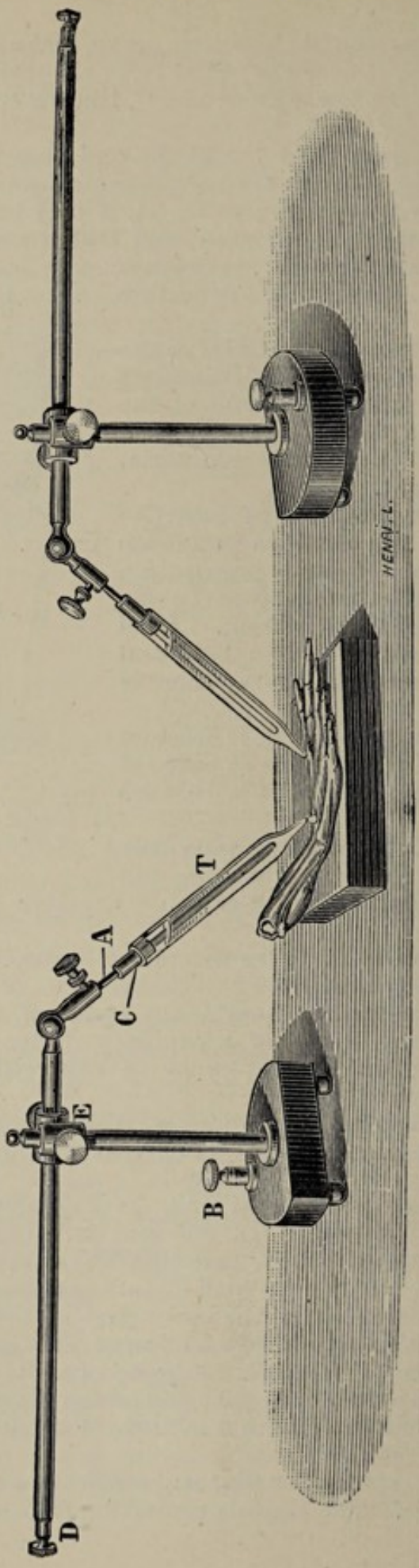


Fig. 204.—D'Arsonval's non-polarisable electrodes, as made by Verdin.

are brought into contact with the tissues. They should be kept in the dark. The wire is held in a suitable stand, while the coated part is placed in a tube tapering below and filled with normal saline. At the lower tapered end there is a small aperture into which is introduced a thick thread. The tube is closed above with a cork, C, through which passes the silver electrode, A.

LESSON XLVI.

CAPILLARY ELECTROMETER—MUSCLE AND HEART CURRENT—MEASUREMENT OF E.M.F.

1. The capillary electrometer is an electrical manometer, and indicates electrical pressure. By it minute differences of electrical potential between two surfaces can readily be detected. It may be used to demonstrate electrical currents, but this is better done with a galvanometer. It has been specially used for measuring the electrical variations of the heart, *e.g.*, of frog or man.

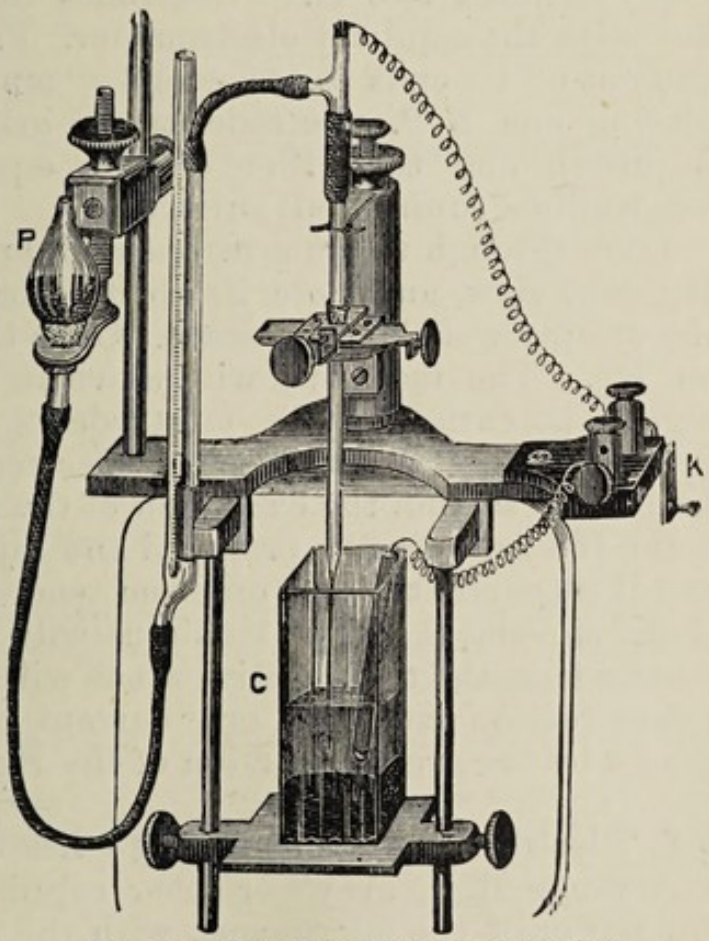


Fig. 205.—Frey's capillary electrometer.

There are several forms of the instrument, but the original form of Lippmann consists essentially of a piece of glass tube, with one end drawn out to a fine point (internal diameter 20-30 μ), which is partly filled with clean mercury and brought into connection with a pressure apparatus. The capillary end of this tube is arranged to dip into a tube containing 10 p.c. H_2SO_4 and mercury. Into the lower end of this tube is fused a platinum wire which establishes one connection with the lower Hg column. A similar platinum connection is made with the capillary tube.

In the capillary tube of the electrometer the mercury is forced along by the pressure bottle, P, until the weight of the column of Hg is balanced by the surface tension, which is inversely proportional to the diameter of the tube. Anything which alters the surface tension will cause the Hg meniscus to move.

The form devised by M. v. Frey (fig. 205) can be fixed on the stage of any microscope provided with a joint in the stand, so that the tube of the microscope is horizontal and stage vertical. It consists of a vessel holding Hg and dil. H_2SO_4 serving as one electrode, a support for the capillary tube, C, the second electrode and a pressure bottle, P, for regulating, and a manometer for measuring the Hg pressure. The wires from the two electrodes pass to a key, K (*du Bois-Reymond's Archiv.*, 1887, p. 204). The mercury meniscus is observed with a low-power microscope provided with an eye-piece having a micrometer scale, to enable one to note the extent of movement of the Hg meniscus.

A convenient form, with the capillary tube horizontal, is made under the direction of Professor Langendorff by Westien of Rostock. It is small and compact, and can be placed horizontally on the stage of any microscope. Waller's form is both simple and practical.

2. Demarcation current of muscle with electrometer.

(*a*) Connect two N.P. electrodes through a short-circuiting key with the capillary electrometer. Prepare a sartorius muscle, and cut off its ends transversely—"muscle prism." Lead off by placing one N.P. electrode on an artificial transverse section (injured), and the other on the equator or middle of the longitudinal (uninjured) surface.

Look through the microscope and bring the meniscus of the Hg into view, and note its position on the micrometer scale in the eye-piece of the microscope. Open the key, and observe effect on Hg. The meniscus will move in a direction indicating a higher potential at the electrodes (+) on the longitudinal surface ("equator"). If the + electrode be moved towards the transverse section the excursion of the Hg will be diminished.

(*b*) If a sartorius be prepared and injured as little as possible, and if its current be led off from tendons and "equator," only a slight movement of the meniscus will be obtained. Injure the muscle near the tendon with a hot wire. Immediately a strong "demarcation current" or "current of injury" is set up, as shown by the great excursion of the Hg meniscus.

3. Electrical variations of frog's heart (*Capillary electrometer*).

—Arrange M. v. Frey's or other capillary electrometer under a low power of the microscope, with the meniscus of the mercury in view, and capable of moving freely. Connect a pair of D'Arsonval's silver electrodes or other N.P. electrodes to the short-circuiting key.

Expose the heart in a pithed frog, and arrest the movements of its ventricle by a Stannius ligature (Lesson LVI.), and by means of moist cotton threads projecting from the electrodes bring the latter into contact with the base and apex of the quiescent ventricle. Excite a contraction by touching the ventricle with a needle (1) near base, (2) near apex. Open the key and note the order and direction of the rapid oscillations of the Hg. The variations in the excised heart can be studied in a similar manner. The oscillations can be photographed on a moving sensitive surface.

4. Measurement of E.M.F. (potential) of Muscle and Nerve (*Compensation method of du Bois-Reymond*).—The principle is simple—viz., that of a balance-compensation where the tissue-current through the galvanometer is balanced by a fractional part of a current of known E.M.F. —*e.g.*, Daniell's battery—sent through the galvanometer in the reverse direction.

(*a*) Arrange a metre bridge with one gap unbridged, or use a monochord or rheochord. Place a commutator with X bars in C circuit with a Daniell's cell, D, and arrange C so that the constant current passes in the wire from B to A.

(*b*) Arrange the muscle or nerve, T, on N.P. electrodes and couple them up with the galvanometer. (Keys omitted in diagram.)

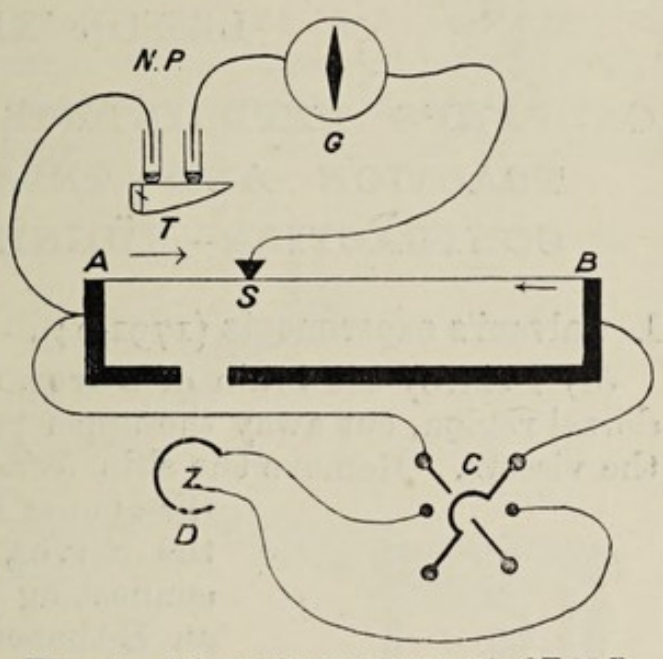


Fig. 206.—Scheme for measurement of E.M.F. of M or N (T).

(*c*) Push the slider towards A (few c.m.), and get smallest deflection of galvanometer. Push it more towards A until the spot of light from galvanometer stands at zero.

With the enormous external resistance in the tissue, T, the difference of potential between A and B is practically that of a Daniell cell. The fall of potential from A to B is uniform, so that the E.M.F. between A and *s* is $\frac{As}{AB}$. E, where E is E.M.F. of a Daniell (= 1·1 volts). It will be found that *s* lies very close to A. The short portion of the wire A*s* compared with the total length of the wire (1000 mm.) gives the E.M.F. of the tissue in terms of a Daniell :—

$$1000 : As :: 1{\cdot}1 \text{ volts} : x = \text{volts of tissue.}$$

N is usually about ·03 volt.

Or, instead of a single wire, use a low, *r* (representing A*s*), and a high resistance, R, rheostat (representing S*b*). The wires from the battery go to the outer binding screw on each rheostat, the two inner screws of

r and R are connected by a wire. The tissue is arranged as before in circuit with G. The tissue current is led into the outer binding screw of r, and the return wire to G is connected to the wire connecting r and R. The potential difference (P.D.) is practically the difference between the terminals of r and R. The P.D. of a muscle is P.D. $= \frac{r}{r + R}$. Suppose the balance to be obtained with 250 and 7250 ohms, then $\frac{250}{250 + 7250}$ = ·033 Daniell (*Waller*).

LESSON XLVII.

GALVANI'S EXPERIMENTS — SECONDARY CONTRACTION AND TETANUS — PARADOXICAL CONTRACTION—KÜHNE'S EXPERIMENTS.

1. Galvani's experiments (1791-94).

(*a*) Destroy the brain of a frog, divide the spine in the mid dorsal region, cut away the upper part of the body, and remove the viscera. Remove the skin from the hind parts, divide the iliac bones and urostyle, avoid injuring the nerves, which remain as the only connecting link with the legs. Push an S-shaped copper hook through the lower end of the spinal column (fig. 207).

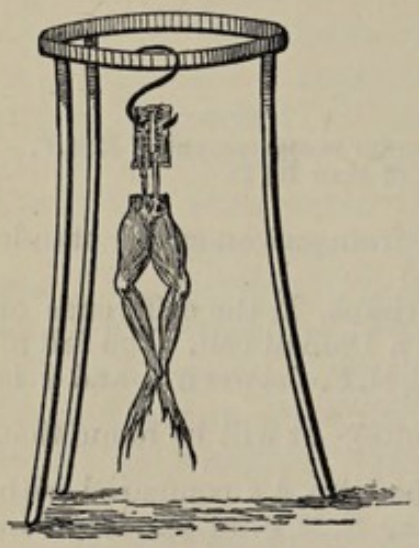


Fig. 207.—Galvani's experiment with metals.

(*b*) Suspend the preparation on an iron tripod. Tilt the tripod so that the point of contact, being made clean by means of a file, comes in contact with one of the legs of the tripod; vigorous contractions occur whenever the frog's legs touch the tripod.

(*c*) **Experiment with metals** (1794).—With the frog hanging perpendicularly without touching the tripod, make a U-shaped piece of wire composed of a copper and zinc wire soldered together. Touch the nerves above with the copper (or zinc) end, and the muscles below with the zinc (or copper), when

contraction occurs at make or break, or both—*i.e.*, make or break currents.

Galvani supposed that the electricity in this case was produced in and by the animal tissues. Volta pointed out that when two dissimilar metals are brought into contact electrical currents are produced. In this experiment the chief source of the electrical energy is due to the contact of the two dissimilar metals with the saline fluids of the living tissues.

2. Galvani's experiment of contraction without metals.

(*a*) Make a fresh, vigorous N.M. preparation, leaving the leg attached to the femur and the sciatic nerve as long as possible. Hold the femur in one hand, lift the nerve on a glass rod moistened with normal saline, and allow it to fall upon the gastrocnemius, when the muscle will contract, or lay the muscle on a glass plate (fig. 208). Contraction occurs because the nerve is suddenly stimulated, owing to the surface of the muscle having different potentials.

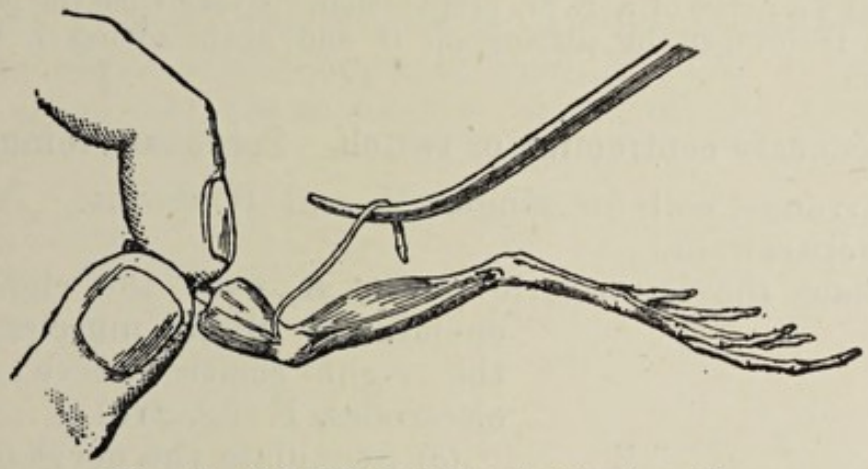


Fig. 208.—Galvani's experiment without metals.

(*b*) Or remove the skin from the hind legs of a frog, and dissect out the sciatic nerve in its whole extent. Divide it at its upper end. If the nerve be lifted on a glass rod and allowed to fall longitudinally on the triceps muscle there is no contraction (fig. 208).

[See "Lectures on Animal Electricity," by A. D. Waller, 1897. A, Volta, *Briefe über thierische Elektricität.* Oswald's *Classiker*, No. 124. Leipzig, 1900. Translation and reproduction of Volta's letters to Galvani.]

3. Demarcation muscle-current to stimulate nerve.

(*a*) Make a cut across the triceps and arrange the nerve so that its cut end rests on the transverse section of the muscle and its longitudinal surface on the longitudinal surface of the

muscle. As soon as this interval is bridged over, the leg muscles contract.

There is a large difference in potential between the transversely-cut muscle and its longitudinal surface—there is a "muscle-current" in the muscle from the artificial transverse section to the longitudinal surface, so when the nerve bridges over these surfaces, there is an external derivation-current passing in the nerve, whereby the latter is stimulated. Thus the "physiological rheoscope" is used to show the presence of electrical currents in muscle under certain conditions.

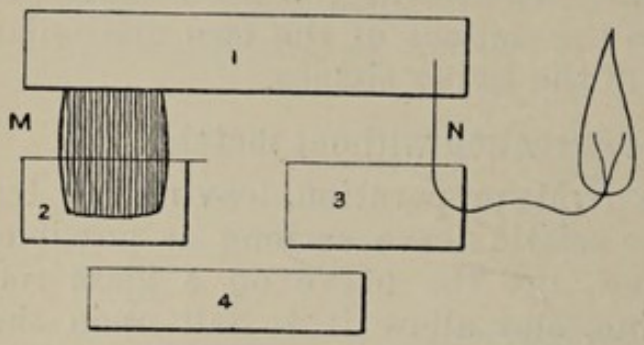


Fig. 209.—Pads of moist blotting-paper, 1, 2, 3, 4. *N*, N.M. preparation; *M*, Muscle. (*Schenck*, after *du Bois-Reymond*.)

(*b*) Arrange on a glass slide folds of blotting-paper, as in fig. 209, laying over 1 and 3 a nerve of a N.M. preparation. Every time the gap between 2 and 3 is bridged by placing on it and again lifting 4, the muscle contracts.

4. Secondary contraction or twitch. Secondary tetanus.

(*a*) Arrange coil for single M and B shocks. Make two N.M. preparations.

(*b*) Place the left sciatic nerve, A, over the right gastrocnemius or thigh muscles, B, and the right sciatic nerve over the electrodes, E (fig. 210).

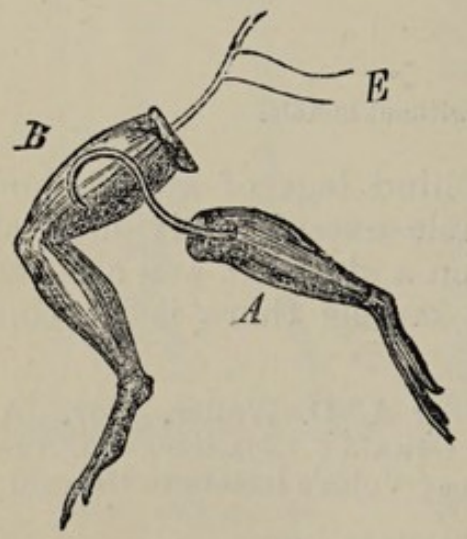


Fig. 210.—Secondary contraction.

(*c*) Stimulate the nerve of B with single induction shocks—the muscles of both B and A contract. The contraction in A is called a **secondary contraction**. A is the **rheoscopic limb**, as by its contraction it shows the existence of an electrical current in B. When B contracts, there is a sudden diminution of its muscle-current, which circulates in the nerve of A. This sudden diminution—negative variation—is tantamount to a stimulus, and so the nerve of A is stimulated.

(*d*) Stimulate the nerve of B with repeated shocks. B is

tetanised, and so is A simultaneously. This is secondary tetanus. The nerve of A is stimulated by the sudden series of negative variations of the muscle-current during the contraction of B. This shows that the electrical change during tetanus is discontinuous and not continuous like the change in form of the muscle, and with 50 shocks per second each electrical change must reach its maximum and subside in $\frac{1}{100}''$.

(*e*) Test for current escape by ligaturing the nerve of A near the muscle, stimulate the nerve of B; there is no contraction of A although B contracts, showing that the result in (*c*) and (*d*) is a true secondary contraction due to excitation of the nerve of A by a negative variation of the current of muscle of B. If on tetanising B nerve, after ligature of A nerve A contracts, there has been an escape of current.

(*f*) Prepare another limb and adjust it in place of A, ligature the nerve of B. On stimulating the nerve of B, no contraction takes place either in A or B.

5. Secondary contraction from nerve.

(*a*) Place a N.M. preparation on a glass slide B. Dissect the sciatic nerve of the opposite side, A. Lay 1 cm. of the isolated nerve, A, on a similar length of the nerve of B (fig. 211).

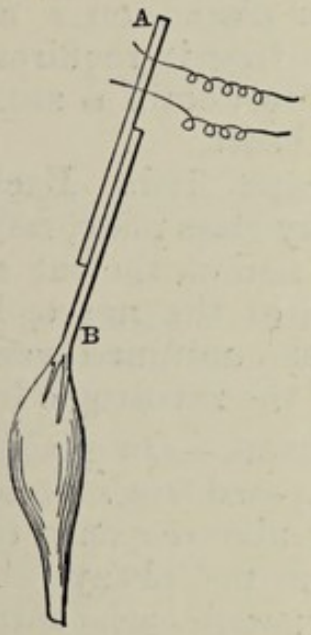


Fig. 211.—Scheme of secondary contraction.

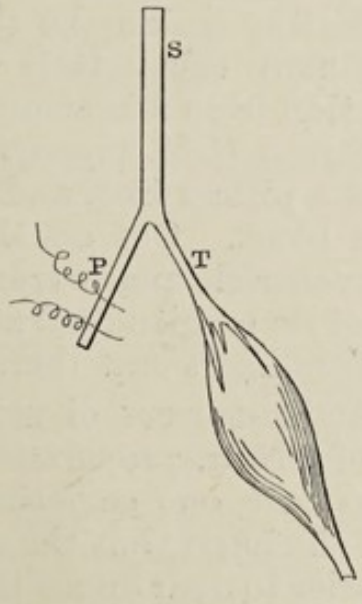


Fig. 212.—Scheme of paradoxical contraction.

(*b*) Stimulate A with (*a*) a single induction shock—B contracts; (*b*) an interrupted current—B is tetanised.

(*c*) Ligature A and stimulate again. B does not contract. Therefore contraction was not due to escape of the stimulating

current. The "secondary contractions" in B are due to sudden variations of the electro-motivity produced in A when it is stimulated.

6. Paradoxical contraction.

(*a*) Use repeated induction shocks or a constant current.

(*b*) Expose the sciatic nerve down to the knee (fig. 212, S). Trace its two branches. Divide the outer or peroneal branch, and stimulate its *central end*, P, by a faradic current. A certain strength of current will be found whereby the muscles supplied by the other division of the nerve are thrown into tetanus, T. The tibial nerve to the gastrocnemius is stimulated by escape or spread of "electrotonic" currents from the excited nerve.

(*c*) Instead of induction shocks, use a shock from a Daniell's cell. There is a paradoxical twitch.

No paradoxical response is produced by stimuli other than electrical—*e.g.*, section, or salt. It occurs after the peroneal nerve is ligatured on the central side of the seat of stimulation.

7. Frog's heart-current (Secondary contraction from heart).

(*a*) Injured heart.—A quiescent uninjured heart gives no current, but an active heart does, and so does an injured one. The action-current of an injured heart is easily shown when a nerve of a nerve-muscle preparation is placed on a rabbit's heart beating inside the thorax. In the frog it requires some care to show this. It is easy, however, to obtain a secondary contraction from a beating injured frog's heart.

Prepare a N.M. preparation or rheoscopic limb. Excise the heart of a pithed frog, and place it on a dry glass plate, removing surplus blood. Cut off the heart apex, and on the cut surface of the ventricle place transverse section of the nerve, letting a part of its longitudinal surface rest on the uninjured ventricle. With each heart-beat there is a twitch of the rheoscopic limb.

(*b*) Action-current of uninjured frog's heart.—On placing the nerve of a N.M. preparation along the exposed frog's heart from apex to base, one sometimes gets a muscular response to each beat of the heart, but the experiment does not always succeed. It is easier to do it on a Stanniused heart; with each contraction of the heart excited artificially, there is a secondary contraction, due to the nerve being stimulated by the electrical current which accompanies each beat of the heart (Lesson LV., 1).

8. Kühne's nerve-current experiment (*Contraction without metals*).

(*a*) Fix a glass plate to an earthenware bowl (fig. 213).

(*b*) Make two rolls of kaolin (moistened with normal saline), about 1 cm.

in diameter and 6 cm. in length (P, P′), bend them at a right angle, and hang them over the glass plate about 6 mm. apart.

(*c*) Place the muscle of a N.M. preparation on the glass plate, and the nerve, N, over the rolls of china clay.

(*d*) Fill a glass vessel, C, with normal saline, and allow the two free ends of the clay to dip into it. With each dip the muscle contracts. In this case the nerve is stimulated by the completion of the circuit of its own demarcation-current, and this in turn indirectly stimulates the muscle.

9. Kühne's muscle-press—Secondary contraction from muscle to muscle.—Prepare two sartorius muscles. Place the end of one muscle over one end of the other, both muscles being in line with each other, and the overlapping portion so arranged that they can be pressed together by means of the small screw-press of Kühne.

On stimulating—by electrical, chemical, or other stimuli—the free end of either muscle, so as to cause that muscle to contract, the second muscle also contracts. The negative variation of the muscle-current stimulates the second muscle. This result does not take place if a thin layer of tin-foil be placed between the two muscles.

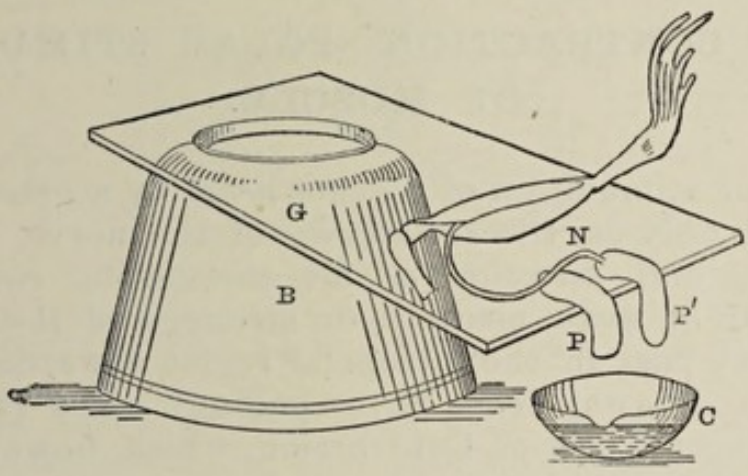


Fig. 213.—Kühne's experiment on nerve current. B, Bowl; G, Glass plate; N, Nerve on P, P′, Pads of clay; C, Capsule.

10. Biedermann's modification of secondary muscular contraction.—If a frog be denuded of its skin and left exposed to the air for 24 hours—the time varying with the temperature, amount of moisture in the air, &c.—on causing one muscle to contract, other muscles contract secondarily. On placing the two sartorius muscles in direct contact with each other, when one muscle is made to contract the other does so secondarily without the use of a muscle-press.

11. Hering's experiment on secondary contraction.—In a pithed frog expose on both sides by removing the urostyle the nerve-trunks forming the sciatic nerve. Divide them with scissors about the level of the middle of the urostyle. Push a pair of wire electrodes down the spinal canal to come into contact with the lower motor nerve roots. On weak tetanisation of the motor nerve roots either in this way, or by their direct exposure above the point of section, the limbs are tetanised. This is due to the contraction of muscles—coccygeal—which are supplied with nerves arising above the point of division. These contracting muscles excite the ends of the sciatic nerves beyond the point of division.

12. Secondary excitation from nerve to nerve (*Hering*).—(*a*) In a pithed, "cooled" frog expose the whole length of the sciatic nerve from hip to knee. Ligature it at the knee and divide it behind the ligature.

(*b*) Tetanise feebly the nerve near its distant cut end, and find a current which brings about contraction of the muscle of the thigh. Stimulate the nerve nearer the spinal cord. These contractions do not increase; they decrease, and disappear. Hence, they were not due to escape of current, and equally so the result is not a "paradoxical contraction." It would, therefore, seem to be due to excitation of the centrifugal nerve fibres to the thigh muscles by action-currents set up in neighbouring fibres.

LESSON XLVIII.

LAW OF CONTRACTION—POLAR STIMULATION OF MUSCLE.

THE result of stimulation of a *motor nerve* by a *constant current* depends not only on the *excitability* of the nerve, but also on the *intensity and direction of the stimulating current.* The intensity may be weak, medium, or strong, and the current in the nerve may pass in the intrapolar region towards the muscle (descending), or away from it (ascending). The result is the so-called Pflüger's Law of Contraction, which, however, is only a statement of facts.

In carrying out the experiment, begin with current ascending —*i.e.*, with anode next the muscle. We may test the effect of M and B for currents of medium and strong intensity—all for ascending currents, and then do the same for descending currents.

1. Pflüger's law of contraction—Polar stimulation of nerve.—Several Daniell cells, commutator with cross-bars, du Bois and Hg-key, rheochord, N.P. electrodes, moist chamber, wires, recording apparatus.

(*a*) Arrange the apparatus (fig. 214). Connect two cells to a Pohl's commutator *with cross-bars*, with Hg-key, K, in the circuit: connect the commutator with the rheochord, R — a metre rheochord will do—and the latter with N.P. electrodes, introducing a short-circuiting key. Fix to a recording lever an N.M. preparation—with a long nerve—in the moist chamber, and lay the nerve over the N.P. electrodes.

(*b*) Begin with all the rheochord plugs in position, and the slider hard up to the brass blocks. Place the commutator to give an ascending current, make and break the current—gradually adjusting the slider—until a contraction occurs at make and none at break. Reverse the commutator to get a descending current, make and break, observing again a contraction at make and none at break. This represents the effect of a *weak* current. Sometimes the current so obtained is not weak enough. A simple rheochord should then be used.

(*c*) Pull the slider farther away and remove one or more plugs until contraction is obtained at make and break, both with an ascending and descending current—the effect of a *medium* current.

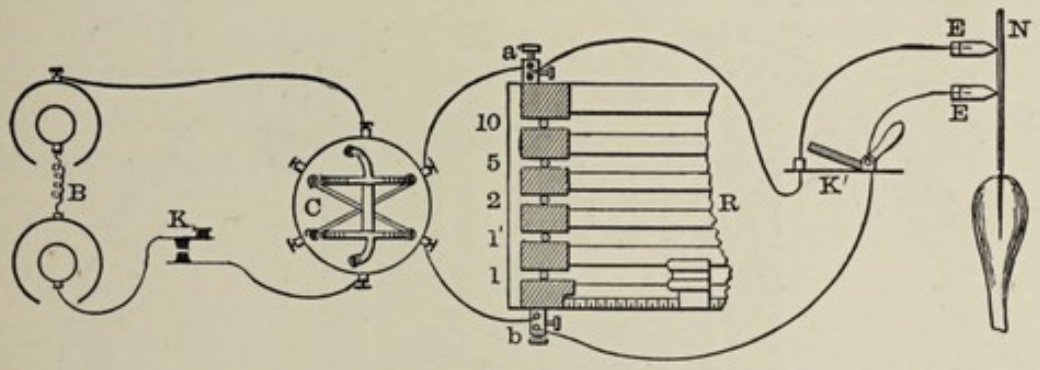


Fig. 214.—Scheme for Pflüger's law. R, Rheochord; B, Battery; C, Commutator; K, Mercury key; K′, du Bois key; E.N.P., Electrodes; N, Nerves.

(*d*) Use six small Grove's cells, take out all the rheochord plugs. With an ascending current contraction occurs at break only; with a descending current at make only = effect of a *strong* current. Tabulate the results.

Fresh strong frogs are necessary, and several preparations may be required to work out all the details. Instead of reversing the commutator after testing the effect of an alteration of the direction of the current, use one preparation to test at intervals the effect of weak, medium, and strong currents when the current is ascending, and a second preparation to test the results with currents of varying intensity when the current is descending. Results—R = rest; C = contraction :—

Strength of current.	Ascending.		Descending.	
	On Making.	On Breaking.	On Making.	On Breaking.
Weak, . . .	C	R	C	R
Medium, . .	C	C	C	C
Strong, . . .	R	C	C	R

Note that contraction is readily obtained at M of a weak ascending current, but not with a strong one. This must be accounted for. Granting that the make contraction is kathodic and the break anodic, there must be some important changes taking place at the poles to account for the result.

That the passage of a constant current along a nerve affects its excitability and conductivity—sets up electrotonus—is dealt with in Lesson XLIX.

That changes occur at the poles is readily shown thus :— Arrange the experiment as for Pflüger's law, but set up a coil with metal electrodes for single, *not* maximal, induction shocks,

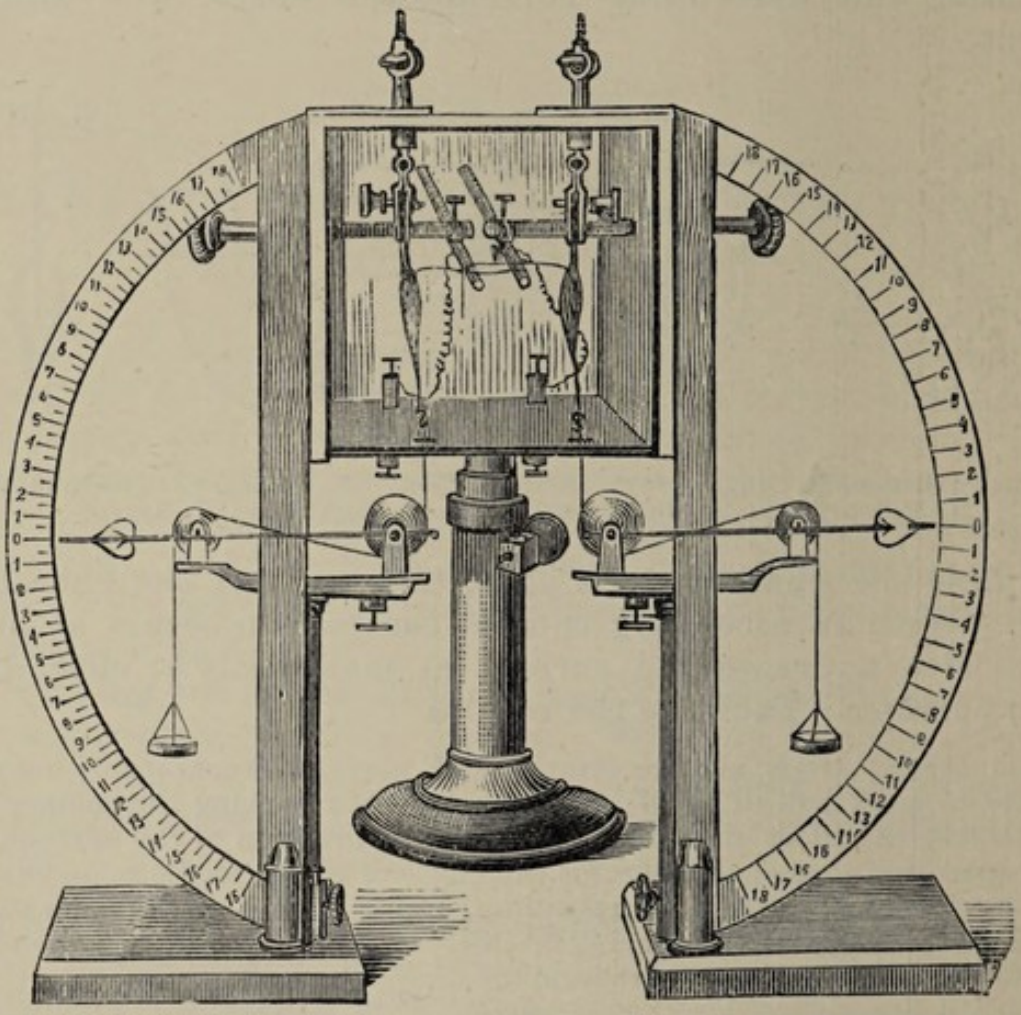


Fig. 215.—Hering's double myograph, made by Rothe of Leipzig.

and place the electrodes on the nerve between the N.P. electrodes and the muscle (Lesson XLIX.). Make the constant current in the nerve (*i.e.*, what is now the polarising current) descending—*i.e.*, kathode next the muscle. Record a break twitch on a stationary drum—(1) with no constant current passing, (2) during passage, (3) after passage of a current. The second twitch will

be the highest—*i.e.*, the excitability of the nerve is increased in the region of the kathode. Change commutator, so that the anode is next the muscle, and do as above. Stimulation of the anodal region gives a diminished or no response, therefore the + has lowered the excitability in the region of its influence.

2. Or arrange the experiment, using fig. 215 to make the results visible. This apparatus is excellent for demonstration purposes.

Polar stimulation of skeletal muscle (**Stimulation by constant current**).—We have already seen (Lesson XXXI.) that a constant current may cause contraction at M or B, or both, but apparently no effect, as a rule, during its passage. The effects produced at M and B differ not only in extent, but the contractions start from different poles. At M of current the contraction starts from the kathode—*i.e.*, *M. contr. is kathodic*—while at B it starts from the anode—*i.e.*, *B. contr. is anodic.* The contraction passes over the muscle from the point stimulated.

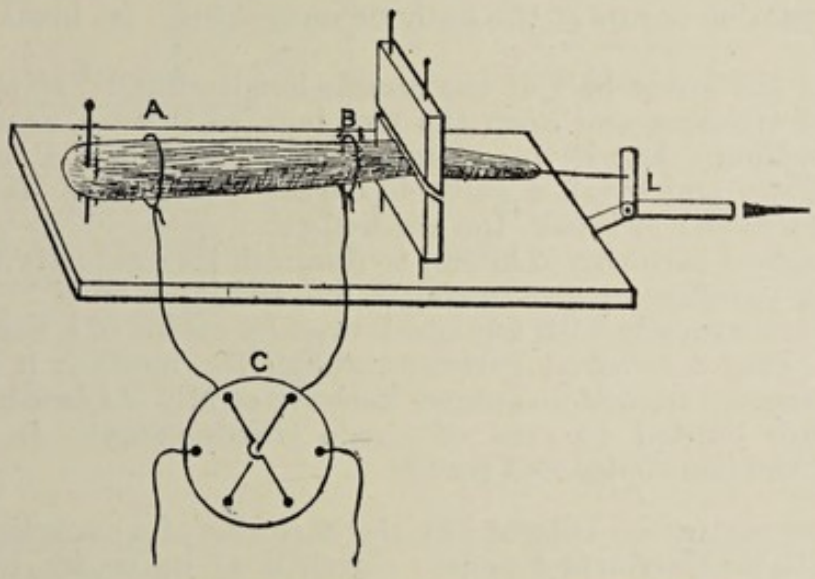


Fig. 216.—Apparatus for polar stimulation of sartorius, fixed lightly near one end with a cork clamp. *L*, Lever; *A* and *B*, Thread electrodes from commutator, *C*, with cross bars.

Constant current.—*In muscle and nerve stimulation occurs only at the kathode when the current is made* (*closed*), *and at the anode when it is broken* (*opened*)—(*von Bezold*, 1861.) This is most readily seen in fatigued muscles.

Instead of B - and + we may speak of the points at which a current enters or leaves a muscle or nerve. It is better to regard the current in the latter way, because the entrance and exit then correspond to the true physiological anode and kathode (p. 333).

3. Fix a curarised sartorius as in fig. 216 (Lesson XXI., 6). Arrange the threads of N.P. electrodes at A and B and connected with a commutator, C, with cross-bars, coupled up with a key and a constant current which gives response of muscle at M and B in either direction, a signal to indicate the moment of stimulation being in the circuit. Record contraction, moment of stimulation, and time-curve. A contraction starting at A will take longer to traverse the muscle than one starting at B. The effects are best obtained in a fatigued muscle. Arrange the commutator so that the current enters at B—B, anodic—and take two contractions, M and B. Reverse commutator, current will enter at A—A is anodic. Take two tracings, one at M and the other at B. Four curves are thus obtained. Measure L.P. and note differences.

The L.P. obtained at M with B anodic (ascending) and at B with A anodic (descending) are practically equal, but longer than those at B with B anodic (descending) and at M with A anodic (ascending). The latter two curves are also nearly equal. Hence contraction at make with an ascending current from A, and at B with a descending current—*i.e.*, in both cases from the kathode. The reverse is the case with the anodes.

4. **Engelmann's experiment.**—(*a*) Suspend vertically a *curarised* sartorius of a frog, and pass a constant current transversely across its pelvic end through its upper extremity by N.P. electrodes. On making the current, the free end of the muscle moves towards the side of the kathode, because contraction occurs at the kathode on making. At break it inclines to the anode.

(*b*) Slit up the lower half of the muscle longitudinally, so that it looks like a pair of trousers, and keep the two legs, as it were, asunder by an insulating medium. Lay it on a glass plate, and apply a N.P. electrode to each leg. Make and break a galvanic current. At make, the kathodic half alone contracts; at break, the anodic half.

Cool a curarised sartorius (6 hours) to diminish its conductivity, or place the muscle on paraffined paper resting on ice, and lay it on N.P. electrodes, and mark it transversely with fine black lines by means of a hair dipped in Indian ink. Pass a constant current through the muscle; it shows sustained, prolonged contraction around kathode at M. At break there may be contracture limited to area of anode (*Biedermann*). Is there also inhibition in the non-contracted part?

5. **Another method.**—Dissect out the sartorius of a *curarised* frog, but remove it with its bony attachments; clamp it at its centre, and arrange it either vertically, attaching its ends to two recording levers placed one above it and the other below it, or fix it on a double crank-myograph. Pass thin wires from the battery through the two ends of the muscle; on making the current the lever attached to the kathode rises before the other—*i.e.*, where the current leaves the muscle. On breaking the current the anodic lever rises first, showing that the anodic half contracts before the kathodic half.

6. Or arrange the experiment as shown in fig. 217 by *Rutherford's method*. This figure and the following one are reproduced from drawings given to the author by the late Professor Rutherford. The method embodies that shown before the Physiological Society several years ago. A curarised sartorius is clamped in the middle, and its ends attached by Kronecker's forceps to levers to record on a drum.

The results obtained are shown in fig. 218.

7. J. S. New's apparatus.—"This consists of a small cork pulley on a spindle over which the muscle strip (curarised) is pinned down to the cork supports on either side. The cork support is grooved to receive the pulley and spindle, so that the upper surfaces of the cork support are tangential to the surface of the pulley. The spindle is itself supported by two celluloid plates attached to opposite sides of the cork support. A light lever or mirror is used on the spindle to render the movements more evident. The muscle is stimulated by the pin electrodes which are used to hold it; a Pohl's reverser and Daniell cell with key complete the circuit" (*Journ. of Phys.*, xxiv., 1899).

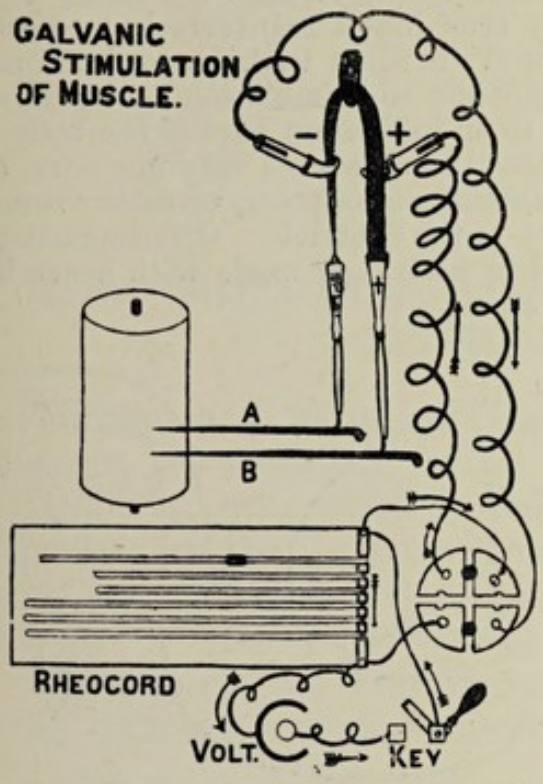


Fig. 217.—Arrangement for polar or galvanic stimulation of muscle (*Rutherford*). C, Clamp; A and B, Levers; K, Kronecker's forceps.

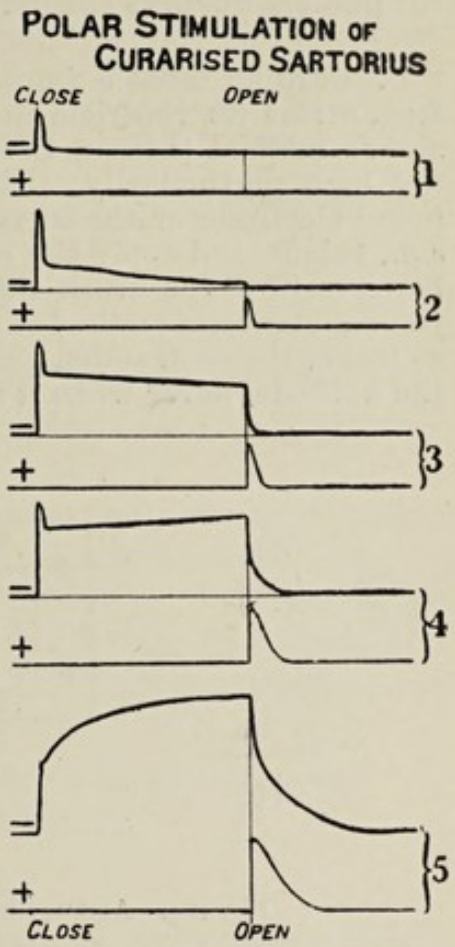


Fig. 218.—Results obtained (*Rutherford*).

8. Physiological anode and kathode.—It is important to notice the difference between the physiological anode and kathode as distinguished from the point at which the electrodes are applied—*i.e.*, the points where the current enters or leaves a tissue. In nerve and muscle with straight parallel fibres the physical and physiological + and − coincide, but it is not so with tissues whose fibres are irregularly disposed.

Remove the rectus abdominis of a frog; lay it on a glass plate, and observe the transverse tendinous intersections across the muscle. Connect the ends of the muscle with N.P. electrodes through a key to a cell. M, the current = contraction on the kathodal side of each muscle near the tendon. At B the thickening is at the anode. Use a hand lens.

Monopolar method.—The stimulating effect of a galvanic current depends on its density, so that if a large indifferent electrode and a small-pointed N.P. electrode are used the current will be diffused at the first point and more concentrated at the latter, so that the "threshold" of stimulation will be reached first at the small electrode. The large "indifferent" electrode may be placed in any part of the body. Use a large metallic electrode covered with cotton cloth or chamois leather dipped in normal saline.

9. Cardiac muscle (monopolar method).—(*a*) Expose the heart of a frog, arrest its ventricle in diastole by crushing it transversely by Bernstein's method (Lesson LIII., 8). The V. is filled with blood, but does not beat rhythmically, although it responds to stimulation. Apply one broad electrode of the current battery to an indifferent part of the body—*e.g.*, thigh—and apply the other fine pencil electrode—a very fine wire, or better still a fine brush electrode—practically a unipolar, or rather *monopolar* punctiform method of stimulation—to the ventricle. On stimulating, at make, the contraction occurs only when contact is made with heart by the kathode, never when it is made by anode.

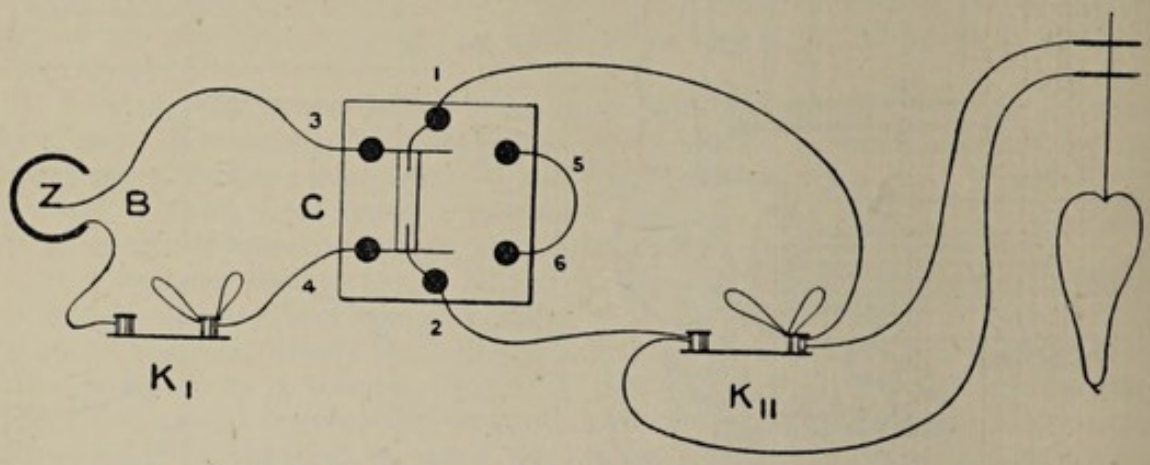


Fig. 219.—Arrangement for showing polarisation of electrodes.

(*b*) Place the "indifferent" electrode over the liver or livers, and the pointed N.P. one on a Stanniused heart. Connect these through a commutator with cross-bars to a rheocord, and the latter through a M and B key to a cell. Arrange the commutator to make the heart electrode +, M and B current; contraction takes place at B only, if it occurs at all. Reverse commutator and make the heart electrode −, M and B current; contraction takes place at M only.

10. Polar stimulation of nerve.—A simple method is that of Jolyet and Sigalas. Prepare a frog after the method of Marianini—*i.e.*, divide the spinal column cord and trunk transversely just above the lumbar plexus, remove viscera, and sever vertically the tissue that unites the two legs, leaving the nerves in connection with the vertebral column. Place a platinum electrode from a constant current under the nerves of the sciatic plexus on each side. Introduce a rheostat and a commutator. It will be found that contraction on closing occurs only at the negative pole, and at break at the positive pole.

11. Polar stimulation of sartorius.—Dip the lower end of an excised sartorius in distilled water for a few minutes. The part immersed becomes shorter and thicker. Dry the muscle and lay it on a glass plate, and apply N.P. electrodes to either end—living and dead. On passing a constant current through the muscle when the dead end is the kathode one obtains a make but no break contraction. The reverse occurs when the part in water rigor is the anode (*Hermann*).

12. Polarisation of electrodes (Lesson XXVII.)—(*a*) A more elegant method is to connect a battery and commutator with a N.M. preparation, as in fig. 219. Pass the current through the nerve for 2 or 3 minutes—*i.e.*, with bridge of C as in fig. 219. Turn bridge to dip into 5 and 6 = contraction. On again raising it, the same result, which can be repeated many times.

LESSON XLIX.

ELECTROTONUS—ELECTROTONIC VARIATION OF EXCITABILITY.

Electrotonus.—When a nerve is traversed by a constant current, its so-called "vital" properties are altered—*i.e.*, its **excitability, conductivity,** and **electro-motivity.** The region affected by the positive pole is said to be in the **anelectrotonic,** and that by the negative in the **kathelectrotonic** condition. Therefore we have to study the—

I. Electrotonic alteration of *excitability* and *conductivity.*
II. Electrotonic alteration of *electro-motivity.*

1. Electrotonic variation of excitability.

A. (*a*) Connect two Daniell or Leclanché cells to a Pohl's commutator *with cross-bars*, introducing a du Bois key to short-

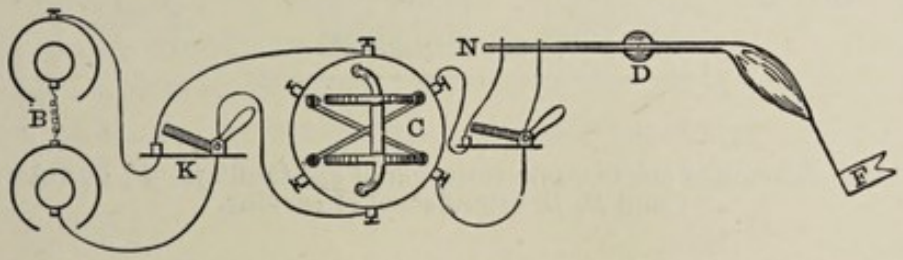


Fig. 220.—Scheme of electrotonic variation of excitability. *D*, Drop of saturated solution of salt on nerve, *N*; *F*, Flag on muscle.

circuit the battery. From two of the binding screws connect wires with two N.P. electrodes, introducing a short-circuiting key in the electrode circuit (fig. 220).

(*b*) Pin a straw flag to the foot of a N.M. preparation, and fix the femur in a clamp. Lay the nerve over the N.P. electrodes —the electrodes being separated from one another about 2 cm. Trace the direction of the current, and make a mark to guide you showing when the current in the nerve is descending or ascending—*i.e.*, whether the + or − pole is next the muscle.

(*c*) Place on the nerve between the electrodes and the muscle a drop of a saturated solution of common salt. In a minute or less the toes begin to twitch, and gradually a more or less complete tetanus sets in. By-and-by the flag is raised and kept in the horizontal position.

(*d*) Turn the commutator, so that the positive pole is next the muscle; the flag sinks—*i.e.*, the excitability of the nerve in the region of the positive pole is so diminished as to "block" the impulse passing to the muscle, showing that the positive pole lowers the excitability.

(*e*) Reverse the commutator—put negative pole next the muscle—the limb becomes tetanic. The negative pole (*kath-electrotonic area*) increases the excitability.

(*f*) The contractions of the muscle may, if desired, be recorded.

2. Another method.—(*a*) Two cells, two pairs of N.P. electrodes, or one pair N.P. electrodes and one pair of platinum electrodes, two du Bois keys, a spring-key, commutator *with cross-bars*. Arrange apparatus (fig. 221). Prepare two pairs of N.P. electrodes for the nerve. If necessary, a rheochord may be introduced in the polarising circuit.

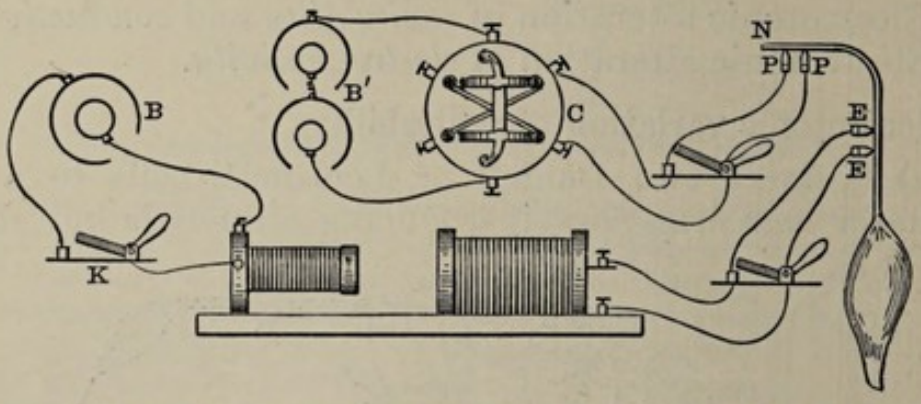


Fig. 221.—Scheme of electrotonic variation of excitability. *P*, *P*, Polarising; and *E*, *E*, Stimulation of current.

(*b*) Connect two cells with a Pohl's commutator *with cross-bars*, C; connect the commutator—a short-circuiting key intervening—to one pair of the N.P. electrodes. This is the "polarising current," P, P.

(*c*) Arrange a coil for tetanising shocks [or single shocks]; use N.P. electrodes or platinum electrodes, and short-circuit the S circuit. This is the "exciting current," E, E.

(*d*) Arrange a N.M. preparation—nerve as long as possible—to record on a drum. Place the nerve on the two pairs of electrodes in the moist chamber, the "polarising" pair being next the cut end of the nerve, P, P, and about 1 centimetre apart. Between the polarising pair and the muscle apply the "exciting" electrodes to the nerve, E, E, as close as possible to one N.P. electrode.

(*e*) With the polarising current short-circuited, push the S from the P coil, and find the minimum distance at which a feeble contraction—weak tetanus—of the muscle is obtained. Push the S coil up until a weak contraction is obtained, and take a tracing. Previously arrange the commutator to send a *descending* current through the nerve. While the muscle is contracting feebly, throw in the descending polarising current; at once the contraction becomes much *stronger*. Reverse the commutator and pass an *ascending* polarising current through the nerve, the contraction ceases.

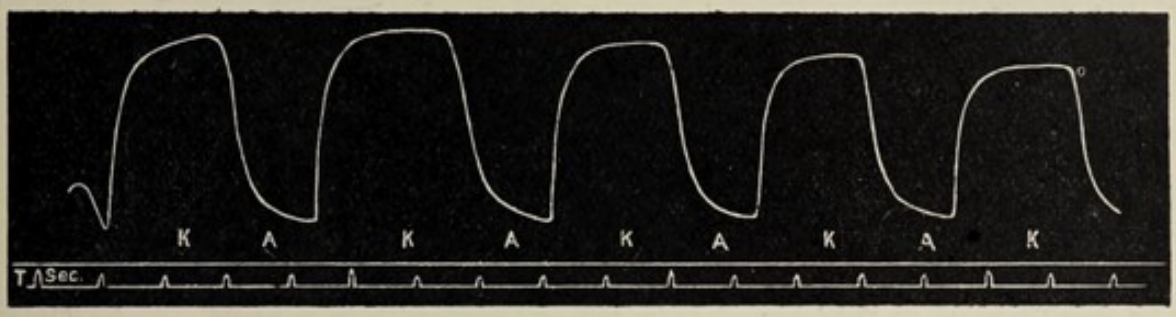


Fig. 222.—Tracing showing effect of anode and kathode on excitability of nerve, the latter stimulated with repeated shocks. *T*, Time in seconds.

(*f*) Repeat the experiment, using Neef's hammer, selecting a strength of stimulus just insufficient to give tetanic response when the + pole of the polarising current is next the muscle. Reverse the commutator, and at once the previously inadequate shocks become adequate and tetanus results (fig. 222), where the effect of + and − poles are shown alternately.

(*g*) Repeat the above experiments, using single break-shocks instead of a break-tetanus.

In the first case, the area influenced by the exciting electrodes was affected by the negative pole—*i.e.*, was in the condition of kathelectrotonus, and the tetanus was increased; therefore, *the kathelectrotonic condition increases the excitability of a nerve.* In the second, the nerve next the exciting electrodes was in the condition of anelectrotonus, and the contractions ceased; therefore, *the anelectrotonic condition diminishes the excitability of a nerve* (fig. 223).

3. **Rheochord—Salt as stimulus.**—A rheochord may be used to graduate the polarising current, salt being used as the stimulus.

(*a*) Arrange two N.P. electrodes in a moist chamber, provided with a recording lever, placing the N.P.'s about 1 cm. apart.

(*b*) Connect the terminals of two cells (arranged in circuit) to the central screws of a Pohl's commutator (with cross-bars, as in fig. 221), placing a

Hg-key in the circuit. Connect the wires, x, y, to the two blocks on the rheochord (fig. 93). By reversing the commutator the current through the rheochord is reversed. Connect one N.P. electrode with one terminal of the rheochord, and the other N.P. with the movable slider, S, of the rheochord.

(*c*) Notice which pole is next the muscle and make a mark to guide you. Make a long N.M. preparation, and arrange the nerve over the electrodes, attaching the muscle to a recording lever (crank).

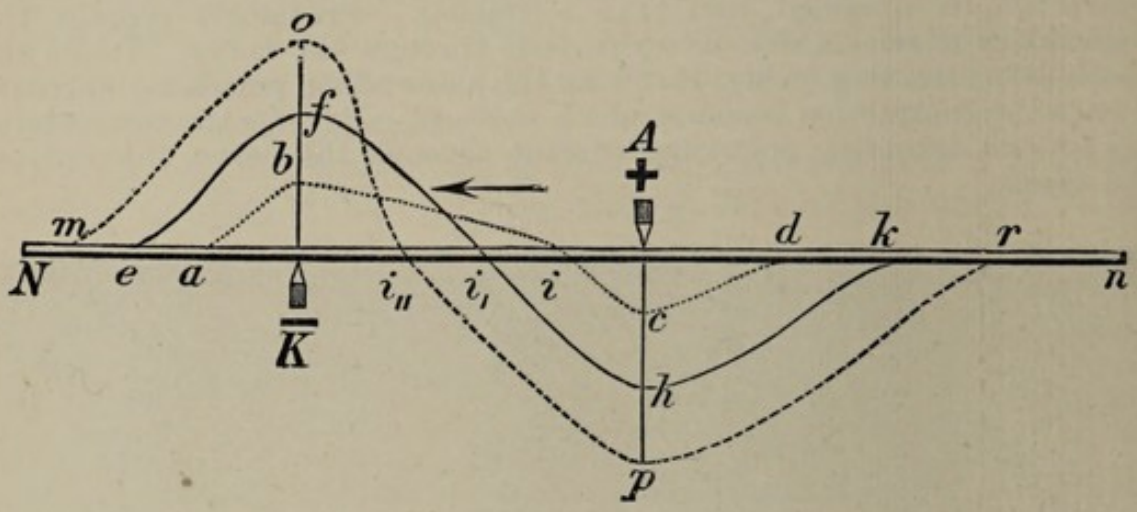


Fig. 223.—Scheme of electrotonic variation of excitability in a nerve. *K*, Kathode; *A*, Anode; *N*, *n*, Nerve. The curve above the line indicates increase, and that below the line decrease of excitability.

(*d*) Begin with the slider, S, close up to zero, and gradually slide it along until, on closing the battery circuit, the muscle responds at make whether the + or − pole is next the muscle—*i.e.*, whether the current is ascending or descending.

(*e*) Break the circuit, place on the nerve near the muscle either a drop of saturated solution of common salt or fine moist crystals of salt. Wait till the salt causes occasional spasmodic movements. Close the key, place the (−) pole next the muscle, at once the limb becomes tetanic owing to the increase of excitability under the influence of the (−) pole (*Kathelectrotonus*). Break the current, the limb becomes quiescent.

(*f*) Open the key, and, after a short time, when the spasms reappear reverse the commutator so that the (+) pole is next the muscle. Close the current, the limb becomes quiescent, due to the fall of excitability under the influence of the (+) pole (*Anelectrotonus*). Break the current, the muscle becomes tetanic. Thus it is shown that the appearance of kath-electrotonus and the disappearance of anelectrotonus are accompanied by increase of excitability, while the disappearance of kathelectrotonus and the appearance of anelectrotonus are accompanied by diminution of excitability.

4. Conductivity is impaired in Intra-Polar Region.—Arrange the experiment as in 3, but place the salt on the nerve as far as possible from the muscle. When the salt causes tetanic spasms, close the current through the electrodes, and, whether this current be ascending or descending, the spasms cease, because the excitatory change is "blocked" in the intra-polar area.

5. Ritter's Tetanus or Opening Tetanus.—(*a*) Connect three cells with N.P. electrodes, short-circuiting with a du Bois key. To the nerve of N.M. preparation apply the electrodes so that the (+) pole is next the muscle—*i.e.*, the current is *ascending* in the nerve. Allow the current to circulate in the nerve for some time (about five minutes), no contraction takes place. Short-circuit, and the muscle becomes tetanic.

(*b*) Divide the nerve between the electrodes, and the tetanus does not cease; but on dividing it between the (+) pole and the muscle the tetanus ceases. Therefore the tetanus is due to some condition at the positive pole —*i.e.*, the stimulation proceeds from the positive pole at break.

(*c*) If the intra-polar current is descending—*i.e.*, kathode next the muscle—when "opening tetanus" appears on breaking the circuit, cut the nerve between the electrodes. Observe, the tetanus ceases—*i.e.*, the anode or source of stimulation is removed.

6. Kathodic stimulus is the more powerful.—(*a*) Let the M and B shocks be made approximately equal by the arrangement shown in fig. 224. In the S circuit place a Pohl's commutator with cross-bars, R. Place one electrode, C, under the sciatic nerve, and the other on a distant part of the body.

(*b*) Suppose C to be the kathode, select a strength of shock—*i.e.*, distance of S from P coil—so that there is response on breaking the primary current. Reverse the commutator so that C becomes the anode. There is no muscular response at break, but it occurs at make, as C is then the kathode.

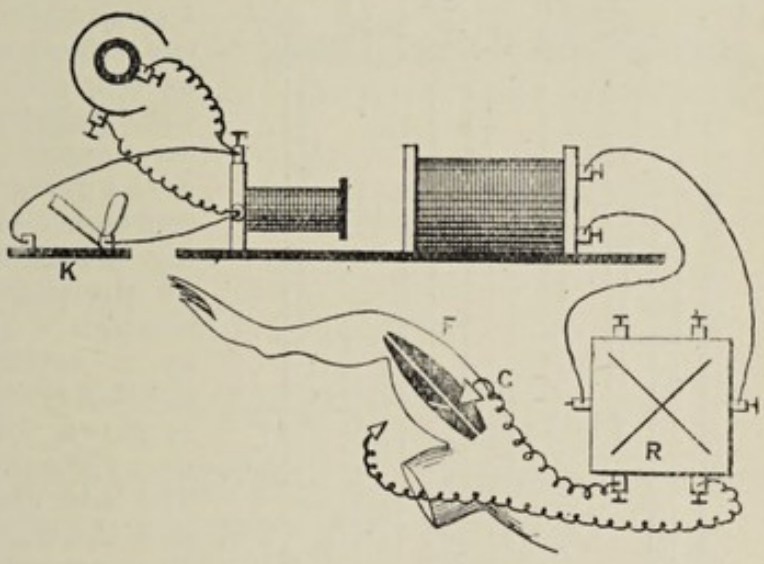


Fig. 224.—Scheme to show that kathodic stimulation is the more powerful. *K*, Key; *R*, Commutator; *F*, Frog's leg; *C*, One electrode.

7. Electrotonic change in conductivity.—Arrange the experiment as in Fig. 221, but place the polarising electrodes next the muscle, and the exciting electrodes near the cut end of the nerve. Tetanise the muscle through its nerve, throw in polarising current either ascending or descending, the tetanus ceases. The polarising current has caused a "block" by lowering the conductivity of the nerve in the intra-polar region.

8. Electrotonic variation of electro-motivity.

(*a*) Arrange a long nerve on N.P. electrodes, as for determining its demarcation-current. Place the free end of the nerve on a pair of N.P. electrodes—the polarising current—arranged as in Lesson XLIX., so that the current can be made ascending or descending.

(*b*) Take the deflection of the galvanometer or demarcation-current when the polarising current is shut off. Throw in a descending polarising current, the spot of light travels towards zero. Reverse the commutator and throw in an ascending current, the spot of light shows a greater positive variation than before. From this we conclude that *kathelectrotonus diminishes the electro-motivity, while anelectrotonus increases it.* In the extra-polar kathodic

region an electrotonic current appears when the polarising current is closed. It has the same direction as the polarising current. In the anodic region the direction is also that of the polarising current; but the electrotonic current is stronger than the kathodic current. If a demarcation-current exists already, the electrotonic currents are superposed on it.

9. Hermann's Schema.

Extra-polar electrotonic currents.—This model ("*Kernleiter*") is merely an apparatus for imitating the polarisation phenomena in medullated nerve. It consists of a glass tube 5 to 10 mm. in diameter, with several vertical branches. Through the horizontal tube passes a platinum wire stretched between two corks at either end. The system is filled with saturated solution of $ZnSO_4$. Two of the vertical tubes represent the polarising current, and two the extra-polar current. Two amalgamated zinc rods—inserted in the corresponding vertical tubes—connected with a Daniell cell represent the polarising current. A similar arrangement of electrodes connected with a galvanometer (*e.g.*, d'Arsonval's) represents the arrangement for studying the extra-polar currents produced by the passage of a galvanic current through a platinum wire surrounded by a saturated solution of $ZnSO_4$. Similar results are obtained to those in nerve, but, as Waller points out, they are not suppressed by anæsthetics. The tube may be filled with 0·6 NaCl, and the wire may be platinum or zinc. The currents obtained are polarisation currents, for if a Zn wire be used with $ZnSO_4$ where no polarisation obtains, there is no extra-polar current.

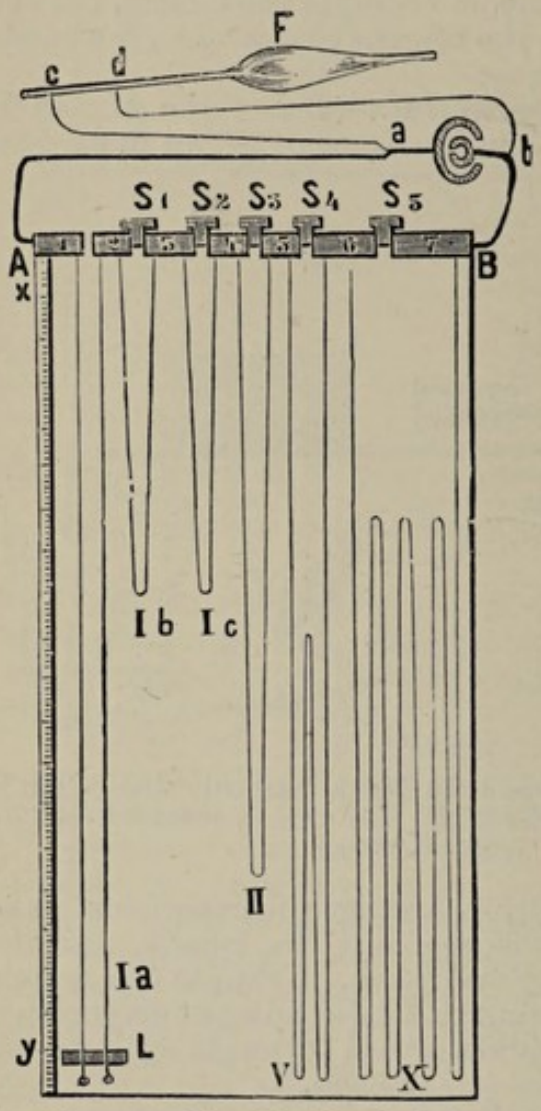


Fig. 225.—Rheochord of du Bois-Reymond.

10. Rheochord of du Bois-Reymond may be used to vary the amount of a *constant current* applied to a muscle or nerve (fig. 225). It consists of an oblong shallow box, with German-silver wire—of varying length, and whose resistance is accurately graduated—stretched in relation to it. At one end are a series of brass blocks disconnected with each other above, but connected below by a German-silver wire passing round a pin. These blocks, however, may be connected directly by brass plugs, S_1S_2 . . S_5. From the blocks 1 and 2 two platinum wires pass from A to the opposite end of the box, Y, where they are insulated. Between the wires is a "slider," L, consisting of two brass cups containing mercury, which slide along the wires.

In using it, connect a Daniell's cell with the binding screws A and B, and to the same screws attach the wires of the electrodes over which the

nerve, *c d*, of the muscle, F, is laid. We have two circuits, *a c d b* and *a* A B *b* ; the wires of the rheochord are introduced in the latter.

Push up the slider with its cups, L, until it touches the two brass plates 1 and 2, and insert all the plugs, S_1-S_5, in their places, thus making the several blocks of brass practically one block. In this position, the zero of the instrument, the resistance of the rheochord circuit is so small compared with that including the nerve, that practically all the electricity passes through the former and none through the latter.

Move the slider away from A, where by resistance is thrown into the rheochord circuit, according to the length of the platinum wires thus introduced. Thus a certain fraction of the current is sent through the electrode circuit. If the plug S_1 be taken out more resistance is introduced, that due to the German-silver wire, I *b*, and, therefore, a certain amount of the current is made to pass through the electrode circuit. By taking out plug after plug more and more resistance is thrown into the rheochord circuit. The plugs are numbered, and the number and length of the German-silver wires are so selected in making the instrument, that the resistances represented by the several plugs when removed are all multiples of the resistance in the platinum wires on which the slider moves. Proceed taking out plug after plug, and note the result. The result and explanation are referred to in Lesson XLVIII.

LESSON L.

VELOCITY OF NERVE-IMPULSE—CONDUCTIVITY AND EXCITABILITY OF NERVE.

1. Velocity of nerve-impulse in motor nerve of frog.

The rate of propagation of a nerve-impulse or excitatory change may be estimated by the pendulum or spring-myograph, or by using a recording surface moving with sufficient velocity. With the spring-myograph it is necessary to use such a coiled spring as will cause the glass plate to move with sufficient rapidity to give an interval long enough for the estimation of the latent period.

(*a*) Arrange the spring-myograph (fig. 226)—*i.e.*, a coil for single break-shocks—with the trigger-key of the myograph, 1, 2, in the P circuit; in the S circuit (which should be short-circuited, not represented in the diagram) place a Pohl's commutator *without cross-bars*, C. Two pairs of wires from the commutator pass to two pairs of electrodes (*a*, *b*), arranged on a bar in the moist chamber. Measure the distance between the electrodes.

(*b*) Use a N.M. preparation with a long nerve, N, clamp the femur, *f*, attach the tendon, *m*, to a writing-lever, and lay the nerve over the electrodes, the distance between them being known. It is well to *cool* the nerve by iced normal saline; the velocity of the impulse diminishes.

(*c*) Arrange the smoked glass plate, adjust the lever to mark on the glass, close trigger-key in P circuit, and open the secondary. Arrange commutator so that the stimulus will be sent through the electrodes next the muscle, *a*. Press the thumb plate; the glass plate shoots across. The tooth, 3, breaks the P circuit, and a curve is inscribed on the plate.

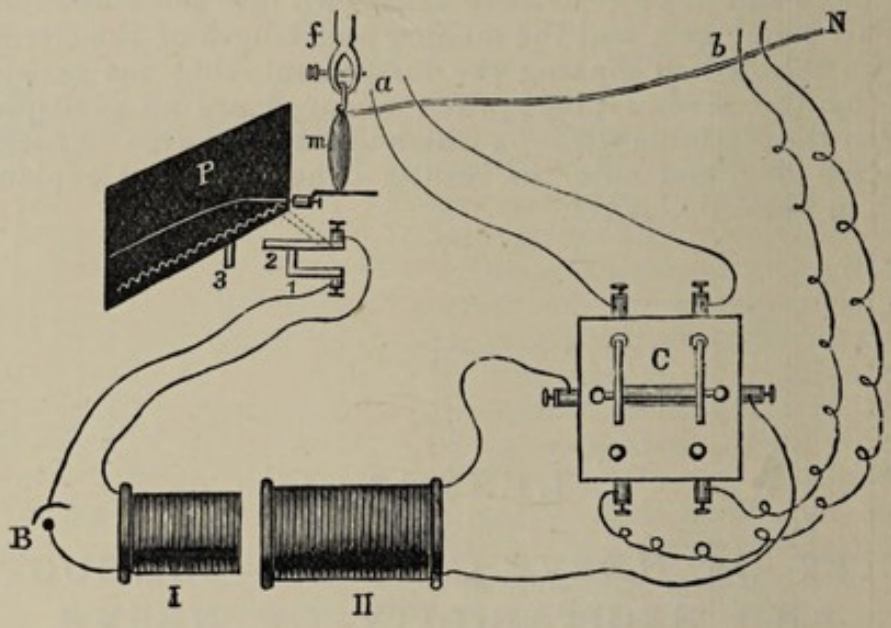


Fig. 226.—Scheme for estimating the velocity of nerve-impulse.

(*d*) Short-circuit again, re-adjust glass plate, close trigger-key, and reverse commutator. This time the stimulus will pass through the electrodes away from the muscle, *b*. Open the S circuit, and shoot the glass plate; another curve is recorded, this time a *little later* than the first one. The contraction also will be slightly higher.

(*e*) Re-adjust glass plate, close the trigger-key, open the S circuit, and shoot the plate. This makes the abscissa.

(*f*) Re-adjust glass plate, close trigger-key, and bring the tooth of the glass plate, 3, just to touch trigger-key; raise the writing-lever to make a mark to indicate the moment when the stimulus was thrown into both points of the nerve.

(*g*) Remove the moist chamber, push up the glass plate, close the trigger-key, and record time by a tuning-fork vibrating 250 D.V. per second. Fix and study the tracing. Draw ordinates

from the beginning of the curves obtained by the stimulation of *a* and *b* respectively, measure the time between them from the time-curve (this gives the time the impulse took to travel from *b* to *a*), and calculate the velocity from the data obtained.

Example.—Suppose the length of nerve between the electrodes to be 4 cm., and the time required for the impulse to travel from *b* to *a* to be $\frac{1}{750}$ second. Then we have 4 : 100 : $\frac{1}{750}''$: $\frac{1}{30}''$, or 30 metres (about 98 feet) per second, as the velocity of nerve-energy along a nerve.

2. With the **pendulum-myograph** practically the same arrangements are necessary. If it be desired to test the effect of **heat** or **cold** on the rapidity of propagation, the nerve must be laid on platinum electrodes fixed in a chamber made of ebonite. The top of the chamber is covered with a lacquered copper plate on which the nerve rests. Through the chamber water at different temperatures can be passed, and the effect on the rate of propagation observed.

3. **Velocity of motor nerve-impulse in man.**

(*a*) *Pendulum-myograph.*—Connect two Leclanché cells with the P circuit of an induction coil and interpose in the circuit the trigger-key of the myograph, which the pendulum opens in swinging past. Short-circuit the S circuit, and to the short-circuiting key attach a pair of rheophores moistened with strong solution of salt.

(*b*) Arrange Marey's "*pince myographique*" (fig. 194*a*) to compress the adductor muscles of the thumb when the latter is in the abducted position. Connect the "pince" by means of an india-rubber tube with a Marey's tambour (fig. 193) arranged to record on the myograph.

(*c*) The nerve supplying the adductor muscles of the thumb must be stimulated first near the ball of the thumb, and secondly at the bend of the elbow. Contraction takes place sooner from the former than from the latter position. Suppose the right thumb to be used, apply one rheophore to the right side of the chest, and the other just over the ball of the thumb. Allow the pendulum to swing. Take a tracing. Replace pendulum, short-circuit the S circuit, and close trigger-key.

(*d*) Open the S circuit. Apply the arm rheophore to the median nerve at the bend of the elbow, and record contraction.

(*e*) Record a base-line, and mark the point of stimulation on the myograph plate. Make a time-tracing under the two muscle curves.

(*f*) Measure the distance between (i.) the two arm electrodes; (ii.) the beginning of the two curves; (iii.) note the time value of (ii.) as indicated by the time curve; and from these data calculate the time the nervous impulse took to travel from the elbow to the nerve supplying the muscles of the ball of the thumb.

4. Conductivity *v.* Excitability (Grünhagen's experiment).

(*a*) Pass the nerve of a N.M. preparation through a glass tube (fig. 227), sealing the ends with clay, but not compressing the nerve. The tube is supplied with an inlet and outlet, to which elastic tubes can be attached and through which vapours or gases can be passed, and also with electrodes so that the nerve can be stimulated within or outside the tube. Use a Pohl's commutator without cross-bars for this purpose.

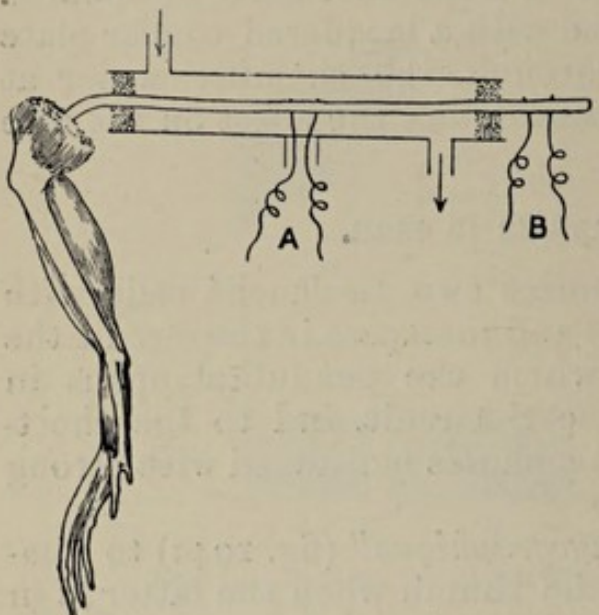


Fig. 227.—Grünhagen's experiment on Conductivity *v.* Excitability.

(*b*) Pass CO_2 from a Kipp's apparatus through the tube; on stimulating the nerve at A with repeated shocks, there is no response, but on stimulating at B there is. Find a strength of stimulus which just excites the nerve at A and B. On passing CO_2 A no longer responds to this stimulus, but requires a stronger stimulus, or it may not respond at all. It would seem that the excitatory change set up at B is propagated through A, although its excitability is very feeble or nil. It thus seems to conduct, even though it is inexcitable to stimuli applied to it directly.

(*c*) Blow away the CO_2, the nerve recovers its excitability or irritability. On passing the vapour of alcohol the conductivity appears to vanish before the excitability. It is better to suck the vapour through by means of any form of exhaust pump. The alcohol itself must not touch the nerve. Stimulate at A and B as before. After a time stimulation of B fails, while A causes response. Thus the conductivity seems impaired, while the irritability is retained. The results, however, may be capable of a different interpretation. (Gad, *du Bois-Reymond's*

Archiv., 1888, 395, and 1889, 350; Piotrowski, "Trennung d. Reizbark. v. Leitungsfäh. d. Nerven," *ibid.*, 1893, 205.)

(*d*) Apply cold to a nerve, lay the nerve over a glass tube through which cold water is flowing. Cold, like CO_2, abolishes or diminishes the excitability but not the conductivity.

(*e*) **Ether.**—An elegant method devised by Gotch consists in exciting the nerve by single induction shocks alternately within the chamber (fig. 227), and also central to or above the ether chamber, the contractions being recorded simply as ordinates on a slow-moving drum. Excitability, as tested by electrodes within the chamber, disappears before loss of conductivity. On blowing away the ether vapour, the excitability of nerve within the chamber re-appears. Re-apply ether vapour until both excitability and conductivity disappear. Blow away ether, conductivity re-appears—*i.e.*, proved by stimulation of nerve inside and above ether chamber, and, later, the excitability of the nerve within the tube returns.

(*f*) *Chloroform vapour* rapidly abolishes both excitability and conductivity, and the result may be final. It is a far more potent poison for nerve tissue than ether (*Waller*).

5. **Double conduction in nerve** (*Kühne's experiment on sartorius*)—*i.e.*, the same nerve may conduct impulses both centrifugally and centripetally. Kühne showed that the sartorius at its ends was devoid of nerve fibres. Not only does the nerve on entering the muscle divide, as shown in fig. 228, but each axis cylinder divides, one branch going upwards and the other downwards.

(*a*) Remove the sartorius with great care, slit it up in the middle line for one-third of its length, beginning at the broad end. Suspend it vertically by its bony attachment in a clamp.

(*b*) Snip the right segment at its lower end. Only the right half contracts; snip again a little higher—same result. Snip higher in the region of nerves, both segments twitch ("Zwei-Zipfel Versuch," 1860).



Fig. 228.—Distribution of nerve fibres in sartorius of frog (*Mays*).

6. **Double conduction in nerve** (*Kühne's gracilis experiment*).—The gracilis is divided into a larger and smaller portion, L, by a tendinous intersection, K, running across it (fig. 229). The nerve, N, enters at the hilum in the larger half, and bifurcates, giving a branch, *k*, to the smaller portion, and another to the larger portion of the muscle, but neither branch reaches quite to the end of either half of the muscle.

(*a*) Remove the gracilis (rectus internus major and minor) (*Ecker*). The method of removing semi-membranosus and gracilis together has already been described (Lesson XXIX., 5). Place a pithed and skinned frog on its back. In order to see the outline of the thigh muscles better, moisten them with blood. The sartorius by its inner margin lies in relation with the gracilis near its lower attachment, the gracilis itself lying on the ventral surface of the inner part of the thigh, having its origin at the symphysis, and its insertion at the tibia. The small part—minor—is attached to the

skin and is usually torn through when the skin is removed. By its other margin it is in contact with the semi-membranosus. The muscle is detached from below upwards. Its tendinous inscription or intersection is readily visible on a black surface.

(*b*) Cut it (fig. 229), avoid injuring nerves, so that only the nerve twig (*k*) connects the larger and smaller halves, and in one tongue, Z, terminates a nerve. After excision lay it on a glass plate with a *black* background, to see clearly the inscription and the course of the nerves.

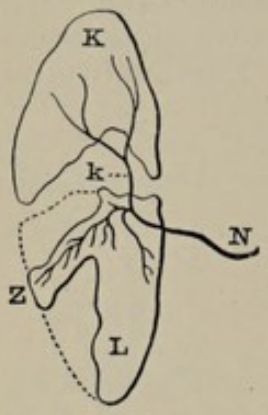


Fig. 229.—Kühne's experiment on the Gracilis.

(*c*) Stimulate the tongue, Z, with fine electrodes, contraction occurs in both L and K. This, according to Kühne, is due to centripetal conduction in a motor nerve. This experiment is adduced by him as proof of double conduction in nerve fibres. Mays finds that the nerve fibre divides and supplies both halves of the muscle.

(*d*) If the muscle be exposed in a curarised frog, on stimulating either half of the muscle with repeated shocks, only that half responds, as the inscription blocks the passage of the muscle-wave.

(*e*) If an uncurarised muscle is used, stimulation of the muscle near its ends causes response only in its own half. Why? Because there are no nerves there; but stimulation near the inscription causes response in both halves. Why? Because they are excited through their nerves, as shown definitely by (*c*).

LESSON LI.

CONDITIONS AFFECTING EXCITABILITY OF NERVE.

1. Unequal excitability of a motor nerve—Apparatus.—Cell, two keys, wires, commutator, coil, either for single or faradic shocks, two pairs of electrodes.

(*a*) Arrange as in Fig. 230. Dissect the whole sciatic nerve with the leg attached, and lay the nerve on two pairs of electrodes, A and B, one near the muscle, and the other as far away from it as possible. Two pairs of wires thrust through a cork do quite well.

(*b*) Stimulate at A to get a minimal contraction—*i.e.*, determine the *threshold* value at A. Reverse the commutator, and stimulate at B, a stronger contraction is obtained. The excitability of a

nerve is greater farther from a muscle or nearer the centre. Instead of using single shocks, repeated shocks may be used.

2. Temperature on excitability of nerve.

(*a*) Fix an N.M. preparation on a crank myograph, so as to record on a revolving drum provided with an automatic break-key in the P circuit of a coil arranged to give feeble break-shocks.

(*b*) Bring a test tube filled with water at 80° to 90° C. near the nerve, where the electrodes lie on it. The contractions rapidly increase, and may become maximal.

(*c*) Remove the tube, the contractions diminish—*i.e.*, the excitability falls.

(*d*) Similar results may be obtained by the direct application of warm normal saline to a nerve.

(For other kinds of nerve fibres see "Effects of stimulation and of changes in temperature upon irritability and conductivity of nerve fibres," by Howell and others, *Journ. of Phys.*, xvi., 298; Gotch and Macdonald, *Journ. of Phys.*, xii., xx., also § 8.)

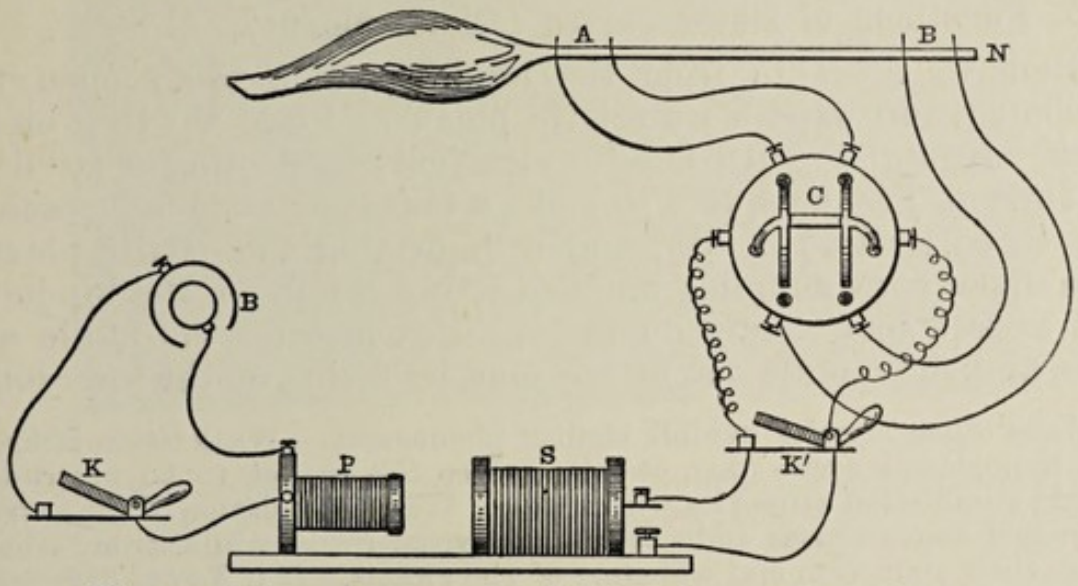


Fig. 230.—Scheme for unequal excitability of nerve (single shocks).

3. Salt increases excitability of nerve.

(*a*) Arrange an N.M. preparation as in 2, and determine the distance of the S from the P coil to obtain a minimal stimulus—*i.e.*, response. Apply a drop of saturated solution of common salt to the nerve between the electrodes and the muscle. Almost at once the excitability of the nerve is increased, as shown by the height of the contraction.

(*b*) After several minutes the muscles begin to twitch. The excitability is increased, but before muscular response to chemical stimulation is elicited a considerable time elapses.

4. Effect of section on excitability of a nerve.

(*a*) Arrange a coil for single shocks, expose the sciatic nerve, and under it, near its central end, place insulated electrodes. Ascertain the distance of the S from the P coil at which the break-shock is just too weak to cause the muscles to respond (sub-minimal).

(*b*) With scissors divide the nerve on the central side of the electrodes. The stimulus (previously sub-minimal) now causes a strong contraction.

(*c*) Ascertain the distance (perhaps several cms.) to which the S coil must be pushed away from the P in order to obtain again a sub-minimal stimulus. The condition of increased excitability lasts for some time.

5. Excitability of flexors and extensors (*Rollett*).

Arrange a coil for repeated shocks. Expose either the sciatic nerve or the sciatic plexus. With a weak current flexion of the leg is obtained; on pushing up the S coil, extension.

6. Functions of motor nerves (sciatic plexus).

Remove the skin from the hind-legs of a frog; open the abdomen, and expose its sciatic plexus. Place the frog on its back. Stimulate with faradic electricity—selecting a strength of current just adequate to yield a muscular response—each of the three cords, 7th, 8th, and 9th, forming the sciatic plexus. The upper cord supplies muscles acting chiefly on the hip-joint, the lowest acts chiefly on the muscles moving the ankle and toes, and the middle one on the muscles acting on the knee-joint.

Many other muscles exhibit similar phenomena. Weak stimulation of the hypoglossal nerve (dog, rabbit) causes the tongue to be protruded, strong stimulation causes its withdrawal. Weak stimulation of the current laryngeal nerve causes adduction of the vocal cords, while strong stimuli cause their abduction and widening of the glottis. Is it due to differences in excitability of nerve fibre, or to variation in the structure—relative amount of sarcoplasm, &c.—in the muscles themselves?

7. Nerve impulse remains in fibre in which it originates (Uni-polar method).—(*a*) Expose the sacral plexus, and denude the hind limbs of skin. Connect to S coil arranged for tetanising currents one wire soldered to a needle, the latter enclosed in a glass tube to act as a "point electrode." Connect the other wire of the S coil to a gas pipe.

(*b*) Touch the sacral nerves here and there with the needle electrode, and observe local contraction in corresponding fibres of the sartorius or other muscle.

(*c*) Stimulate local fibres of sartorius directly, only those fibres in contact with needle contract.

8. Influence of localised cold upon excitability (*Gotch*).—A. *Upon nerve.* The influence of changes in temperature upon excitability can be investi-

gated by arranging in the moist chamber a glass tube placed at right angles to the nerve of a N.M. preparation, and situated so that a small portion of the nerve shall lie athwart the tube. Through the tube water at temperatures varied at will from 10° to 30° C. is allowed to flow. Arrange two beakers on a suitable support—or place two glass funnels in a double filter stand—with elastic tubing and clips, to deliver a flow of hot or cold water through the thin glass tube on which the nerve is placed (see also p. 348).

The alteration in temperature *causes a marked alteration in the electrical resistance of the tissue*, this being lowered by warmth and raised by cold ; in order to get rid of this purely physical change, it is essential that a large resistance should be introduced into the exciting circuit. This is most simply done by using N.P. electrodes with threads attached to the ends of the electrodes kept moist by normal saline. The threads are now arranged so as to touch the nerve where it lies on the tube, one thread being placed so that the contact shall be on the edge of the cooling tube nearest the muscle. The simplest method of exciting the nerve is by means of a weak galvanic current. For this purpose the rheochord is used and a weak current employed of such direction that it shall descend the nerve and thus excite this at the cathodic contact on the distal edge of the glass tube.

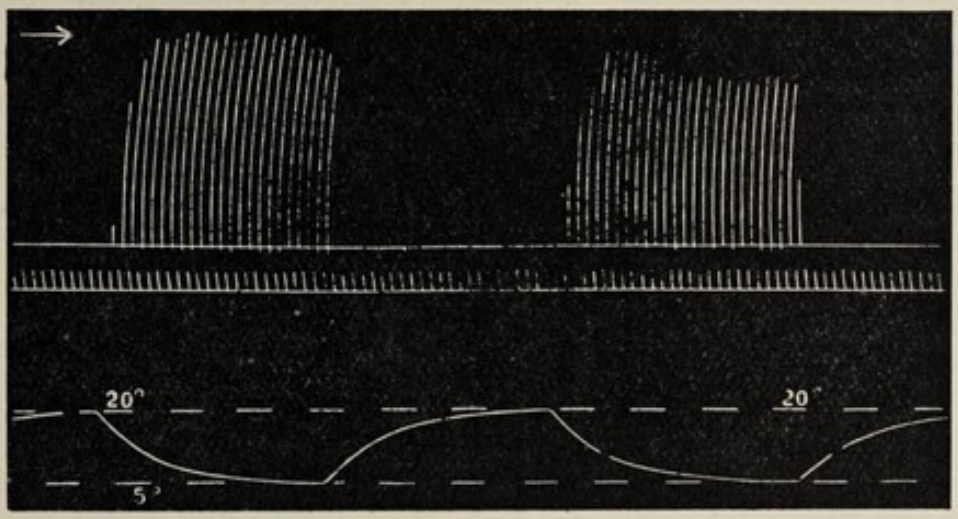


Fig. 231.—Effect of local alteration of T° upon nerve excitation by galvanic currents 100,000 ohms in exciting circuit. Galvanic current descending nerve with kathodal seat of excitation on temperature tube. Current closed every 10″ for 0·5″. Time relations and T° change indicator (*Gotch*).

In order to ensure that the galvanic current is always of the same duration, it is desirable to close the current by an automatic arrangement—either a revolving drum carrying a striker which shall at each revolution strike a stretched wire, or a metronome ; but the influence of the temperature alteration may be obtained without this arrangement, the closure being effected by a Pohl's reverser without cross lines as a double make mercurial key worked by the hand.

The N.M. preparation having been made and the muscle attached to an appropriate lever, so as to record its contraction upon a very slowly moving surface, an intensity of surface is ascertained, which, with the nerve at the normal temperature of the room, is only just adequate to evoke a very weak minimal muscular response whenever the circuit is closed.

The temperature of the nerve is now raised by allowing water at 30° C.



Fig. 232.—Effects of ether on nerves (*Gotch*).

to pass through the tube, when the response will disappear; the temperature is now lowered by allowing water at 10° C. or less to flow—the response is now very marked. *Localised cold* thus *increases the excitability of nerve* to a *galvanic stimulus* (fig. 231). Similar effects can be obtained with the condenser discharge, with mechanical and with chemical stimuli. If the induction current is used instead of the galvanic current, a reverse effect is obtained, the N.M. preparation responding better when the excited nerve is at 30° C.; and this favourable influence of warmth persists even when a very large external resistance is introduced into the circuit.

B. *Upon muscle.*

The sartorius muscle of the frog is used for the experiment, the threads of the exciting electrodes being placed upon the broad "nerveless" pelvic end of the muscle under which the tube of the cooling arrangement is fixed. It is then found that the muscle responds better when cooled to every form of stimulus applied to the cooled region, including the induction current. If the electrodes be shifted to the "nerved" portion of muscle, the response, being indirect, is disfavoured by cold when the induction current is used.—(*Communicated by Professor Gotch.*) See also Gotch and Macdonald, "Temperature and Excitability," *Journal of Phys.*, xii., xx., 247, 1896.

9. **Ether on nerve.**—Arrange a N.M. preparation in a moist chamber to record on a very slow drum. With an automatic break apply single B shocks to the central end of the nerve. Lay middle portion of nerve in a small trough made with china clay covered with a cover slip, and provided with an inlet and outlet tube, the former connected to a vessel containing dilute ether and supplied with the bellows of a spray producer.

Record several contractions, and then subject the part of the nerve in the trough to the action of ether vapour; gradually the contractions diminish until they finally cease. Remove ether

and the nerve recovers. The nerve impulses are completely blocked by the ether.

10. Influence of length of nerve on excitation (response) by voltaic current (*Gotch*).—A galvanic current causes a greater response the larger the stretch of nerve it flows through.

In a moist chamber a frog's sciatic nerve with segment of spinal column prepared from origin to termination, with muscle attached, and arranged to record, is placed on two N.P. electrodes—one (A) near the spinal column, the other (B) near the recording muscle. Adjust the nerve on A by transfixing the spinal column with a pin, which also fixes the preparation on the plate of the myograph. In the middle, between the two electrodes, on a cover glass, is placed a wedge of kaolin, C. Between the upper electrode, A, and C is stretched a piece of sciatic nerve from the other leg of the frog. The nerve is in air between the contacts.

Arrange that the muscle shall record. Connect the N.P. electrodes A and B through a rheochord with a Daniell's cell, a Hg-key intervening.

Call the position of the nerve as described (I.)—*i.e.*, with long intra-polar region, the nerve resting on A and B. The positions are easily altered by moving the pin transfixing the spinal column so that the nerve of the N.M. preparation is off A and on C.

In position I. (with long intra-polar region) M excitation by descending galvanic current (or undried current) is more effective than with II.

The electrotonic changes at B are far more pronounced (*Gotch*).

11. Influence of length of nerve on response.— Dissect out two N.M. preparations, and lay the nerve of the one over the nerve of the other, close to the origin of the branches for the thigh. Two N.P. electrodes are applied to the nerves, one immediately below the crossing, and the other electrode to the other nerve much lower down. The nerves are to be stimulated by tetanising shocks. On pushing up the secondary coil, that muscle contracts first which is connected with the longer nerve, although intensity and density of the current are the same in both nerves (*Hermann*).

LESSON LII.

SMOOTH MUSCLE—CILIARY MOTION—CHEMOTAXIS—GALVANOTAXIS.

A. Smooth muscle.—The differences between this and skeletal muscle, although at first sight very marked, are in most cases differences in degree only. All the phases of its activity are more sluggish. The latest period may be 0·2 to 1·0 sec., and the contraction may last from 0·5 to 3 mins. It is far less dependent on the central nervous system, and often exhibits rhythmical contractions, especially when subjected to tension.

While nerves proceed direct to the cord from skeletal muscle, smooth muscle is usually supplied with non-medullated fibres, and there is a nerve-cell station between the cord and the smooth muscle. There are often ganglion cells and nerve plexuses interwoven amongst its fibres.

Owing to its slow reaction, stimuli of long duration are more effective in exciting it than rapid prompt ones. The make slow induction shock is more effective than the rapid short break, while the M or B of a constant current causes greater response than either. The make of a constant current is more effective than the break; the M excitation starts from the kathode, and the break excitation from the anode, just as in voluntary muscle.

Double innervation.—Smooth muscle has a certain independent activity, and it may be relaxed or contracted quite apart from its innervation from the central nervous system. It is supplied with *two* kinds of functionally different nerve fibres. One set *motor*, and the other *inhibitory*, the latter causing relaxation or cessation of motor activity. Both sets are connected with nerve cells in their course, and their ultimate peripheral terminals are non-medullated.

In skeletal muscle there is no propagation of the excitatory state laterally from one fibre to one lying next it. In smooth muscle, a stimulus applied to one part may set up an excitatory process which is propagated all over the sheet of tissue. It is readily susceptible to heat—heat, as a rule, causing relaxation, and cold a tonic contraction, though the result varies with the rapidity of change of temperature and many other conditions.

No absolute distinction can be drawn between striated and smooth muscle physiologically, so that v. Uexküll has suggested the classification under the headings of skeletal and visceral.

Our chief knowledge has been derived from a study of the œsophagus and stomach of the frog, ureter, intestine, bladder, and retractor penis (dog, cat).

1. Cut out a ring of muscular tissue from about the middle of a frog's stomach by making two cuts across it about 5 mm. apart (Bernstein's preparation). Adjust this ring to a muscle lever by means of two metallic hooks, or fix it in Wild's apparatus (fig. 196), arrange to stimulate the tissue, and record the result on a slow drum. Let the writing point be applied to the drum as in Pflüger's myograph, or as shown in Fig. 144, where the pen swings on a horizontal axis attached to the free end of the writing lever, and at right-angles to the direction of the lever. There is then no distortion of the curves, the curvilinear motion

of the ordinary lever being avoided. The smooth fibres are nearly all arranged circularly.

(*a*) *Spontaneous contractions* appear, and may continue for a considerable time—many hours—but they vary exceedingly in every respect, strength, shape of curve, and length of intervening intervals. The tracings often show a compound rhythm, but its individual tracings are not maximal—*i.e.*, smooth muscle has no "all or none" law as obtains for cardiac muscle.

(*b*) Stimulate with single induction shock, which would be effective for skeletal muscle = no contraction.

(*c*) Use a constant current—make shocks—keeping the current made, say $\frac{1}{5}$ sec.—slight response. If the current be kept closed 1 to 5 secs., the shortening will be greater.

(*d*) If the current be made and broken very rapidly there may be no response.

(*e*) Note that, during the passage of a strong galvanic current, the muscle remains contracted. A similar result, though less marked, occurs in skeletal muscle stimulated by a galvanic current, a phenomena more marked in fatigued muscle.

(*f*) In the above, determine latent period and duration of phases of contraction.

(*g*) Try to produce tetanus with slow shocks.

(*h*) Heating from 15° to 30° diminishes the size and increases the frequency, but reduces the tonus, while cold increases the tonus.

2. **Œsophagus of toad.** — Use the excised, slit-up, non-stretched œsophagus of a toad, which consists of smooth fibres arranged in one direction, and record its spontaneous contractions when weighted with 3 to 4 grms. (*Bottazzi* and *Grünbaum*). Both ends are fixed to separate pieces of cork, and one of these is fixed in a clamp, the other being attached to a recording lever. Record the spontaneous automatic beats, the effect of load, heat, drugs, &c.

L. Ranvier, *Leçons d'Anat. gén. sur le système musculaire*, Paris, 1880. See *Leçons* xxix. and xxx. for R.'s experiments on frog's stomach. R. always used a fasting frog, because the spontaneous movements—systole, diastole, pause—are more regular. G. H. Cooke, *Action of Various Stimuli on Non-Striped Muscle. Studies from the Physiol. Lab. of Owens College*, 1890. Morgen, *Halle Untersuchungen*, 1890. H. Winkler, *Pflüg. Arch.*, lxxi., 357, 1898. P. Schultz, *Arch. f. Anat. v. Phys.*, 1 and 307, 1897. "On Plain Muscle," Phil. Bottazzi and O. F. F. Grünbaum, *Journ. of Phys.*, xxiv., 51, 1899. "Contraction of Smooth Muscle," R. S. Woodworth, *Amer. Journ. of Phys.*, iii., 26, 1899. A. Grützner, *Pflüg. Arch.*, lxxxiii., 187, 1901.

3. **Polar stimulation of smooth muscle** ("physical" and "physiological" anode and kathode).

Results as regards contraction, similar to those obtained with skeletal muscle (Lesson XLVIII., 7), are obtained with the excised ureter of a

rabbit kept warm and moist by being placed on a glass slide over a beaker containing hot water.

The exposed small intestine of a rabbit just killed may also be used. The kathode electrode is placed on an indifferent point—*e.g.*, liver—and the anode on the intestine. At M the intestine at (+) contracts and remains constricted during the passage of the current. This would seem to be the reverse of what happens with the ureter, but it is only apparently so, for where the electrode touches the peritoneum of the intestine is not the true physiological kathode or anode—*i.e.*, where the current leaves or enters the muscular coat—so that the physical (+) or (−) is not at the same time the physiological (+) or (−).

(*a*) Expose the small intestine of a rabbit just killed. The muscular coat consists of two layers of smooth muscle—an outer longitudinal and an inner circular. Apply one, "the indifferent electrode," over the liver, and a N.P. electrode connected through a commutator and key with two cells to the intestine. Or apply two N.P. electrodes to the intestine at some distance apart. Note which is anode. At make there will be apparently a strong constriction of the circular coat, which is propagated upwards and downwards, and a longitudinal contraction at the kathode. This seems anomalous, but what actually occurs is that, at the (+), both coats are relaxed, with contraction of the circular coat on each side, while at (−) there is contraction of both coats.

At the anode the current leaves the tissue by a series of virtual kathodes, and similarly near the kathode a series of virtual kathodes (Lesson XLVIII).

4. Proteids of smooth muscle.—These may be obtained in the same way as those of striped muscle by extracting with saline solutions, 5 per cent. $MgSO_4$, &c., from the smooth muscle of the stomach of the sheep or calf (Lesson XIV., 11). *Rigor mortis* sets in, but *post-mortem* acidity is slight. The saline extracts contain little, if any, paramyosinogen (Coag T° 47° to 50°), but much myosinogen (55° to 65°). (Munk, also Swale Vincent and Lewis, *Proc. Phys. Soc.*, 1901.)

B. Ciliary motion.

1. Microscopical.—(*a*) Observe under the microscope a drop of hay infusion containing *Paramœcium*. Note the character of the movements and the cilia on the surface of the animal, which in this case act as motor organs.

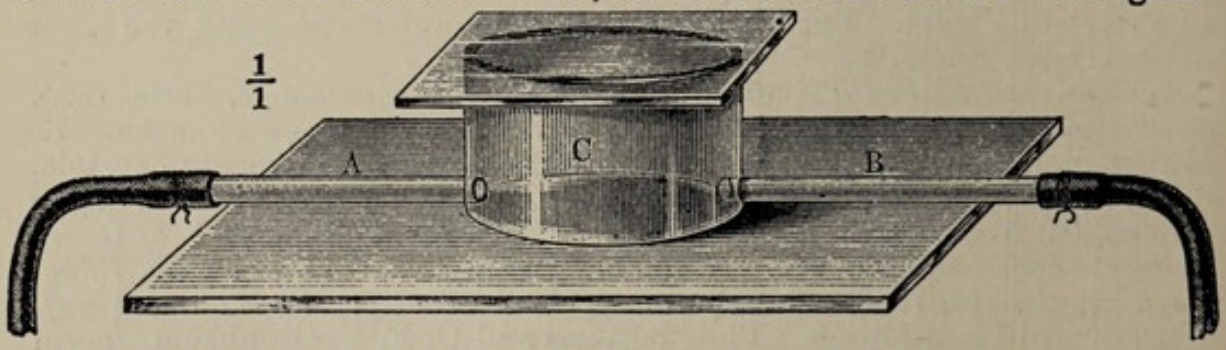


Fig. 233.—Gas chamber for making a "hanging drop" of ciliated organs or organisms, with inlet and outlet tubes for passing gases or vapour through the chamber.

(*b*) Snip off a fragment of the gill of a living mussel or oyster, separate slightly the bars of the gill with needles, place it on the under surface of a cover-glass, moisten with normal saline, and insert the latter over a circular cell of a gas chamber (fig. 233), so as to form a "hanging drop preparation."

Examine with a low power (× 100) and high power (× 300). Note the movement of the cilia attached to the cells covering the bars of the gills, the resultant movement of the fluid, and the onward movement of any particles suspended in the fluid.

(*c*) Scrape off some of the ciliated epithelium from the palatine mucous membrane of a frog, and examine its cilia and their action.

Ether.—Observe the effect of a trace of a dilute solution of chloroform, or ether, placed on the floor of the chamber. Lift off the cover-slip, give time for the narcotic to evaporate, and replace cover-slip in gas chamber. Do the cilia resume their movements? There comes in here the relative toxicity of ether and chloroform on protoplasm.

As it is possible to narcotise cilia—*e.g.*, by ether and chloroform—and as they may resume their activity after ventilation, it is important to determine the relative toxicity of these drugs. Try which is the more powerful. Try the effect of a stream of CO_2.

2. Mechanical effects.

(*a*) Destroy the brain and spinal cord of a frog. Place the frog on its back on a frog-plate. Divide the lower jaw longitudinally, carrying the incision through pharynx and œsophagus and right along the stomach, or cut off the lower jaw transversely as far back as possible. Divide skin in medium plane as far as pubes, and do the same with the sternum, xyphoid cartilage, and abdominal walls. Separate the cut parts, fix them with pins. Dissect out stomach and œsophagus, and cut off the head with the alimentary organs attached. Open œsophagus and stomach and pin them, mucous surface upwards, on a cork board. Pin back flaps. Moisten the mucous membrane with normal saline.

(*b*) Place a small cork flag—or piece of liver—on the mucous membrane covering the palate between the eyes. It will be carried backwards by ciliary motion. Repeat the experiment, and determine the time the flag takes to travel a given distance. Note that it does not pass over boundary line between œsophagus and stomach. Why? Does it move over surface of stomach. If not, why not?

(*c*) Heat. The load is carried faster.

(*d*) Grains of charcoal, Berlin blue, or a straw in æsophagus are similarly carried backwards.

(*e*) With a hot wire cauterise superficially a small area of the mucous membrane in a preparation bestrewn with charcoal. Ciliary motion stops not only at the cauterised area, but also in a triangular area whose apex is at the burned point, and whose base is next the œsophagus. The movements of the cilia in individual cells appear to be independent of the movements in neighbouring cells.

(*f*) *Apparatus for study of ciliary motion.*—Omitting the Flimmeruhr and Mühle of Engelmann (*Pflüg. Arch.*, xv., 493, 1877), the simplest and most instructive experiment is that easily made on the apparatus of Ranvier, shown in fig. 234. (Ranvier, *Leçons sur le système musculaire*, Leçon

xxxii., Paris, 1880.) The palatal mucous membrane is carefully removed and pinned to a cork, ciliated surface uppermost. As shown, this surface rests on the under surface of a glass disc, moving on a vertical axis with a horizontal pointer attached. The apparatus is placed in normal saline. Study the effects of heat and cold on the rate of movement which can be read off on the index.

(*g*) *Work done by cilia.*—As in (*c*), remove the whole extent of the palate-œsophageal mucous membrane. Pin it, mucous surface upwards, on a frog-plate (10 × 5 cm.), with long axis of œsophagus in long axis of plate.

A convenient weight is made from sheet-lead, 1 mm. in thickness—pieces of 5 mm., 3 mm., and 2 mm. square are supplied. Place one of the squares of known weight on the palatine mucous membrane near the œsophagus. Note if the weight moves. If it does so, incline the plate by resting it on a block of wood (5 cm. × 5 cm.), but keep the œsophageal end of the mucous membrane at the base. Find the angle at which the lead weight is carried upwards—*i.e.*, from palate towards stomach. Note rate of progress of lead weight upwards. Vary the incline of the plate, and therefore of the ciliated membrane, by altering its angle in relation to the wood block. Vary the weight, and ascertain which is carried up more rapidly at a given angle.

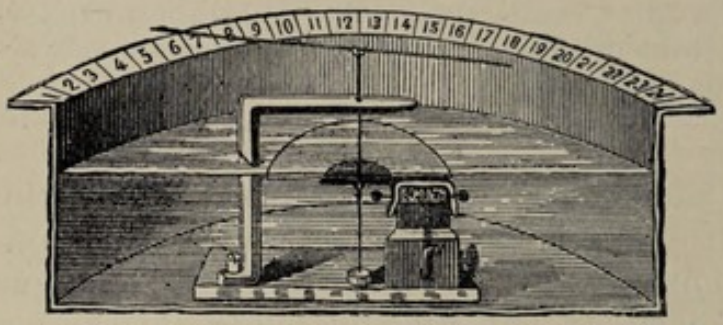


Fig. 234.—Ranvier's apparatus for measuring the velocity of movement of cilia, made by Verdin.

Under certain conditions the lead weight is carried up the inclined plane. Ascertain distance travelled per minute with a given incline, and with a millimetre rule ascertain the actual height (*h*). The actual work done is expressed by the formula, W (work done) = *w* (weight lifted) + *h* (height in millimetres)—*i.e.*, W = *w* × *h* = work in milligram-millimetres.

C. Chemotaxis indicates "that property of organisms that are endowed with the capacity of active movement, by which, under the influence of chemical stimuli acting unilaterally, they move toward or away from the source of the stimulus. Where there is an approach to the source of the stimulus, there is *positive* chemotaxis; where there is a removal from the source, *negative* chemotaxis" (*M. Verworn*).

It is a phenomenon manifested by many bacteria, leucocytes, swarm spores, and many infusoria.

1. Paramæcium—*e.g.*, aurelia or caudatum. The recent investigations of Verworn and his pupils, H. S. Jennings, and others, have given us simple methods of studying this phenomenon in ciliated infusoria.

(*a*) Paramæcia are placed upon a glass slide and covered with a long cover-glass, supported near its two ends by bits of capillary glass-tubing; a pretty thick layer of water containing the paramæcia lies between the slide and the cover, so that the animals can swim about freely (fig. 235). Observe how paramæcium swims nearly straight forward in a slightly

sinuous course, the narrower, blunter end directed forwards. At the same time it revolves on its long axis.

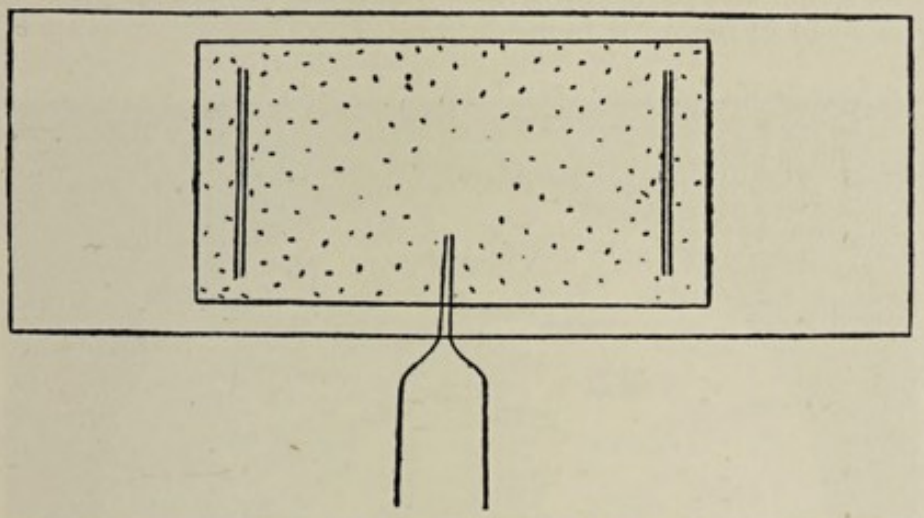


Fig. 235.—Paramæcia. How to apply a fluid from a pipette (*Jennings*).

(*b*) Introduce beneath the cover-glass—by means of a pipette drawn out into a capillary tube—a drop of any solution towards which the method of reaction is to be tested—*e.g.*, a dilute acid, $\frac{1}{50}$ p.c. acetic acid. At once the paramæcia react in a characteristic manner, and gather in a dense swarm within the drop (fig. 236)—*i.e.*, they are *positively* chemotatic to feeble acids.

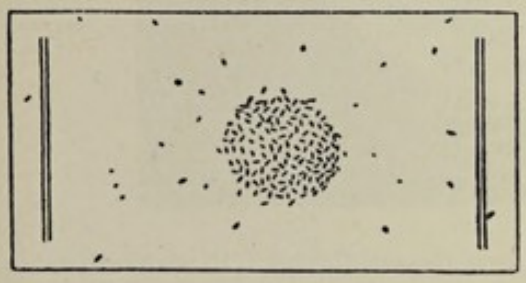


Fig. 236.—Paramæcia gathered round a drop of H_2SO_4 ($\frac{1}{100}$ p.c.) (*Jennings*).

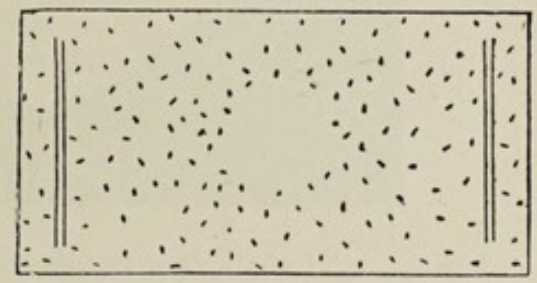


Fig. 237.—Paramæcia. Preparation into which was introduced 10 mins. before a drop of $\frac{1}{120}$ p.c. Na_2CO_3 (*Jennings*).

(*c*) In a fresh preparation introduce a weak solution of sodium carbonate, or $\frac{1}{5}$ p.c. NaCl solution, or $\frac{1}{200}$ p.c. NaHO; the drop remains entirely empty—*negative* chemotaxis (fig. 237).

D. Galvanotaxis. — The galvanic current, as already shown, causes polar excitation. Hermann, in 1885, showed the directive effect on tadpoles, which turned their heads towards the anode and their tails towards the kathode, their long axis being parallel to the curved lines of flow of a galvanic current passed through the fluid in which they were placed. Use paramæcium, as for chemotaxis.

1. Make a *stimulation-trough* as follows:—Cement, parallel to one another and about 1 cm. apart, two oblong strips of unbaked earthenware —such as is used for the porous cell of a battery—to a large glass slide by

means of sealing wax. Complete the side walls with sealing wax (fig. 238). Depth of cell, about 1 mm. Apply N.P. electrodes—fixed in suitable holders—to touch the porcelain slips. Soak the apparatus in the fluid to be experimented in before it is used.

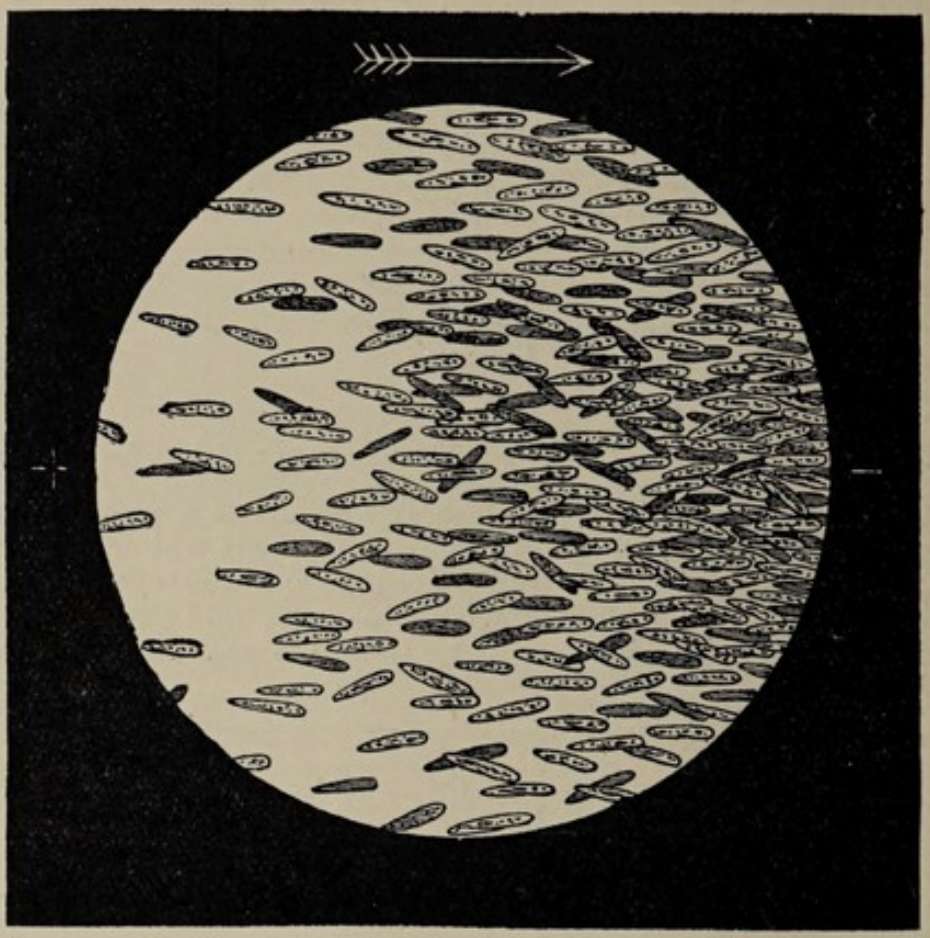


Fig. 238.—Galvanotaxis of Paramæcium. Arrow indicates direction in which they swim. Microscopic picture (*Verworn*).

2. Place paramæcia in the trough, pass a constant current through the fluid, placing commutator in the circuit to change the poles. Observe the effects (fig. 239).

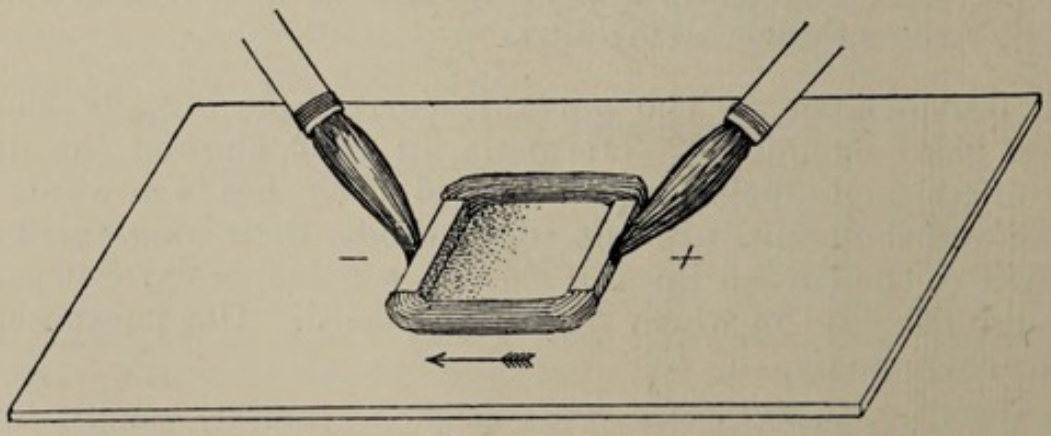


Fig. 239.—Stimulation trough for Galvanotaxis of Paramæcia, and how electrodes are applied. Naked eye appearance (*Verworn*).

"At closure the paramæcia turn altogether at the word of command, with the anterior pole of the body towards the negative electrode, and swim in this direction with uniform speed" (*M. Verworn*). If the animals are killed by chloroform, no such result occurs, so that the living state is essential to this phenomenon. The effect of the constant current on the direction of the head of tadpoles, or on any other organisms, may be tried.

3. *Infusoria from frog.*—As is well known, many free, swimming, ciliated infusoria live in the frog's intestine. Dale has experimented with these. Select the contents of the rectum, and numerous examples of *Opalina ranarum* are sure to be found. Compare its behaviour with Paramæcium.

4. Similar results were obtained by O. Carlgren with the composite *Volvox aureus*, in which the several individuals are held together by "protoplasmic jelly."

E. Thermotaxis.—Place paramæcia in a trough (10 cm. × 1 cm.). Heat one end to 28° C. They move towards the cooler side (Mendelssohn, *Pflüger's Arch.*, lx., 1895).

"Studies on Reactions to Stimuli of Unicellular Organisms," by H. S. Jennings—*Journ. of Physiology*, xxi., 258, 1897; *American Journ. of Phys.*, ii., 311 (1899) *et seq.* In same journal, see Loch. O. Carlgren, *Arch. f. Anat. u. Phys.* (Phys. Abth.), 1899, 465, 1900. Boris Birukoff, *Pflüg. Arch.*, lxxvii., 555, 1899. "Galvanotaxis and Chemotaxis of Ciliate Infusoria," by H. H. Dale—*Journ. of Phys.*, xxvi., 291, 1901.

CIRCULATION OF THE BLOOD.

LESSON LIII.

FROG'S HEART—HEAT AND COLD—SECTION OF HEART—GRAPHIC RECORD OF BEAT.

1. Heart of frog. Expose it.

(*a*) Pith a frog; lay it on its back and pin out its legs on a frog-plate. Make a median incision through the skin over the sternum; continue the incision upwards and downwards, and from the middle of the sternum make transverse incisions.

(*b*) Reflect the four flaps of skin, raise the episternum, and cut through the sternal cartilage just above its lower end, to avoid wounding the epigastric vein. With a pair of scissors remove the sternum, and divide it above. Do not injure the heart. Observe it beating within its transparent, glistening pericardium.

(*c*) With fine forceps carefully lift up the thin transparent pericardium; open it, thus exposing the heart.

2. Frog's heart.

(*a*) Observe its shape, noting the two auricles above (fig. 240, *Ad*, *As*), and the blunt conical apex of the single ventricle below, *v*, the auricles being mapped off from the ventricle by a groove—auriculo-ventricular—which runs obliquely across its anterior aspect. The ventricle is continued anteriorly into the bulbus aortæ, B, which projects in front of the right auricle, passing across the auricles from below upwards, and from right to left, and then divides into two aortæ—right and left—the left being the larger. Each trunk is divided by two longitudinal septa into three channels, which soon become independent as three arteries:—

(1) Most anterior, *Ductus caroticus* (fig. 240).

(2) Middle, *D. aorticus*, passes on each side to form the aorta (2).

(3) Posterior, *D. pulmo-cutaneus*, which gives off the pulmonary artery and a large cutaneous artery. Note how in this way the skin of the frog forms an important respiratory organ.

(*b*) Tilt up the ventricle, and observe the sinus venosus (fig. 241, *s.v.*) continuous with the right auricle, and formed by the junction of the large inferior vena cava, *c.i.*, and the two (smaller) superior venæ cavæ, *c.s.s*, *c.s.d.* The frænum is seen as a fine thread containing a vein; divide it. Observe a white transverse crescentic line, "crescent," where the S.V. joins the left auricle—the site of Remak's ganglion.

3. Note the sequence and course of contraction of the several parts—viz., sinus venosus (*s.v.*), auricles (*As*), ventricle (*v.*), and bulbus arteriosus.

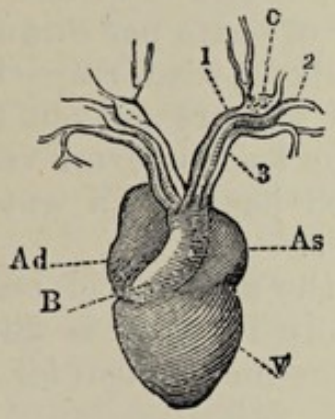


Fig. 240.—Frog's heart from the front. *v*, Single ventricle; *Ad*, *As*, Right and left auricles; B, Bulbus arteriosus; 1, Carotid; 2, Aorta; 3, Pulmo-cutaneous arteries; C, Carotid gland.

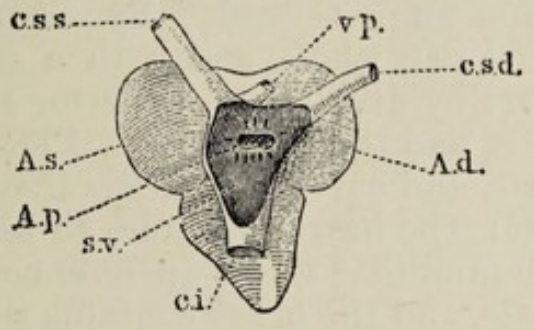


Fig. 241.—Heart of frog from behind. *s.v.*, Sinus venosus opened; *c.i.*, Inferior, *c.s.d.*, *c.s.s.*, Right and left superior venæ cavæ; *v.p.*, Pulmonary vein; *Ad*, and *As*, Right and left auricles; *Ap*, Communication between the right and left auricle.

This sequence of events is difficult to note, but what can be easily observed is the relative condition of vascularity of the ventricle. The frog's ventricle has no blood-vessels supplying its muscular walls. When it is at rest it is soft, but when it beats it becomes hard. Note that during systole of the ventricle —*i.e.*, during its contraction—it becomes pale, the blood being driven out of its spongy and elegantly-arranged system of muscular fibres, which are pale in colour; and immediately thereafter, during its diastole, it is suddenly distended with blood and has a red appearance, the blood flowing into it being propelled quickly by the contracting auricles. Notice also how the position of the auriculo-ventricular groove moves upwards and downwards during each cardiac cycle.

Note the rate—*i.e.*, the number of beats per minute—by timing it with a stop-watch. The rate varies greatly in different frogs, 20 to 80 per minute.

4. Effect of temperature (Heart *in situ*).—(*a*) With a pipette drop normal saline at 20° to 25° C. on the heart, and note increase in number of beats, and that each individual beat is quicker.

(*b*) Apply normal saline at 10° C. or 5° C. Note the slowing of rate or rhythm, with slower contraction of each beat.

5. Excised heart beats.—(*a*) With a seeker tilt up the apex of the ventricle, and observe that a fine thread or "frænum" passes from the pericardium to the posterior aspect of the ventricle. Divide it. Tie a fine silk thread round the frænum dorsal to the ligature. Count the number of beats per minute. Enter in note-book. By means of the silk thread raise the heart as a whole, and with a sharp pair of scissors cut out the heart by dividing the inferior vena cava, the two superior venæ cavæ, and the two aortæ. Place the excised heart in a watch-glass, and cover it with another watch-glass.

(*b*) The heart continues to beat. Count the number of beats per minute. Enter in note-book. Therefore its beat is automatic, and the heart contains within itself the mechanism for its rhythmical beats.

(*c*) Place the heart on a glass slide; during diastole it is soft and flaccid, and adjusts itself to any surface it may rest on. During systole—*i.e.*, when it contracts—its apex is raised and erected, and the heart muscle becomes hard.

6. Heat and cold on excised heart.—(*a*) Place the watch-glass containing the beating heart on the palm of the hand; the heart beats faster; or on a beaker of warm water, which must not be above 40° C., as the temperature of the heart rises, it beats faster—there are more beats per minute—therefore each single beat is faster.

(*b*) Place the watch-glass and heart over a beaker of iced water, the number of beats diminishes, each beat being executed more slowly and sluggishly. Enter all results in note-book.

7. Section of heart.—(*a*) Expose a heart, divide the pericardium, and ligature the frænum, and by means of it gently raise the heart. Excise the whole heart, including the S.V. The heart still beats.

(*b*) Cut off the heart from the sinus; the latter continues to

beat. The rest of the heart ceases to beat for a time, but by-and-by it commences to beat rhythmically.

(*c*) Sever the auricles from the ventricle; the V ceases to beat. The V, however, has not lost the power of beating rhythmically. Prove this by stimulating it mechanically—*e.g.*, prick it with a needle. After an appreciable latent period, it executes one—generally several—beats, and then is quiescent. Stimulate with a single induction shock, it discharges one or more beats.

(*d*) Cut off the apex of the ventricle; it remains quiescent; but if it be stimulated, either mechanically or electrically, it makes a single beat—not a series, as in the case of (*c*).

(*e*) Divide the V of another heart below the auriculo-ventricular groove. The As, with the upper part of the V attached, continue to beat, while the lower two-thirds cease to beat spontaneously. Pricked with a needle, the detached part contracts as often as it is thus stimulated. It responds by a single contraction to a single stimulus, but a single stimulus does not excite a series of rhythmical contractions.

(*f*) With scissors divide longitudinally the auricles with the attached portion of the ventricle. Each half contracts spontaneously, although the rhythm may not be the same in both.

Record in your note-book the result of your observations on rate and sequence of beat of heart, opening pericardium, excising heart, section of heart.

Time.	Observation.	Rate of Heart-beat and of S.A.V.

8. **Isolated apex—Bernstein's experiment.**—(*a*) Instead of cutting, one may use a ligature, or the heart apex may be separated by Bernstein's method—viz., compress the heart halfway between base and apex by forceps, or ligature sufficiently lightly to break the physiological continuity but not the physical, both parts remaining connected with each other. Remove ligature. In a pulsating heart, all pulsates except the isolated apex.

(*b*) The apex remains red, quiescent, and filled with blood,

while the rest of the ventricle pales during systole. If the bulbus aortæ be compressed with forceps so as to raise the pressure within the apex, the apex also beats. This is an important experiment in its bearing on the theory of cardiac action.

(*c*) *Mechanical stimulus.*—Touch the isolated apex with the point of a pin. It gives a single beat on response, but after an appreciable latent period.

(*d*) *Chemical stimulus.*—Place a crystal of NaCl on the apex; it pulsates rhythmically.

9. **Movements of heart.**—Expose the heart of a freshly-pithed frog, or destroy only the brain and curarise lightly. Observe

(*a*) That the auricles contract synchronously and force their blood into the ventricle, which, from being pale and flaccid, becomes red, turgid, and distended with blood.

(*b*) Immediately thereafter the ventricle suddenly contracts, and forces the blood into the bulbus aortæ, at the same time becoming pale, while its apex is tilted forwards and upwards. As the auricles continue to fill during the systole of the ventricle, on superficial observation it might seem as if the blood were driven to and fro between the auricles and ventricles, but careful observation will soon satisfy one that this is not the case. Observe how the position of the auriculo-ventricular groove varies during the several phases of cardiac activity.

(*c*) The slight contraction of the bulbus aortæ immediately following the ventricular systole.

(*d*) The diastolic phase or pause when the whole heart is at rest before the auricles begin to contract. Ligature the frænum and divide it, gently raise up the ventricle by the ligature attached to the frænum, and observe the sinus venosus.

(*e*) The peristaltic wave, or wave of contraction, begins at the upper end of the vena cava inferior and sinus venosus; it extends to the auricles, which contract, then comes the ventricular systole and that of the bulbus aortæ, and finally the pause; when the whole sequence of events begins again with the systole of the sinus.

(*f*) Before the ventricular systole is complete the sinus is full, while the auricles are filling.

All this is easier to describe than to observe, and it requires patient and intelligent observation to assure one's self of the succession of events.

10. **Heart currents.**—Lay the nerve of a N.M. preparation along the beating heart—pericardium removed—from base to apex. Observe a secondary twitch in the muscle with each heart-beat. (Lesson XLVII.)

Some inferences.—The foregoing experiments clearly show that the rhythmical beat of the heart is not dependent on stimuli reaching it along the nerve paths, or on the presence of blood. It contains in its own walls the secret of its rhythm. The rhythm may be due to (1) an independent inherent rhythmical property of its muscular fibres, or (2) rhythmical discharges from some of its nerve cells, which are abundant in certain regions of the heart. The ventricular apex is devoid of nerve cells, and under certain conditions it does beat rhythmically. This observation is more easily made on the heart of the tortoise. Other observations support the theory of inherent rhythmical qualities of the heart muscle. In the embryonic beat rhythm occurs before it contains nerve cells. A heart poisoned with nicotine, which is said to paralyse nerve cells, still beats spontaneously. The severed apex beats under a certain internal pressure.

But it is also obvious that the several parts of the heart are not equally endued with rhythmical properties. It is greatest in the sinus venosus, and roughly there is a descending scale from S.V. to ventricle, and it is least in the ventricle apex; Bernstein's experiment, however, seems to indicate that a constant stimulus—in this case raised intra-cardiac pressure—may cause rhythmical contractions in an organ habituated to rhythmical discharges.

11. Graphic record of beat (direct registration with lever)—Figs. 242, 243.—(*a*) Destroy the brain only; curarise; expose heart, still within its pericardium, and arrange a heart-lever to rest lightly on the pericardium over the heart. Adjust the lever to write on a revolving cylinder, moving at the rate of 5 to 6 cm. per sec. Take a tracing.

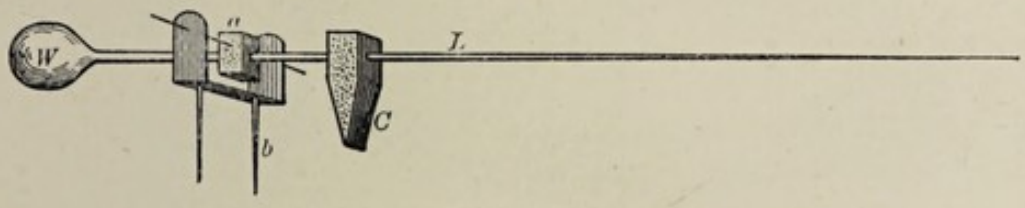


Fig. 242.—Simple frog's heart-lever. *a*, Fulcrum; *L*, Glass lever with knob to act as counterpoise; *b*, To fix into the cork of a frog-plate; *C*, cork to rest on the heart.

(*b*) An isotonic heart-lever may be made with a straw about 12 inches long, or a thin strip of wood about the same length. Thrust a needle transversely either through the straw or through a piece of cork slipped over the straw about 2 inches from one end of the lever. The needle forms the fulcrum of the lever, and works in bearings, whose height can be adjusted. To the end of the lever nearest this is attached, at right angles, a needle with a small piece of cork on its free end. The lever is so

adjusted that the cork on the needle rests on the heart. The long arm of the lever is provided with a writing-style of copper-foil, or a writing point made of parchment paper, fixed to it with sealing-wax. By using a long lever a sufficient excursion is obtained. Another form of heart-lever is shown in fig. 242. It consists of a thin glass rod, fixed as shown in the figure. The frog is laid on its back on a frog plate covered with cork. The heart-lever is fixed into the cork by means of the two pins, *b*, while C is so adjusted as to rest on the heart.

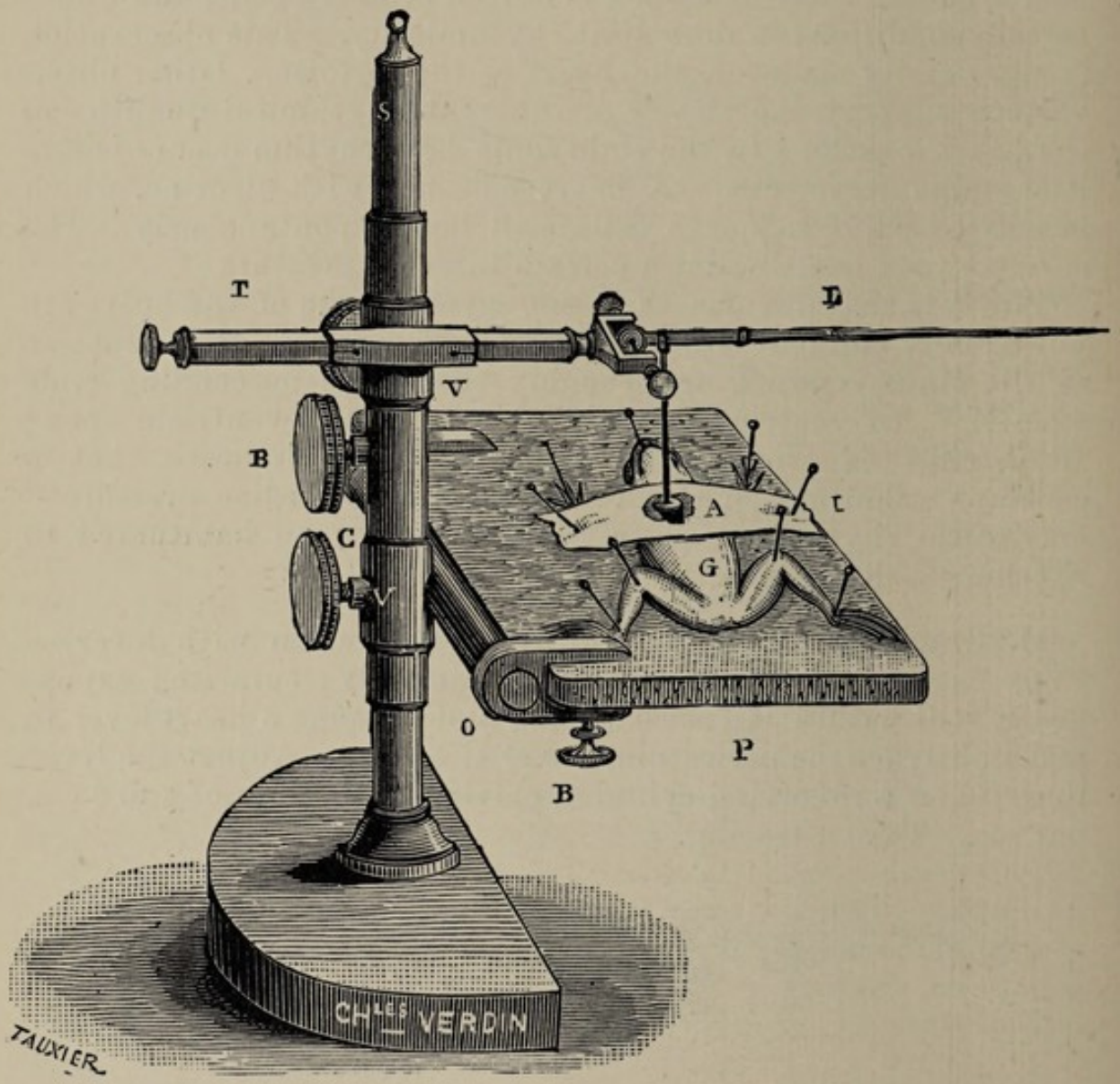


Fig. 243.—Lever resting on frog's exposed heart, direct registration (made by Verdin). *A*, Thin sheet of caoutchouc.

12. Excised heart (*Gotch's arrangement*).—By this method all the parts are fixed to a T-piece which is clamped in a stand, so that all the apparatus, collectively and individually, can be easily adjusted (fig. 244).

Suspend an excised heart by clamp-forceps, F, to a horizontal rod attached to a T-piece, T.P. On the T-piece is a cork into which the electrodes are fixed, while the heart pulls on a counterpoised lever.

We can show (1) with a Stannius heart (i.) the latent period of cardiac muscle or cardiac delay, (ii.) the delay of transmission of an impulse from A to V in the groove; (2) with a beating heart, the refractory period, rhythm, inhibition from the sinus (crescent), effect of atropin, muscarin, &c.

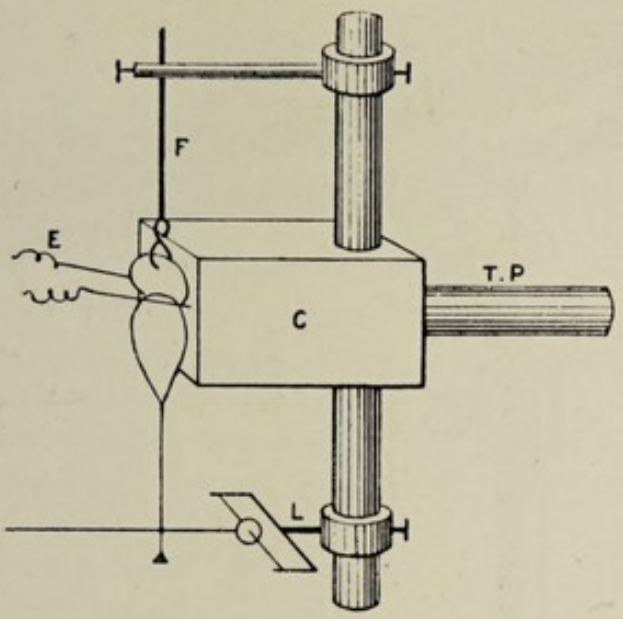


Fig. 244.—Gotch's arrangement for excised heart. All parts are fixed on one T-piece, *T.P.* *F*, Clamp-forceps for heart; *C*, Cork; *L*, Lever.

13. Place a frog on a crank-myograph, attach the apex of the heart still *in situ* to a very light crank-lever and record its movements.

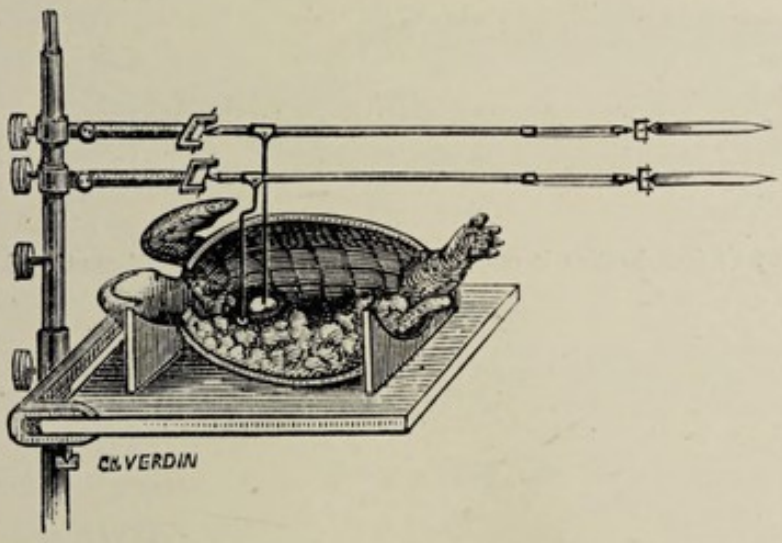


Fig. 245.—Auricular and ventricular lever for the heart of a tortoise (*Verdin*).

14. In order to record simultaneously the contractions of As and V, and to study the relations of these events one to the other, a lever must be placed on the As and another on the V, and the points of both must be arranged so that the one writes directly over the other as shown in fig. 245, in the heart of a turtle or tortoise.

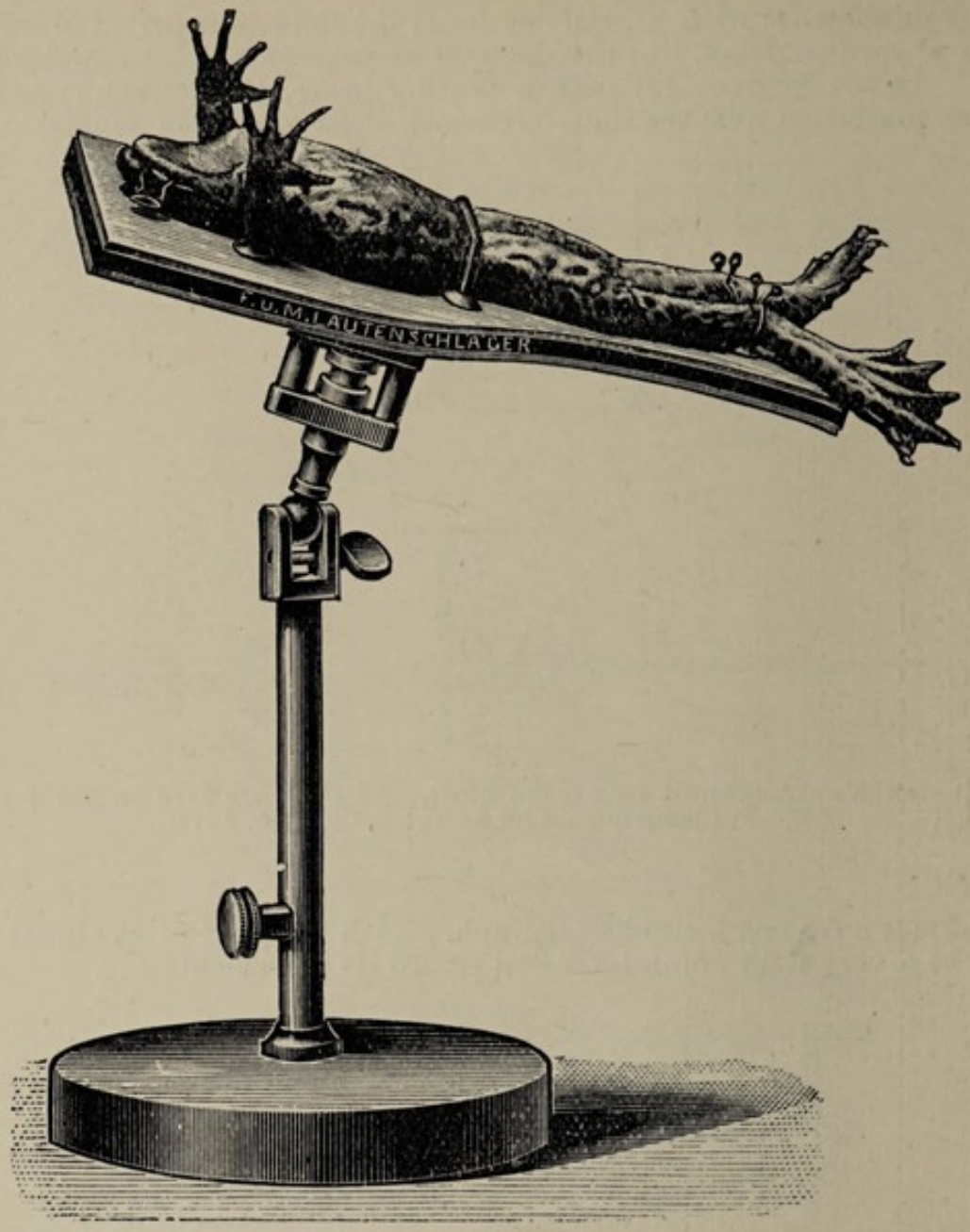


Fig. 246.—Cowl's frog-holder is convenient when a delicate dissection has to be made.

LESSON LIV.

SUSPENSION METHODS FOR HEART—GASKELL'S HEART-LEVER AND CLAMP.

1. Gaskell's heart-lever (Suspension method).

(*a*) This lever is very convenient (fig. 247). Expose the heart of a pithed frog, ligature and divide the frænum, tie a fine silk thread, moistened with normal saline, to the tip of the apex of the ventricle, and attach the thread to the writing-lever placed above it, or pass a bent pin as a hook through the apex of the ventricle. The lever is kept in position by a thin thread of elastic or weak spiral spring (fig. 249), which raises the lever after the contraction of the heart has depressed it. Adjust the tension to get the maximum excursion of the lever; the latter must be horizontal.

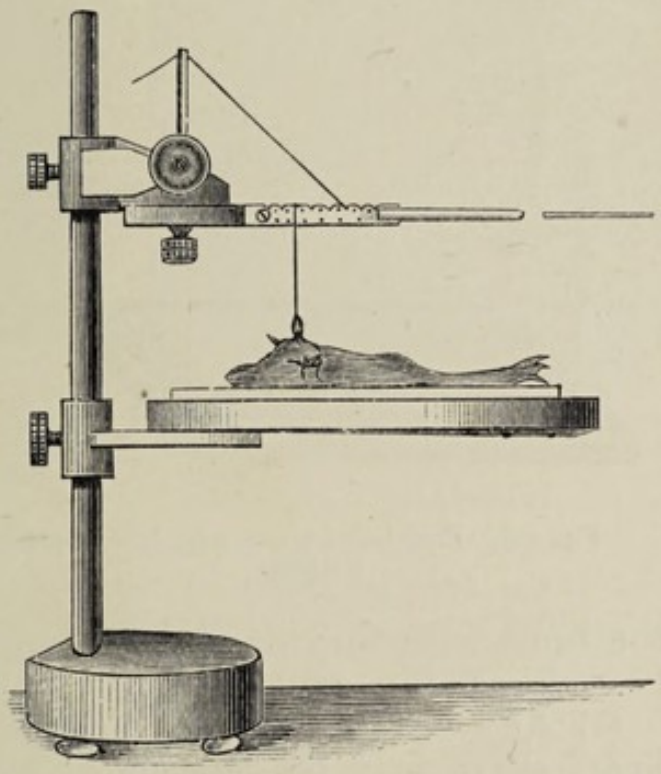


Fig. 247.—Frog heart lever. Gaskell's suspension method.

(*b*) Record on a slow drum. The contraction, of course, is recorded downwards. The writing-style must have a parchment paper point tipped with a glass filament, as described by Bayliss (p. 376). The pressure must be accurately adjusted to get the maximum excursion of the lever. Record time in seconds.

(*c*) First the sinus contracts, but this may not be obvious; then the auricles contract and pull down the lever slightly, then the greater contraction of the ventricle pulls the lever down further, and when the ventricle relaxes, the lever is raised by

the elastic thread. Fig. 248 shows tracing obtained when the heart is free, full of blood, and no clamp is applied.

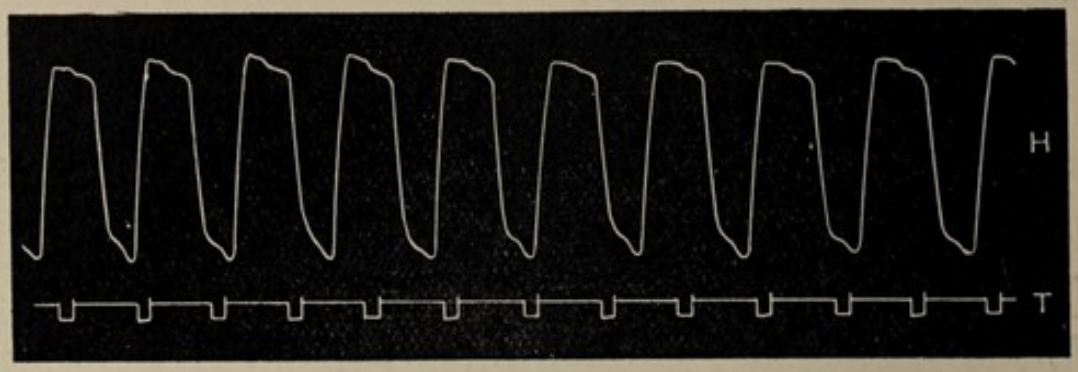


Fig. 248.—Tracing of a frog's heart taken with apparatus shown in fig. 247. *H*, Heart-tracing; *T*, Time in seconds. (Compare with fig. 253.)

By this method, also, the effect of heat, cold, drugs on the heart can be ascertained.

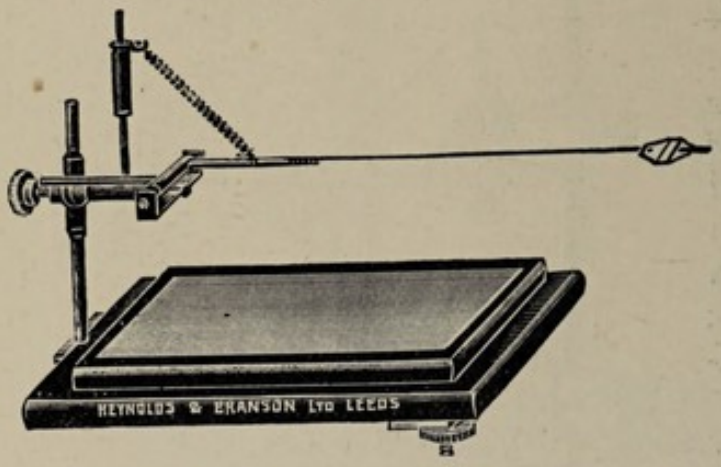


Fig. 249.—Frog heart-lever, with spiral spring tension.

If it is desired to ascertain the action of a drug on the heart by this method, make a snip in the heart so that the blood may flow out and the drug act directly on the cardiac muscle.

2. Varying speed of drum with effect of temperature.

(*a*) With Gaskell's lever or method record the beats with varying speed of drum, marking time in seconds in each case (fig. 250).

(*b*) *Effect of temperature* on the rate of beat and form of heart curve may be determined by applying normal saline or diluted blood (sheep's blood 1, normal saline 4), say at 0°, 15°, and 30° C., directly to the heart (fig. 251).

Allow either of these fluids or Ringer's fluid at the desired temperature to flow on the heart from a pipette with capillary point. In this way the ventricle may be heated without affecting the S.V. or As. Use a slow drum.

The effect of heat applied to S.V., As, or V by the pipette method shows that—

(i.) Heat localised to S.V. affects the rhythm more than the character of each beat.

(ii.) While temperature variations of V affect rather force and character of the beat, and to a less extent the rhythm.

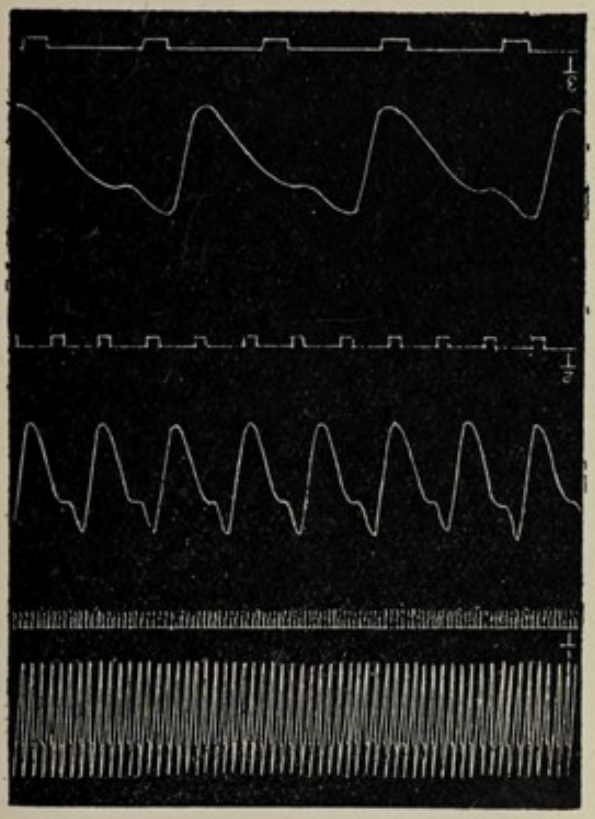


Fig. 250.—Shows how heart curve varies with rate of drum. In 1, 2, 3, *T*=time in seconds. Gaskell's lever. Read tracing from above downwards.

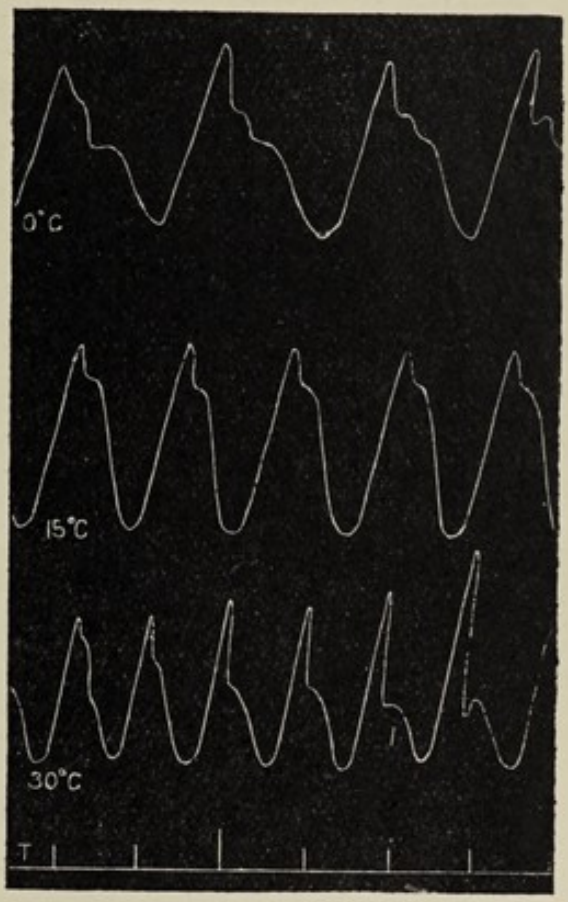


Fig. 251.—Shows the effect of normal saline directly applied to the heart (at 0°, 15°, and 30° C.) *T*, time in seconds. Gaskell's lever. Read from above down

3. Suspension method (*fulcrum of lever between power and weight*).—A light straw recording-lever working on a horizontal axis may be used. The lever is placed above the heart, and the latter is attached to one end of the lever by a bent, thin, metallic hook thrust through the apex of the heart, or by a thread tied to the tip of the ventricle. The power is thus at one end the fulcrum or axis, and between it and the recording end of the lever, which is weighted near the axis, suppose the relative lengths of the arms to be 3 cm. and 18 cm., then the magnification is 6 times.

Attach the frog's heart by the lever of the cardiograph (fig. 252) and record a tracing on a slow drum, 1 cm. per second, and also record a time tracing of 4 or 8 vibrations per second (fig. 253). Vary rate of drum. In this case, of course, the

ascent of the lever is due to contraction of a part of the heart, and the curve is read from below upwards.

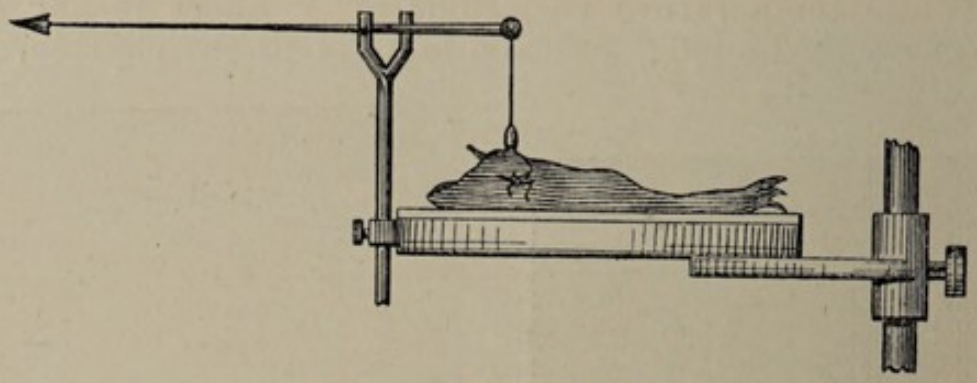


Fig. 252.—Frog's cardiograph.

(*a*) Record the heart-beats of a vigorous frog, with the brain only destroyed, so that the heart is well filled with blood. Four more or less marked undulations may be seen on the ascent in the curve of each cardiac cycle, indicating (i.) contraction of

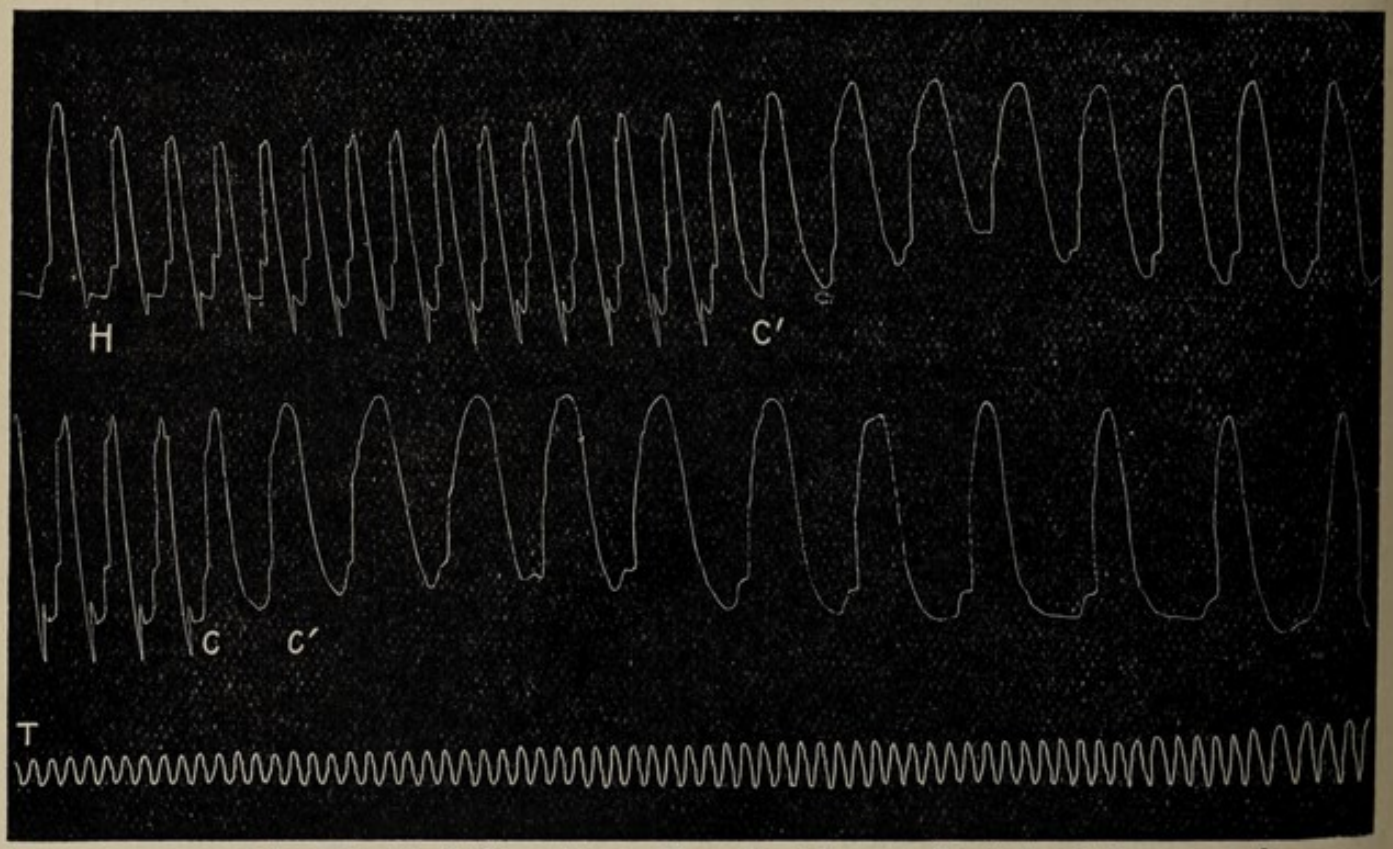


Fig. 253.—Effect of cold, *C*, *C′*, and heat, *H*, on frog's heart. Read from below upwards. Recorded with lever like fig. 252. Time, 0·5 second (*Stirling*).

S.V., perhaps followed by a short descent ; then (ii.) contraction of the auricles, followed by a slight descent due to their relaxation ; then (iii.) contraction or systole of the ventricle, at first rapid, and then (iv.) slower at the latter part of its systole, as the ventricle at that time contracts against greater resistance.

These effects are fairly well shown in the first part left end of fig. 253, before the application of cold. The one phase gradually merges into the other. Fig. 254 shows the same effect with the S.V. record absent. Notice the long oblique systolic plateau at the upper part of the curve, as the ventricle has now got up pressure and is emptying its contents against the increased resistance in the arterial system.

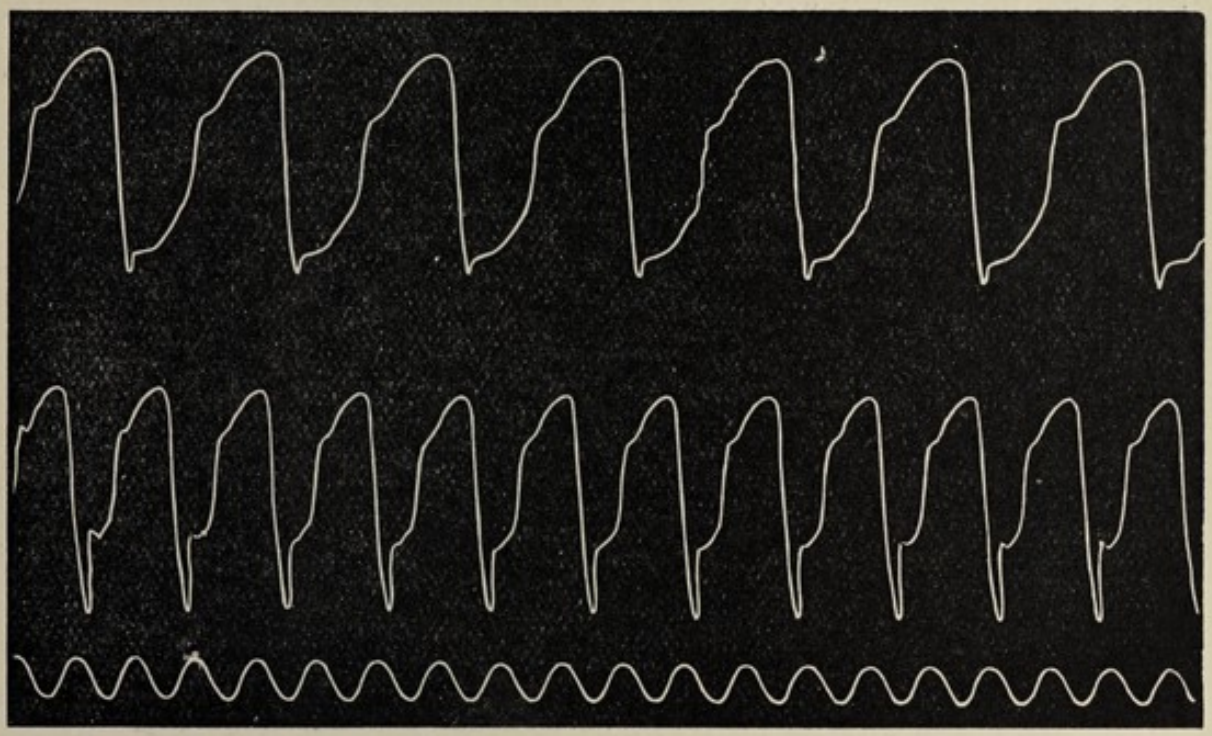


Fig. 254.—Record of frog's heart-beat (lever of first order). Read each beat from below up. Upper curve quickened speed of drum (*Stirling*).

(*b*) *Effect of snip in heart.*—This flatter part of the systolic plateau disappears if both aortæ are cut, or if a snip is made into the S.V. or As to allow the blood to escape (fig. 255).

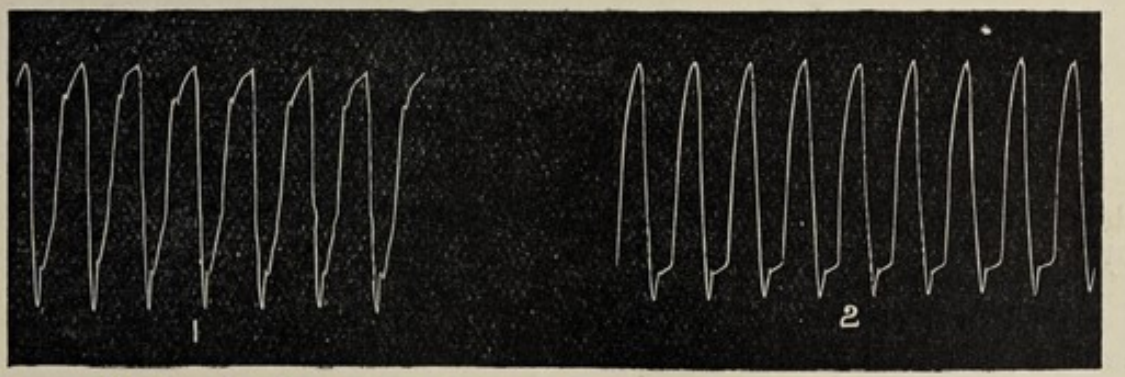


Fig. 255.—Tracing frog's heart. Read each beat from below upwards. Notice in 1, at apex, oblique form of systolic plateau, which disappears (2) when snip is made in heart to allow blood to escape. In these curves the ascent represents systole and the descent diastole (*Stirling*).

Transmission of wave of excitation from As to V.—The wave of contraction sweeps over the heart from S.V. to As, and As to V. As it passes from As to V there is a distinct delay—"auriculo-ventricular interval"—as if its progress were impeded by a "block," and then the wave reaches the V. If a greater obstacle or block be placed between the As and V in the auriculo-ventricular groove, then the delay will be increased, and the excitation wave may or may not pass. The As may beat at one rate, and the V at another, just as the excitation wave can overcome the block. A mechanical "block" of greater or less resistance can be introduced by the use of Gaskell's clamp.

4. Gaskell's frog heart clamp is shown in its actual size in Fig. 256. By it the heart, recording by a suspension method, can be clamped at the auriculo-ventricular groove. The apparatus can be fixed in any suitable stand. By turning the milled head a sliding piece is gradually forced forwards, and any part between the two jaws of what is practically a vice with bevelled jaws is compressed. A spring draws the sliding piece back when the screw is slackened.

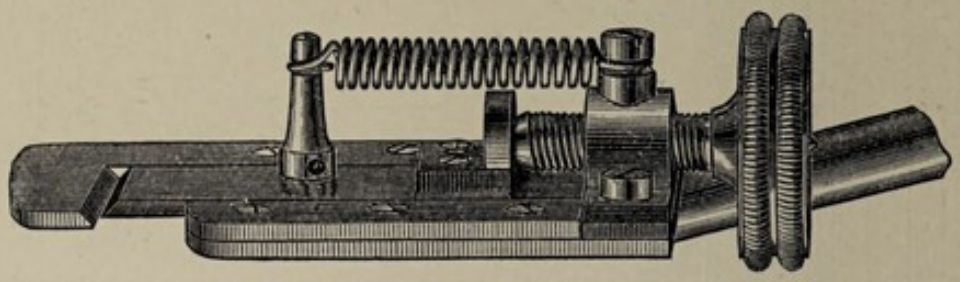


Fig. 256.—Frog heart clamp of Gaskell (Cambridge Scientific Inst. Co.).

(*a*) Record the heart beats by a suspension method, and adjust jaws of Gaskell's clamp over the auriculo-ventricular groove. Cautiously turn the screw, and gently compress the tissues in the A to V region. Note the effects; the As will continue to beat practically unchanged, but as the compression is increased the V may respond only after two or three beats of the As. The conductivity has been so diminished that only every second or third excitation wave passes. It is usually recorded thus—$\frac{A}{V}, \frac{1}{1}, \frac{2}{1}, \frac{3}{1}$.

Repeat the above experiment, but clamp the ventricle. There will be delay in transmission of the impulse from one part of the ventricle to the other.

The natural delay in the passage of the excitation wave from As to V is ascribed to the small number, and perhaps the embryonic character of the muscle cells uniting As with V.

Cut across the auricles so that only a small bridge of muscular tissue connects them. Stimulate one half, the other will contract after an interval, which will be longer the smaller the bridge of tissue left. Many of these experiments are best done on the heart of the tortoise.

(*b*) *Clamp excised heart* (*Phil. Trans.*, iii., 993, 1882).—Arrange two equally long light levers with their writing points exactly in the same vertical line to record on a slow drum, the levers being about 10 cm. apart; counterpoise the lower lever. About midway between the two place a modified Gaskell's clamp consisting of two narrow strips of brass like the points of a fine pair of forceps, which can be approximated by means of a screw. Support the upper lever with an india-rubber thread. Expose the heart of a pithed frog. Tie a fine silk thread to the apex of the ventricle, and another to the upper part of the auricles, and excise the heart. Attach the auricular thread to the upper lever, and the ventricular one to the lower lever.

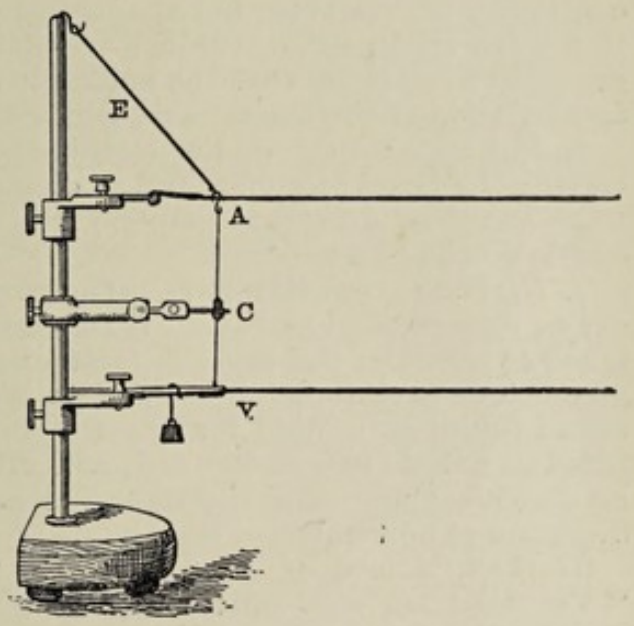


Fig. 257.—Gaskell's clamp. *C*, Heart in clamp; *A*, Auricular, and *V*, Ventricular lever; *E*, Elastic to raise *A* after it is pulled down.

Adjust the clamp to the heart in the auriculo-ventricular groove, but at first do not tighten it too much, just sufficient to keep the heart in position. After fixing the heart make both levers horizontal, and adjust the elastic thread attached to A, so that when its elasticity is called into play by the auricles pulling down the lever, it will, when they relax, make A horizontal.

Cause a time-marker to write exactly under the writing points of the two levers. Moisten the heart from time to time with dilute blood.

After obtaining a tracing with As and V contracting alternately (fig. 258), clamp the auriculo-ventricular groove slightly until the ratio of A to V

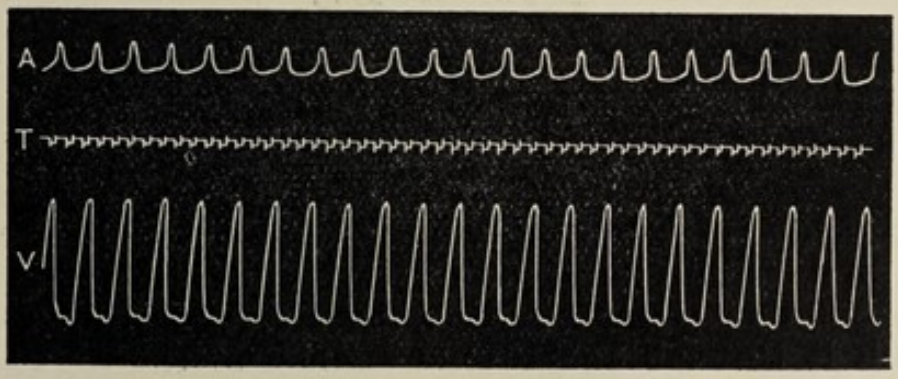


Fig. 258.—Tracing from auricle (*A*) and ventricle (*V*) of excised heart by Gaskell's method. *T*, Time in seconds.

contraction alters—*i.e.*, until, by compressing the auriculo-ventricular groove, the impulse from the auricles to the ventricle is "blocked" to a greater or less extent. The auricles will then contract more frequently than the ventricle — $\frac{A}{V}, \frac{1}{1}, \frac{2}{1}, \frac{3}{1}$, &c.

5. Transmission of contraction wave in ventricle (Engelmann's incisions). Normally, the contraction wave passes from the base to the apex of the heart, preceded by the active current of the heart. Is the conduction in the ventricle due to the presence of nerve fibres, or does it depend on the muscular tissue?

Expose a heart. Beginning at the apex, snip the ventricle transversely, alternately on opposite sides, to get a zigzag strip, so that the muscular continuity is preserved, but the nerves are cut (*Engelmann*). The contraction wave still passes over the mutilated ventricle. This experiment is regarded as showing that the muscular tissue itself is the main medium for transmission of the excitatory state.

6. Put a glass tube in the œsophagus, cut away the surrounding parts leaving the heart attached, and so dispose it that it rests on the œsophagus. Pass water at different temperatures through the tube and observe its effects on the heart.

7. Writing point (Bayliss).—The writing point of Bayliss is unsurpassed —*i.e.*, on a smoked surface. When it is necessary to diminish friction as much as possible, this style is most excellent. Fix to a straw a piece of gummed or parchment paper, and to this attach a bit of peritoneal membrane (same as is used for oncometers) and a bit of capillary glass tube fused to a little ball at the end, and attached to the peritoneal membrane by Prout's glue. The membrane is made broad to give rigidity in the direction of movement of the lever.

(Gaskell, *Journ. of Phys.*, iv., 43, 1883 (tortoise heart); Engelmann, "Versuche am suspendirten Herzen," *Pflüger's Archiv.*, lii., lvi., lix.; Kaiser, *Zeits. f. Biol.*, xxxii., 1895.)

LESSON LV.

STANNIUS' EXPERIMENT—ALL CONTRACTIONS MAXIMAL.

Intrinsic ganglia of heart.—The heart is connected to the central nervous system by two kinds of physiologically-distinct nerve fibres—inhibitory (vagus) and augmentor (sympathetic). Both sets reach it through the cardiac branch of the vagus (Lesson LVII.), which is really the vago-sympathetic. The two cardiac branches of the vagus enter the heart behind on the venæ cavæ, and run to the sinus, S.V., to come into relation with a group of ganglionic nerve-cells lying between the S V. and R.A., and called *Remak's ganglion* or "crescent" (1844). The nerve fibres pass along the septum between the auricles—septal nerves—and in their course are many nerve-cells—the septal ganglia or

Ludwig's ganglion (1848). The septal nerves pass to aggregations of nerve-cells that lie in the auriculo-ventricular groove, *Bidder's ganglion* (1852). No nerve-cells have been found in the lower two-thirds of the ventricle, although, like the rest of the heart muscle, it has an abundant supply of nerve fibres.

1. Stannius' experiment (1851).—(*a*) Expose the heart of a pithed frog (brain only); with a seeker clear the two aortæ from the auricles; pass a moist cotton thread between the two aortæ and the superior venæ cavæ; turn up the apex of the heart, divide the frænum, and raise the heart to expose its posterior surface, and the "**crescent**" (Remak's ganglion) or line of junction of the sinus venosus and the right auricle.

First Stannius ligature.—Bring the two ends of the ligature round the heart—call this, for convenience, No. 1 ligature—tie them, and tighten the ligature just over the "crescent," so as to constrict the line of junction of the S.V. with the R.A. Before tightening the ligature, see that the heart beats freely. Tighten the ligature, the auricles and ventricle usually cease to beat, and remain in a state of relaxation, while the S.V. continues to beat at the same rate as before. After a time, if left to itself, the ventricle may begin to beat, but with an altered rhythm. If the relaxed V be pricked, it executes a single contraction—*i.e.*, a single stimulation produces a single response.

(*b*) *Second Stannius ligature.*—When the heart is still relaxed, place a second ligature (No. 2, different colour) round, and tighten it over the auriculo-ventricular groove. Immediately the V begins to beat again, while the auricles remain in diastole.

(*c*) Instead of applying No. 2 ligature, the V may be cut off from the auricles. Immediately it is severed, the V beats.

(*d*) *Record graphically the effect of No. 1 ligature.*—A fresh frog's heart being arranged by a suspension method (fig. 252). On suddenly tightening the ligature the As and V cease to beat and the lever records a line higher up and nearly parallel to the abscissa, with slight elevation due to the beats of S.V. (fig. 259). Prick the V, it responds by a single contraction. The As similarly respond, and an induction shock elicits a single response. Try to record the effect of the *second ligature* (fig. 260).

Stannius ligature is of *practical importance* (i.) for arresting the uninjured ventricle to measure its electro-motivity (Lesson XLV.), (ii.) for ascertaining the latent period and other features of a single contraction of cardiac muscle in the same way as one

investigates a simple twitch on skeletal muscle (p. 258). (Hofmann, "Function d. Scheidewandnerven d. Froschherzens," *Pflüger's Archiv.*, Bd. 60, p. 139.)

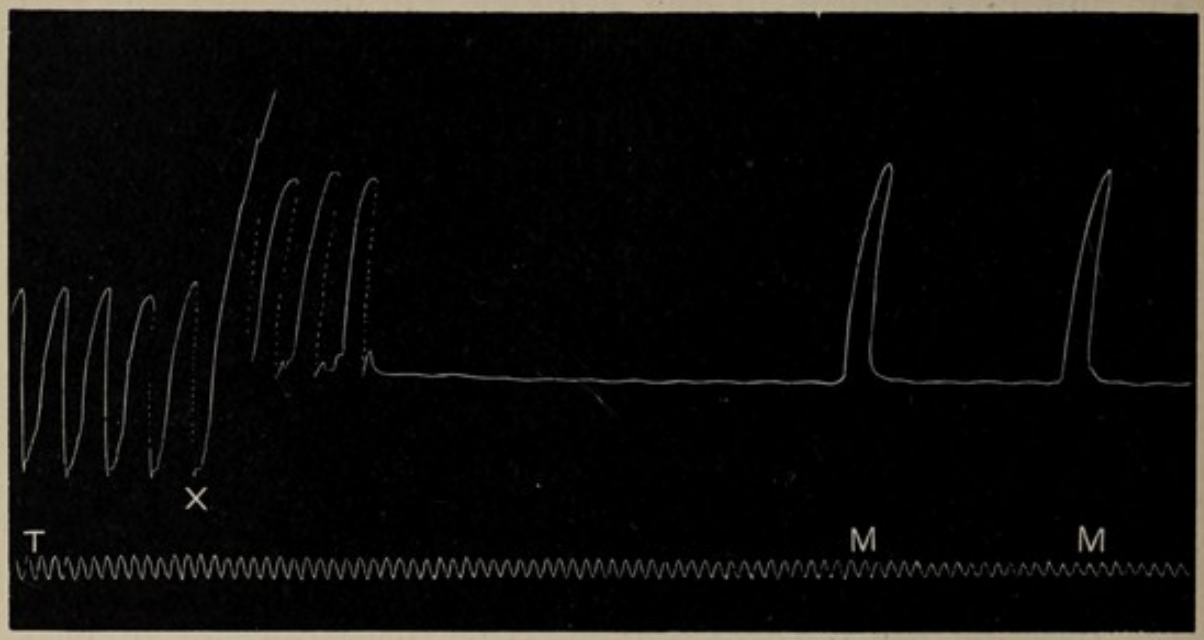


Fig. 259.—Effect of first ligature of Stannius. The beats, *M*, are due to mechanical stimulation of the quiescent ventricle. Read from below upwards. ×, Moment of application of ligature. *T*, Time, 100 D.V. per second (*Stirling*).

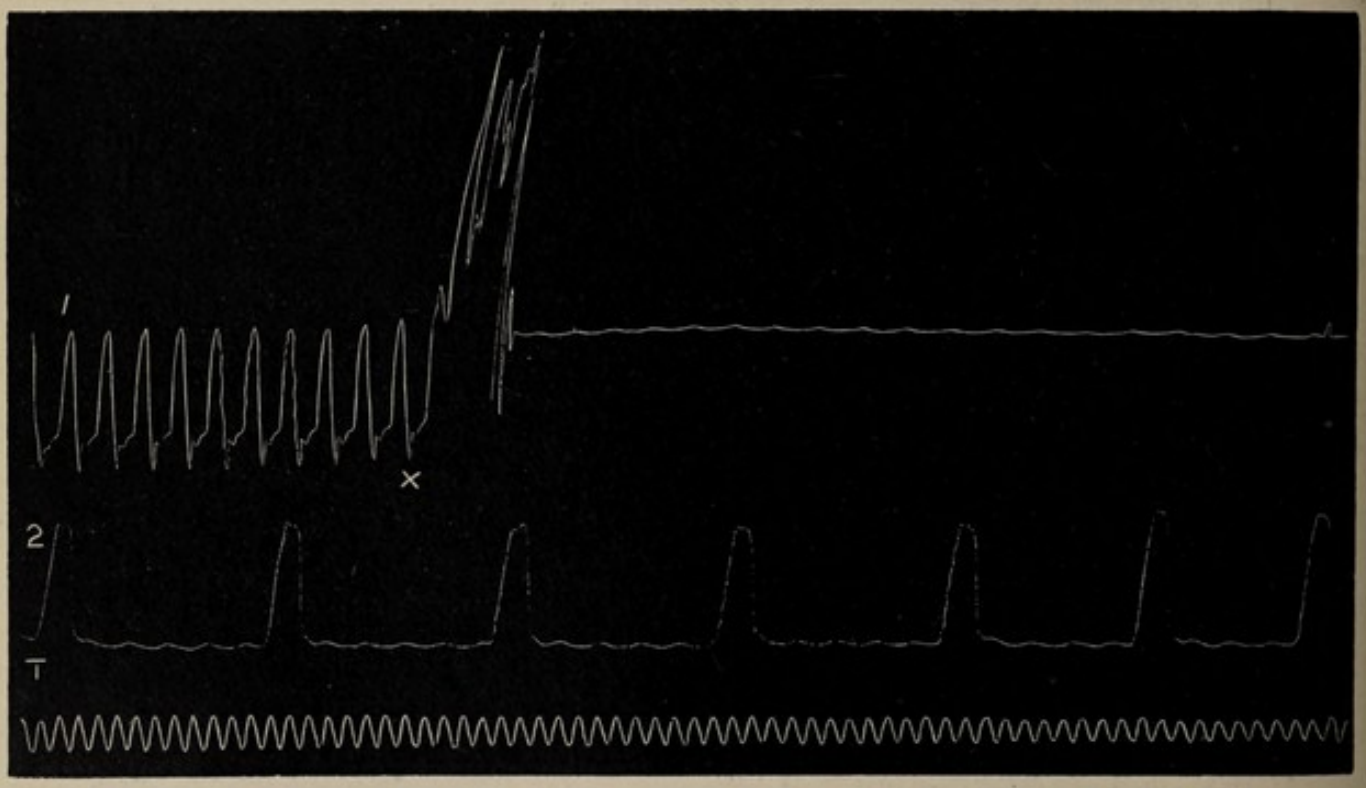


Fig. 260.—1. Normal heart-beat (frogs). ×, First ligature of Stannius; the smaller beats are due to S.V., which continues to beat. 2. Effect of second ligature; the large beats are due to V, the smaller to As and S.V. Note difference of rate of V and S.V.-As (*Stirling*).

2. **Minimal stimulus causes maximal contraction** ("all or nothing"). "All contractions maximal." Stannius a heart attached to a recording suspension lever to record only height of contraction. Arrange single shocks of a coil to give ineffective break-shocks. Push up S coil until a minimal break shock elicits a contraction. Move drum 1 mm.; wait a minute and push up S coil 1 cm.; stimulate again = a response of equal height. Increase the strength of the break-shock, and record result. There is no increase in the height. The heart either contracts or it does not, and if it does, it does its best. It differs in this respect from skeletal muscle in that a minimal stimulus causes a maximal contraction, there being no graduated relation between strength of stimulus and the response as in skeletal muscle. The reason why about a minute is allowed to elapse after each stimulation is explained by the following experiment:—

3. **Staircase character of heart-beats.**

Attach the apex of the heart by means of a silk thread to a recording lever, as in fig. 252, and record on a slow drum.

Stannius a heart as above—*i.e.*, arrest the beating of the auricles and ventricle by tightening a ligature over the sino-auricular groove.

The heart is quiescent. Stimulate it with single effective break induction shocks at intervals of 5 seconds. Notice that the first beat is taller than the second, the second than the third, so that each beat exceeds its predecessor in amplitude until a maximum beat is obtained—usually about the fifth or sixth. The amount of increase in height gradually decreases towards the end of the series—*i.e.*, the increment of height of 2 above 1 is greater than 3 above 2, and 4 above 3, &c. This is the "Staircase" or "Treppe" of Bowditch (fig. 261). A similar result is obtained with skeletal muscle (fig. 188). The form of the curve is said to be a hyperbola (1871).

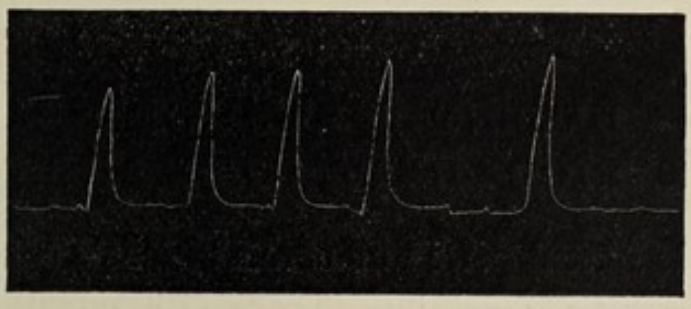


Fig. 261.—Staircase of heart-beat (frog) produced by single shocks applied to quiescent Stanniused heart (*Stirling*).

4. **Load on ventricular contraction.**—(*a*) Record by a suspension method the contractions of the unopened frog's ventricle—well filled with

blood—clamp the aortæ with forceps (jaws covered with india-rubber) to increase the intra-ventricular pressure—*i.e.*, the load.

(*b*) Note great diminution of frequency of beat, but each individual contraction is increased in force.

5. Tonus of auricles.—Record the beats of one auricle of a tortoise heart on a slow drum, so that the beats are close together. Note (1) the individual beats; (2) a secondary rhythm—rise and fall—involving twenty or more beats, due to the oscillations of the "tonus" of the auricle. Not infrequently one gets such a rhythm in a "suspended" frog's heart when the ventricle has been exposed for a time and has nearly ceased to beat. These curves occur most markedly in fatigued hearts or those about to die. Fano (who has specially studied this phenomenon) attributes it to stimulation of the ends of the vagus fibres in the auricles. (*Arch. ital. de Biol.*, xxxiv.) Cold is apt to produce it, and so do drugs that stimulate the terminations of the vagus.

6. Inhibition by galvanic current—Heart.—Connect a cell through a M and B key with a commutator (cross-bars), and the latter with a rheochord. Connect N.P. electrodes with the slider and positive binding screw of the rheochord. Expose the beating heart of a pithed frog. Arrange one electrode over the liver and the other over the apex of the heart, making the latter the anode. Make current. The part of the ventricle under the influence of the + remains at rest, and is filled with dark red blood during systole of the rest of the heart—*i.e.*, localised diastole.

Reverse commutator, so that the heart electrode is now −, break the current. At the next systole the kathodal region remains relaxed during the contraction of the ventricle.

LESSON LVI.

LATENT PERIOD OF HEART—REFRACTORY PERIOD—HEAT, COLD, AND CONSTANT CURRENT.

1. Latent period of cardiac muscle (Cardiac delay)—*Record single beat.*—The latent period is ascertained in the same way as in skeletal muscle, but there is this difference, the heart beats rhythmically while the skeletal muscle is at rest until excited. Therefore the heart-beat must be brought to a standstill. This can be done by a Stannius ligature.

(*a*) Arrange a coil for single effective break-shocks, putting in the P circuit an electro-magnet to record its movement on a slow drum to indicate the moment of stimulation.

(*b*) Expose and Stannius the heart of a pithed (brain) frog. Tie a silk thread to the apex of the ventricle, and attach the

thread to a Gaskell's heart-lever, arranged to record exactly above the electro-magnet.

(*c*) Adjust a time lever over the electro-magnet lever.

(*d*) Two horizontal lines are recorded. Stimulate the base of the ventricle with a single break induction shock (fine electrodes); the moment of stimulation will be indicated by the second lever, and shortly after the heart will respond. The interval represents the "latent period," usually about $\frac{1}{6}$ second, but it may be nearly half a second according to temperature and other conditions (fig. 262).

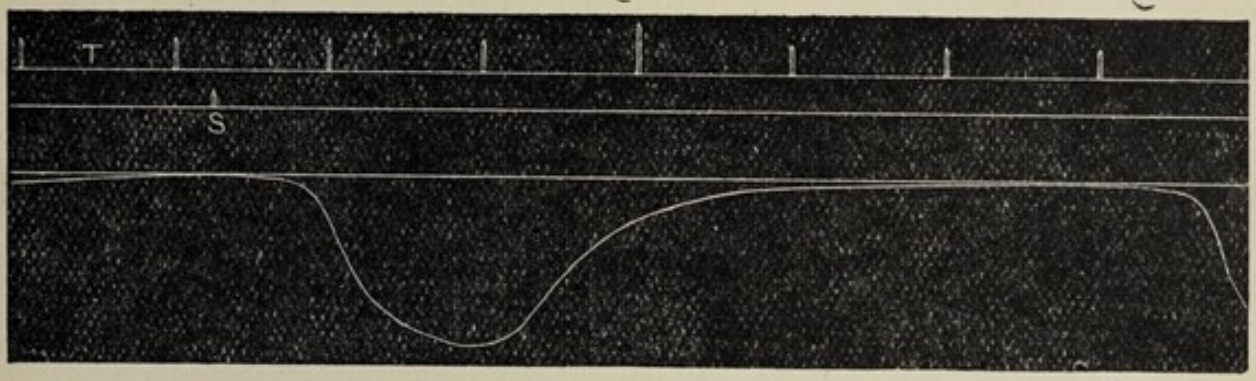


Fig. 262.—Tracing of Stanniused heart of frog, stimulated at S with a single maximum induction shock. *T*, Time in seconds. Gaskell's lever (*Stirling*).

(*e*) Stimulate the auricle, and observe the longer "delay"; the wave of contraction takes longer to travel, and is delayed at the groove.

2. Latent period of heart.—Arrange the experiment exactly as for a skeletal muscle (fig. 137), substituting suspension method for the crank-myograph, and for the time-tracing record 50

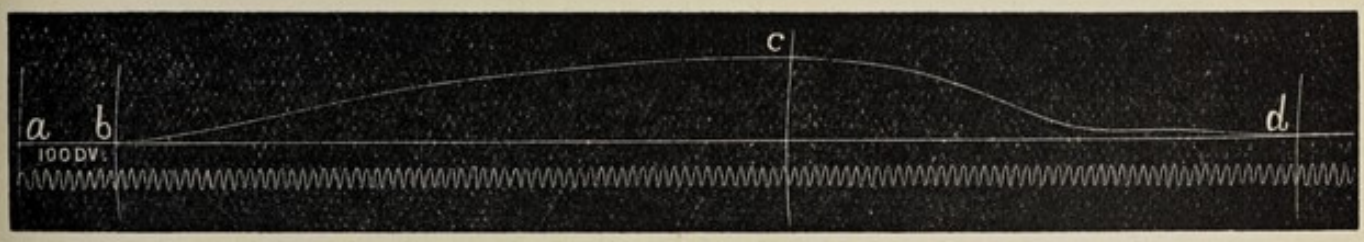


Fig. 263.—Single heart-beat (frog), record on rapid drum. *a b*, Latent period; *b c*, Shortening; *c d*, Relaxation (*Stirling*).

vibrations per second, or less, according to circumstances. Mark (1) moment of stimulation (automatic break); (2) when contraction begins (L.P.); (3) when it reaches its maximum; and (4) when relaxation ends (fig. 263). Notice the flat top of the heart-curve. Measure the several phases, and record in your note-

book. Compare the relative duration of the several phases with your records of a skeletal muscle.

L.P. = ; Contraction = ; Relaxation = second.

3. Heat and cold on single beat.—Arrange a heart to record as a Stannius preparation; rate of drum 2 cm. per second. Arrange electrodes—single automatic break-shocks—to stimulate the ventricle. Bathe the heart with diluted blood (1 blood, 4 normal saline), at varying T°—*e.g.*, 5°, 15°, 20°, 30° C.—each for about 1 minute; then record a contraction at each of the above T°, all on the same abscissa. Record point of stimulation, and measure the curves. Compare the results as regards L.P., period of contraction, and relaxation with those for skeletal muscle. They agree pretty closely (p. 258),

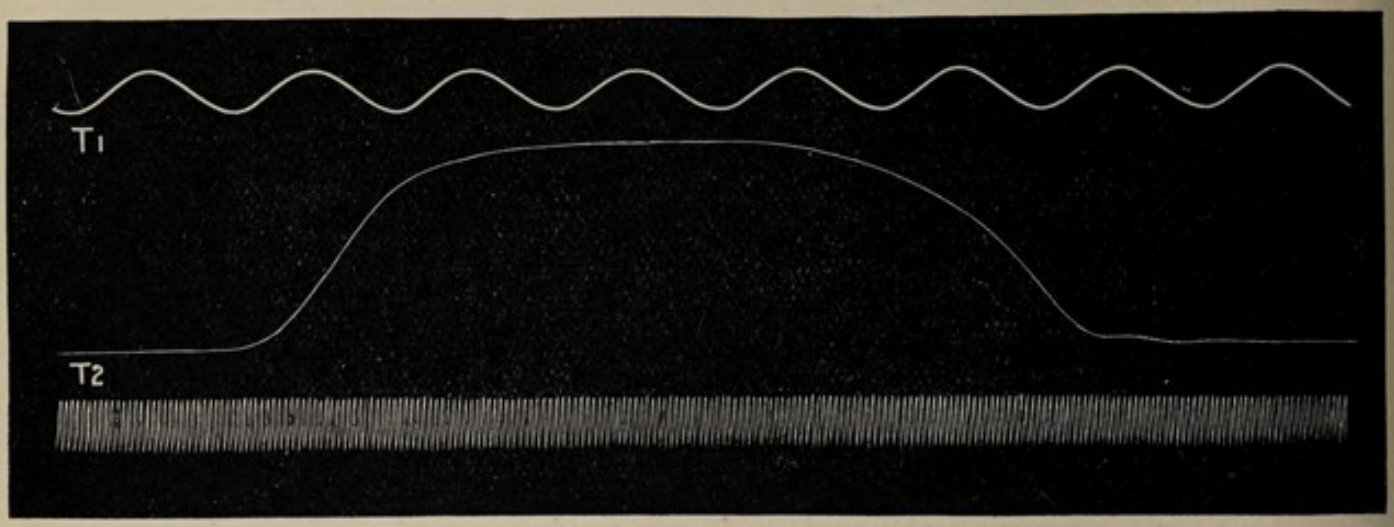


Fig. 264.—Single conrtaction of ventricle (frog). T_1, Time 0·5″; T_2, 100 D.V. per second.

Measure curves, and tabulate as follows:—

Temp.	L.P.	P. of Contr.	P. of Relax.	Total.	Height.
5° C.					
15°					

The effects are more readily seen if the results be plotted on "millimetre paper"—*i.e.*, paper ruled into areas 1 mm. square. T° is marked on the abscissa, and time or height on the ordinates.

(i.) L.P., P. of contraction, and P. of relaxation shorten with rise of T°, but not necessarily, on the whole, in the same ratio.

(ii.) The duration of the whole beat also shortens with rise of T°.

(iii.) The height of the contraction shows greatest diminution with high T°.

4. Refractory period of heart—*i.e.*, the time during which a stimulus produces no obvious mechanical response. The heart has a *long refractory period* which lasts through the whole period of its contraction (shortening). We have already studied the effect of two successive stimuli applied to skeletal muscle and found that its refractory period practically coincides with its latent period. (Lesson XL., fig. 178.)

(*a*) Arrange a drum (rate, 3 cm. per second) to record by automatic break two successive shocks (Lesson XL.).

Arrange Stanniused heart and recording lever as in previous experiments.

(i.) Record a single beat, one contact used, marking L.P. on an abscissa.

(ii.) Arrange second contact so that the second stimulation shall fall well on the descent (relaxation) of curve. Mark points of stimulation (fig. 265). An "extra contraction" is obtained.

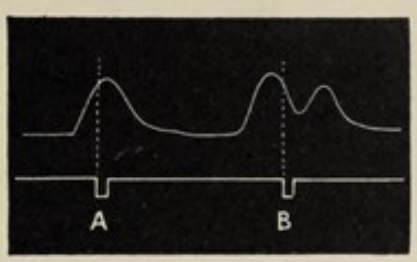


Fig. 265. — Refractory period of heart. Shock applied at *A*, ineffective; at *B*, effective and causing an "extra contraction."

(iii.) Repeat (ii.), but bring the second contact-striker nearer to the first, so that the second stimulus is thrown in just after the beginning of the descent (relaxation). Mark points of stimulation.

(iv.) Bring the two contacts nearer, and repeat the double stimulation so that the second shall fall during the contraction (shortening) period. Record points of stimulation.

The fact of physiological importance is that the heart has a long refractory period—*i.e.*, during the whole of the period of shortening. This is not to be interpreted that there is a fundamental difference between skeletal and cardiac muscle, but only that there is a difference in degree.

5. Compensatory pause.—If the heart executes a second contraction before the completion of its beat, then there is a short delay before the next beat. This is the "compensatory pause" (fig. 266).

6. Repeated shocks.—To the same Stanniused heart apply tetanising shocks, but vary the strength of the stimulus. Arrange to record on a slow drum.

With repeated rapid strong stimuli the heart is not thrown into tetanus. It beats with an altered rhythm and may not relax completely. There is no complete fusion of its contractions, as in skeletal muscle, so that it is said "the heart knows no tetanus"—*i.e.*, complete tetanus.

7. Constant current on heart.

(*a*) Pith a frog. Cut out the heart, dividing it below the auriculo-ventricular groove, thus obtaining an "**apex**" preparation which does not beat spontaneously.

(*b*) Fix a cork to a lead base, cover the upper end of the cork with sealing-wax, and thrust through it two wires to serve as electrodes, about 4 mm. apart (fig. 267), or by means of sealing-wax fix two fine wires upon an ordinary microscopic glass slide to act as electrodes. Cover the whole with a beaker lined with moist blotting-paper. Place the heart apex with its base against one electrode, and its apex against the other.

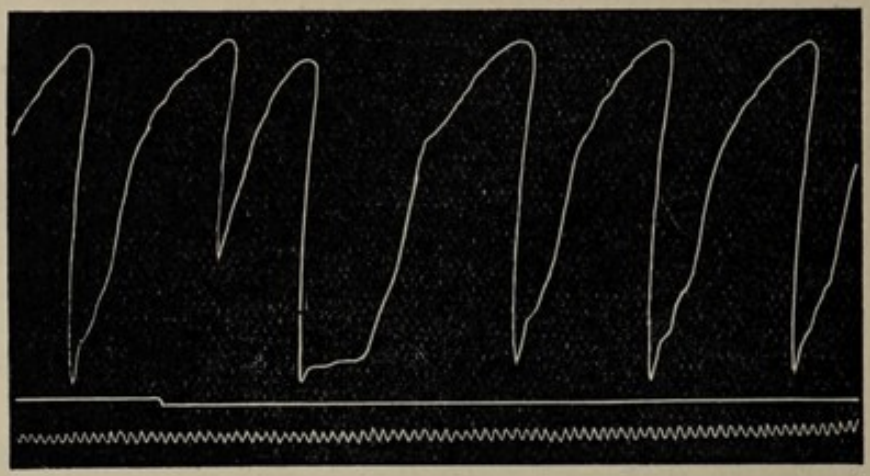


Fig. 266.—An "extra-systole" comes in before the second has completed. This is followed by a longer interval before the next beat occurs—the "compensatory pause."

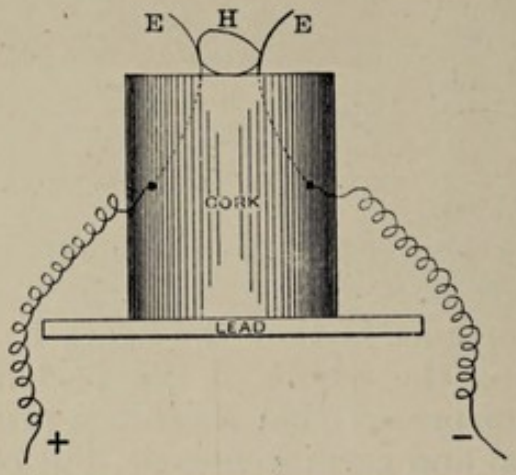


Fig. 267.—Support for frog's heart. *E*, Electrodes; *H*, Heart.

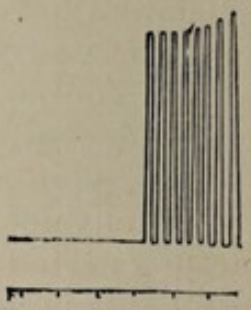


Fig. 268.—Staircase character of heart-beat.

(*c*) Arrange two cells in circuit, connect them with a key, and to the latter attach the electrodes. Pass a continuous current in the direction of the apex. The heart resumes its rhythmical beating, and continues to do so as long as the constant current passes through the living preparation.

8. Staircase of apex preparation.—(*a*) To a microscopical glass slide fix with sealing-wax two copper wires in the long axis of the slide, their free ends being about 3 millimetres apart. They will act as electrodes. Connect the other ends of the wires to a du Bois key introduced in the S circuit of an induction machine. Arrange the P coil for single induction shocks, introducing a Morse key in the circuit.

(*b*) Make an "apex preparation," and place it on the electrodes on the glass slide. Rest on the heart a light heart-lever properly counterbalanced and arranged to record its movements on a slow-moving drum (5 mm. per

second). The preparation does not contract spontaneously, but responds to mechanical or electrical stimulation.

(*c*) Stimulate the apex preparation with single-break induction shocks at intervals of about 10 seconds. To do this, open the S circuit, depress the Morse key, short-circuit the S circuit, and close the Morse key again. Repeat this at intervals of 10 seconds, and note that the amplitude of the second contraction is greater than the first, that of the third than the second, the fourth than the third, and then the successive beats have the same amplitude (fig. 268). Allow the heart apex to rest for a few minutes, and repeat the stimulation—same result. From the graduated rise of the first three or four beats after a period of rest, the phenomenon is known as the "staircase." The increment is not equal in each successive beat, but diminishes from the beginning to the end of the series.

(*d*) If, while the apex is relaxing, it be stimulated by a make shock, it contracts again, so that the lever does not immediately come to the abscissa. Observe the effect of the "extra-contraction" on the next beat. It is delayed; there is a compensatory pause (fig. 266).

LESSON LVII.

INNERVATION OF FROG'S HEART—INTRACARDIAC NERVES — VAGUS — AUGMENTOR NERVES.

Innervation of the frog's heart.—The heart is connected with the central nervous system by two sets of physiologically distinct nerve fibres—inhibitory and augmentor—antagonistic in their action, having separate origins, but both reach the heart through the splanchnic branch of the vagus.

The augmentor fibres leave the spinal cord in the anterior root—chiefly of the third spinal nerve—and pass *viâ* the ramus communicans to the third sympathetic ganglion, and thence up the cervical sympathetic to join the vagus and proceed with it to the heart. It is probable that the augmentor fibres end around the nerve cells in the sympathetic ganglion (indicated by nicotin method), and that new fibres proceed from these ganglionic cells. The important fact is that the augmentor or sympathetic fibres reach the heart *viâ* the vagus, so that the so-called vagus in the frog is really the **vago-sympathetic**, consequently stimulation of the vagus or its cardiac branch does not necessarily yield

a true or pure vagus effect. The course of the vagus—inhibitory nerve—is shown in fig. 269.

The positive relations of the intracardiac ganglia are given in Lesson LV.

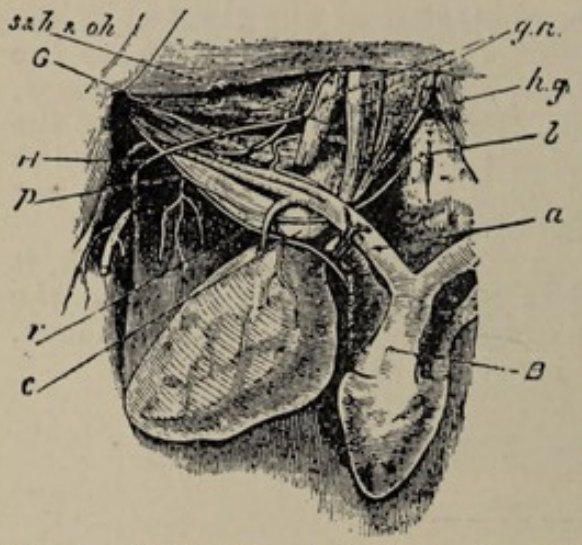


Fig. 269.—Dissection of frog's heart, right lung, and chief cranial nerves in relation with them. Œsophagus distended with glass tube. *a*, Right aorta; *b*, Larynx; *c*, Posterior horn of hyoid; *gh*, Geniohyoid; *hg*, Hyoglossus; *p*, Lowest of three petrohyoids; *H*, Hypoglossal; *G*, Glosso-pharyngeal; *r*, Vagus; *B*, Bulbus aortæ.

1. **Intracardiac inhibitory mechanism** (*Atropin*). — (*a*) Expose a frog's heart. By means of a fine ligature attached to the frænum gently raise the whole heart upwards to expose the somewhat whitish V-shaped "crescent" (Remak's ganglion) between the sinus venosus and right auricle.

(*b*) Arrange previously a coil for fairly strong repeated shocks, using a short-circuiting key. Place the electrodes—which must be fine, and their points not too far apart (2 millimetres)—upon the crescent, and faradise it for a second; if the current be sufficiently strong, after a latent period—one or two heart-beats—the auricles and ventricle are relaxed and cease to beat for a time, but they begin to beat even in spite of continued stimulation (vagus escape). The thin copper wire or pin electrodes are conveniently supported and fixed by modeller's wax on a short cylinder of lead.

(*c*) Stimulate the auricles; no inhibition or arrest.

(*d*) Apply a solution of sulphate of atropin (Lesson LIX.) to the heart, having previously surrounded its base with small pieces of blotting-paper. Stimulation of the crescent no longer arrests the heart. The atropin paralyses the inhibitory fibres of the vagus. Had nicotin been used, the heart would be inhibited, at least at first, by stimulation of the crescent.

2. **Record inhibitory (crescent) arrest.**

(*a*) Take a tracing with Gaskell's lever. Stimulate the crescent for 1 to 2 seconds with weak tetanising induction shocks as in suspension method, and observe the arrest of the beat (fig. 270). The lever remains horizontal. Sometimes at first slight acceleration is obtained, due to stimulation of the accelerans or aug-

mentor fibres which run in the vagus of the frog—*i.e.*, it is really the vago-sympathetic (*Gaskell*). In the P circuit place an electro-magnetic signal. This vibrates when the P circuit is closed, and marks the period of stimulation as a white patch on the black surface. Make its point record exactly under the heart-lever. Take a time-tracing in seconds.

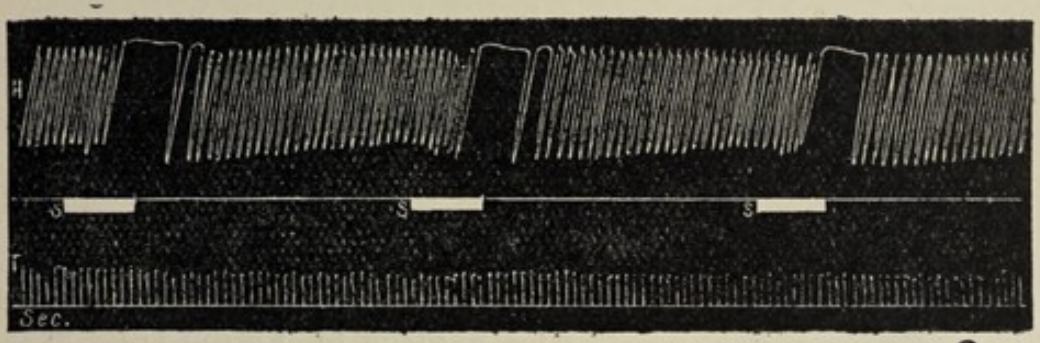


Fig. 270.—Arrest of frog's heart-beat by electrical stimulation of crescent. *Sec.*, Time in seconds; *H*, Heart-beats; *S*, Stimulation.

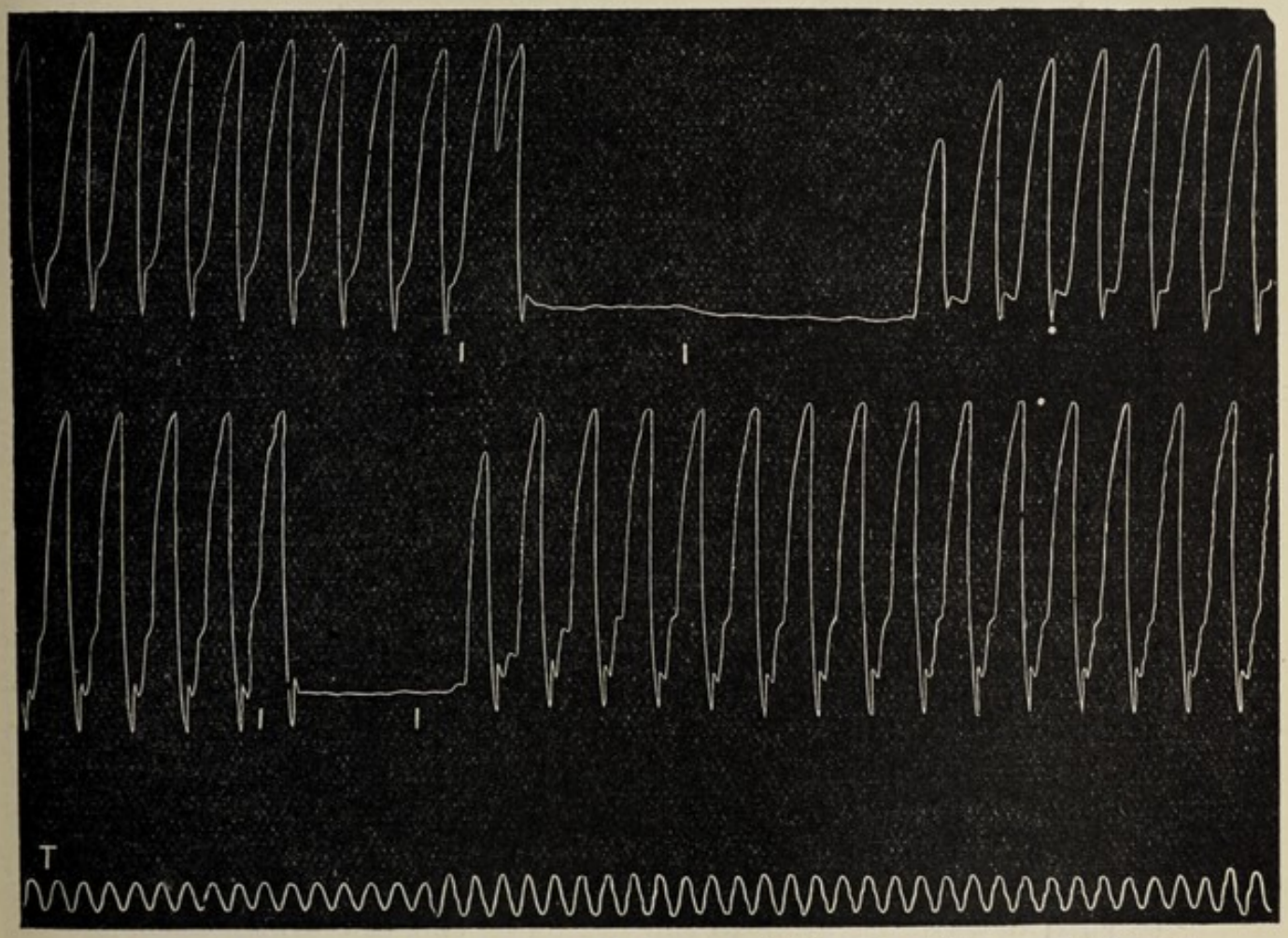


Fig. 271.—Strong stimulation of crescent between vertical marks. Note arrest of As and V in diastole, and staircase of beats after arrest. The tracing shows very well the successive systole and diastole of S.V., A, and V. Read from below up. Time, 0·5 sec. Both tracings from same heart (*Stirling*).

(*b*) After a pause the beat begins, the contraction travelling as a wave from sinus, through auricles to ventricle.

(*c*) Stimulate the auricles. During inhibition the sinus beats, but the auricles and ventricle do not, because the excitability of the auricles is so lowered that they do not propagate the excitatory process.

(*d*) Stimulate the ventricle mechanically, the heart beats in the reverse order from ventricle, auricles to sinus.

(*e*) Do the same with a suspension method, when the heart acts on end of a lever, as in A, and record the effect of stimulation of the crescent (Fig. 271).

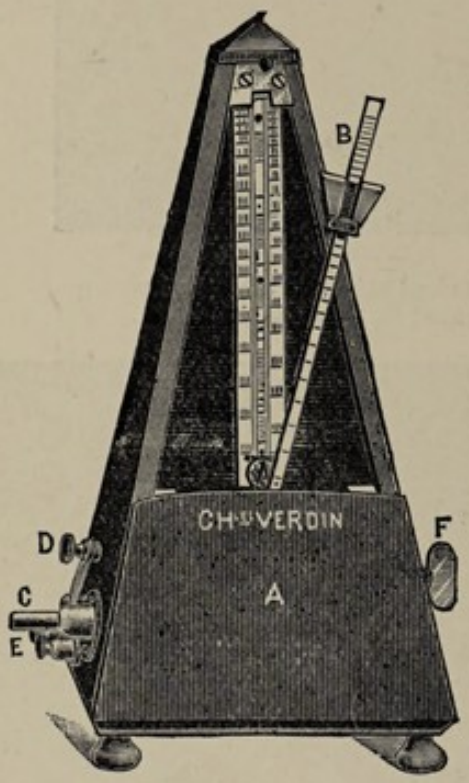


Fig. 272.—Registering metronome (*Verdin*). It can be used either for electric currents, or be coupled up through *C* with a recording tambour.

A simple chronograph for recording time is made by Verdin (Fig. 272) from a modified metronome. It can be used either for electrical contact, or with a Marey's tambour.

3. Cardiac vagus of frog.—Make a preliminary dissection before attempting to stimulate the vagus.

Lay a frog on its back on a frog-plate. Expose the heart, and pull the fore-legs well apart. Introduce a small test-tube or stick of sealing wax into the œsophagus, as the nerves leaving the cranium are better seen winding round from behind when the œsophagus is distended. Remove the structures covering the petrohyoid muscle, which reaches from the petrous bone to the posterior horn of the hyoid bone (Figs. 269, 273), and are the guide to the vagus. Three nerves, or rather four, are seen coursing round the pharynx parallel to these muscles. The lowest, nearest the middle line and superficial, is the hypoglossal (Hg) easily recognised by tracing it forward as it crosses the petrohyoid muscle (P.H.) and the other structures to pass to the tongue.

More laterally placed and anteriorly is the glosso-pharyngeal (G.P.), which is seen at the angle of the jaw, and crossing P.H. to run forward parallel to the ramus of the jaw under cover of the hyoglossus to the tissues in the floor of the mouth. Running along the lower border of P.H. in close relation with the cutaneous

branch of the pulmo-cutaneous artery is a fine nerve—the vagus—the lowest of all.

The vagus gives off its laryngeal branch near the angle of the lower jaw, and this branch runs nearly parallel to the visceral branch of the vagus. It is, however, easily seen lying on the petrohyoid, and passing upward and onward to the larynx. Now trace the vagus as it runs along the superior vena cava to the right auricle, but before it reaches the heart it gives off a branch to the lungs. Now trace the vagus to its origin at the skull. Cut away the entire lower jaw by a cross-cut from angle to angle. Remove the mucous membrane from roof of mouth, expose levator anguli scapulæ (Fig. 275), and cut it at its attachment to the parasphenoid of the skull. Observe at the angle of the skull a pigmented mass—vagal ganglion—and from it trace the glosso-pharyngeal and vagus running ventrally. Trace the latter onwards towards the heart. Observe the fine laryngeal branch of the vagus (L) as a separate nerve running for part of its course parallel to V. The vagus divides near the heart; one branch goes to the heart—cardiac—and the larger backwards to the lung. The vagus outside the cranium is the **vago-sympathetic**. The G.P. and V leave the cranium through the same foramen in the ex-occipital bone, and through the same foramen the sympathetic enters the skull. *N.B.*—The cardiac vagus contains both vagal and sympathetic fibres, and is, therefore, the **vago-sympathetic**.

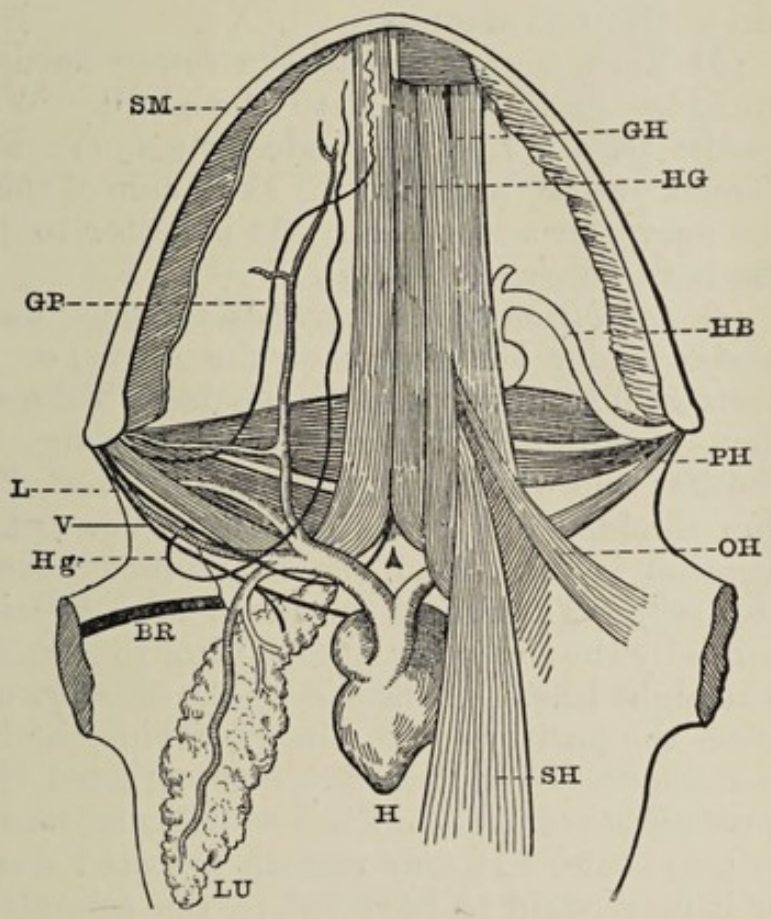


Fig. 273.—Dissection of frog's vagus. *SM*, Submentalis; *LU*, Lung; *V*, Vagus; *GP*, Glosso-pharyngeal; *Hg*, Hypoglossal; *L*, Laryngeal; *PH*, *SH*, *GH*, *OH*, Petro-, Sterno-, Genio-, Omo-hyoid: *HB*, Hyoid; *HG*, Hyoglossus; *H*, Heart; *BR*, Brachial plexus.

It is convenient to remove the frog from the frog-plate, and study the disposition of the nerves from the side.

4. Stimulation of vagus (vago-sympathetic in frog—*i.e.*, cardiac inhibitory fibres).—(*a*) Arrange a coil for weak tetanising shocks with short-circuiting key in the S circuit, and an electric signal in the primary, the latter arranged to record on a drum. Adjust a suspension heart-lever to record on a slow drum (2 mm. per sec.). Record seconds.

(*b*) Find the vagus at the lower border of the petrohyoid muscle. Trace it back to the skull. With a fine aneurism needle pass a ligature under the nerve near the skull, tie the thread, cut the nerve on the skull side of the ligature, and isolate the nerve for about 1 cm. It is better to do this than to isolate the nerve near the heart.

(*c*) *Inhibition of the heart-beat.*—Place well-insulated fine electrodes under the trunk of the nerve, or place a thin piece of india-rubber under the electrodes. Take a normal tracing, and then stimulate the vagus with strong tetanising induction shocks; the whole of the heart is *arrested in diastole.* Use the fine shielded electrodes shown in fig. 343. Although the faradisation is continued, the heart recommences beating ("vagus escape"). The arrest, or *period of inhibition*, is manifest in the curve by the lever rising or falling to zero, and recording merely a straight line. If the laryngeal muscles contract, and thereby affect the position of the heart, divide the laryngeal branches of the vagus. The heart, when arrested, is soft and flaccid—*diastolic standstill*—but before it is arrested it usually makes one or two beats. It may remain arrested during the stimulation, or it may begin to beat before the stimulation—if prolonged—ceases. Instead of arrest there may be merely slowing of the beats. The diastolic arrest may continue after stimulation has ceased.

(*d*) When the heart begins to beat the beats are small at first, and gradually rise to normal, but sometimes they are more vigorous (fig. 274).

(*e*) When the vagus is found to be effective, test the effect of *strength of current*, beginning with the S coil at 20 or 25 cm. from the primary coil. A strength of current will be found when the *rate* is slowed.

(*f*) Note any change in the rhythm of As and V, and in the height (force) of the beats.

(*g*) When complete inhibition is obtained, touch the ventricle sharply with the point of a seeker. The ventricle gives a single response, showing that the *inhibited heart is still excitable.*

It is important to remember that both vagal (inhibitory) and sympathetic (accelerator or augmentor) fibres are stimulated; therefore the results are complex. The vagus fibres have a short latent period and the sympathetic a much longer one, so that apparent "after-effects," really due to stimulation of the sympathetic, will be found. Moreover, the vagi may be unequal in their effect on the heart.

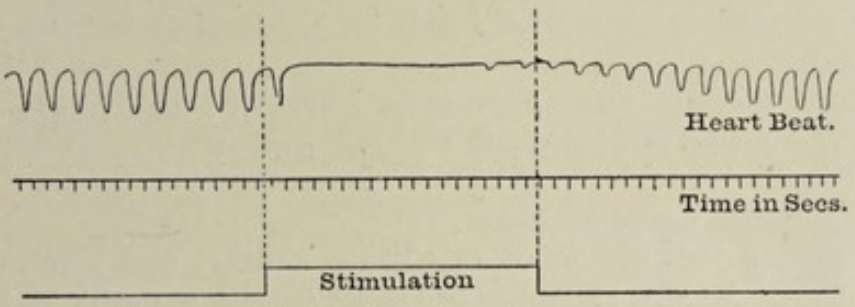


Fig. 274.—Stimulation of cardiac vagus of frog.

Taken broadly, however, **stimulation of the vagal fibres** brings about:—

(i.) Slowing or arrest (inhibition) of the heart-beat, according to the strength of stimulus.

(ii.) Weakening of the force of the beat.

(iii.) The condition of the wave of contraction from As to V is favoured.

(iv.) After-effects, which have been differently interpreted, indicating increased force of beat.

The only way to get a pure vagal effect is to stimulate the vagus within the cranium before it is joined by the sympathetic.

Besides these effects, it can be shown that vagus stimulation increases the intra-ventricular blood pressure, lessens the inflow and outflow of blood from the ventricle, and lowers the tone of the heart muscle.

5. **Stimulation of vagus *v.* "crescent."**—In "winter frogs" the student may sometimes find what may appear atypical results in connection with vagus action. But, on the same heart, it is well to compare the effect of stimulation of the vagus with the marked, prompt, and decided effect produced by stimulation of the crescent (Lesson LVII., i.), noting the effect on rate, sequence, force, and extent of contraction of As and V with different strengths of current—using, of course, interrupted shocks.

6. **Latent period or delay of vagus.**—(*a*) For this purpose a time-marker and an arrangement to indicate when the stimulus is thrown into the nerve are required. Arrange the heart-lever and a time-marker to write exactly over one another.

(*b*) Arrange coil for repeated shocks with an electro-magnetic

signal in the primary circuit, and so adjusted that its writing-style writes exactly under the heart-lever.

(*c*) Close the primary circuit vigorously; the magnet vibrates, and the style records when the heart is beating and recording steadily. Stimulate the vagus, and observe the effect. Move up the secondary coil until a sufficient strength of stimulus is obtained. The heart may be only slowed, and not arrested. The heart is not arrested immediately, but a certain time elapses —*latent period*—about one beat (1·5 second) before the heart is arrested.

(*d*) Short-circuit the secondary current; the heart gradually resumes its usual rhythm—S.V., As, and V.

7. Sympathetic nerve on heart.

(*a*) Arrange a coil for weak tetanising shocks, and place an electro-magnetic signal in the primary circuit to record moment of stimulation exactly under the heart-lever.

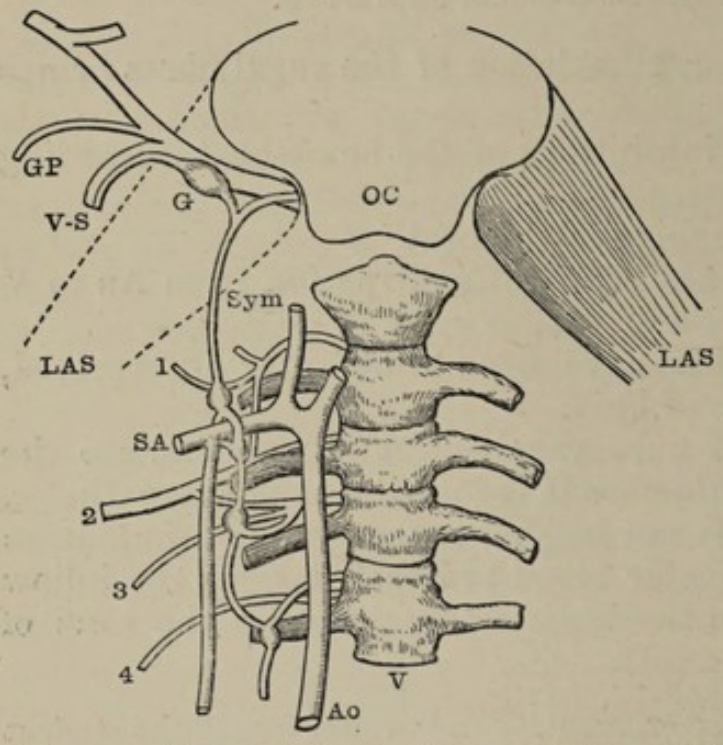


Fig. 275.—Scheme of the frog's sympathetic. *LAS*, Levator anguli scapulæ; *Sym*, Sympathetic; *GP*, Glosso-pharyngeal; *V-S*, Vago-sympathetic; *G*, Ganglion of the vagus; *Ao*, Aortæ; *SA*, Subclavian artery (*Gaskell*).

(*b*) Pith a frog, or preferably a toad, and cut away the whole of the lower jaw by a transverse cut. Remove the mucous membrane covering the roof of the mouth, and bring into view the triangular levator anguli scapulæ. The sympathetic is found before it joins the vagus emerging from the cranium (fig. 275). Carefully isolate the sympathetic. It lies immediately under the levator anguli scapulæ, which must be removed with fine forceps, when the nerve comes into view, usually lying under an artery. The nerve is usually pigmented. Trace the nerve downwards, when it will be seen crossing the second spinal nerve and forming the annulus of Vieussens round the subclavian artery just above this. The augmentor, accelerator, or sympathetic fibres come out chiefly in the anterior root of the third nerve, and in less numbers in the fourth. If the petrohyoid muscles are removed, the whole course of the vago-sympathetic is disclosed. Put a ligature round it as far away from the skull as practicable, and cut the nerve below the ligature.

(*c*) Record heart-beat by a suspension method for several contractions, and then stimulate the sympathetic with weak interrupted shocks by

means of fine electrodes. The heart beats quicker, and may not relax completely. If the heart is beating quickly, reduce the number of beats by *cooling* it with ice placed near it, or drop on it cooled normal saline. In the tracing observe the long latent period, and that (i.) the beats are quickened—*i.e.*, increased rate; (ii.) force of the beats is increased; (iii.) conduction from As to V is slowed, thus leading to alteration in sequence of beat of As and V; and (iv.) observe the after effects. These effects are exactly the opposite of those of vagal stimulation.

(*d*) Isolate and stimulate the vagus and compare the effects of the two nerves (the vagus outside the skull is really the vago-sympathetic). Stimulation of the **intracranial vagus**—*i.e.*, before it is joined by the sympathetic—is omitted here. The vagus effect varies with the season of the year. In some animals one vagus is inactive.

8. Stimulation of vagus from mouth.—In the frog the vagus, after leaving the cranial cavity, passes across the levator anguli scapulæ superioris and touches the cartilaginous capsule of the middle ear. The nerves may be here reached from the inside of the mouth if suitable bent electrodes be applied to the posterior margins of the Eustachian tubes. A weak current produces a strongly marked vagus action on the heart. This is effective even after complete destruction of the whole central nervous system. (Muskens, *Amer. Journ. of Phys.*, i., 486, 1898.)

9. Seat of motor centres.—(*a*) Expose the heart in a pithed frog, cut out the ventricle with the auricles attached to it, observe that the heart continues to beat. Divide the ventricle vertically by two parallel cuts into three portions. The middle portion contains the auricular septum, in which lie ganglionic cells. It continues to beat while the right and left lateral parts do not beat spontaneously, but respond by means of a single contraction if they are stimulated.

10. Effect of destruction of central nervous system on heart and vascular tonus (*Goltz*).

(*a*) Destroy the brain of a frog, expose heart, avoid loss of blood; heart fills well with blood (*red*).

(*b*) Suspend the frog, or leave it on its back, introduce a stout pin into the spinal canal, destroy the spinal cord, and leave the pin in the canal. The heart still beats, but is *pale*, *collapsed*, and apparently *empty*; it no longer fills with blood. The blood remains in the greatly dilated abdominal blood-vessels, and does not return to the arterial system. If the belly be opened, the abdominal veins are seen to be filled with blood.

(*c*) Amputate one limb at the thigh, perhaps not more than one or two drops of blood will be shed. A frog with intact spinal cord bleeds freely after amputation of a limb.

LESSON LVIII.

ENDOCARDIAL PRESSURE—FROG MANOMETER—TONOMETER.

THE endocardial pressure is measured by the frog-manometer of Kronecker (figs. 276, 277), which registers the changes of pressure in the excised heart, fed with a nutritive fluid circulating through its cavities. Two preparations of the heart are used. One called a *heart preparation*, which includes the ventricle and part of the auricles, and therefore the ganglionic cells of the auriculo-ventricular groove and some auricular nerve cells. The other consists of the lower two-thirds of the ventricle, and contains no nerve cells. It is called an *apex preparation*. By using the latter preparation, one can eliminate the nerve cells.

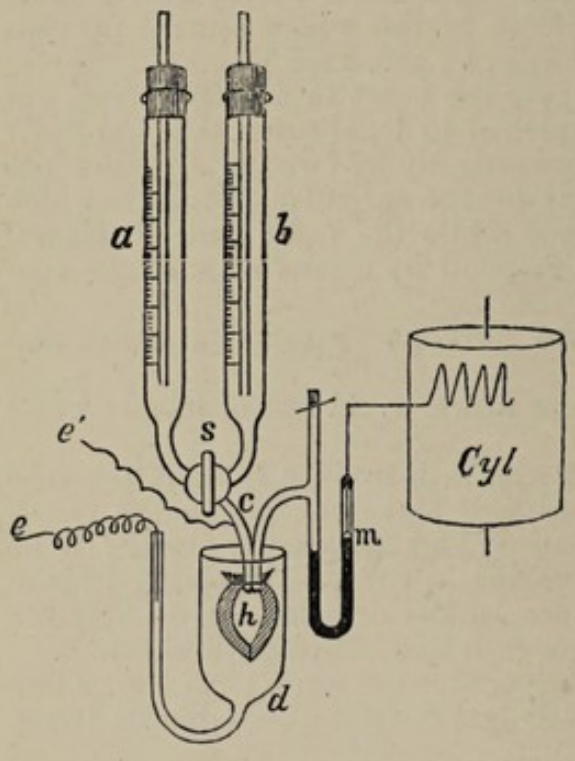


Fig. 276.—Scheme of Kronecker's frog manometer.

1. **Frog manometer.**—A two-way cannula (figs. 276, 277) is used to perfuse fluids through the heart, the end, *d*, being tied into the heart, while *a* is connected with a stopcock, so that either of the two reservoirs —Marriotte's flasks—can be made to communicate with the heart. The other limb, *b*, is connected with the Hg-manometer, which is provided with an exit tube to permit of the whole system being filled with the fluid to be perfused, when it is clamped. The manometer has a float and recording style in its open limb arranged to record on a slow drum. To keep the heart moist it is placed in a vessel, *d*, filled with the perfusion fluid. The letters *e* and *e'* indicate where electrodes can be attached if it be desired to stimulate with electrical shocks.

When S is turned so that *a* can communicate with the cavity of the heart, *f* being open, the fluid simply flows through the heart. When, however, the communication with *a* is closed and *f* is also closed, the beat of the heart exerts its pressure on the Hg column and records on the drum. All air must be absent from the system.

By using two Marriotte flasks, each communicating with the inlet tube and capable of being shut off or opened by clamps, two different fluids may be perfused. Further, by adding atropin, muscarin, spartein, or other drug to one reservoir, one can readily ascertain the effect of these drugs on the heart, or the antagonism of one drug to another.

Marriotte's flask keeps the pressure of the fluid in the inflow tube constant.

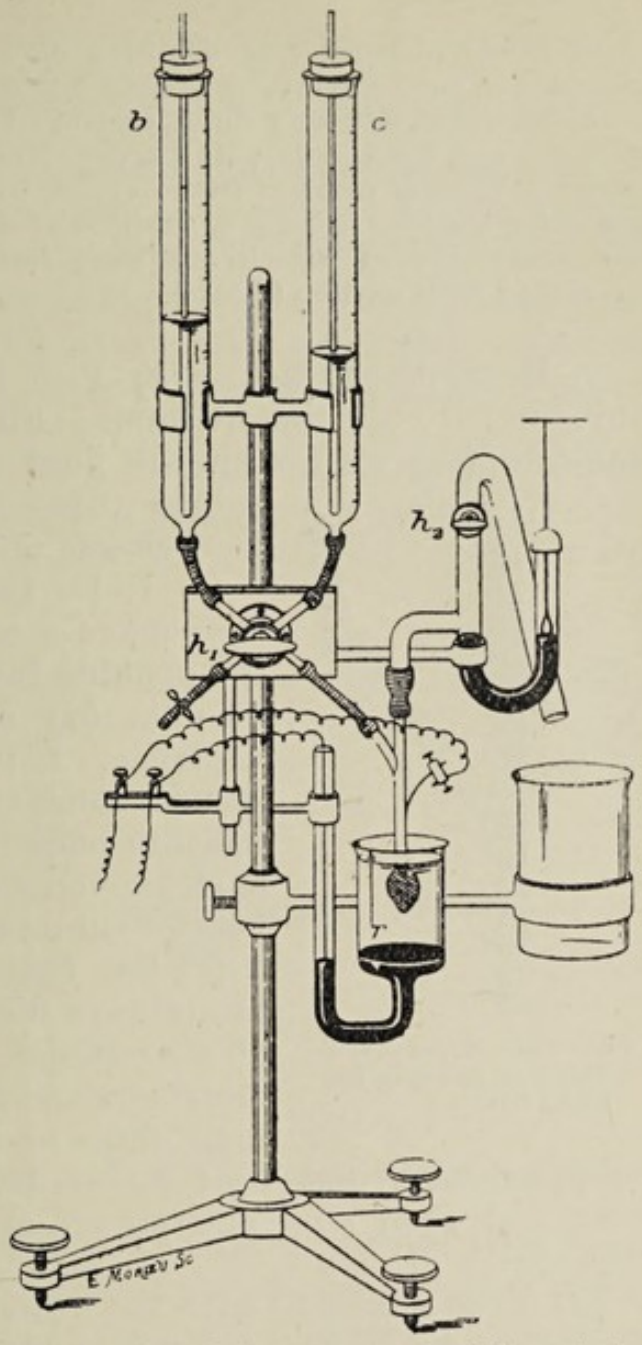


Fig. 277.—Complete arrangement of Kronecker's frog manometer.

2. The perfusion fluid must be capable of nourishing and sustaining the energy of the cardiac tissue.

(*a*) **Fluids.**—To two volumes of normal saline add one of defibrinated sheep's blood, well shaken up with air, mix, and filter through linen.

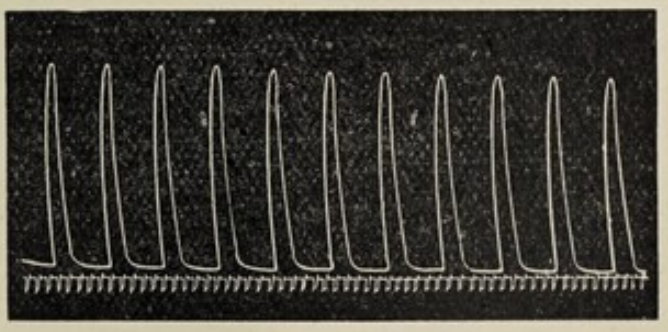


Fig. 278.—Tracing of frog's heart perfused with dilute blood. The lower line indicates seconds.

(*b*) **Ringer's fluid.**—Saturate 0·5 p.c. NaCl solution

with calcic phosphate, and add 2 c.c. of a 1 p.c. solution of potassic chloride to each 100 c.c.

(*c*) Modified Ringer's fluid [Lesson LIX., 3 (*d*)].—Locke's fluid consists of NaCl 0·9, $CaCl_2$ 0·024, KCl 0·042, $NaHCO_3$ 0·01 to 0·02 p.c. in *distilled* water. This, *if charged with oxygen*, will keep an excised heart of rabbit going for hours. A 0·1 p.c. solution of dextrose rapidly "knocks down" the heart's beats, which are soon restored by the above fluid when saturated with oxygen (*Locke*).

3. **Heart-preparation.**—(*a*) Pith a frog, expose its heart, ligature and divide the frænum. Fill the tubes of the two-way cannula (fig. 279) with the fluid to be perfused. Pinch the rubber tubes with bull-dog forceps to prevent escape of the fluid.

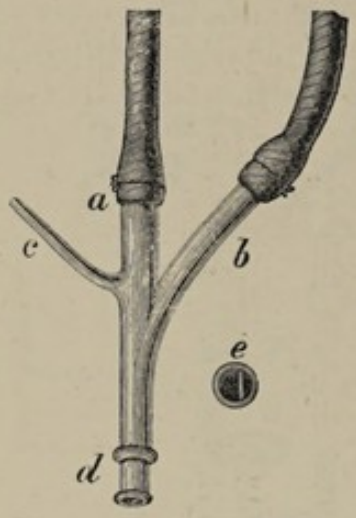


Fig. 279.—Kronecker's two-way cannula for frog's heart.

(*b*) Raise the heart by the frænum ligature, make a cut in the sinus, and through the opening introduce the lower end of the cannula (*d*) until it is well within the ventricle. If it is desired to have a "heart-preparation," tie it in with a ligature—the ligature constricting the auricles above the auriculo-ventricular groove. Cut out the heart with its cannula.

(*c*) See that the heart and tubes of the cannula are filled with the perfusion fluid—no air bubbles—then tie it on to the supply reservoirs and manometer. Open *f*. Perfuse some fluid, and then close *f*. The heart so prepared may not beat spontaneously for a time, or it may beat and then stop and go on again, giving what is called "Luciani's groups."

(*d*) Take a tracing with the outflow tube, *f*, and Marriotte's flask shut off, so that the whole effect of the contraction of the heart is exerted upon the mercury in the manometer. Take another tracing when the fluid is allowed to flow continuously through the heart. The second Marriotte's flask shown in the figure is for the perfusion of a different fluid. By means of the stopcock, *s*, one can pass either fluid through the heart.

4. **Apex-preparation.**—Only the apex of the heart is used. As a rule, it does not beat spontaneously until sufficient pressure is applied to its inner surface by the fluid circulating through the heart.

(*a*) Prepare the heart as for the last exercise, but place the cannula deeper into the ventricle, and tie the ligature round the

ventricle *below the auriculo-ventricular groove.* Excise the heart and cannula, and attach it to the heart apparatus as in the previous experiment.

(*b*) If the "heart apex" preparation does not contract spontaneously after getting up pressure, stimulate it by, *e.g.*, single induction shocks, attaching wires from the S coil to *e* and *e'*.

(*c*) By introducing an electric signal into the P circuit, and having a time-marker recording at the same time, one can determine the latent period of the apex preparation. It is about 0·15 sec.

(*d*) If desired, the effect of a constant current may be studied in this way instead of by the method described in Lesson LVI., 7. The apex beats rhythmically under the influence of the constant current.

5. Piston-recorder or Heart-plethysmograph of Schäfer (fig. 280).

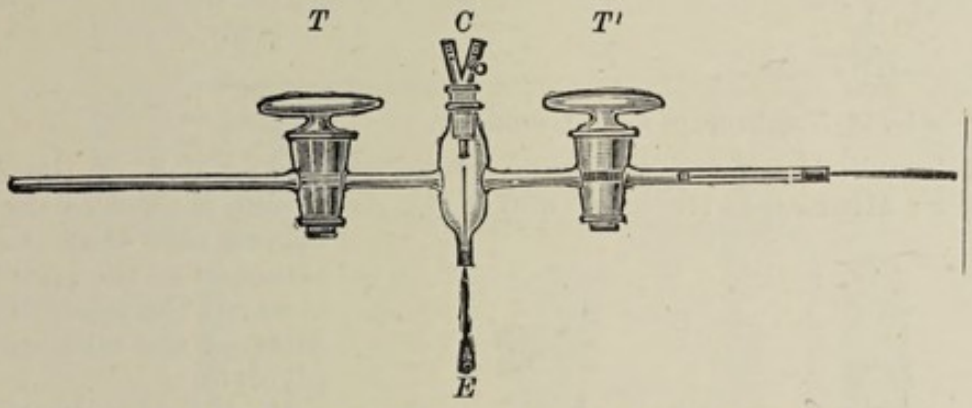


Fig. 280.—Schäfer's heart-plethysmograph. *T*, *T'*, Taps; *C*, Cannula; *E*, Electrode.

The heart is tied to a two-way cannula as before, introduced into a horizontal tube with a dilatation on it, and fitted on either side with a tap. The tap on the left is for adjusting, the other is in relation to the piston. The bulb is fitted with electrodes. The bulb and tube are filled with oil, and as the heart dilates it forces the oil along the tube and moves a light piston resting on it. When systole takes place, the oil recedes and with it the piston. The piston records on a slow drum placed horizontally, and gives excellent results. (*Journ. of Phys.*, v., 130.)

6. Roy's Tonometer (*Frog Cardiograph*).—This apparatus registers the change of volume of the contracting heart. Fig. 281 shows a scheme, and fig. 282 shows the apparatus itself. It consists of a small bell-jar resting on a circular brass plate about 2 inches in diameter, and fixed to a stand adjustable on an upright. In the brass plate are two openings—the small one leads into an outlet tube, *e*, provided with a stopcock. The other is in the centre of the plate, and leads into a short cylinder 1 cm. in length by

1 cm. in internal diameter. A groove runs round the outside of this cylinder near its lower edge, to permit of a membrane being tied on to it. In this cylinder works a light aluminium piston, *p*, slightly less in diameter than the cylinder. Around the lower aperture of the cylinder is tied a piece of flexible animal membrane, the ligature resting in the grooved collar. The free part of the membrane is tied to the piston, from the centre of whose under-surface, *p*, a needle passes down to be attached to a light writing-lever, *l*, fixed below the stage. The bell-jar is filled with oil, *o*, while in its upper opening is fitted a short glass stopper, perforated to allow the passage of a two-wayed heart-cannula with the heart attached, *h*.

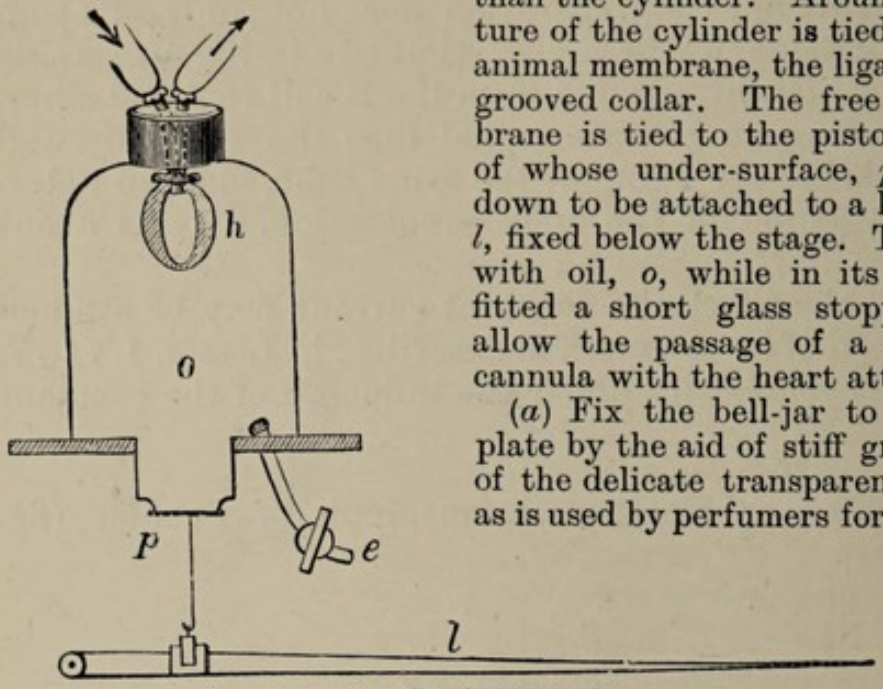


Fig. 281.—Scheme of Roy's tonometer.

(*a*) Fix the bell-jar to the circular brass plate by the aid of stiff grease. Tie a piece of the delicate transparent membrane—such as is used by perfumers for covering the corks of bottles—in the form of a tube round the lower end of the grooved cylinder; afterwards the lower end of the membrane is fixed to the piston, taking care that the needle attached to the piston hangs towards the recording lever. Moisten the membrane with glycerin.

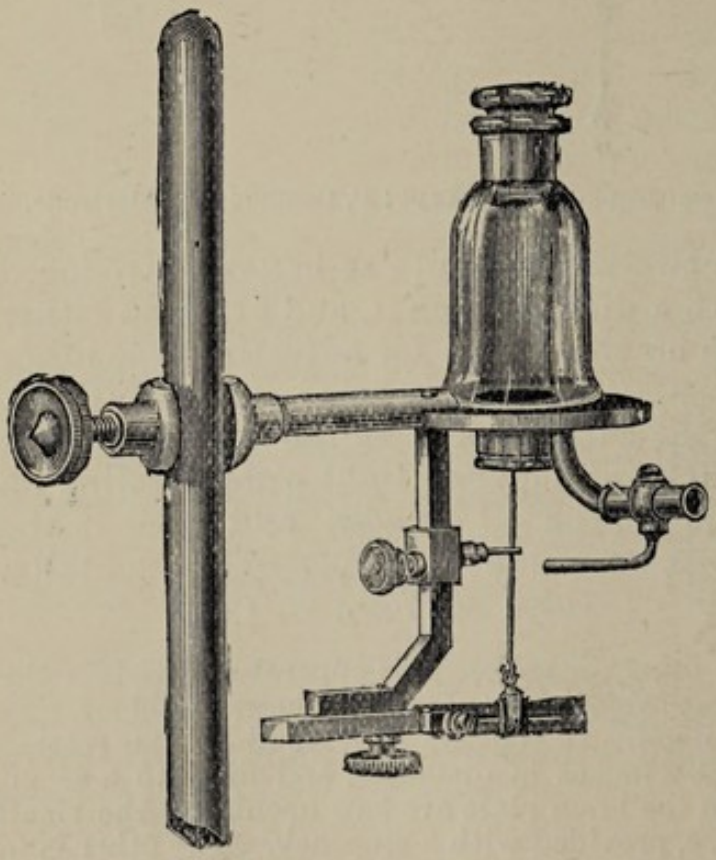


Fig. 282.—Roy's tonometer (Cambridge Sc. Instrument Co.).

(*b*) Fill the jar with olive oil, and have the recording apparatus ready adjusted. Prepare the heart of a large frog, the cannula used being one fixed in the glass stopper of the bell-jar, and attach the inlet tube of the cannula to the reservoir of nutrient fluid, while the outlet tube is arranged so as to allow fluid which has passed through the heart to drop into a suitable vessel.

(*c*) Introduce the cannula, with the heart attached, into the oil, and see that the stopper is securely fixed. Open the stopcock, *e*, and allow some oil to flow out of *o*, thus rendering the pressure within sub-atmospheric;

and, as soon as the pressure has fallen sufficiently and the little piston is gradually drawn up to the proper height, close the stopcock. Attach the needle of the piston to the recording light lever, and take a tracing (*Journ. of Phys.*, i., 452, 1879).

For a modification by Gaskell, whereby the heart beats in normal saline and not oil, see *Journ. of Phys.*, iii., 50, 1880.

7. **Arterial blood-pressure in frog.**—(*a*) Expose the heart of a decerebrated and lightly curarised frog. Make a glass cannula with its tip sufficiently small to pass into one aorta. Tie a short length of rubber tubing to the other end of the cannula and fill it with 1 p.c. sodium carbonate solution. Close the tube with bull-dog forceps or with a glass rod.

(*b*) Prepare a small heart Hg-manometer, like that in fig. 283, and see that its proximal limb is filled with the soda solution, and that the float is arranged to record on a slow drum.

(*c*) Isolate one aorta, and ligature it as near the head as possible. Put a second ligature round the aorta, as near the heart as possible ; do not tie it. Between the second ligature and the heart clamp the aorta with fine forceps. With sharp scissors make an oblique slit in the aorta—apex of slit towards heart—between the first ligature and the forceps, introduce the cannula with its narrow end towards the heart, and tie it in position with the second ligature.

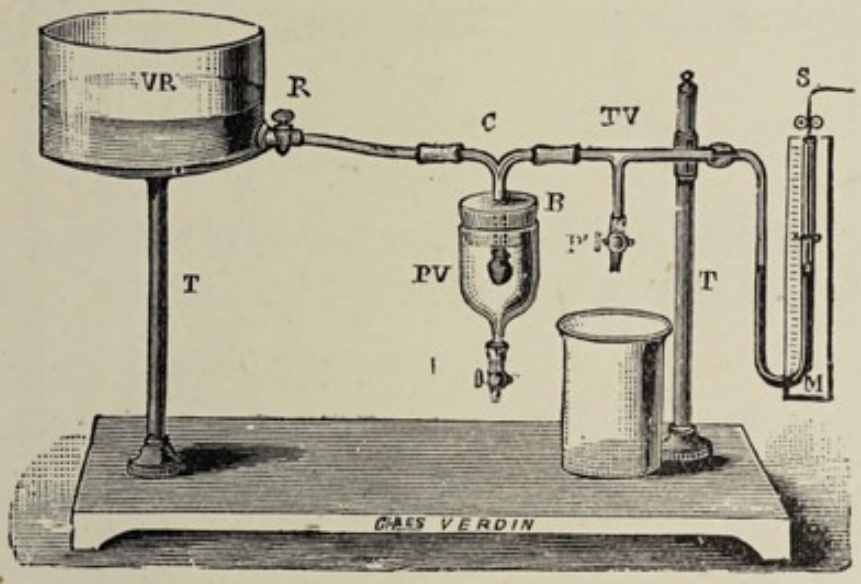


Fig. 283.—Simple form of frog's heart manometer (*Fredericq*).

(*d*) Connect the elastic tube of the cannula with the tube of the proximal limb of the manometer, see that the whole system is filled with solution of sodium carbonate—no air. The carbonate prevents the blood from coagulating. Remove the forceps between the cannula and the heart, and record the beats of the heart. (For the details of the method used to estimate the pressure in mammals, see Lesson LXVI.)

This preparation may be used to show the effect of various conditions on the arterial—*i.e.*, aortic—blood-pressure—*e.g.*, stimulation of the central end of any sensory nerve—*e.g.*, the sciatic, or compression of the abdomen (*Fredericq*).

Fig. 283 shows a simple form of frog heart manometer for the excised heart that can readily be made in any laboratory.

8. **Williams' heart apparatus** (frog's heart) is used largely by pharmacologists. The excised heart (fig. 284, *h*) is "perfused" and the arterial pressure acts on the Hg in the manometer, *m*, provided with a recording pen, *s*. The heart is, as in fig. 284, connected with a Marriotte's flask to obtain a constant pressure. It differs from Kronecker's form in that the two tubes leading from the perfusion cannula are each provided with ingenious membranous valves (fig. 285) which permit the perfused fluid to pass readily towards the heart and manometer, but prevent its reflux. If

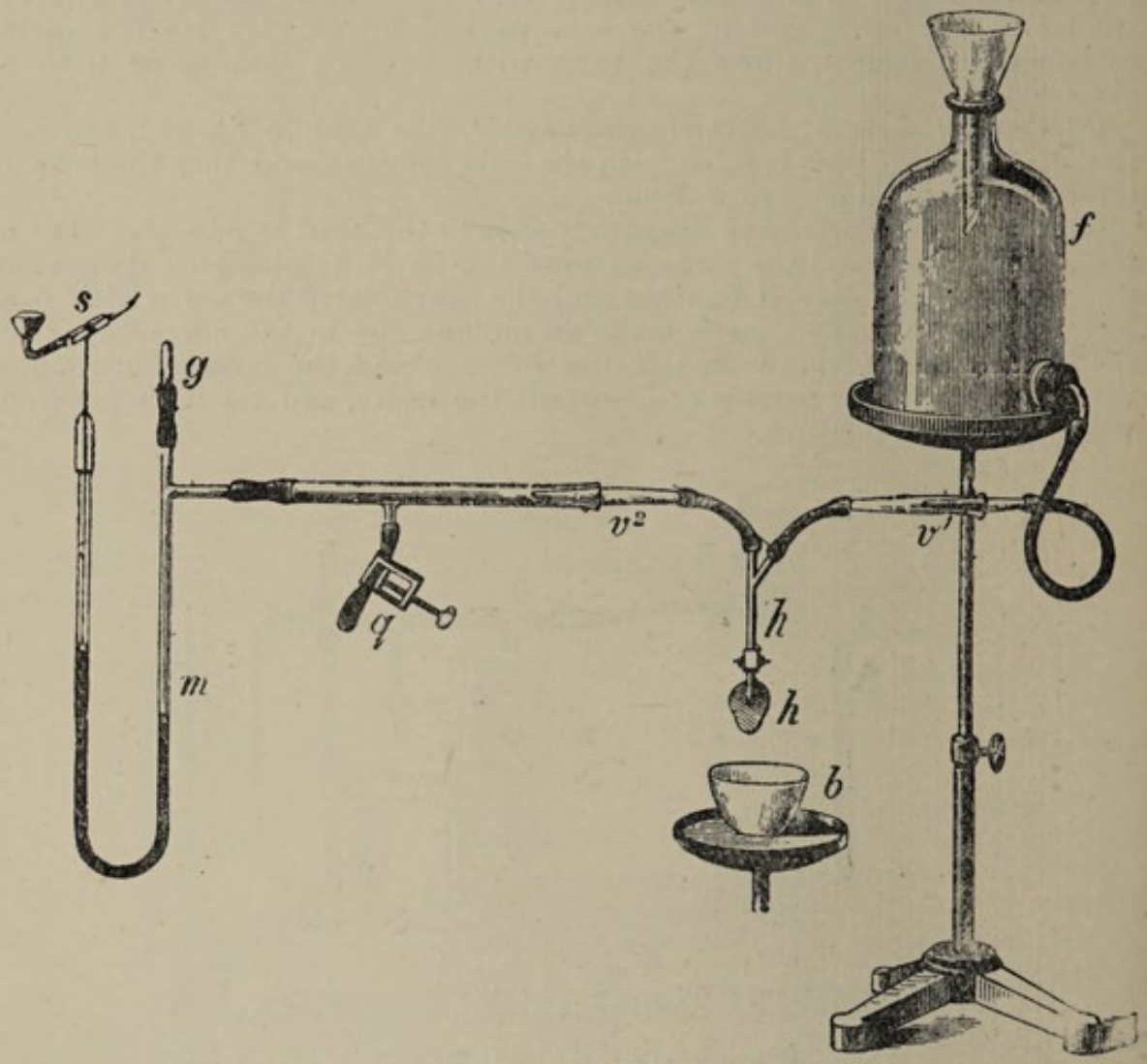


Fig. 284.—Williams' frog-heart apparatus. *h*, *h*, Heart and cannula; v_1 and v_2, Special valves; *g*, *m*, *s*, Hg-manometer and writing style; *f*, Pressure bottle.

the outlet tube, *q*, be closed, then a two-way cannula, *k*, is introduced through one aorta until its point lies in the ventricle. Ligature the cannula and the veins. The two tubes from the cannula are connected to the valves, v_1 and v_2, the former connected with a Marriotte's flask placed at a suitable height, and the other with *f*, the manometer, the whole system being filled with the fluid to be perfused. Fig. 285 shows the arrangement of valves of natural size. A thin membrane is tied over the slit, *s*—a piece of the intestine of a frog may be used.

If the valves are arranged as in fig. 285, the apparatus acts as a "**maximum manometer**," so that it may be used not only to register

the heart-beats, but also to determine the absolute force exerted by the beating heart. To do the former, the outflow tube must be regulated, and then the heart beats with constantly renewed or perfused fluid (*Archiv. f. exp. Pharmakol*, xiii., 1880).



Fig. 285.—Plan of valves used for fig. 284.

9. Glass perfusion cannula.—A glass perfusion cannula is easily made. First make a cannula like fig. 344, with a T attachment and shoulder of the requisite size. Pass through the long tube a narrower tube, and over the end removed from the shoulder fix rubber tube to keep the inner and outer tubes in position, the inner one, of course, projecting beyond its containing tube (fig. 292).

LESSON LIX.

ACTION OF SALTS, ALKALOIDS, DRUGS, AND EXTRACTS ON FROG'S HEART.

Action of drugs on heart.

Either the excised heart, placed in a watch-glass, or the heart *in situ* may be used. The heart should be made to record its beats by a suspension method (fig. 252). The best plan is to record by a suspension method — having previously exposed the vagi—if it be desired to test the effect of vagus action on the heart after the action of a drug. Set up a coil for tetanising shocks, and an arrangement for recording time. The apparatus of Klemensiewicz is very convenient. The pendulum of a metronome beats alternately against two receiving tambours, and by air-transmission through communicating with a recording tambour time is recorded.

1. Distilled water.—We have seen that this is a powerful poison for muscle and nerve, and, indeed, for all protoplasmic tissues. Record the beats of a heart by a suspension method, and allow distilled water to drip on the heart from a pipette.

Make a snip in one auricle, which allows the blood to escape, and the distilled water to act on the heart. The beats become slower, less frequent, and the prolonged action of distilled water stops the beat; but if its salts be restored by bathing it with normal saline made with tap-water, it may begin to beat.

2 Normal saline.—If made with tap-water it sustains the heart-beat for some time, as it contains several salts, especially of calcium. As many drugs are dissolved in normal saline, before testing the effect of such a drug it is important to note the effect, if any, of the normal saline used as a solvent.

"Normal saline" cannot permanently maintain the beat. The heart ceases to beat, and passes into a condition of "apparent death," from which it may be restored by the action of normal saline containing blood, or Ringer's fluid, or a mixture composed of 0·7 p.c. NaCl, 0·026 p.c. $CaCl_2$, and 0·03 p.c. KCl (*C. W. Greene*). The contractions ultimately recorded under normal saline present the appearance of fatigue; such an appearance is not due to fatigue, but to the inorganic salts necessary to contraction having been removed. The phenomenon of so-called exhaustion or apparent death is not due to exhaustion of the contractile substance of the heart (C. W. Greene, *Amer. Journ. of Phys.*, ii., 1898).

3. Inorganic salts on heart muscle (*W. T. Porter*).—This is best studied on a circular strip 2·3 mm. wide, cut from the ventricle of a tortoise heart; the ring is severed, and the strip is fixed to a lever in the apparatus shown in fig. 252, or a similar arrangement.

(*a*) *Sodium.*—Immersed in 0·7 p.c. NaCl, after a time the strip may beat rhythmically, but the beats soon cease, the muscle loses its tonus, so that NaCl alone cannot maintain the heart-beat.

(*b*) *Potassium.*—If a non-beating strip of heart muscle be immersed in KCl (0·9 p.c., which is isotonic with 0·7 p.c. NaCl) no contractions result. This solution even arrests a beating strip.

(*c*) *Calcium.*—Calcium chloride alone (1·0 p.c. isotonic with 0·7 p.c. NaCl) also causes a beating strip to cease beating. If added to NaCl, however, it prolongs the beating, and increases strength of the contraction.

(*d*) *Modified Ringer's fluid—i.e.*, the combined action of Na, Ca, and K. Porter gives the following solution used by C. W. Greene—NaCl (0·7 p.c.), $CaCl_2$ (0·026 p.c.), KCl (0·035 p.c.)—as one which secures long-continued rhythmic contraction.

4. Muscarin, prepared from *Agaricus muscarius*, is very expensive, so that the artificial muscarin nitrate ($C_5H_{15}NO_3$) prepared from cholin ($C_5H_{15}NO_2$) is usually employed (10 p.c. solution).

(*a*) Pith a frog, expose its heart, and record its movements by a suspension method. To get the full effect of the drug on cardiac action snip the heart to allow the blood to run out. With a fine pipette apply a few drops of normal saline containing a trace of **muscarin** to the recording heart (artificial muscarin nitrate may be used). The beats rapidly become slower, the force diminishes, and the rhythmical vigorous beating ceases until the heart ceases to beat—"standstill"—in the phase of complete *diastole*. If the heart is not cut, this is a good method of collecting a considerable quantity of frog's blood when it is wanted for any purpose from the heart (*Kühne*).

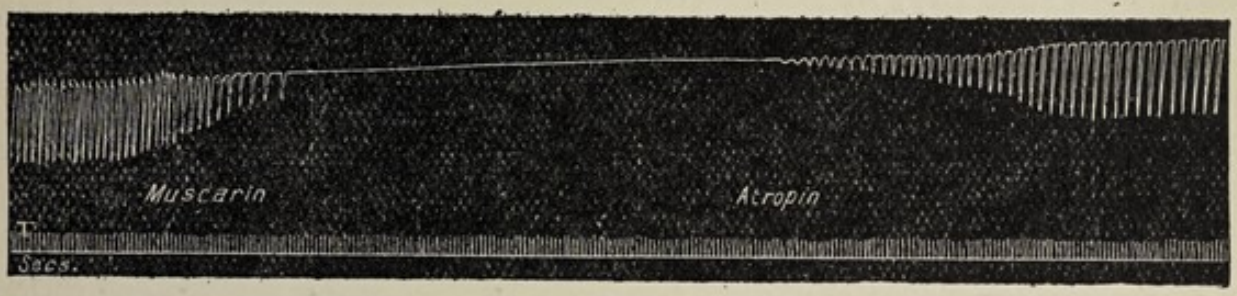


Fig. 286.—Tracing of heart (Gaskell's lever) arrested by muscarin, and rhythm restored by atropin. *T*, Time in seconds.

(*b*) When the heart is completely relaxed in the diastolic phase, it is very inexcitable—mechanical or electrical—responding only to strong stimuli, and perhaps the auricles not at all.

In a frog's heart poisoned with a drop of 0·1 p.c. of muscarin-platinum chloride, it would seem that such a heart summates its contractions, and may yield a tetanus, but this effect is undone by atropin (fig. 286) (*A. Walther.*)

5. Atropin ($C_{17}H_{23}NO_3$).—To the heart arrested with muscarin —*i.e.*, after a sufficient interval, and when the "muscarinised" heart shows no tendency to recovery.

(*a*) Apply a few drops of a 0·5 p.c. solution of **sulphate of atropin** in normal saline. The heart gradually begins to beat; sometimes the extent of the beat is increased. Atropin undoes the effect of muscarin; sometimes spoken of as "antagonistic action" of drugs—*i.e.*, *physiological* antagonism (fig. 286).

(*b*) Faradise (i.) the vagi, and (ii.) the crescent or inhibitory centre of the atropinised heart; the heart is no longer arrested,

because the atropin has paralysed the intracardiac inhibitory mechanism.

(*c*) Or after finding the vagus is effective, apply a few drops of a 0·5 p.c. solution of sulphate of atropin in normal saline to a normal heart recording as above. Record the result to show that neither stimulation of the crescent nor of vagus arrests the beat. Atropin paralyses the intracardiac terminals of the vagus or so-called inhibitory mechanism.

6. **Nicotin** ($C_{10}H_{14}N_2$).—Apply **Nicotin** (2 milligrams). Stimulation of the vagus no longer arrests the heart's action, but stimulation of the sinus venosus does; so that nicotin paralyses the fibres of the vagus, and leaves the intracardiac inhibitory mechanism intact.

Arrange a heart as before, see that vagus is active, record normal beats, apply to the heart 0·1 p.c.—*i.e.*, 1 per 1000—nicotin (fig. 287) in N.S. Note—

(i.) First effect is slowing of heart.

(ii.) Stimulation of vagus is without inhibitory effect—may be acceleration—but

(iii.) Crescent (S.V.) stimulation arrests the beat (fig. 287).

This drug is of supreme importance in nerve-physiology, and has been extensively used by Langley and others to determine when a nerve cell is intercalated in the course of a visceral or peripheral nerve path. In (ii.) the pre-ganglionic terminations of vagus have been paralysed, and in (iii.) the post-ganglionic are still active.

It appears first to stimulate certain nerve cells, or arborisations around these cells, and then to paralyse them, but the prolonged application of large doses ultimately affects the whole nervous apparatus of the heart. On this supposition the experiments (ii.) and (iii.) are explained by assuming that the terminations of the vagus fibres "arborise" around the nerve cells of the "crescent" (Remak's ganglion).

(iv.) After the full nicotin effect, again stimulate the crescent with strong currents; there may be marked acceleration of the beat. Why?

The interpretation given is that the accelerator (augmentor) fibres are stimulated—that they have no nerve cells in their intracardiac course, and run direct to their terminations.

The effect varies with the dose. Try effect of applying a drop of pure nicotin on the heart.

It is not necessary to record the result in order to determine the seat of action of nicotin. Apply 0·2 p.c. solution to the

heart. After a few minutes stimulate the vagus. No inhibitory effect. But raise heart and stimulate the "crescent;" the heart is inhibited.

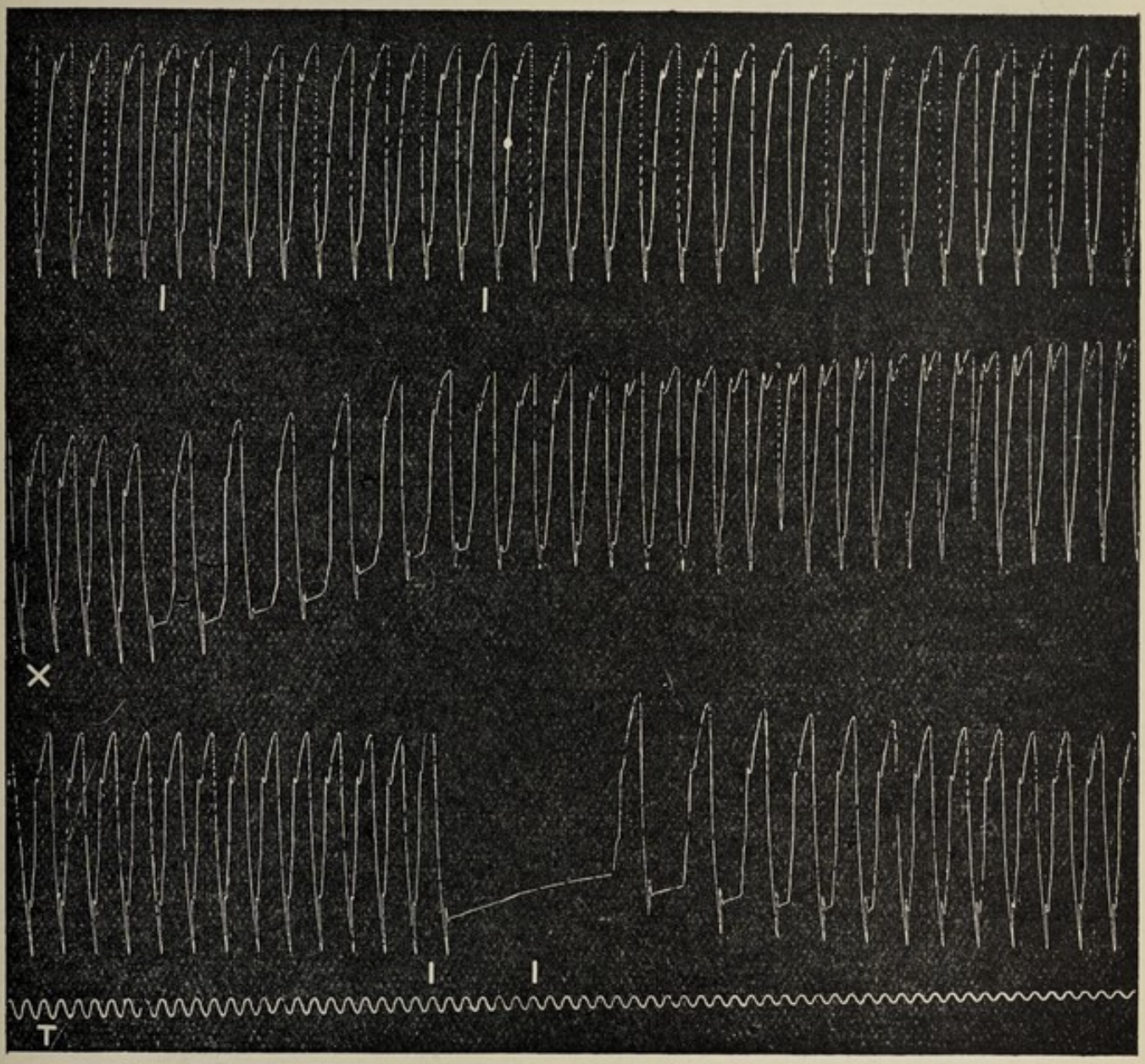


Fig. 287.—Stimulation of crescent between vertical lines. In lowest curve arrest of As and V. Nicotin applied at × (middle curve). In uppermost curve stimulation of crescent; no arrest.

It is evident, then, that as we know nicotin does not paralyse nerve trunks in this stage of its action, it must have paralysed some mechanism between the vagus and the intracardiac inhibitory nerves. This renders it probable that the vagus does not pass directly to cardiac muscle, but ends around nerve cells—probably in Remak's ganglion.

Prolonged action of nicotin ultimately also paralyses the intra-cardiac inhibitory mechanism, so that stimulation of the crescent no longer produces inhibition.

7. **Pilocarpin** ($C_{11}H_{16}N_2O_2$, and P. nitrate, $C_{11}H_{16}N_2O_2.HNO_3$; sol. 1 : 9 parts water).—Arrest [or slow] the heart with 0·5 p.c. [rapid with 10 p.c.] solution of pilocarpin nitrate. The heart is arrested in diastole. Apply atropin to antagonise it, observing that the heart beats again after the action of the atropin. Atropin restores the beat.

8. **Curare** (alkaloid curarin, $C_{10}H_{15}Na$, soluble in water).—Record normal contraction. Apply a few drops of 1 p.c. in N.S. solution to heart. After a few minutes, stimulate the vagus; no effect (perhaps acceleration). But stimulation of the S.V. or crescent arrests the heart in diastole—*i.e.*, the pre-ganglionic but not the post-ganglionic fibres of the vagus are paralysed. A very strong dose ultimately paralyses the latter, and the V is more affected than As or S.V.

9. **Digitalin** ($C_{50}H_{40}O_3$.—Merck's preparation consists of a mixture of several glucosides).—Apply a 1 or 2 p.c. solution to a fresh heart. The V contracts strongly, but the relaxation phase during diastole of the V beat becomes less, until the heart ceases to beat, the heart itself being in a state of prolonged or permanent contraction. The tone of the heart muscle is increased, as shown by the height of the curve above the abscissa, and at first the V beat is most affected. Ultimately the whole heart passes into a permanent systolic phase and is dead.

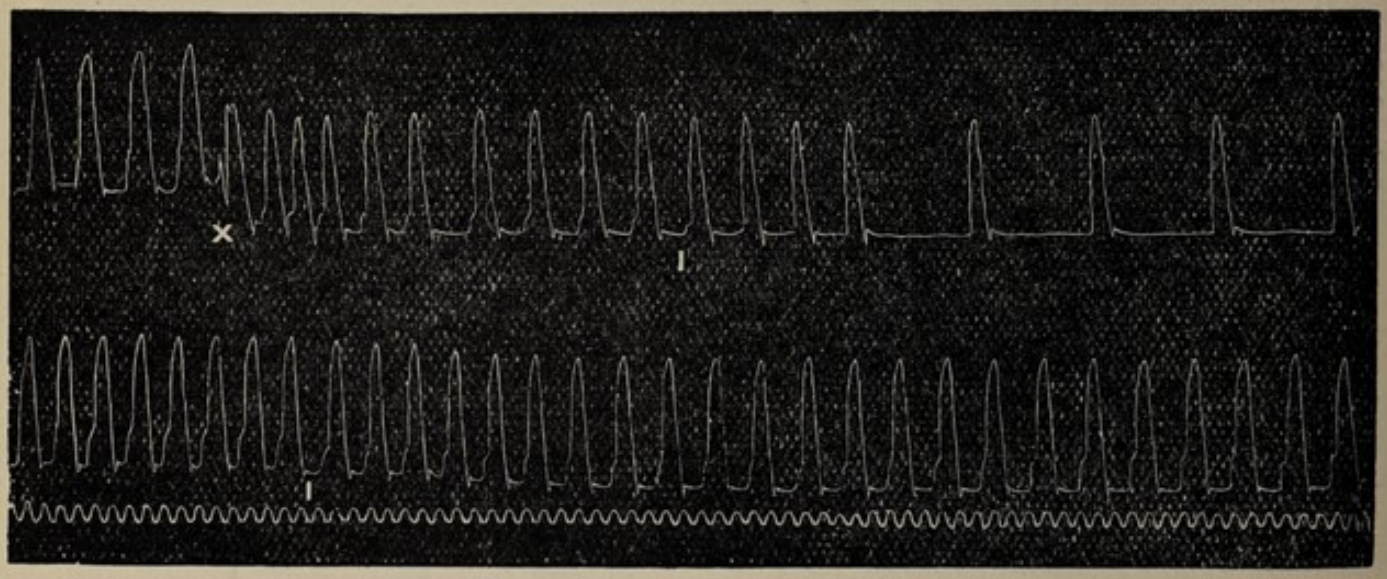


Fig. 288.—In lower curve a normal tracing with supra-renal extract applied direct to beating heart filled with blood. and recording by a suspension method. Read curves from below upwards. Very slight effect. At ×, snip in As or S.V., extract again applied. Note marked effect. Time, 0·5″.

10. **Cocain** ($C_{17}H_{21}NO_4$. Hydrochlorate, $C_{17}H_{21}NO_4 : HCl$)—(0·2 p.c. solution, 1 in 500 N.S.).—Apply this to heart. The V does not respond to each beat of S.V. and As. Vagus stimulation without vagus effect. Large doses abolish V beat.

11. **Caffein** ($C_8H_{10}N_4O_2 . H_2O$; soluble 1 : 80 in N.S.).—If applied directly to the heart it rapidly slows the beat, increases tone, so that the ventricle does not undergo full diastolic expansion, but continues to contract more

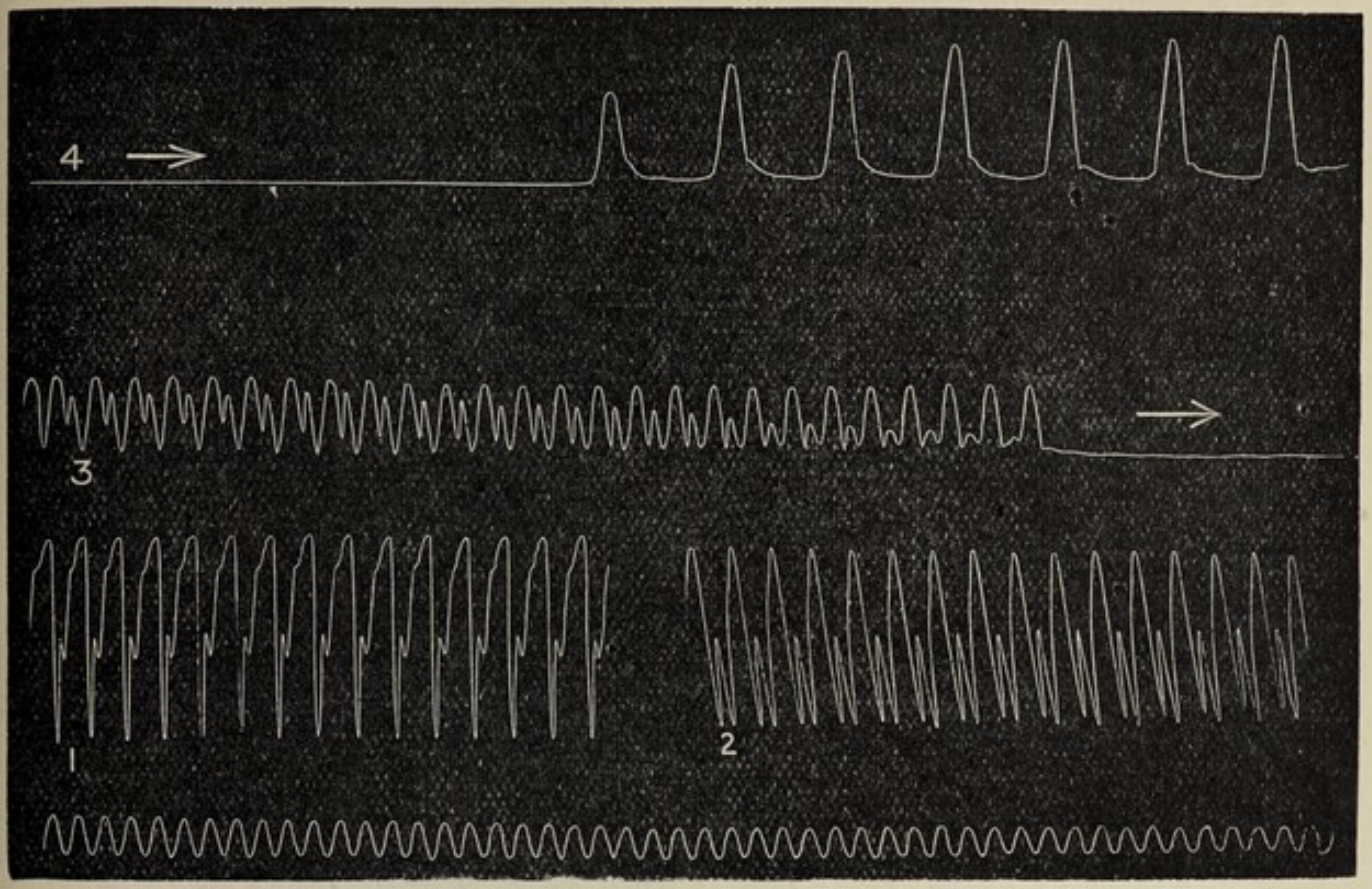


Fig. 289.—1. Normal heart-beat. Read upwards. 2. Effect of snip in S.V. 3. Effect of direct application of supra-renal extract, with arrest of beat, and then, later, in 4, renewal at different rate, and with renewed extent of contraction of ventricle. Notice staircase character of renewed beat. Time, 0·5″.

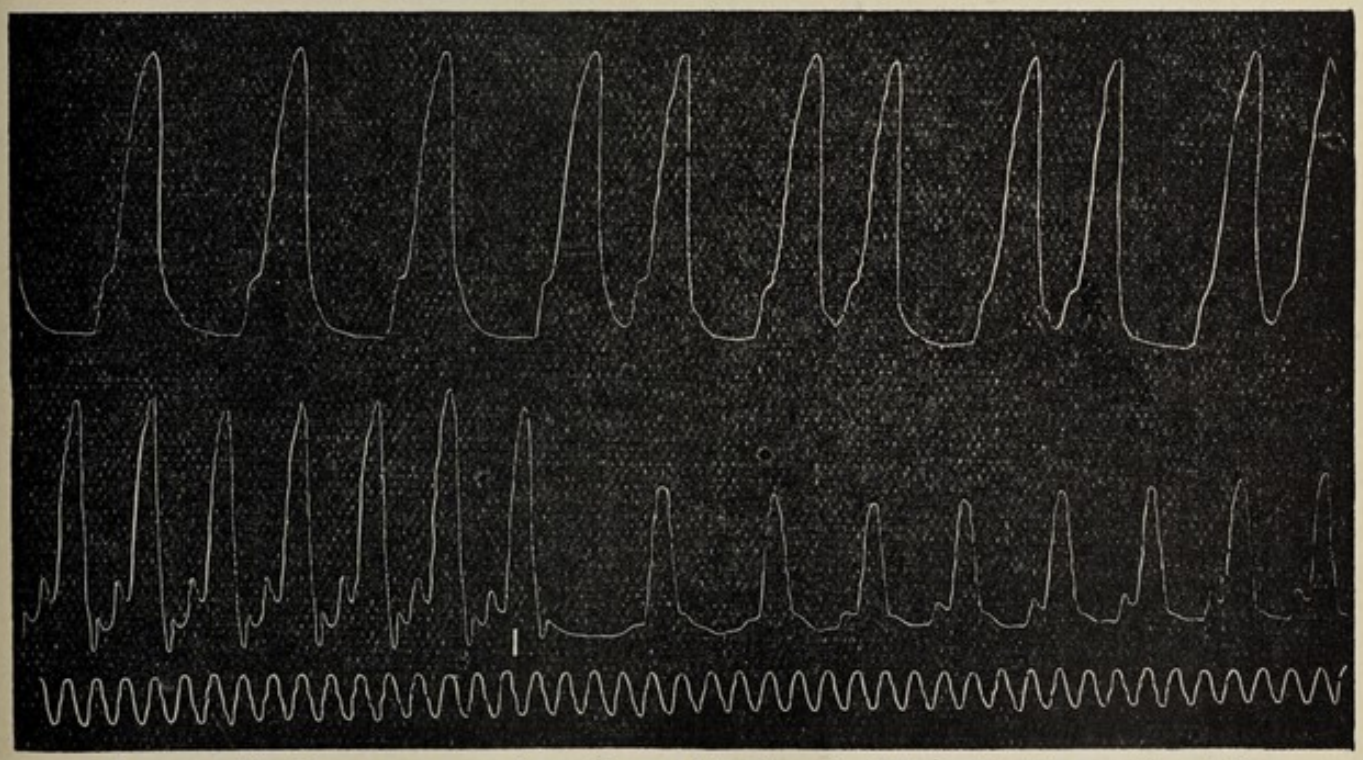


Fig. 290.—The lower curve, read up, shows a bloodless (snip) heart-beating, with systole and diastole of S.V., As, and V well marked. At the vertical mark supra-renal extract has applied directly to heart. Note immediate effect. The upper curve from the same heart shows a later result. Note two groups of beats, longer interval. Is it compensation? Time, 0·5″.

and more feebly until the drug causes "rigor" of the ventricle, while the S.V. and As still continue to beat.

12. Acids and alkalies.—Dilute solutions may be applied, and their effects studied (Gaskell, "Tonicity of heart," *Journ. of Phys.*, iii., 1890).

13. Supra-renal extract.—The effects of this remarkable substance—a watery extract of the medulla portion of the gland—not the cortex—are shown in figs. 288, 289, 290. For the enormous, but temporary, rise of blood-pressure produced by it when injected into the circulation (see Lesson LXX.). The pressure-raising substance is called **Epinephrin**.

14. Extract of meat.—Test the effect of a watery or saline solution of extract of meat—Liebig's or Armour's—and record the result.

See the "Physiological action of drugs," by M. S. Pembrey and C. D. F. Phillips, London, 1901, for many excellent tracings which show the action of all the drugs already mentioned, and many more; also, the effect of drugs on a nerve-muscle preparation.

LESSON LX.

MAMMALIAN HEART AND ITS NUTRITION—PERFUSION THROUGH BLOOD-VESSELS.

1. Mammalian heart-valves.—Use the heart and lungs of a sheep, with the pericardium still unopened.

(*a*) Open pericardium, observe its reflection round the blood-vessels at the base of the heart. Cut off the lungs moderately wide from the heart. Under a water-tap wash out any clots from the heart. Prepare from glass tubing, 15 mm. in diameter, a tube 8 cm. long and another 60 cm. Make a flange on one end of both. Fix a ring to hold a large funnel on a retort stand.

(*b*) Tie the short tube in the S.V.C., the flanged end being inserted in the vessel and tied in with well-waxed stout twine. In the pulmonary artery, P.A.—separated from its connections with the aorta, which lies behind it—tie the long tube, the flange securing it completely. Ligature the inferior vena cava, and the left azygos vein opening into the R.A. Connect the short tube by means of rubber tubing with the funnel in the retort stand. Keep the level of the water in the funnel below the upper surface of the P.A. tube. Fill the funnel with water; it passes into the R.V., and rises to the same height in the P.A. as the fluid in the funnel. Compress the R.V. with the hand,

the fluid rises in P.A. tube; on relaxing the pressure the fluid remains stationary in P.A. tube, as it is supported by the closed semilunar valves. They are said to be "competent." If the R.V. be then compressed rhythmically, the fluid rises higher and higher, until it is forced out of P.A. tube. The column of fluid is supported by the semilunar valves. Observe the three bulgings—sinuses of Valsalva.

(*c*) Repeat (*b*) on the left side, tying a long tube in the aorta and a short one in a pulmonary vein, ligaturing the veins.

(*d*) Cut away the right auricle, hold the heart in the left hand, and pour water from a jug into the tricuspid orifice. It passes into the R.V. and floats up the three cusps of the tricuspid valve; the three segments come into apposition, the upper surfaces of the valves themselves being nearly horizontal.

(*e*) With forceps tear out one of the three segments of the semilunar valve of the P.A. Tie a short tube in the P.A., and to it attach a rubber tube communicating with a funnel supported on a tall retort stand. Pour water into the funnel (avoid entrance of air); it flows into the R.V., floats up, and securely closes the tricuspid valve. The injured (incompetent) semilunar valves allow the fluid to flow into the ventricle. Turn the heart any way you please, fluid escapes through the tricuspid valve, provided, of course, the heart be not raised too high.

(*f*) Cut out the aorta with its semilunar valves from a sheep heart, leaving a considerable amount of adjacent tissue. Tie the narrow end of a funnel-shaped glass tube (flanged) in the aorta. Place the funnel and aorta in a filter stand. Pour water into the funnel, much of it escapes through the coronary arteries; ligature these. The semilunar valves allow no fluid to pass between their segments. Hold a lighted candle—or better, of course, an electric lamp—under the valves, and observe how they come together and close the orifice, the triradiate lines and the lunules in apposition projecting vertically.

The same result is easily obtained by tying a similar preparation on the end of a tube and blowing through the latter. An observer can readily study the play of the sigmoid valves. It is by no means difficult to project the "play" of the valves on a screen.

(*g*) Slit open the P.A., and observe the form and arrangement of the semilunar valves and the sinuses of Valsalva behind each cusp. If the aorta be similarly slit up longitudinally, a corresponding arrangement is seen. Note in addition the orifices of origin (*f*) of the coronary arteries *in the region* of their respective sinuses.

(*h*) *T.S. ventricles.*—Examine a T.S. through both ventricles. Compare the shape of the two cavities, their disposition—(Why is the cavity of the R.V. crescentic?)—and the relative thickness of their respective walls.

(*i*) *Ligature.*—Ligature any large vessel attached to the heart; one feels the sensation of something giving way when the ligature is tightened. Cut away the ligature, open the blood-vessel, and observe the rupture of the coats produced by the ligature.

(*j*) *Casts of heart.*—Study Ludwig and Hesse's casts, showing the ventricles (dog) in (1) diastole, (2) systole.

Endocardial nutrition of mammalian heart.—It is generally stated that the nutrition of the mammalian heart depends on the blood it receives through the coronary arteries, and that without a due supply of blood *via* these vessels the sustained rhythmic contractions of the cardiac muscle cease. F. H. Pratt ("Nutrition of the heart through the vessels of Thebesius and the coronary veins," F. H. Pratt, *Amer. Journ. of Phys.*, i., 86, 1898) has shown that this is not an accurate statement of the whole truth, for the heart will beat for hours whilst its arteries are empty and no longer supplied from the aorta. How is such nutrition of the cardiac muscle possible? (1) Either "through the vessels of Thebesius, which open from the ventricles and auricles into a system of fine branches communicating with the cardiac capillaries; or (2) through the coronary veins, which may convey a backward flow of blood from the auricle into the tissues of the heart."

2. Nutrition of heart through vessels of Thebesius.—These vessels may supply the cardiac muscle when the coronary supply is absolutely cut off; the mammalian ventricular beat under proper conditions may be maintained for hours.

(*a*) Anæsthetise a cat with ether, perform tracheotomy, and bleed it from a cannula tied in the carotid artery. Defibrinate the blood, and filter it through glass wool.

(*b*) Expose heart, and sever it from its connections. The still beating heart is rinsed in saline (0·9 p.c.). Tie a cannula about the size of an ordinary quill into the right ventricle, introducing it through either the right auricle or pulmonary artery. Tie it in with a ligature passing tightly around the base of the heart in a line parallel to the auriculo-ventricular groove, and below the coronary sinus. Thus, fluid introduced into the right ventricle through the cannula can find no outflow except through the vessels of Thebesius.

(*c*) Place heart vertically, and fill it through the cannula with defibrinated blood.

The blood must be kept warm, and a perfusion cannula must be used—

the latter practically one tube within another, with a side outflow tube from the wider tube (fig. 292).

Very soon regular strong co-ordinated contractions of the ventricle are observed, and they may continue for hours, even after fibrillar contractions set in. Attach a Marey's tambour with a rubber tube to the top of the cannula, and take a tracing on a slow drum.

As the blood flows from the outflow tube of the perfusion cannula it is venous.

The beat is not due to increased intra-ventricular pressure, for Ringer's fluid [for mammalian tissues NaCl 0·9 p.c., $CaCl_2$ 0·02 p.c., KCl 0·01 p.c. water distilled in glass (*F. S. Locke*)] under equal pressure fails to sustain the beat, while blood does. [Also, p. 396.]

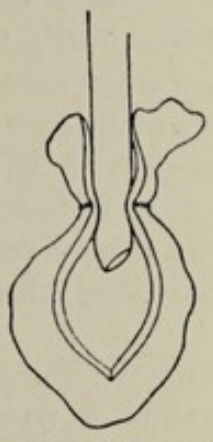


Fig. 291 illustrates the mode of securing the cannula in the ventricular cavity for nutrition through the vessels of Thebesius (*Pratt*).

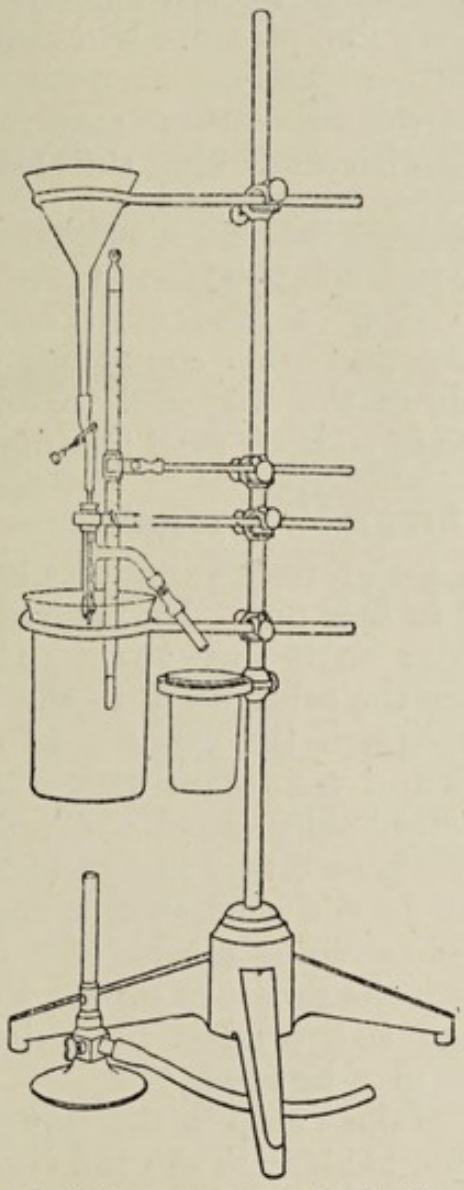


Fig. 292.—Perfusion apparatus for feeding heart cavities. Blood placed in the funnel passes slowly—regulated by clamp—through the fine glass tube into the ventricle, which is tied upon the cannula (Fig. 291). Each beat of the ventricle forces the blood through the space between the finer inner tube and the walls of the cannula, out through the side branch, and thence into the receiving beaker. The large beaker is filled with saline solution kept at normal T° (*Pratt*).

Obviously this experiment has important bearings on the question of cardiac nutrition, perhaps both in health and disease. In any case, the vessels of Thebesius are capable of

bringing from the ventricular cavities to the heart muscle sufficient nutriment to maintain long-continued rhythmical contractions. Coupled with the observation of W. T. Porter that "even isolated portions of the mammalian ventricle supplied through their nutrient arteries with a small quantity of serum at a very low pressure will maintain rhythmical, long-continued, forceful contractions when surrounded by oxygen at high tension," it is obvious that these researches add much to our knowledge of the condition affecting the activity of cardiac muscle.

Perfusion through blood-vessels.—By perfusing blood or other fluids through the blood-vessels of the body, or through isolated "surviving" organs, much may be learned regarding the action of drugs and other conditions on the blood-vessels. The blood-vessels of the frog and tortoise, the excised kidney, and other organs have been used for this purpose.

3. Frog's blood-vessels.

(*a*) Pith a frog, expose its heart, snip one aorta, and allow the blood to flow out. Previously make a fine glass cannula with a shoulder on it (fig. 293). Tie the cannula in one aorta, and include the other aorta in the ligature.

(*b*) Attach the cannula to a rubber tube containing normal saline and connected with a glass funnel filled with the same fluid and held in a suitable holder—*e.g.*, a ring on a retort stand, placed about 6 to 7 inches above the heart. See that there is no air in the connections, and that the cannula is filled with normal saline by means of a fine pipette before it is connected up with the pressure tube. Put a clip on the pressure tube, or arrange two funnels in a double filter stand, so that either funnel may be used.

(*c*) Make a snip in the sinus venosus. Hang up the frog on a suitable holder. Take the clip off the pressure tube to allow the normal saline, or Ringer's fluid, to flow into the blood-vessels and thus to wash out all the blood. Collect the outflow fluid in a funnel placed in a graduated measure.

(*d*) After the fluid runs clear, collect, measure, and record the amount every five minutes.

(*e*) Substitute normal saline or Ringer's fluid, to which some drug has been added, and perfuse it. Note the effect. If there is an increased outflow, the blood-vessels, chiefly the arterioles, have been dilated. If less, they have been contracted. Record the results, and, if necessary, plot a curve to show the result.

The water-tortoise may be used. The perfusion cannula is fixed in the third or fourth aorta, the others being tied. It may be placed in a glass funnel during perfusion.

In the frog, after a time, there is considerable distension of the lymph-sacs.

The student should keep a note of his results, and plot a curve on paper divided into squares. Make the base line represent time, and the vertical lines, or ordinates, the amount of outflow.

Some substances greatly *contract* the blood-vessels — *e.g.*, extract of the suprarenal capsules and very dilute nitric acid. The former is specially powerful in constricting the arterioles. (*Schäfer* and *Oliver.*) Others *dilate* the vessels, especially the nitrites, $\frac{1}{1000}$. Test the effect of a solution of "extract of meat."

PERFUSION EXPERIMENTS.

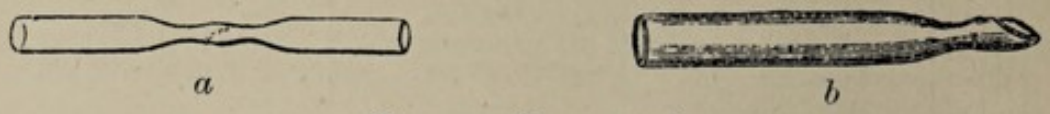
Water-Tortoise. Fluid been running 60′. Pressure, 18 cm.				Water-Tortoise. Fluid been running 60′. Pressure, 18 cm.				Frog. Fluid been running 20′. Pressure, 18 cm.			
Time.	Amount of Outflow in c.c.			Time.	Amount of Outflow in c.c.			Time.	Amount of Outflow in c.c.		
1.0	=	0	Normal saline.	Cord intact. 3.0	=	15	Normal saline.	2.50	=	14·5	Normal saline.
1.10	=	12		3.5	=	13		2.55	=	15·5	
1.15	=	15		3.10	=	11		3.0	=	15·5	
1.20	=	15		3.15	=	8		3.10	=	15·5	
1.25	=	11	NHO_3 $\frac{1}{1000}$.	3.20	=	8		3.15	suprarenal extract.[1]		
1.30	=	7		3.25	=	8		3.20	=	2·5	
1.35	=	7		3.30	=	8		3.25	=	2·0	
1.40	=	6·5	Normal saline.	3.35	=	8		3.30	=	2·0	
1.45	=	5·5		3.40	=	8		3.35		ceased.	
1.50	=	4·5		Cord destroyed. 3.45	=	18	Sodium nitrite $\frac{1}{1000}$.				
1.55	=	4·5		3.50	=	24					
2.0	=	5		3.55	=	24					
2.5	=	6·5		4.0	=	22	Normal saline.				
2.10	=	5·5		4.5	=	22					
2.15	=	5		4.10	=	21					
				4.15	=	19					
				4.20	=	17					
				4.25	=	14					
				4.30	=	12·5					
				4.35	=	11					
				4.40	=	9·5					
				4.45	=	8					
				4.50	=	9	Sodium nitrite.				
				4.55	=	10					
				5.0	=	10					

[1] Made by Messrs. Willows, Francis & Butler, chemists, Holborn, London.

4. **Wash-out blood-vessels** (frog).—(*a*) A pithed frog's heart is exposed. Place a ligature under both aortæ. Tie a cannula in the peripheral end of one aorta and ligature so as to include both aortæ. Arrange on a stand a beaker of normal saline. In it place a glass tube with rubber tube attached to it as a siphon to be fixed in cannula. No air bubbles. Let the saline flow into the aorta, and when the heart is distended make a snip in an auricle and allow the saline to flow freely until it is colourless and all the blood is washed out.

(*b*) Observe almost colourless condition of the muscles, the yellow liver. Such a frog is still reflex. Pflüger and Oertmann (1877) showed that such a frog still breathed, and this experiment formed an important link in the evidence that the respiratory exchanges between the blood and the tissue were not primarily due to changes in the blood itself, but were effective in and by the living tissues.

5. **Make a glass cannula.**—Heat in the flame of a blow-pipe a piece of hard glass tubing about 5 mm. in diameter. When it is soft, take it out of the flame, draw it out gently for about 3 cm. Allow it to cool; make the gas-jet smaller, heat the thin drawn-out part of the tube, and draw it out very slightly. This makes a shoulder between the two (fig. 293, *a*). Draw it out on the opposite side. With a triangular file just scratch the narrow part obliquely between the two constricted parts, and break it across. Two cannulæ with a shoulder and an oblique narrow orifice are thus obtained. Round off the oblique edges either by a file, rubbing them on a whetstone, or heating slightly in a gas flame. Tie a piece of india-rubber on the other end, and the cannula is complete (fig. 293, *b*).



a *b*

Fig. 293.—Glass cannula.

LESSON LXI.

CARDIAC IMPULSE—SOUNDS—CARDIOGRAPHS—ILLUMINATED HEART.

THE phenomena discoverable in connection with the action of the heart by means of the hand, the stethoscope, and cardiograph applied directly over the pre-cordial region deserve the closest attention of the student.

1. **Cardiac impulse ("apex beat") Stethoscope. Heart-sounds.**—(*a*) In a quiet room let the person to be examined stand erect and expose his chest. Apply the flat of the hand and feel the cardiac impulse or so-called "apex beat" in the fifth intercostal space to the left of the sternum, apply the small end of the stethoscope over this spot, and the ear to the opposite end of the instrument, or use a bin-aural stethoscope. The left hand may be placed over the carotid or radial artery to feel the pulse in either of those arteries; compare the time-relations of the

pulse with what is heard over the cardiac impulse. Count the number of heart beats in relation to the respirations. Auscultate both when the person breathes normally, and when he holds his breath.

(*b*) Two "heart-sounds" are heard—the *first* or *systolic* coincides with the impulse, and is followed by the *second* or *diastolic*. Then there is a pause, and the cycle again repeats itself. The first sound corresponding with ventricular systole and largely caused by the rapid increase of tension of the ventricles, is longer and deeper than the second, which is of shorter duration and sharper, and is caused by the tension of the aortic and pulmonary valves. Note pitch, quality, and duration of sounds.

(*c*) Place the stethoscope over different parts of the præcordia, noting that the first sound is heard loudest towards the apex, where the ventricle comes nearest the surface, while the second is heard loudest at the second right costal cartilage at its junction with the sternum—*i.e.*, where the aortic arch comes nearest the surface. In this case the sound is conducted from the places where it is produced—*i.e.*, at the sigmoid valves towards the part of the aorta nearest the surface.

(*d*) The student can study his own heart beats by using a wooden tobacco pipe with a rubber tube attached to its stem. The mouth-piece of the pipe is fixed into the free end of the tube. The open bowl of the pipe is placed over the heart, and the pipe mouth-piece in the ear.

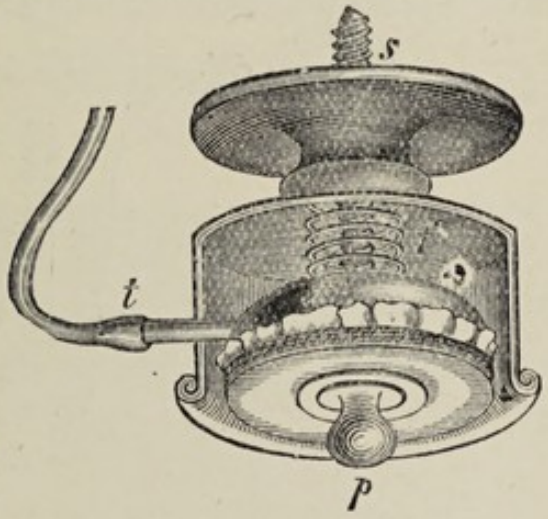


Fig. 294.—Marey's cardiograph. *p*, Button placed over cardiac impulse; *s*, Screw to regulate the projection of *p*; *t*, Tube to other tambour.

2. **Cardiographs.**—There are many forms. Use Marey's (fig. 294).

(*a*) Place the patient recumbent with his head supported on a pillow. The body may be turned towards the left. Sometimes good results are obtained when the person sits near the table. Feel for the cardiac pulse between the fifth and sixth ribs on the left side, and about half an inch inside the mammary line. Mark the spot.

(*b*) Arrange cardiograph by connecting it with thick-walled rubber tubing to a recording Marey's tambour adjusted to write

on a drum. It is held in position by a band or wide tape passing round the body. Arrange between the receiving and recording tambours a valve (rate 0·5 cm. per sec.) or T-tube capable of being opened and closed, to allow air to escape if the pressure be too great.

(*c*) Place the button of the cardiograph, *p*, over the region where the cardiac impulse is best felt. Take a tracing or **cardiogram**. Study it. It shows the steep ascent corresponding to the sudden contraction and hardening and increase in tension of the ventricles, the sudden descent due to ventricular diastole, and between the two a gently sloping line—*systolic plateau*—which marks the period of outflow from the ventricles. The auricular contraction may be recorded. It is small, and precedes the ventricular systole (fig. 296).

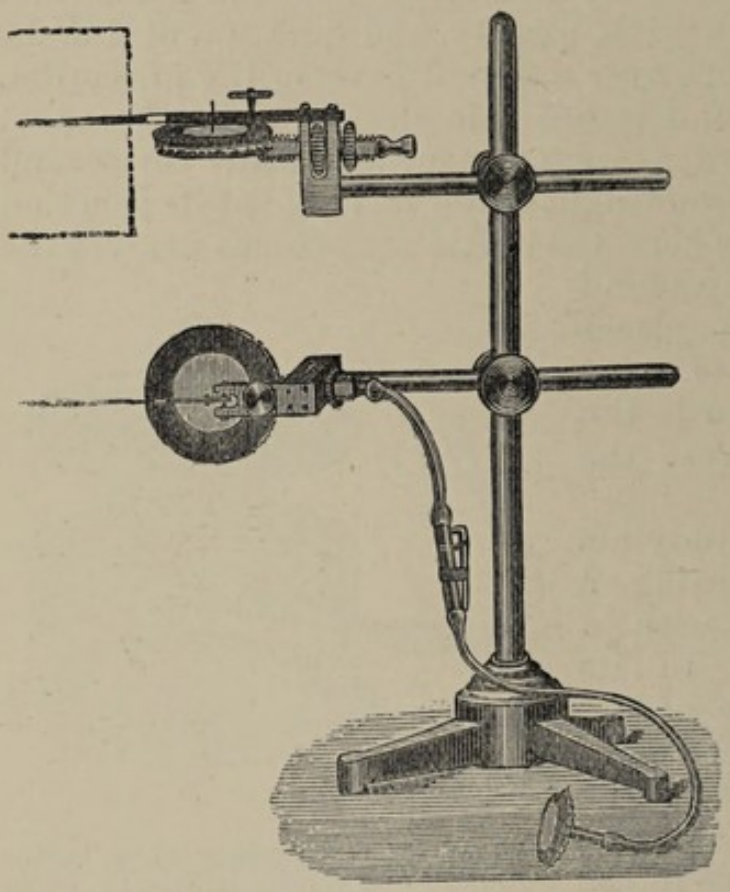


Fig. 295.—Shows a receiver tambour or cardiograph coupled to a recording tambour with air transmission and valve in elastic tube. The tambour is so made that it can record either on a vertical (upper figure) or horizontal drum. Made by Petzold.

While the cardiogram is being recorded, listen to the heart-sounds with a stethoscope and try to determine with which part of the cardiogram the first and second sounds respectively correspond. Compare the cardiac sounds and impulse phenomena in time with the radial pulse.

Compare the tracing with that taken from other members of the class. In locating the position of greatest cardiac impulse, use the bony landmarks rather than the nipple. Compare this position in different individuals.

3. **Swallowing—Effect on heart-beats (man)** (*Reflex augmentation*).—With a watch in front of you count your own pulse-beats

per minute, and then slowly sip a glass of water, still keeping your finger on the pulse. Note the increase. It is better when two students work together—one counting the pulse, while the other swallows the fluid. This is due to the inhibitory action of the vagus being set aside reflexly (fig. 297).

If the pulse-beats be recorded it is found that not only the rate but also the force of the beat is modified (fig. 297). In fact, stimulation of almost any afferent nerve can reflexly modify the force and frequency of the heart-beat—*e.g.*, stimulation of central end of vagus, sciatic.

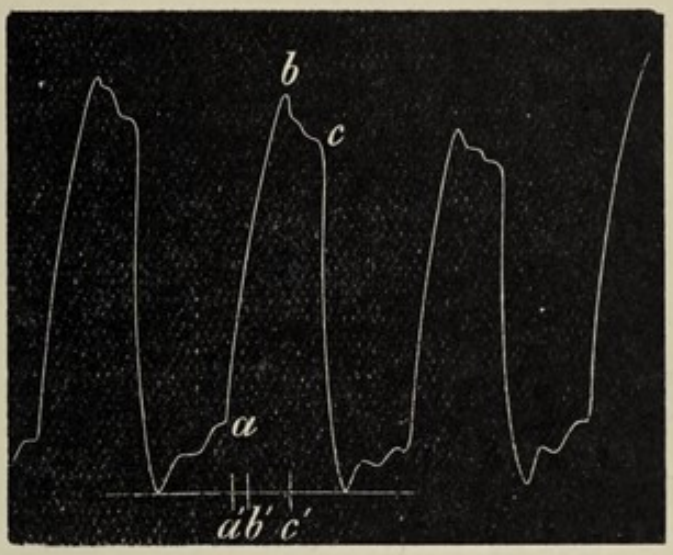


Fig. 296.—Human cardiogram. *a b*, Systole of ventricles indicating increased tension; *b c*, "Systolic plateau;" *b' c'*, Duration of outflow time; *a' b'*, Duration of systole of ventricles.

4. Reflex inhibition of heart (rabbit).—Place one hand over the chest of a rabbit and feel the beating of the heart. With the other hand suddenly close its nostrils, or bring a little ammonia or chloroform near the nostrils, so as to cause the animal to close them. Almost at once the heart is felt to cease beating for a time, but it goes on again.

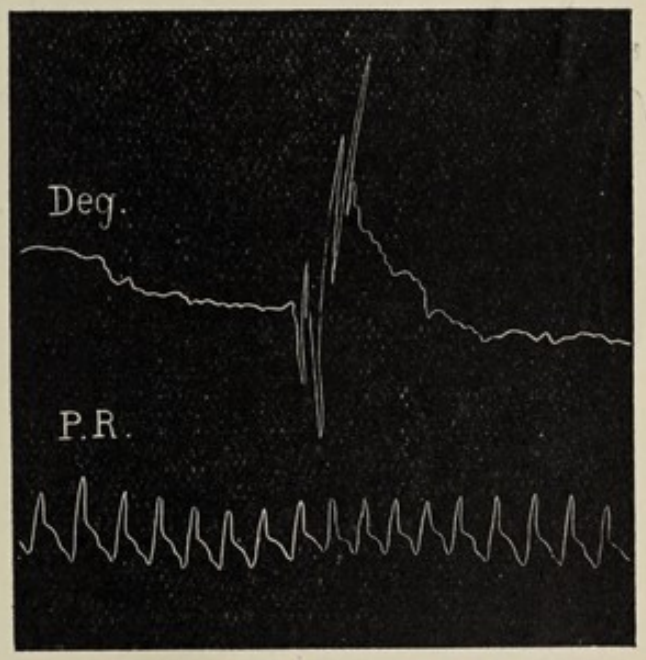


Fig. 297.—Effect on pulse-beat of swallowing fluid. *Deg*, Deglutition; *P R*, Pulse-beats.

5. Reflex cardiac inhibition (frog) (*Goltz's tapping experiment*).

(*a*) Destroy the cerebrum and optic lobes of a frog, or lightly etherise an intact frog. Pin it out on a frog-plate, attach the heart to a suspension lever, or count the number of beats. The lever method, however, is far more impressive. Open the abdominal cavity, expose the intestines and tap them rapidly with the handle of a scalpel. The heart either ceases to beat for a time, being arrested reflexly, or its frequency is diminished. The afferent (centripetal) nerve is the sympathetic in the abdomen, and the

efferent (centrifugal) the vagus (*Bernstein*). In a lightly etherised frog the experiment fails when both vagi are cut. Tapping arrests the heart more promptly if the intestines are slightly inflamed by exposure to the air (*Tarchanoff*).

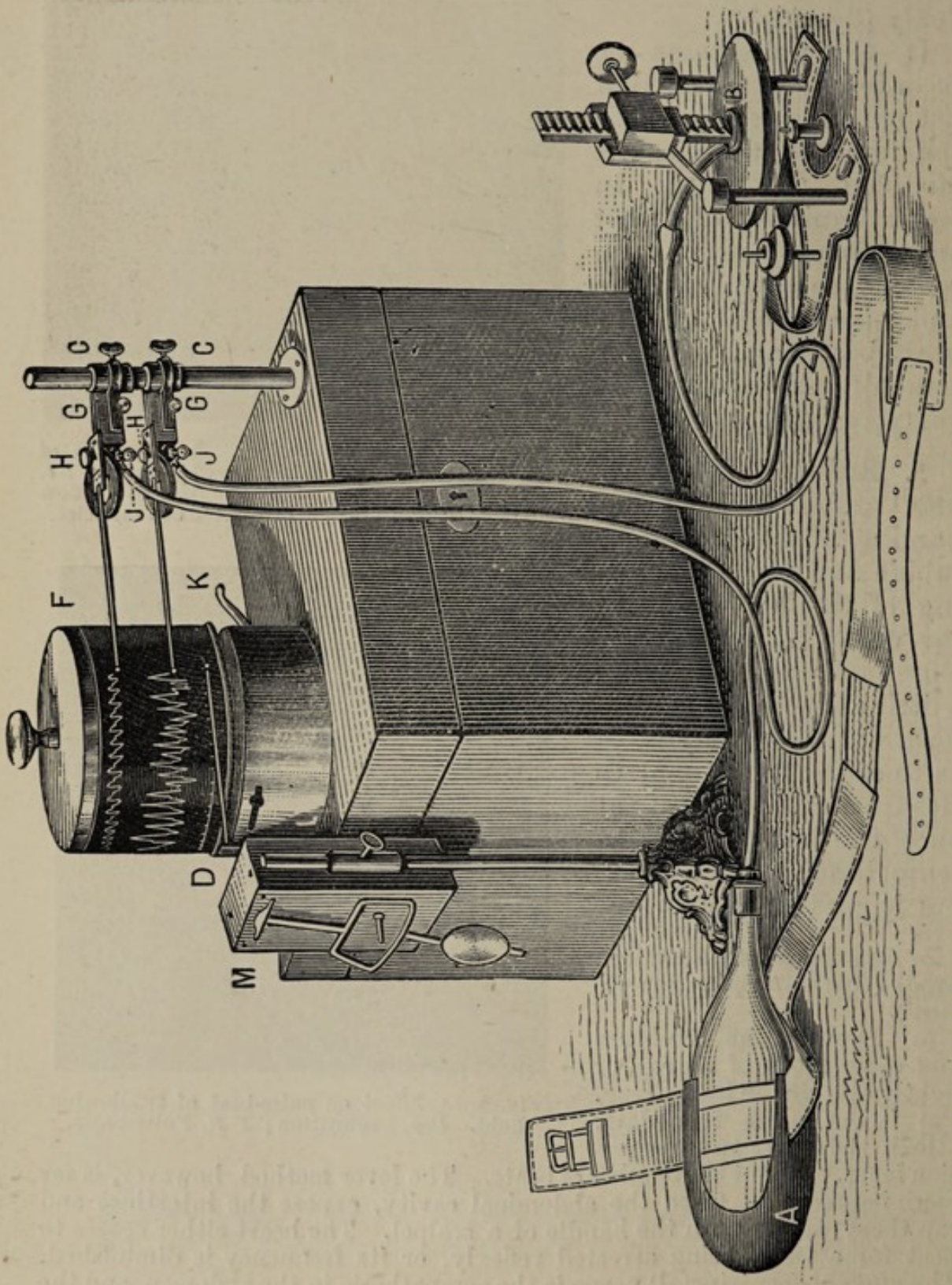


Fig. 298.—Polygraph of Rothe. *A*, Bag for respiratory movements; *B*, Receiving tambour; *C, C*, Supports for *H, H*, the recording tambours; *F*, Drum; *D*, Clockwork; and *K*, Detent for liberating *D*; *M*, Time-marker.

(*b*) It suffices to exert firm pressure over the abdomen to produce this reflex arrest of the heart.

6. **Polygraph of Knoll and Rothe.**—It can be used to take simultaneously cardiac impulse and a pulse tracing, or respiratory movements and a pulse tracing, or two pulse tracings. It consists of a drum, F, moved by clockwork within the box, D. K is a catch for setting D in motion. M is a time-marker beating seconds. H, H are two Marey's registering tambours adjustable on the stand, C. B is a tambour which can be fixed over an artery or over the cardiac impulse, while A is a bottle-shaped caoutchouc bag which can be strapped to the body for studying the respiratory movements (fig. 298).

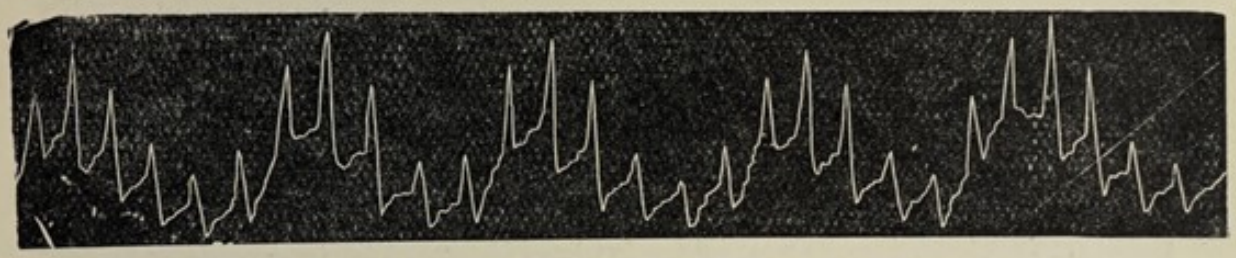


Fig. 299.—Effect of thoracic respiratory movements on cardiac impulse.

(*a*) Adjust the tambour, B, over the cardiac impulse, and fix the bag, A, on the abdomen so as to record simultaneously the cardiac impulse and the respirations (fig. 299). The experimenter may also take a tracing of the cardiac impulse while the respiration is arrested.

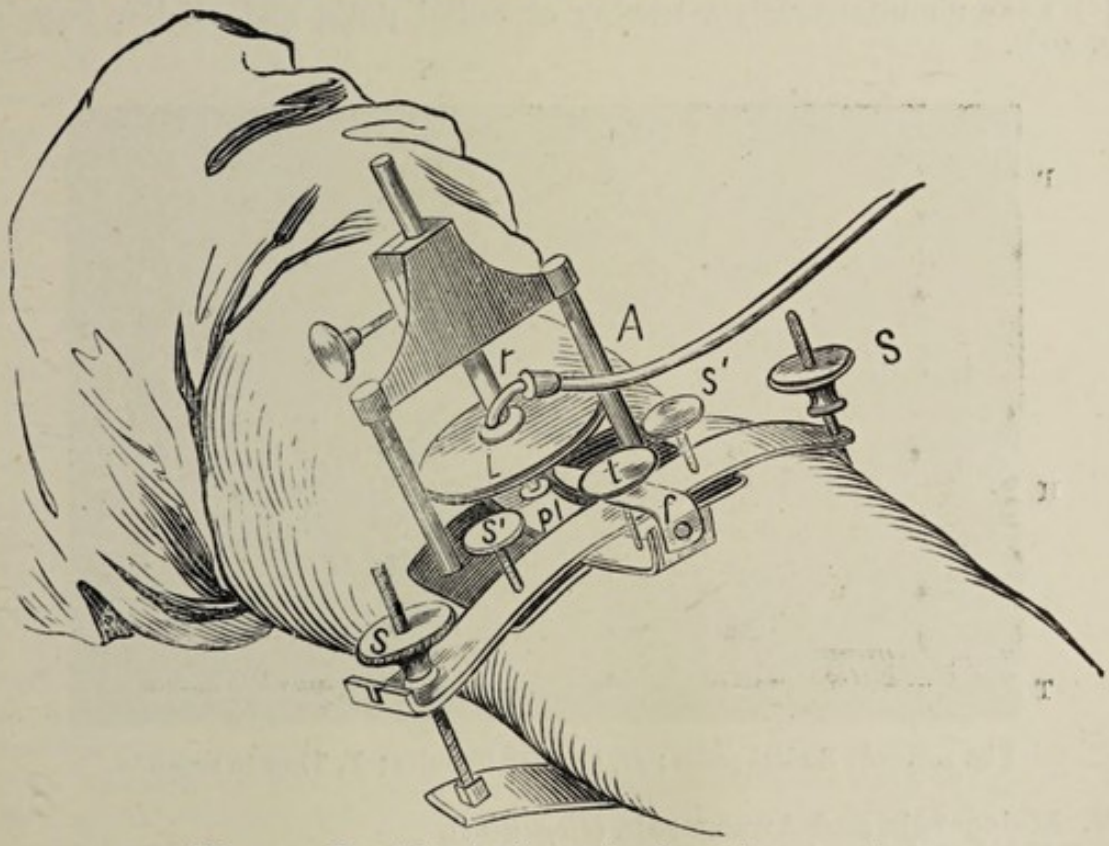


Fig. 300.—Receiving tambour of polygraph on an artery.

(*b*) Take a tracing of the radial pulse and the respiratory movements. Fig. 300 shows how the receiving tambour is adjusted over an artery. At

the same time record the respirations, and note in the tracing (fig. 301) how the number and form of the pulse-beats vary during inspiration and expiration—the number being greater during inspiration.

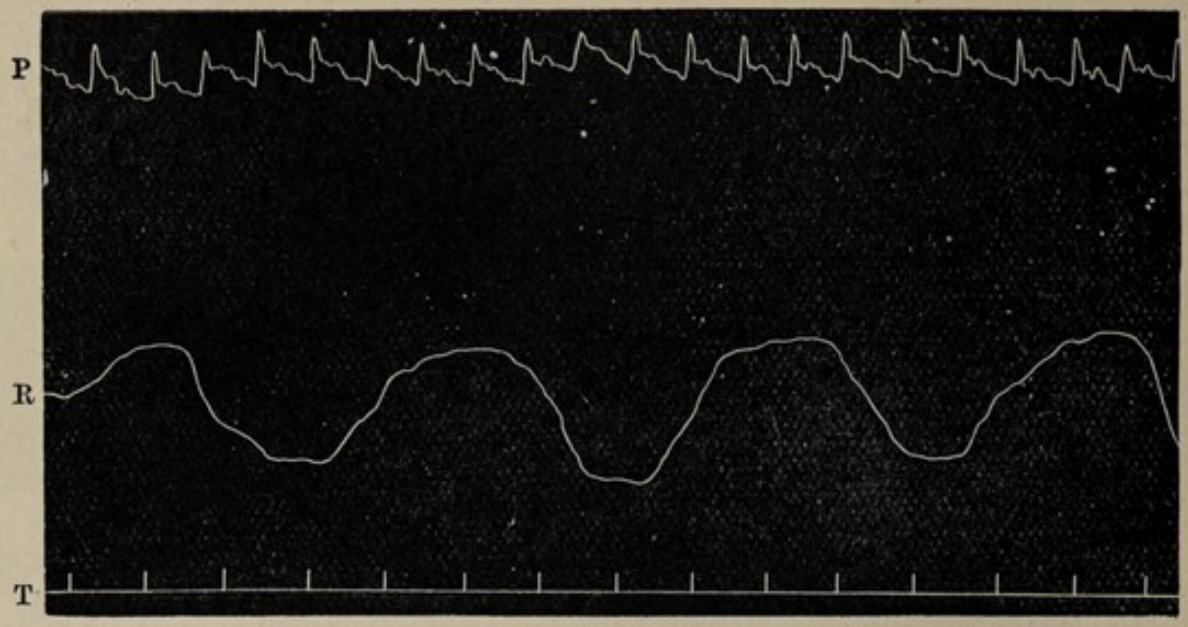


Fig. 301.—*P*, Tracing of radial pulse; *R*, Respirations; *T*, Time in seconds.

(*c*) Take simultaneously a tracing of radial pulse and cardiac impulse (fig. 302).

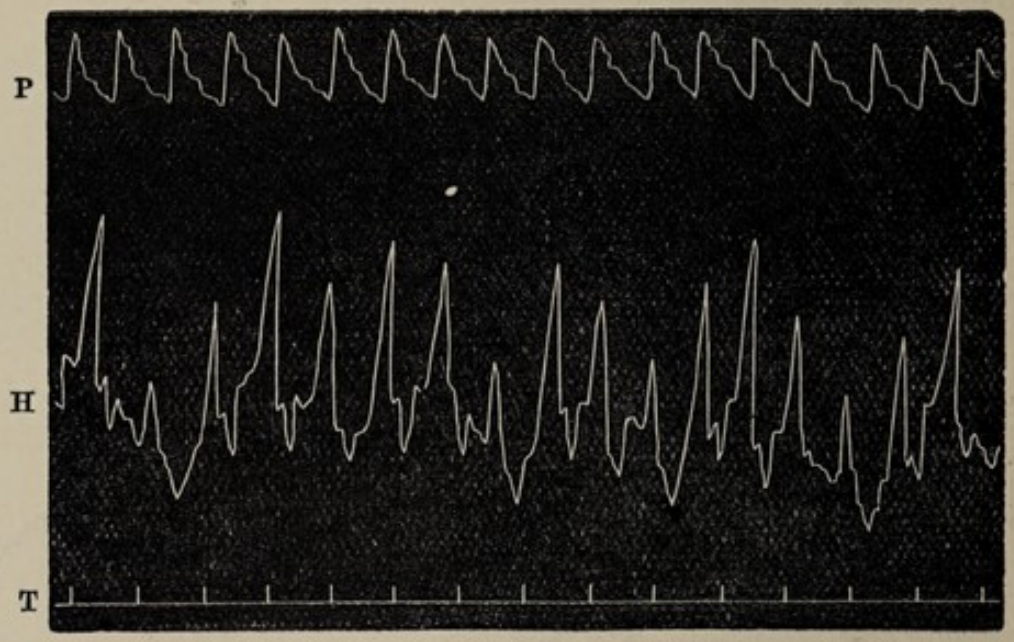


Fig. 302.—*P*, Radial pulse; *H*, Cardiac impulse; *T*, Time in seconds.

7. Meiocardia and Auxocardia (*Ceradini*).

(*a*) Bend a glass tube about 20 mm. in diameter into a semicircle, with a diameter of about 6 to 8 inches. Taper off one end in a gas-flame to fit a nostril, and draw out the other end of the tube to about the same size. Round off the edges of the glass in a gas-flame.

(*b*) Fill the tube with tobacco-smoke. Place one end of the tube in one nostril, close the other nostril, cease to breathe, but *keep the glottis open*. Observe that the smoke is moved in the tube, passing out in a small puff during auxocardia—*i.e.*, when the heart is largest—while it is drawn farther into the tube during meiocardia—*i.e.*, when the heart is smallest.

These movements, sometimes called the "cardio-pneumatic movements," are due to variations in the size of the heart during its several phases of fulness, whereby the volume of air in the lungs is altered (S. J. Meltzer, *Amer. Journ. of Phys.*, i., 1898).

8. Simple cardiograph.—This may be made by tying a rubber membrane over the wide end of a thistle glass tube or glass funnel, and fixing a bone collar-stud (button) on the centre of the membrane, connecting this up by means of rubber tubing with a recording tambour, and introducing a T-shaped tube to act as a valve, which can be closed when the record is being taken. The method of air-transmission is largely used for clinical purposes (*Porter*).

9. Rutherford's scheme of heart-circulation.—The heart of a sheep is prepared (*Lancet*, February 17, 1872). Funnels are tied in the receiving chambers and tubes in the outlet vessels, P.A. and A. P.A. tube discharges into the L.A.—this represents the pulmonary circuit—the A tube into the R.A.—this represents the systemic course. On pouring coloured water into the R.A. and rhythmically compressing the heart, the fluid passes in an orderly course through the heart. Study the competency of the valves.

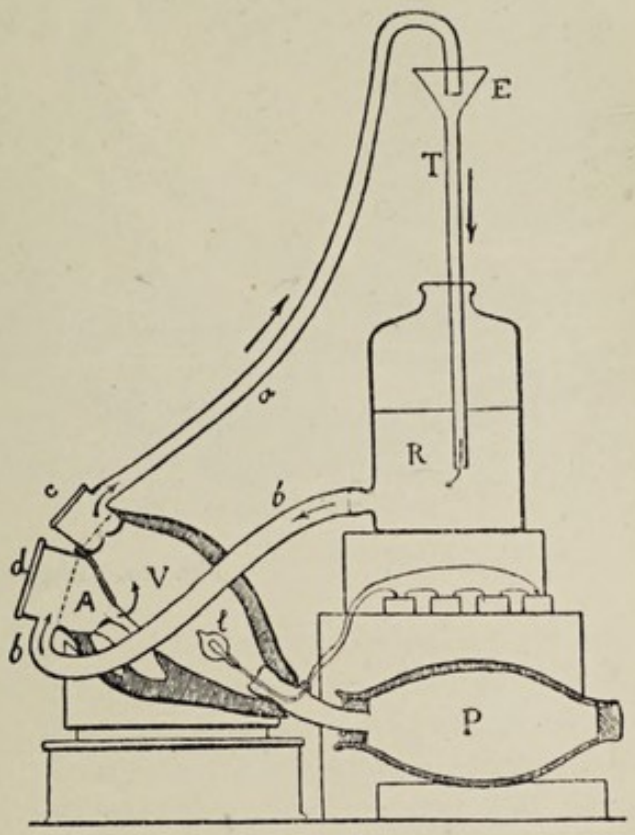


Fig. 303.—Scheme of Gad's apparatus to show the play of the heart-valves. *A*, L. auricle; *d*, Its window, and communicating with *b*, the inlet tube for water from the reservoir, *R*; *V*, L. ventricle, illuminated by an electric lamp, *l*, and communicating with the elastic bag, *P*; *c*, Glass window fixed in tube in aorta; *a*, Tube carrying fluid to the reservoir.

10. Illuminated Ox-Heart (*Gad*) arranged previously (fig. 303).—Two brass tubes with glass windows are tied, one in the L.A., *d* (7 cm. diameter), and the other, *c*, in the aorta (5 cm. diameter). These are connected with a reservoir, R. The interior of the heart is illuminated by an electric lamp, *l*, introduced through the apex. In the apex is tied a brass tube, which is connected with a large rubber bag with thick walls, P. Fill the cardiac system with water. Compress the elastic bag, fluid is driven onwards, when the play of the valves can be seen. On relaxation, the mitral cusps open and the aortic cusps close.

Remove the glass windows of the rubber tubes, and preserve the heart in 10 p.c. chloral hydrate.

11. Phonendoscope (fig. 304).—It is shaped like a large watch, the front part being composed of two discs, one lying above the other. The interior disc is the more sensitive; the second disc can, if necessary, be easily taken off, leaving the interior one uncovered. To examine limited parts (for instance, the sounds of the arteries and the heart), screw the small rod into the outer disc, and press it cautiously and gradually against the part to be examined. The ends of the tube are inserted into the auditory meatus. By using Y-tubes, three students can simultaneously listen to any sound.

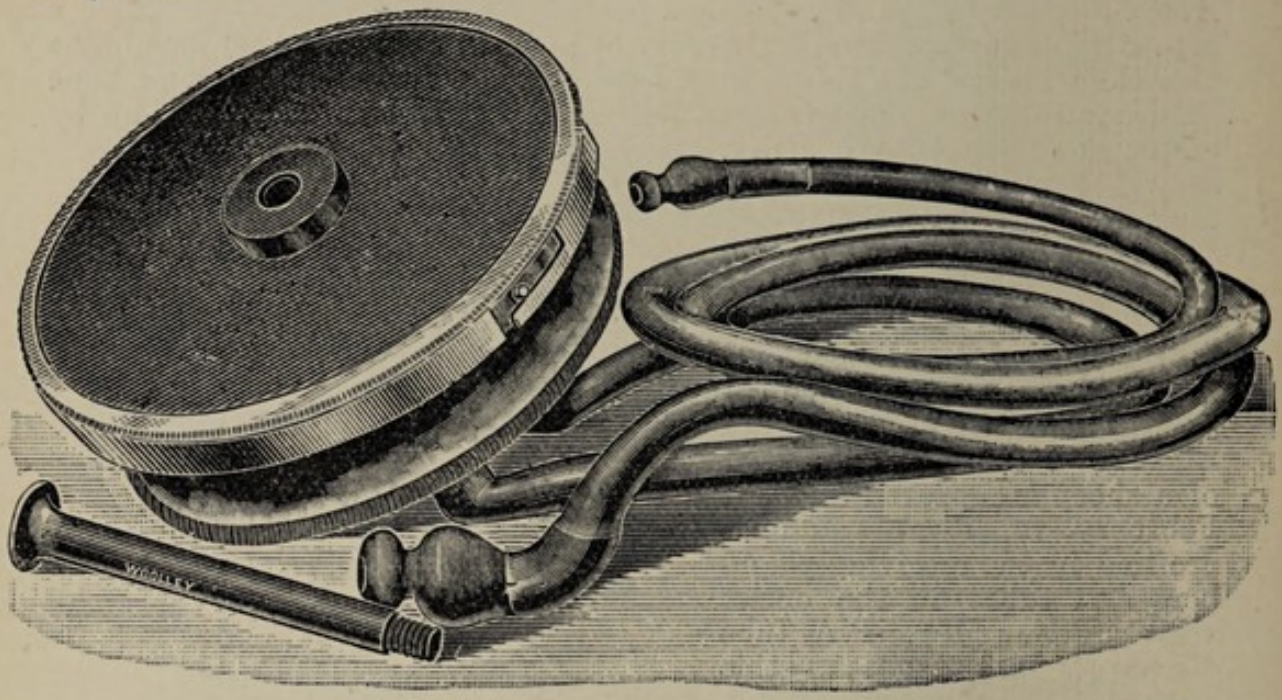


Fig. 304.—Phonendoscope.

LESSON LXII.

PULSE—SPHYGMOGRAPHS—SPHYGMOSCOPE.

1. Feel radial pulse—*i.e.*, Pressure pulse.

(*a*) Feel the radial pulse of a fellow-student, count the number of beats per minute; compare its characters with your own pulse, including its volume and compressibility. Feel the artery with the ball, not the tips of the first, second, and third fingers. Observe how its characters and frequency are altered by (1) muscular exercise; (2) a prolonged and sustained deep inspira-

tion; (3) prolonged expiration; and (4) other conditions—*e.g.*, recumbent, sitting up, standing erect, effect of swallowing fluids.

(*b*) Feel the radial pulse and cardiac impulse simultaneously. Note that the former is not synchronous with the latter; the wrist pulse occurs about $\frac{1}{6}$ second after the heart-beat—*i.e.*, the pulse-wave takes this time to travel from heart to the radial artery.

(*c*) Listen to the heart-sounds and at the same time feel the radial pulse. The pulse is felt after the first sound about midway between the first and second sounds.

(*d*) By appropriate recording apparatus one can readily show that the pulse is not simultaneous throughout the arterial system; thus the carotid precedes the femoral, &c. (Lesson LXIV.)

2. Sphygmograph.—Many forms have been constructed and discarded. Study the two chief methods of transmitting the pulsatile movement of the artery—

(i.) Direct.
(ii.) Transmission.

In the former the movement of the artery is transmitted directly to a recording lever; in the latter the radial movement can be transmitted to a distance by air-transmission and recorded.

I. Direct sphygmographs.

Marey's sphygmograph.—(*a*) Let the patient seat himself at a table, and place his forearm on the double-inclined plane (fig. 305), which, in the improved form of the instrument, is the lid of the sphygmograph case. Semiflex the fingers, so that the back of the wrist, resting on the plane, makes an angle of about 30° with the dorsal surface of the hand.

(*b*) Mark the position of the radial artery with an aniline pencil. Wind up the clock, H, apply the ivory pad exactly over the artery where it lies on the radius, and fix it to the arm by the two elastic straps, K, K. The sphygmograph must be parallel to the radius, and the clockwork next the elbow. Cover the slide with glazed paper, smoke it with camphor soot, fix it in position, and arrange the writing-style, C′, to write upon the smoked surface, G, with the least possible friction. Regulate the pressure upon the artery by means of the milled head, L—*i.e.*, until the greatest amplitude of the lever is obtained.

(*c*) Set the clockwork going, and take a tracing. Fix and

study the tracing (fig. 306). Join the bases of the respective beats, and note the form of the curve or "respiratory line."

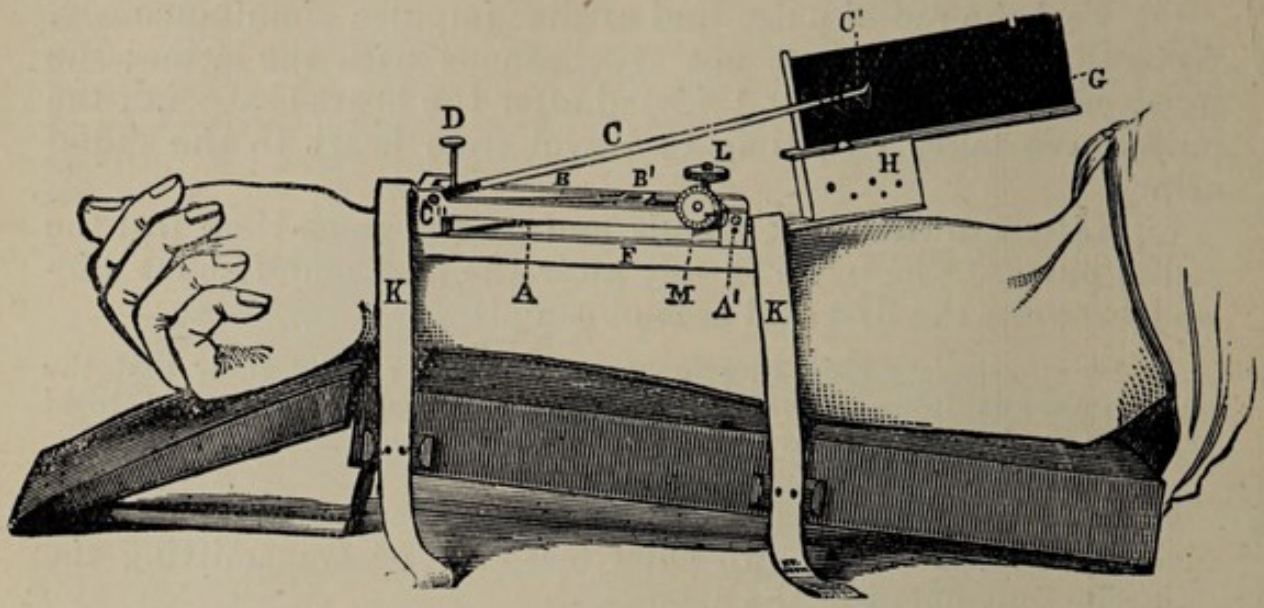


Fig. 305.—Marey's sphygmograph applied to the arm.

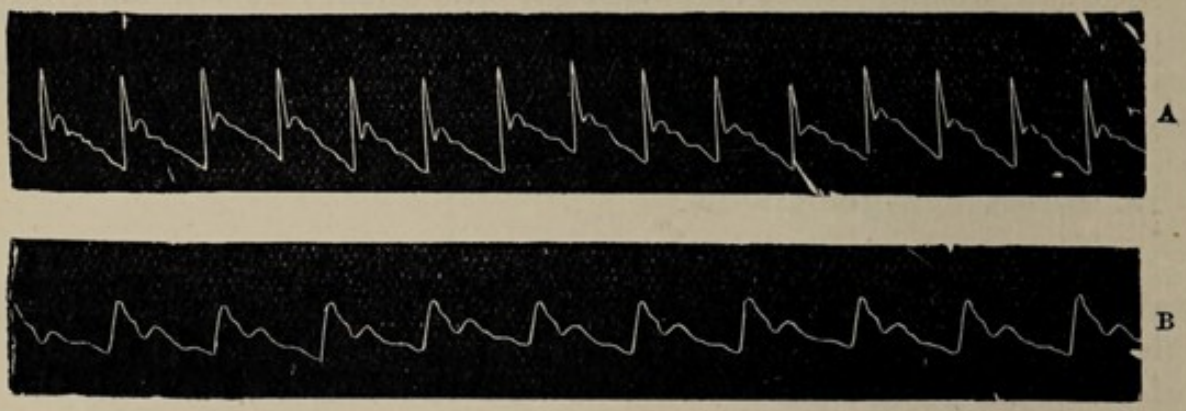


Fig. 306.—Radial artery pulse-tracings (Marey's sphygmograph). *A*, hard ; *B*, soft pulse.

3. Dudgeon's sphygmograph (fig. 307).

(*a*) Fix and adjust the instrument on the radial artery. Smoke the band of paper, insert it between the rollers, and take a tracing. The magnification from the arrangement of the lever is 25-fold. Study the tracing (fig. 308).

(*b*) *Richardson's modification.*—In this the strip of blackened paper is carried between two revolving rollers, but the upper one is furnished with toothed flanges, which record a series of interrupted lines, 2 mm. apart, on the blackened paper (fig. 309).

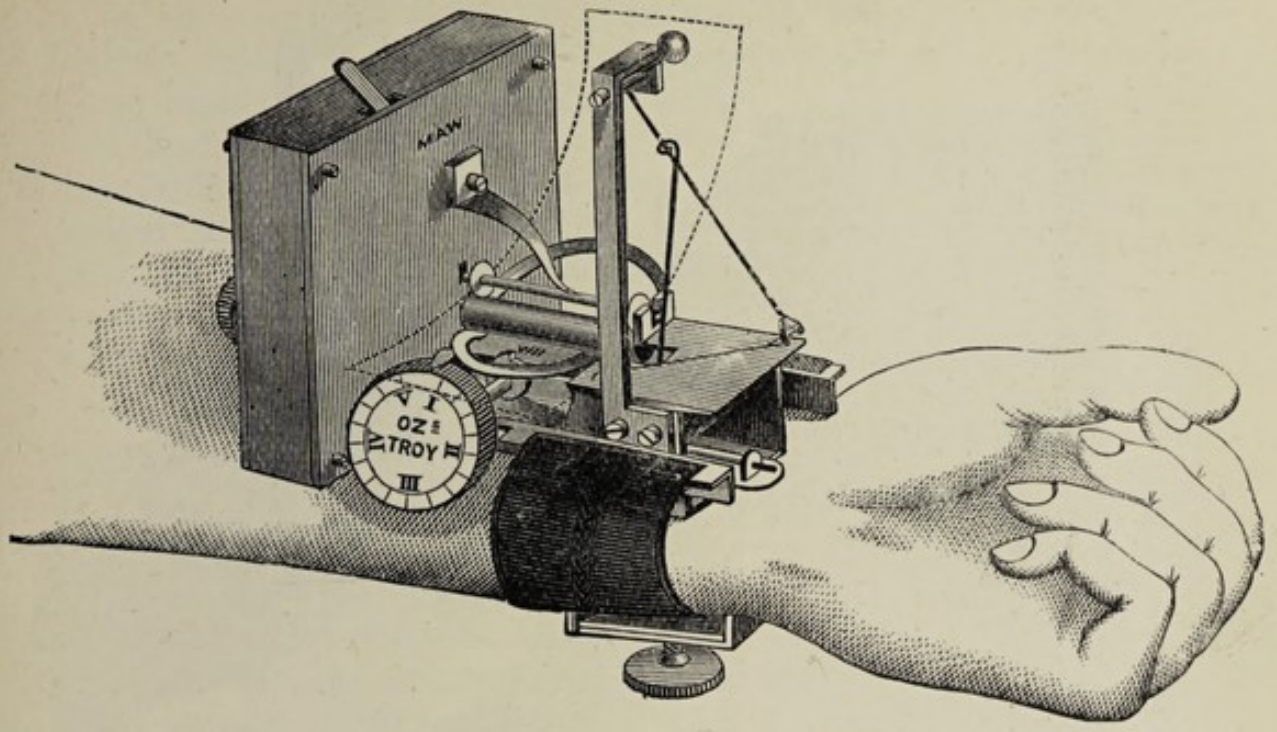


Fig. 307.—Dudgeon's sphygmograph.



Fig. 308.—Tracing of radial pulse taken with Dudgeon's sphygmograph.

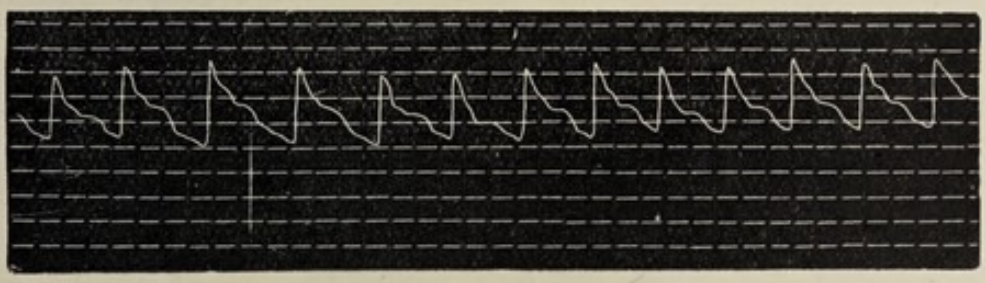


Fig. 309.—Sphygmogram taken with Richardson's modification of Dudgeon's sphygmograph.

4. **Ludwig's sphygmograph** (fig. 310) is modified from Dudgeon's, but a larger framework ensures its application to the forearm, while by the arrangement (fig. 311) the arm is kept in the same position.

In a normal pulse tracing (figs. 308, 312) observe the unbroken ascent and the more gradual but diversified descent.

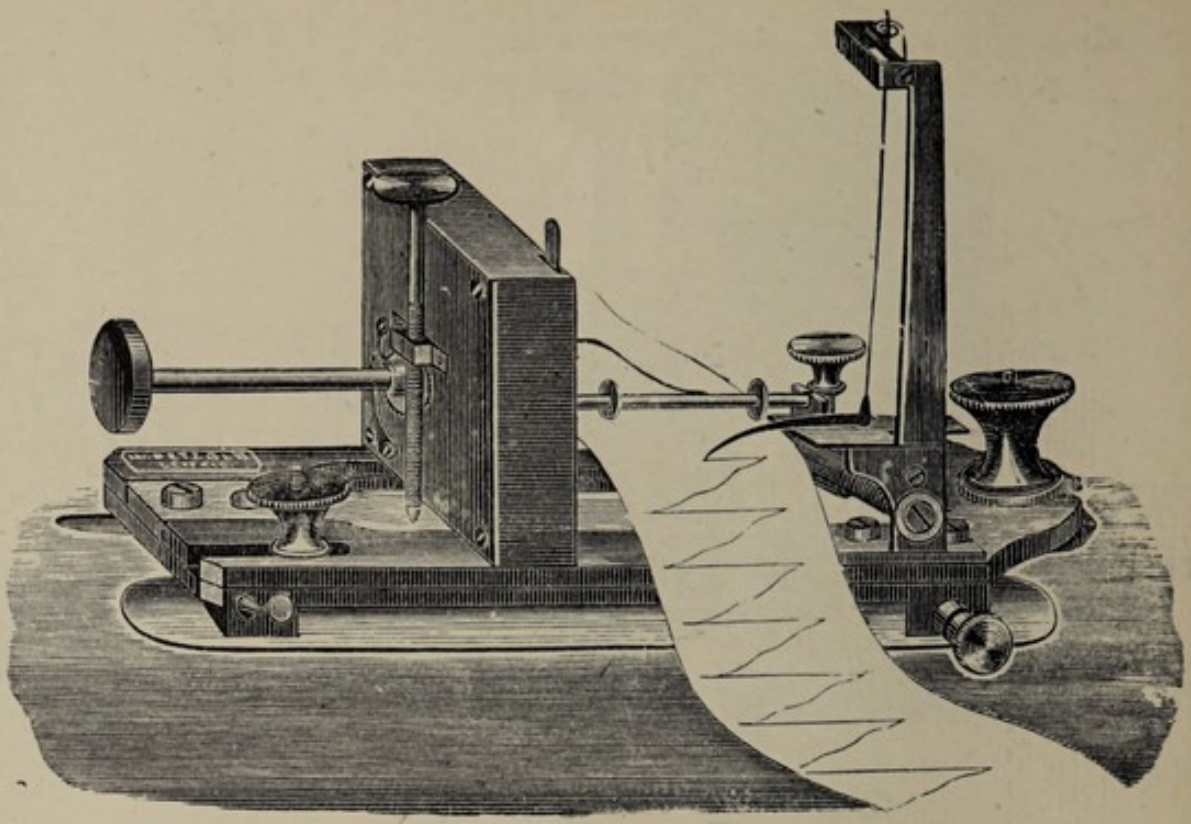


Fig. 310.—Ludwig's sphygmograph, made by Petzold of Leipzig.

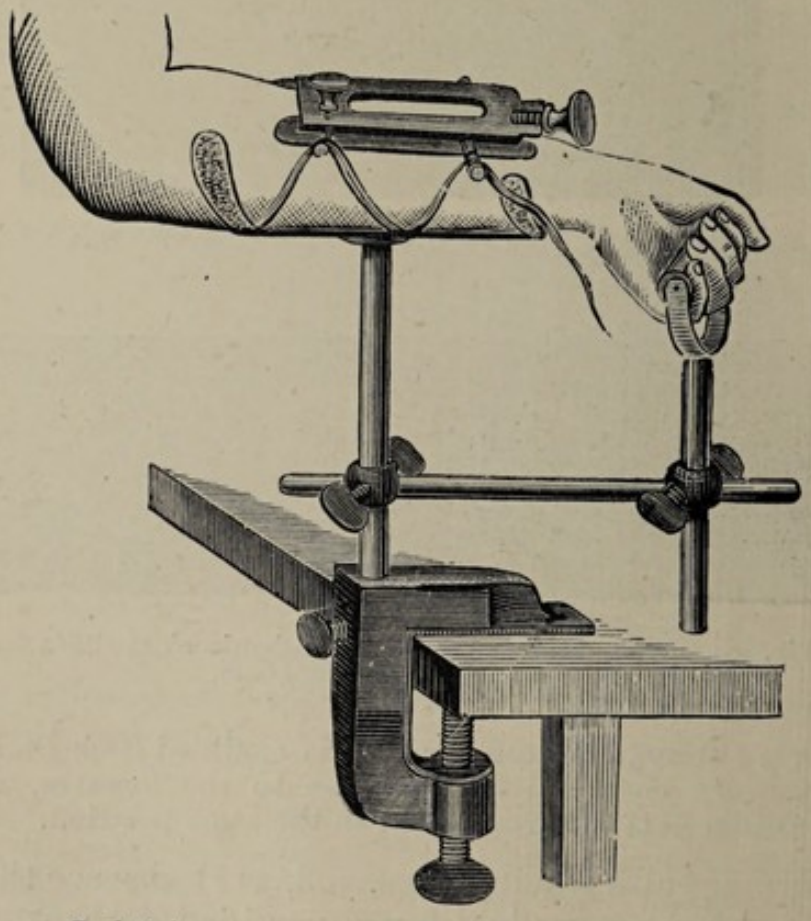


Fig. 311.—Ludwig's arm support for sphygmograph (Petzold, Leipzig).

In the descent note the predicrotic wave if present, the dicrotic notch and dicrotic and post-dicrotic waves if present. Note also differences between a soft and a hard pulse. In the former the predicrotic wave may be absent or very small. In fig. 301 join the bases of all the percussion waves, and note the character of the curve = "respiratory line." These bolder and longer curves are due to the effect of the respiratory movements.

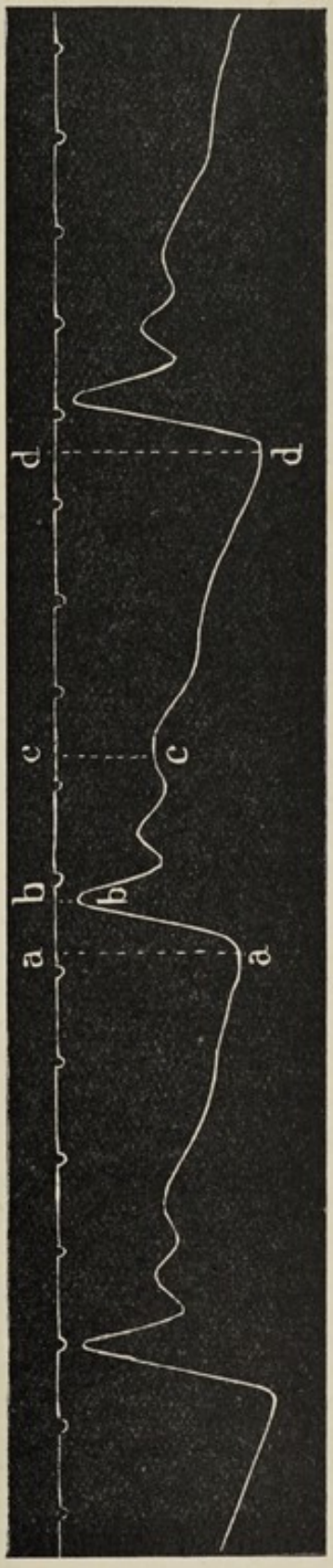


Fig. 312.—Pulse-tracing taken with Jacquet's large sphygmograph and time recorded above in $\frac{1}{10}''$. *a b*, Ascent; *b*, Apex; *c*, Dicrotic wave preceded by dicrotic notch; *d*, End of single pulse-beat. The elevation between *b* and *c* is the predicrotic wave.

II. Transmission Sphygmographs.—These are most useful, because one can combine a record of the pulse with a cardiograph record or a record from another artery. The principle is the application of a tambour of some kind over the artery, which is connected to a recording tambour by means of a rubber tube—*i.e.*, "air transmission."

5. Marey's transmission sphygmograph.

Fig. 313 shows the form used by Marey. A slightly modified form was made by Brondgeest, and called "pansphygmograph."

6. Hürthle's transmission sphygmograph.—Most sphygmographs are so adjusted to the arm as to interfere with the return of venous blood, and this is found to affect the pulse-curve, a defect avoided by this instrument. It consists of (i.) a spring clutch, *Kl Kl*, fixed to the wrist, and (ii.) a tambour, *T*, fixed to *Kl* by the angle-plate, *R*. On the spring, *Kl*, are two leather-covered pads, $P_{,}$ and $P_{,,}$, grasping the lower end of the radius and ulna, which can be regulated by the screw, $Sch_{,}$. Thus the veins on the volar and dorsal surfaces are free and uncompressed.

The knob, *K*, is adjusted over the radial artery, the tambour is lowered till *K* compresses the radial artery, when the whole is fixed in position by the

screw, Sch_{II}. Connect the receiving tambour with the recording tambour in the usual way. This is a most efficient transmission sphygmograph. As the membrane, *M*, of the tambour represents the spring of the sphygmograph, and has to compress the artery, it must not be made of too thin rubber (*Pflüg. Arch.*, lxxii., 583, 1898).

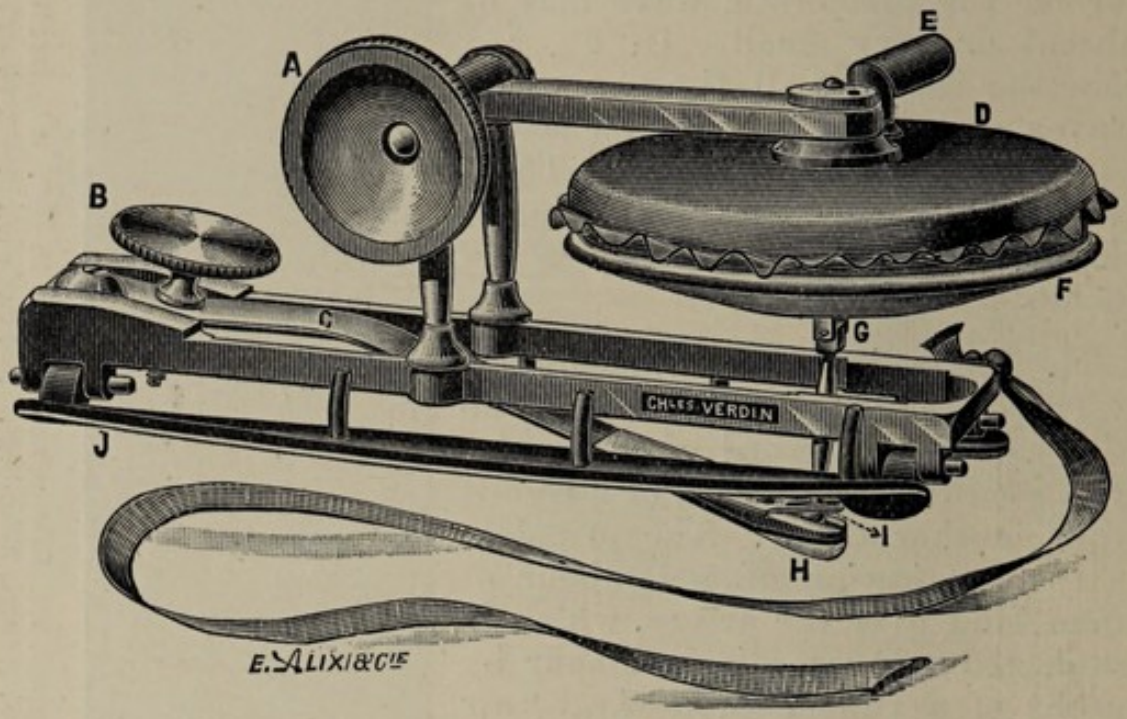


Fig. 313.—Marey's transmission sphygmograph, made by Verdin. The pad, *H*, of the sphygmograph rests on the artery, and conveys its movement to the adjustable receiving tambour, *GFD*, and through *E* communicates with a recording tambour, *A*.

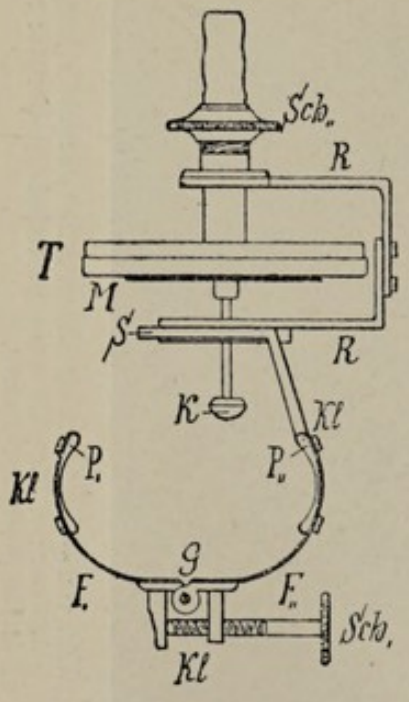


Fig. 314.—Hürthle's transmission sphygmograph for the radial pulse.

7. **Nail Pulse.** — Digital sphygmograph ("Onchyograph"). Several forms of this instrument are now in use. Laulanié (1899) allows a light-weighted lever to rest upon the finger nail so as to be set in motion by the pulsating vessels of the nail bed. It seems to be a pressure pulse of the arterioles. Waller has modified this, and his instrument is shown in Fig. 315. It consists of a rather stiff spring, *S*, and a movable support, *A*, between which the tip of the finger is inserted. The lever, *L*, magnifies the movements. The method of its application is obvious. Try Valsalva's experiment or Müller's experiment (Laulanié, *Journ. of Phys.*, xxiii., 1899; Herz, *Wiener Klinik*, 165, 1896; Waller, *Brit. Med. Journ.*, 1900.)

8. **Amyl Nitrite.**—(*a*) With the sphygmograph adjusted, take a tracing, and then place *two drops—not more*—of amyl nitrite on a handkerchief, and inhale the vapour. Within fifteen to thirty seconds it affects the pulse, lowering the tension, the tracing present-

ing all the characters of a soft-pulse tracing, with a well-marked dicrotic wave.

9. Gas sphygmoscope (fig. 316).—Connect the inlet tube of the instrument with the gas supply. Light the gas flame, *b*. Apply the rubber membrane, *a*, over the radial artery, and observe how the flame rises and

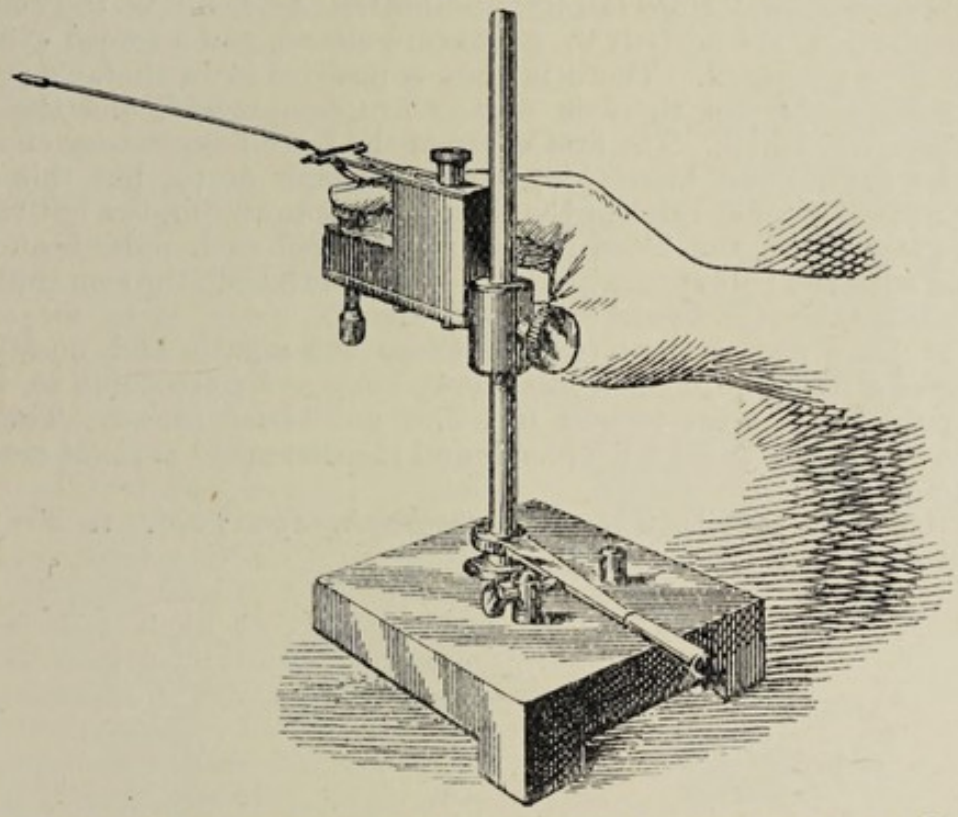


Fig. 315.—Digital sphygmograph of Waller applied to the nail of the fore-finger.

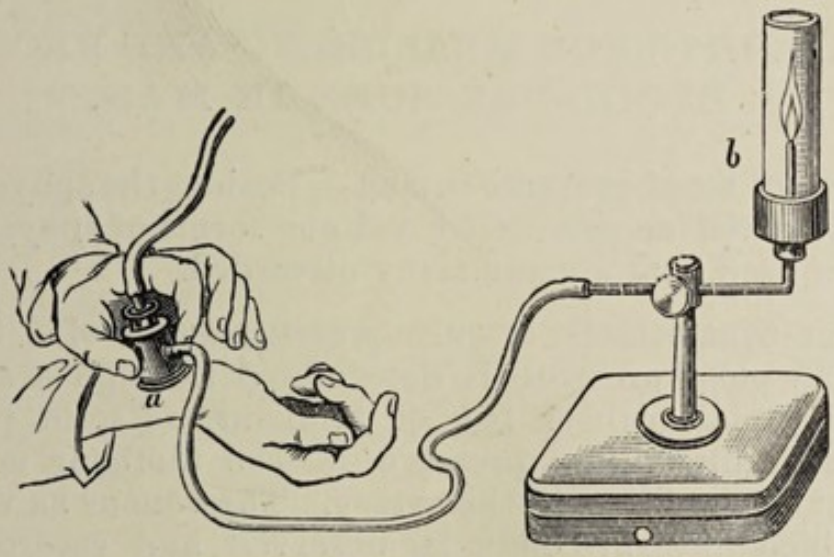


Fig. 316.—Sigmund Mayer's gas sphygmoscope, made by R. Rothe (Leipzig).

falls with each pulse-beat. Take a deep expiration, and observe the dicrotism in the gas flame. Delépine's form is convenient (*Brit. Med. Journ.*, 1891).

10. Piston recorder—Plethysmograph (volume pulse).—Use the air-piston recorder of Ellis, and take a plethysmographic tracing of the

variations of the volume of a finger. The piston of the recorder must be lubricated with an essential oil—*e.g.*, clove (*Journ. of Phys.*, vii., 309, 1886.)

11. Influence of respiration on pulse.

(i.) Valsalva's Experiment.—Arrange as in (*b*), but make a prolonged vigorous *expiration*. Note fall in pulse-beats. After filling the chest by a deep inspiration, the nostrils or glottis are closed, and a forced expiratory effort is then produced. There is thus a positive intra-thoracic pressure, and consequently the thoracic organs are compressed, and the venous return interfered with. The first effect of the forced expiratory effort is to raise the B.P., more blood forced out through aorta, but this is only temporary. The great veins in the thorax are compressed, the left ventricle receives less blood, the blood-pressure falls, and each pulse-beat is less. Try this with the nail apparatus. It is difficult to keep the arm sufficiently still while making the forced expiratory effort.

(ii.) Müller's Experiment (1838).—Close the mouth and nostrils and then make a forced prolonged *inspiratory* effort. Before doing so, feel the radial pulse, and keep feeling it. The pulse-beat ceases. The intra-thoracic vessels are filled with blood, and the distended auricles are unable to contract.

[See Graham Steell, *The Sphygmograph*, 1900; M. v. Frey, *Die Untersuchung des Pulses*, 1892.]

LESSON LXIII.

INSTRUMENTS FOR MEASURING AND RECORDING BLOOD-PRESSURE IN MAN.

Estimation of blood-pressure in man.—Besides the sphygmograph, when calibrated or graduated, various forms of sphygmometers have been used, and a great many discarded.

1. Hæmo-dynamometer or pulse-pressure gauge of G. Oliver (fig. 317).—The blood-pressure is determined through a stratum of *fluid* in a small rubber bag or tambour, as solid pads yield fallacious results. The pressure in the tambour is made to equal that of the blood in the artery. The venous as well as the arterial blood-pressure may be observed and recorded. The instrument is watch-like in form, with a dial 2 inches in diameter, and a spiral pressure scale ranging from 0 to 250 or 300 mm. Hg. Each 10 mm. equals a space of about 1 cm.

(*a*) *Instrument.*—Apply the pad (fig. 318) over the site of the vessel (artery or vein). Pressure is then made by pushing forward the body of

the instrument, when the indicator will rise on the dial and show the exact degree of pressure brought to bear on the pad and the vessel.

(*b*) *To observe arterial blood-pressure.* — Determine the *mean arterial pressure* and the *obliterating arterial pressure*. The former is by far the more important in clinical work. To measure the pressure required to arrest the pulsations of an artery (*the obliterating arterial pressure*) press the pad over the radial artery until the pulsation ceases to be felt by the finger placed on the distal side of it.

(*c*) For the *mean arterial pressure* select any superficial artery — *e.g.*, radial. The wrist is bent at an angle of about 45°, so as to slightly project the vessel on its bed, while the arm rests on its ulnar side. The wrist should be on a level with the ensiform cartilage in the erect (sitting or standing) postures, and with the back in the recumbent posture. The pad is placed over the site of the artery, and the instrument is pushed gently forward in the horizontal position (dial uppermost). The indicator will then rise on the scale, and begin to pulsate when measuring 50 mm. or more. The pulsations gradually increase in size until a point is reached when they attain their largest excursion, beyond which they as gradually diminish. The reading of the mean arterial pressure is made when the pulsations have acquired their fullest development; then the observer has raised the fluid pressure in the bag so that it just balances the blood-pressure in the artery. The mean of this maximum excursion indicates the mean arterial pressure, and the reading is made at that point.

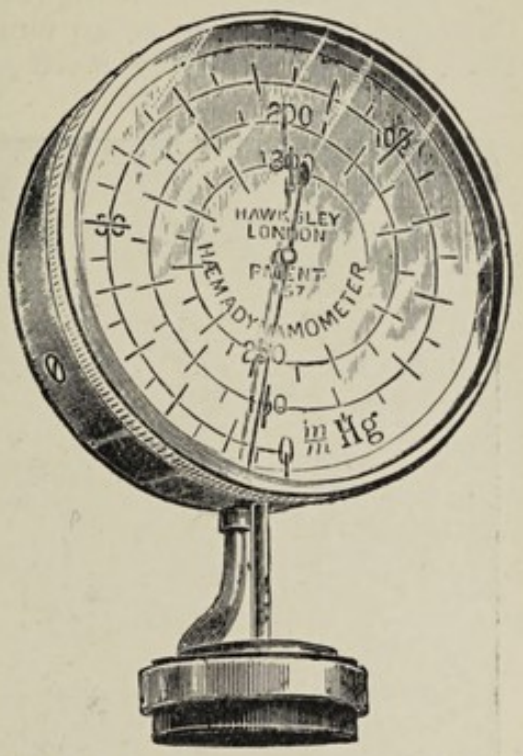


Fig. 317.—Hæmo-dynamometer of G Oliver, as made by Hawksley.

(*d*) *To observe venous blood-pressure.*—Select a piece of vein free from branches or anastomosis on the back of the hand or on the arm. Press the pad over the peripheral end of it until 50 to 100 mm. are recorded. Empty the vein by drawing the finger over it in the direction of the heart. The vein will then remain collapsed if provided with an efficient valve; but if it refills a finger must be kept over the central end of the collapsed vein, or another length of vein furnished with a valve must be selected. Then gradually reduce the pressure on the pad until a point is reached when the vein refills, when the exact pressure indicated must be read.

Dr. Oliver finds (i.) that there is considerable *uniformity of the mean arterial pressure*, provided the observation is made at the same hydrostatic level.

(ii.) *Gravitation* affects equally the arterial and venous blood-pressures.

(iii.) *Posture.*—In healthy and vigorous subjects recumbency affords a lower reading than obtains in the erect postures, arterial pressure being 10 or 15 mm.—*i.e.*, 10 or 15 p.c.—less, and the venous pressure being as much as 50 p.c. less.

(iv.) *Exercise* raises both the arterial and venous pressures; but rest (and especially recumbent rest) causes it to quickly subside.

(*e*) *The mean arterial pressure* in the recumbent posture (the wrist being extended on a level with the back) is 90 to 110 mm. Hg; and in the erect (sitting or standing) postures (the wrist being on a level with the ensiform cartilage) 100 to 120 mm. Hg.

(*f*) *The venous pressure* in recumbency is from 10 to 20 mm. Hg, and in the erect postures 20 to 40 mm. Hg (modified from *Edin. Med. Journ.*, 1898, and *The Blood and Blood-pressure*, G. Oliver, 1901).

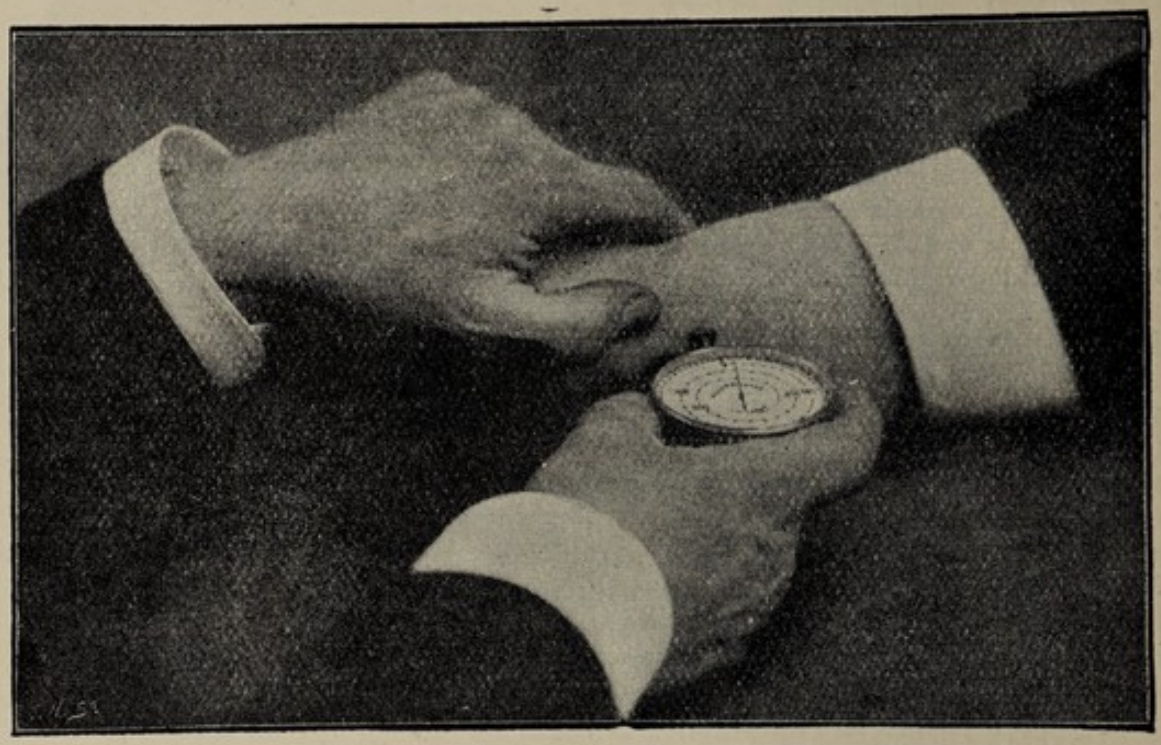


Fig. 318.—Mode of applying instrument over radial artery.

2. Sphygmometer (pocket form) of Hill & Barnard (fig. 319).

A. Pocket form.

(*a*) *Arterial pressure.*—Place the wrist on the same level as the heart. Note the pulse frequency.

(*b*) Hold the sphygmometer in the vertical position. Open the tap and press the edge of the rubber bag upon the table until the fluid rises to zero; then close the tap.

(*c*) Having set the fluid at zero, press the rubber bag upon the radial artery until the maximal pulsation of the fluid is obtained. Read the pressure indicated on the scale graduated in mm. of Hg at that point. Suppose the pulsation is maximal between 110 and 120 mm. Hg, then the mean arterial pressure equals 115 mm. Hg. The normal average pressure in young men is 110 to 120 mm. Hg in the standing, and 100 to 110 mm. Hg in the recumbent posture. The pulse frequency does not, as a rule, vary more than five beats per minute in the two positions.

(*d*) *Venous pressure.*—On the back of the hand choose a length of vein free from anastomosis, and press the sphygmometer upon the peripheral end of this. Then empty the vein centrally with the finger. Next, gradually reduce the pressure of the sphygmometer, and note the exact

pressure at which the vein refills with blood (abbreviated from Hill's description).

(*e*) *Capillary pressure.*—Place the hand, palm down, on a table. Place a small glass square with a vertical glass stem and top piece attached on the thin skin at the root of the nail of the middle finger. Press the sphygmometer upon the upper end of the glass rod until the skin just blanches, and read the pressure.

B. Another form.

The instrument is fastened to the arm as shown (fig. 320).

"When the rubber bag presses upon the outside of the arterial wall, with a pressure equal to the mean pressure exerted within, the wall is able to oscillate with the greatest freedom. In systole the artery is fully expanded, while in diastole it is collapsed by the pressure of the bag.

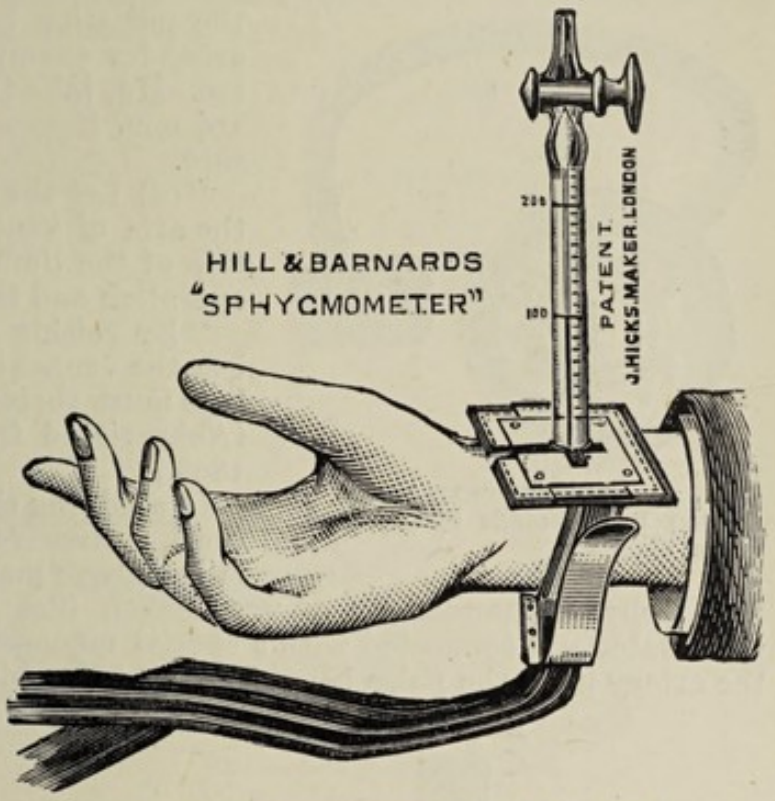


Fig. 319.—Sphygmometer of Hill & Barnard.

"(*a*) Screw valve, A, to pump tightly, screw the other end of valve to instrument, and fix the rubber tube of armlet to instrument as shown in illustration—now fasten armlet close and firmly round the right arm just above the elbow.

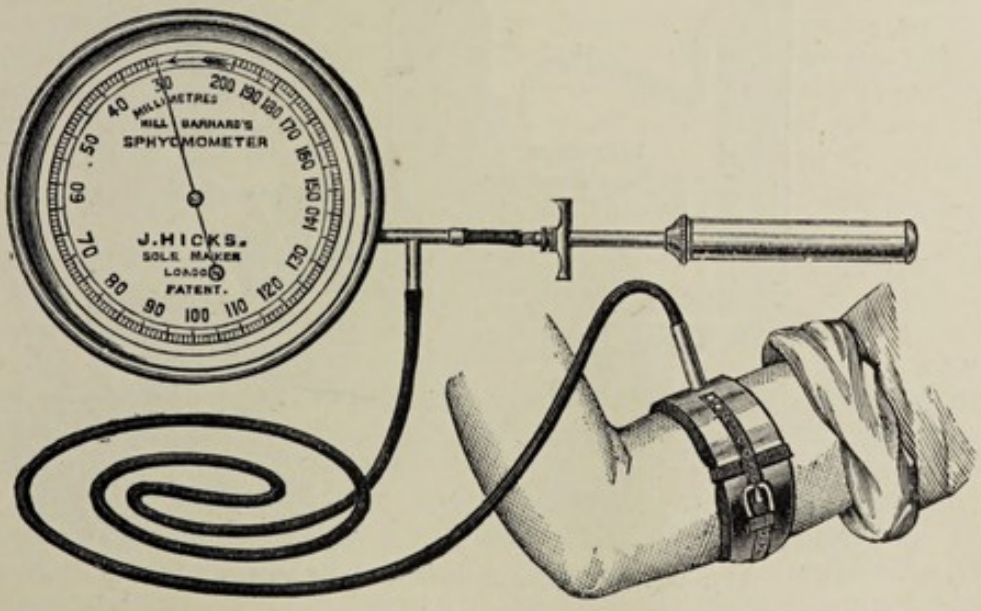


Fig. 320.—Hill & Barnard's sphygmometer arterial and pressure gauge for clinical use.

"(*b*) Force up the pressure rapidly till pulsation appears, and continue to force up the pressure till pulsation disappears or obviously becomes lessened.

"(*c*) Slightly open the valve and allow slow leakage. As the pressure falls, note where the pulsation becomes maximal. The maximal pulsation indicates the mean arterial pressure. If the pulsation be maximal over a certain area, for example, between 104 and 110 mm. Hg, take the mean of these figures—106 mm. Hg—as the mean arterial pressure.

"(*d*) Let the air out entirely and empty the arm of venous blood, either by elevation of the limb or friction. Repeat the operation and take another reading.

"By raising the pressure within the bag the venous outlets are blocked. Readings must therefore be taken rapidly."—(Abbreviated from Hill, *Journ. of Phys.*, 1898.)

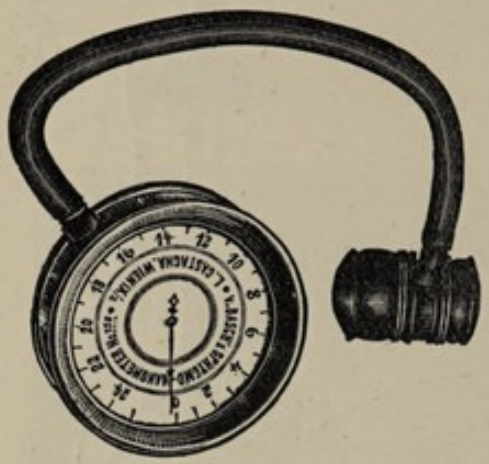


Fig. 321.—Sphygmomanometer of von Basch, made by Castagna, of Vienna.

In studying the effect of varying conditions, successive readings must be taken in the above manner.

3. Sphygmomanometer of von Basch (fig. 321).—The capsule filled with water and connected with a special manometer gauge is pressed upon the artery until the pulse beyond ceases to be felt.

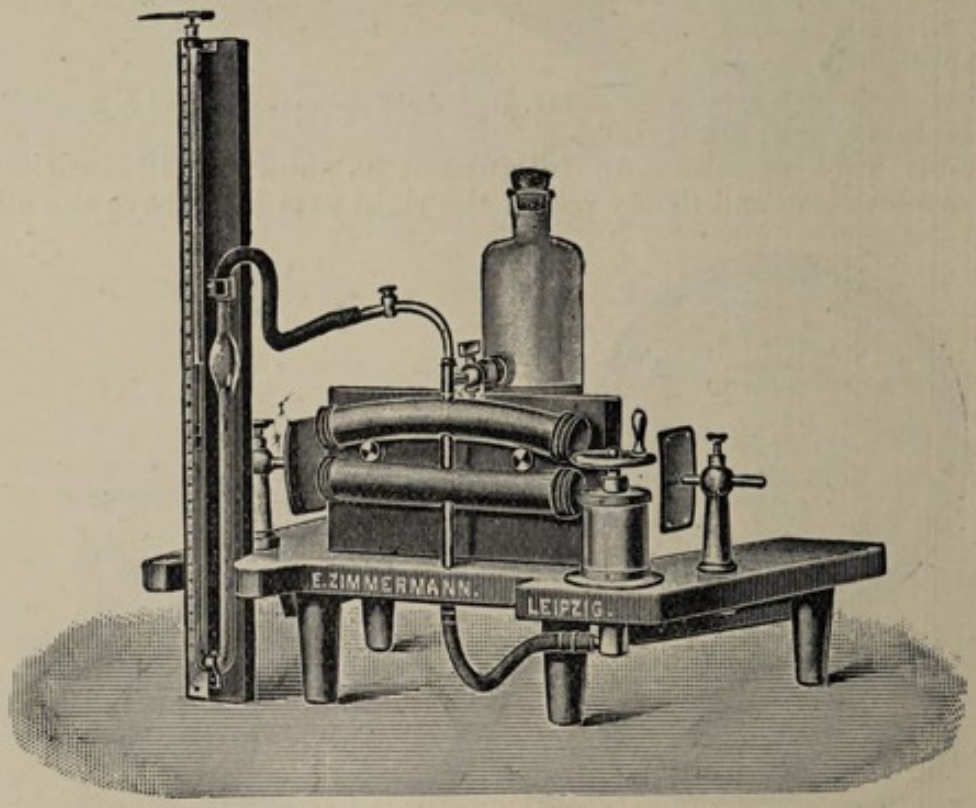


Fig. 322.—Sphygmomanometer of Mosso.

4. Sphygmomanometer of Mosso (figs. 322, 323).—It is obvious that, if the pressure outside an artery is increased so as to exactly balance the

internal pressure, then the oscillations of the arterial walls will be greatest as they are then freest to move. This is effected in Hill's form by air, in Mosso's by water-pressure, as shown in fig. 323, where the two middle fingers of each hand are placed in rubber capsules, inside metallic tubes, and the pressure can be regulated to get the greatest excursion of the Hg-manometer. The oscillations of the Hg can be recorded on any convenient moving surface (*Arch. ital. de Biologie*, xxiii.).

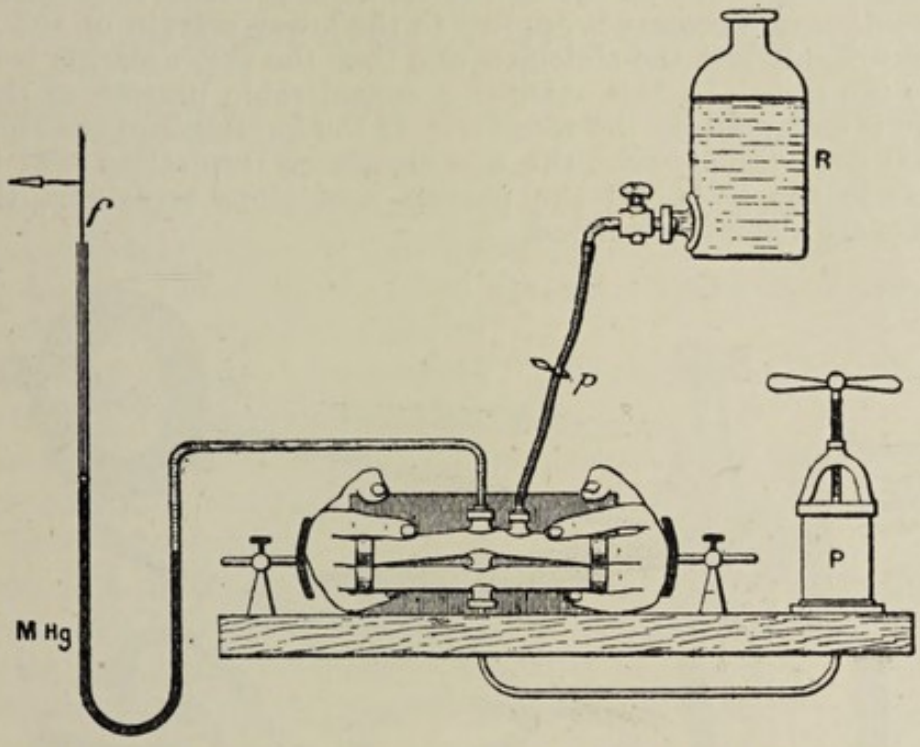


Fig. 323.—Scheme of Sphygmomanometer of A. Mosso. *M Hg*, Mercury manometer *f*, Float; *R*, Reservoir for water; *p*, Clip; *P*, Pressure apparatus.

5. Sphygmochronograph (A. Jacquet, *Zeitsch. f. Biol.*, xxviii., 29, 1891), so-called because it records simultaneously both pulse-beats and a time tracing. It is really a modified Dudgeon's sphygmograph with clock-work added to mark 0·2 second, and recording on a strip of paper that may be 50 cm. long. There is also an arrangement by which the velocity of movement of the paper can be changed from 10 mm. to 40 mm. per second. There is a special arrangement for fixing the apparatus to the forearm.

6. Clinical Polygraph of J. Mackenzie (fig. 324) can be used for taking, at the same time and on the same recording surface, tracings of the radial pulse, apex beat, carotid, venous or liver pulse, or the respiratory movements. It consists of (1) a small cup for receiving the pulsations, (2) a tube for transmitting the impulses through air to (3) the recording tambour and lever, the tambour being fixed to (4) a Dudgeon's or Jacquet's sphygmograph.

The cup or "receiver" is a shallow circular vessel, $1\frac{1}{4} \times 1\frac{1}{2}$ inch. The open mouth is applied over the pulsating part in such a manner that its edges are so closely adapted to the skin that no communication is kept up with the outer air. From the roof of the receiver rises a narrow pipe half an inch in length. To this pipe is fitted an india-rubber tube 3 to 4 feet in length, the other end of which is connected with the recording tambour.

A modification of this receiver is required when tracings of the *liver pulse* are taken. The "liver receiver" is larger, being 5 inches in length, 2 inches in breadth, and 1 inch in depth, its open edges slightly curved on their long axis. A small air-hole is made at one end, near the roof. In employing the liver receiver, the position of the lower margin of the liver having been ascertained, the receiver, held in the right hand, is laid lengthwise across the abdomen, its lower edge being 2 inches below the liver margin and the end with the air-hole towards the middle line. Steady continuous pressure is applied to the lower margin of the receiver till it sinks deeply into the abdomen, and then the upper margin is adapted closely to the skin. In this manner a considerable portion of the lower liver edge is embraced by the receiver. If the forefinger of the right hand be applied over the air-hole, the movements of respiration will be communicated to the liver. If the patient now stops breathing, the liver movements are alone transmitted.

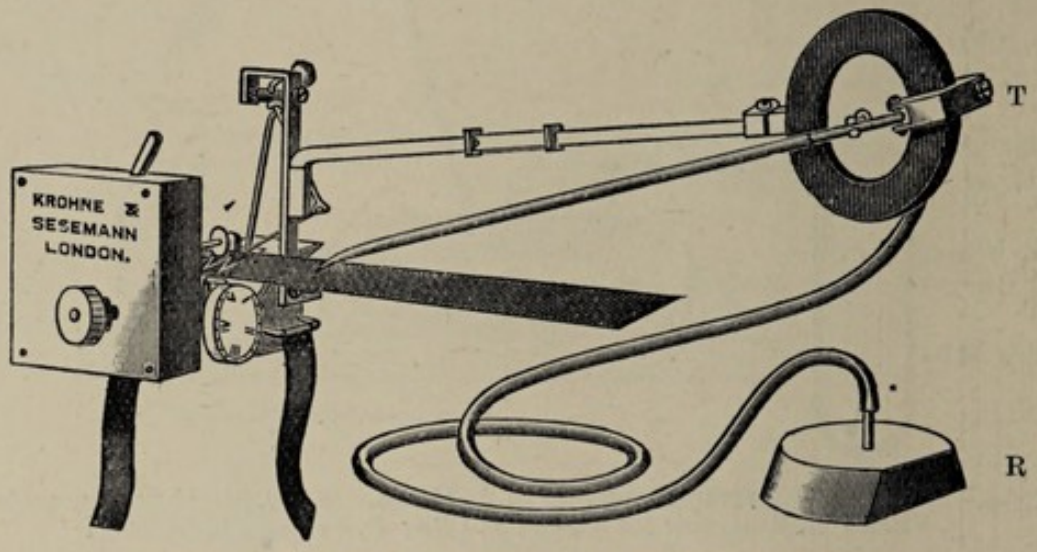


Fig. 324.—Clinical Polygraph of J. Mackenzie, of Burnley.

The tambour supports a writing lever about 6 inches in length, and is itself securely fixed, with its movable surface vertical, on a support fixed to the upright of the sphygmograph. The writing point records horizontally across the paper. Thus any movement can be recorded at the same time and on the same paper as the radial sphygmogram. For convenience of study, more events can readily be recorded within the limits of a short sphygmographic paper; and a record of apex beat, venous, carotid, and liver pulses may be taken at the same time as the radial sphygmogram.

It can also register the respiratory movements at the same time as the radial pulse. The receiver is placed over the episternal notch, or above the clavicle, the inspiration then causes a depression in the tracing. Or a bag tied round the chest or abdomen may be used. Inspiration then causes an upward movement in the tracing (J. Mackenzie, *Journ. of Path. and Bact.*, 1894.

7. **Arteriometer** (*Oliver*) measures the internal diameter of the radial artery. Study this instrument, *Pulse-gauging*, by G. Oliver, 1895; *Blood and Blood Pressure*, by G. Oliver, 127, 1901.

8. **Volume pulse.** See *Plethysmograph*.

LESSON LXIV.

PRINCIPLES OF CIRCULATION—SCHEMATA—PULSE DELAY—STROMUHR.

Hydraulic principles of the circulation.—With each systole of the L ventricle a certain amount of blood is expelled with a certain force or pressure into the aorta (and pulmonary artery) at regular intervals—*i.e.*, energy of heart-beat which

(i.) Exerts pressure in blood-vessels = blood-pressure (B.P.);
(ii.) Moves forward the blood.

The blood is forced in against "peripheral resistance," which is relatively greatest in the arterioles.

A large stretch of elastic, extensible and in part contractile, tubing extends between the L.V. and the innumerable arterioles at the periphery.

There is a great increase in sectional area from aorta to the systemic capillaries (1 : 800) and a diminishing sectional area from the capillaries through the veins to the R.V. The following facts are readily explicable from these data:—

(i.) High pressure and pulsatile flow in large arteries.
(ii.) Steady, slow uniform stream in capillaries.
(iii.) Low pressure and *pulseless* flow in veins.
(iv.) Continued flow from aorta to venæ cavæ.

The "peripheral resistance" in the arterioles due to the state of greater or less tonic contraction of their smooth muscular fibres—governed and controlled by the vascular or vaso-motor nerves—is a most important factor in maintaining and regulating the blood-pressure in the arteries, and also in bringing the elasticity of the large arteries into play, whereby the rhythmical pulsatile flow in the large vessels is converted into the uniform, equable, and pulseless flow in the capillaries.

Part of the heart's energy, therefore, is concerned in maintaining the B.P. spent in moving the blood.

The effect on the blood flow of the enormous increase in sectional area of the capillary regions is, of course, to correspondingly diminish the *velocity* of the flow.

1. Scheme of the circulation.—Use either Rutherford's scheme (*Lancet*, 1871) or the major schema. In the latter the heart is

represented by an elastic pump (Higginson's syringe), the arteries by long elastic tubes dividing into four smaller tubes with clamps on them; two of the tubes leading into tubes filled with sponge to represent the capillaries. The capillaries lead into a tube with thinner walls representing the veins. The inflow tube into the heart and the outflow tube at the vein are placed in a basin of water, and the whole system is filled with water.

(*a*) Use two mercury manometers; connect one with the arterial, and the other with the venous tube. Adjust a float on each, and cause the writing points of the two floats to write exactly one below the other in the same vertical line on a drum.

(*b*) Unclamp all the arteries and work the pump, regulating the number of beats by a metronome beating thirty per minute, and compress the heart to the same extent each time with a lemon-squeezer. Both manometers will oscillate nearly to the same extent with each beat. Take a tracing on a slow drum.

(*c*) Gradually clamp the arteries to increase resistance, and continue to pump; the pressure in the arterial manometer rises with each beat until it reaches a mean level with a slight oscillation with each beat. The pressure in the venous manometer rises much less, and the oscillations are slight or absent.

(*d*) While the mean arterial pressure is high, cease pumping; this will represent the arrest of the heart's action, brought about by stimulation of the peripheral end of the vagus; the arterial blood-pressure falls rapidly and the venous rises.

(*e*) Pump again and restore the mean arterial pressure, then unclamp gradually the small arteries. The steady fall in the arterial manometer represents the fall of B.P. when the central end of the depressor nerve is stimulated (vagi divided).

2. **Rigid and elastic tubes.**—To the single vertical stem of a glass Y-tube or similar arrangement of gas-tubing, 1 cm. in diameter, fix a Higginson's syringe, whose opposite end dips into a vessel of water. To one of the bifurcations connect a glass tube of similar diameter and about 90 cm. in length, and to the open end of this tube attach a small short piece of india-rubber tubing with a screw clamp over it to regulate the outflow. To the other limb attach an india-rubber tube of the same diameter and length as the glass tube, and fix a clamp over its outflow end. Pump water through the system. The pump may be compressed directly by the hand, or it may be placed between the two blades of a "lemon-squeezer," and the extent of the excursion of the latter regulated by a screw. See that all joints are secure.

(*a*) **Rigid tube.**—Clamp the elastic tube near the Y-piece. Work the pump about forty beats per minute, and force water into the glass tube. The water flows out *in jets* in an intermittent stream with each beat. Gradually clamp the outflow tube and keep pumping; the water still flows out in an intermittent stream, and no amount of diminution of the outflow orifice will convert it into a continuous stream; as much water flows out as is forced in.

(*b*) **Elastic tube.**—By means of a clamp exclude the glass tube near the Y-piece, and unclamp the flexible one so as to have no resistance at its outflow orifice. Work the pump; the outflow takes place in jets with each beat of the latter. Pump as rapidly as possible; the outflow is still intermittent. While pumping gradually clamp the tube at its orifice, so as to introduce resistance there—to represent resistance in the arterioles. When there is sufficient resistance at the outflow, the stream becomes a uniform and *continuous* one. Feel the tube, with each beat a pulse-beat is felt. The resistance at the periphery brings the elasticity of the tube into play between the beats, and thus converts the interrupted into a uniform flow. This apparatus serves also to demonstrate why there is no pulse in the capillaries, and under what circumstances a pulse is propagated into the capillaries and veins.

3. **Pulse delay.**—That a certain time elapses between the beat of the heart and the pulse at the wrist is well known. E. H. Weber (1834) estimated this interval, and calculated the rate of propagation of the arterial pulse-wave at 28½ feet (*i.e.*, about 9 metres) per second.

(*a*) Arrange a cardiograph to record on cylinder (rate 50 mm. per sec.). The cardiograph placed over the cardiac impulse and coupled up by rubber tube with a recording Marey's tambour.

(*b*) Place a tambour—explorer—with its button on any convenient artery—*e.g.*, carotid or radial—and record the pulse-beat, with same length of tubing intervening. See that the cardiac and arterial levers record in the same vertical line, or use the radial Hürthle's tambour (fig. 314). Record time also.

Record simultaneously heart and carotid, then heart and radial tracings. Measure the distance between the heart and carotid (about 6 inches), heart and radial, and heart and femoral. Note that the interval between heart-beat and carotid may be 0·1 sec., in the radial 0·17, and in the femoral it may be 0·18. From the distance calculate the velocity (A. Waller, *Journ. of Phys.*, iii.).

4. Velocity of a pulse-wave.—(*a*) Take 3 metres of india-rubber tubing 6 mm. in diameter. To one end of the tube attach a Higginson's syringe (elastic pump) to imitate the heart, while the other end of the tube is left open, with a clamp lightly fixed on it. Arrange to pump water through the tube. Arrange two light recording levers of equal length on one stand, and place a part of the tube near the pump under the lower lever, and resting on a suitable collar support, while part of the tube near the outflow end is similarly arranged under the upper lever. Regulate the pressure of the lever upon the tube by means of lead weights.

(*b*) Arrange on the same stand a Desprez's chronograph to record the vibrations of an electric tuning-fork (30 or 50 D.V. per second), with the writing points of the two levers and chronograph writing upon the drum in the same vertical line.

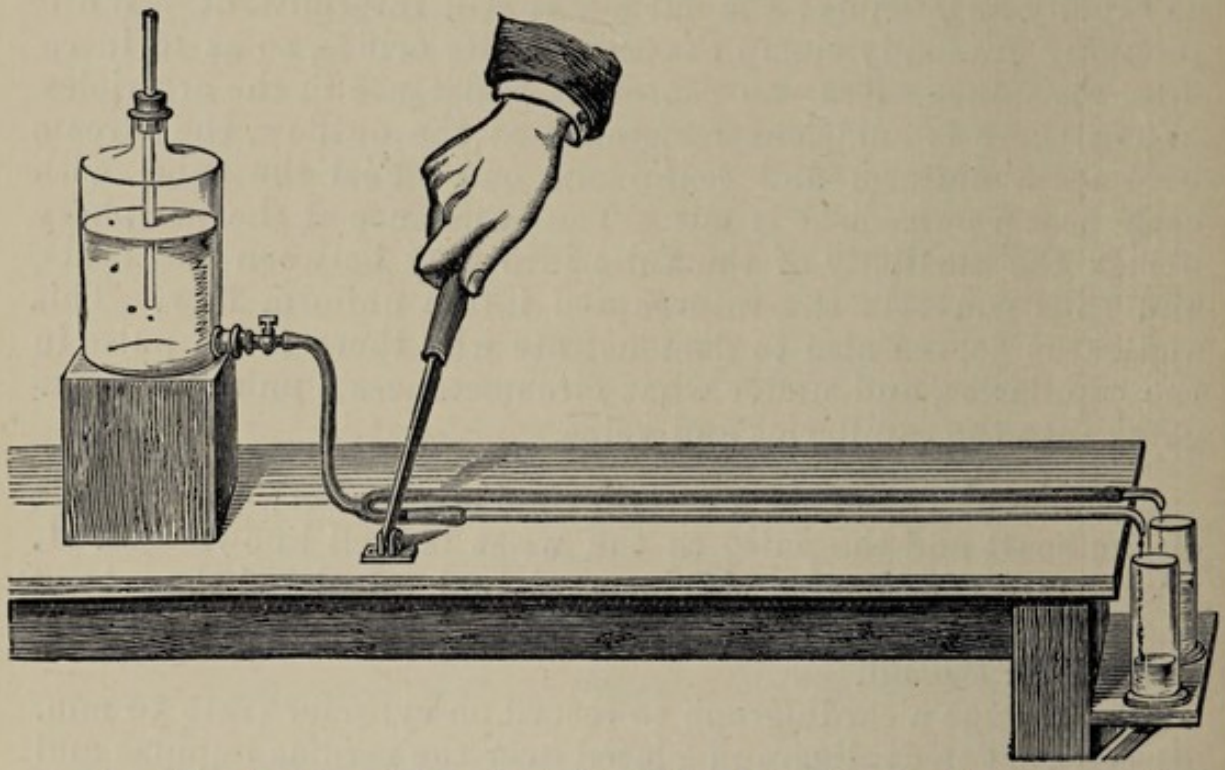


Fig. 325.—Marey's scheme to show that with rigid and elastic tubes of the same calibre, under certain conditions, the elastic tube delivers more fluid than the rigid one.

(*c*) Set the tuning-fork vibrating and the drum moving, compress the elastic pump interruptedly—to imitate the action of the heart—and propel water through the tube. The compression may be done by means of a lemon-squeezer, the extent of the excursion being regulated by a screw, and, to ensure regularity, arrange the number of pulsations to the beat of a metronome. On doing so, as one pumps in the water, the tube distends and raises the lever; in the interval between the beats, as the water flows out at the other end, the tube becomes smaller, and the levers fall. Feel the tube; with each contraction of the pump, a beat—the pulse-beat—can be felt.

(*d*) Fix and study the tracing. The tracing due to the rise of the lever next the pump begins sooner, and is higher than the one from the lever near the outflow. Make two ordinates to intersect the three tracings, one where

the lower pulse-curve rises from the abscissa, and the other where the upper curve begins. Count the number of D.V. of the tuning-fork between these lines. Measure the length of the tube between the two levers, and from these data it is easy to calculate the velocity of the pulse-wave in feet per second. Suppose the distance be represented by D cm., and the time interval by T, then the velocity of the wave in cm. is the quotient of $\frac{D}{T}$. The wave length is determined by dividing its velocity by the time taken by the wave to pass over any one point.

5. **Rigid and elastic tubes.**—Arrange an experiment as shown in fig. 325—*i.e.*, with outflow tube leading to a Marriotte's flask, two tubes—one of glass and the other of caoutchouc—of equal diameter, and outflow orifices of the same size. The glass tube is attached by a short elastic tube to the lead tube coming from the reservoir. As the fluid flows into the tubes, they are compressed rhythmically to imitate the interrupted beat of the heart. More fluid is discharged by the elastic tube.

It is evident, therefore, that the elasticity influences

(1) Character of flow.

(2) Amount of fluid discharged.

6. **Rheometer** (figs. 326, 327) is used to measure the amount of blood flowing through a blood-vessel in a given time. The nozzles of the instrument are inserted

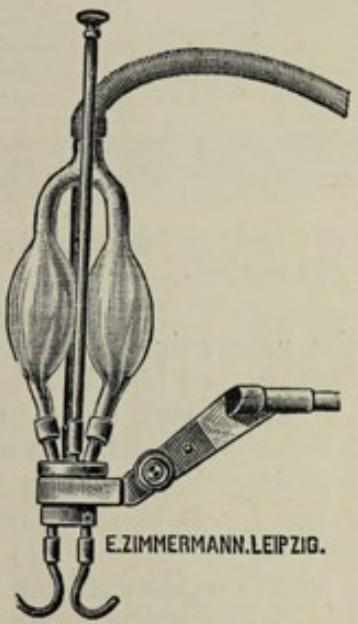


Fig. 326.—Rheometer of Ludwig and Dogiel, showing how it is held in position.

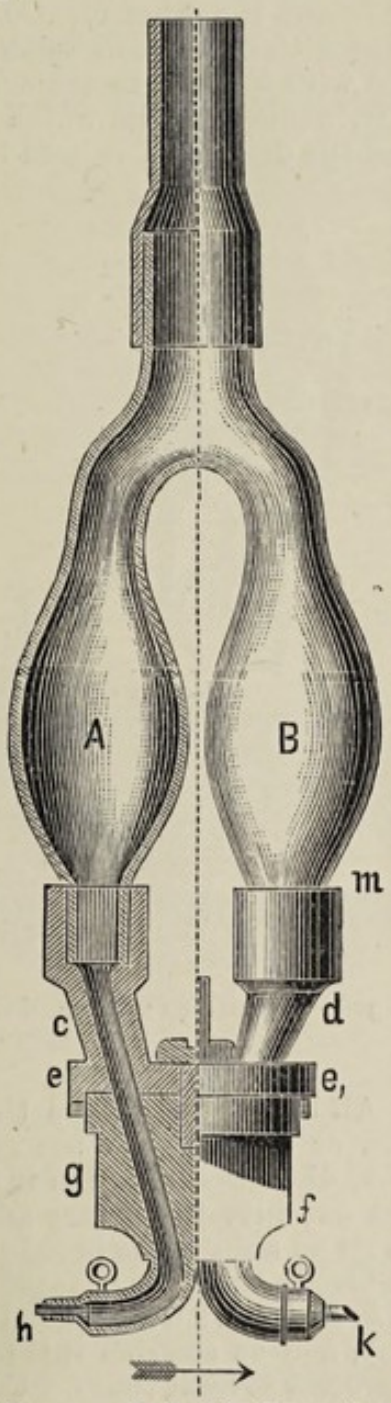


Fig. 327.—Ludwig's rheometer or "Stromuhr."

and tied into the artery of an animal. A rubber tube with a pressure bottle attached may be used to represent the artery.

(*a*) To represent the heart—or the weight of a column of fluid—arrange a Marriotte's flask on a stand, and to the outflow tube attach a narrow rubber tube, and clamp it after filling it with normal saline (to represent defibrinated blood). Fill one bulb with defibrinated blood, the other with almond oil, and close the top of the instrument with a glass plug.

(*b*) Suppose the tube to represent an exposed artery; about the middle of the tube apply two ligatures an inch apart (or two clamps). Divide the part of the tube between the two ligatures, and tie into either end the nozzles provided with the instrument. Call the one next the reservoir or heart *h*, and the other *k*. Connect to the instrument the nozzles, the bulb A filled with oil in connection with *h*, B with defibrinated blood and connected with *k*. The instrument is fixed in position by a support provided with it, while a handle which fits into two tube-sockets on the upper surface of the disc, *e*, e_1, is used to rotate the one disc on the other.

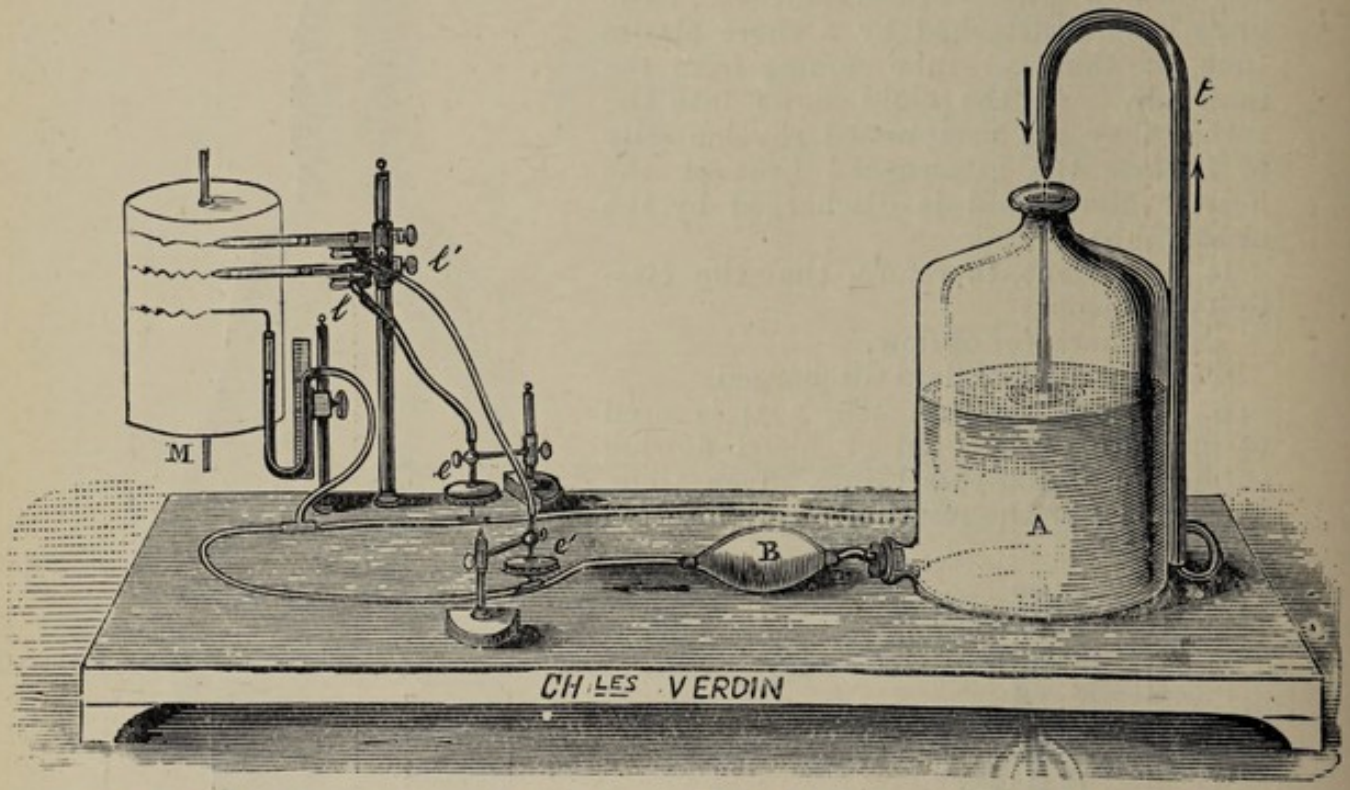


Fig. 328.—Fredericq's scheme of the circulation. *B*, Heart manometer and recording tambours, *l* and *l'* (arterial and venous).

(*c*) All being ready, take the clamp off the reservoir of blood and the clamps or ligatures off the artery. The defibrinated blood flows into the bulb A, displaces the oil in it towards B, the defibrinated blood of B being forced out into the artery and caught in a suitable vessel. In an experiment on an animal the blood passes into the artery. As soon as A is filled with blood, the disc is rapidly rotated by hand. B then communicates with *h*, and A with *k*. Blood now flows into B, displacing the oil in it into A, and as soon as this takes place the disc is again rotated. Repeat this process several times. Count the number. The bulbs have the same capacity and are exactly calibrated.

The time is measured by connecting the rheometer with an electromagnet registering on a drum each rotation of the disc, and under this a time-marker records seconds.

Example.—Suppose each bulb holds 5 c.c., the bulbs to be filled ten times during 100 seconds—*i.e.*, ·50 c.c. flowed from the tube in one second. Suppose the diameter of the tube to be 2 mm.—*i.e.*, radius = 1 mm.—this would give a sectional area of 3·14 mm.

The velocity, V, is calculated by the ratio of the quantity discharged, Q, to the sectional area, S—*i.e.*, the quantity of fluid flowing across any section in unit of time ÷ area of that section. Hence—

$$V = \frac{Q}{S}$$

5 c.c., or what is the same thing, 500 cmm., are discharged in one second; therefore the velocity is $= \frac{500}{3{\cdot}14} = 159$ mm., or about 6 inches per second.

Attach the tube to a litre-bottle—with outflow tube—arranged as a Marriotte's flask. Estimate the amount of fluid discharged in a given time, and from this calculate the velocity of flow in the tube.

7. Schema of Fredericq (fig. 328).—In this scheme, filled with water, B represents the ventricle, with a long stretch of elastic tubing for the arteries, terminating in a narrower tube to represent the peripheral resistance. A represents the venous system. A Hg-manometer is connected with the arterial system and two "exploring" Marey's tambours register the pressure-pulse and rate of propagation of the pulse-wave. The movements of the heart are represented by rhythmical contractions of B made by the hand.

LESSON LXV.

CAPILLARY PRESSURE — LYMPH HEARTS AND SACS—PLETHYSMOGRAPHS.

1. Capillary blood-pressure.—(*a*) To the under surface of a disc of glass, 2 cm. long, 3 to 4 mm. broad, and 1 mm. thick, fix with cement on its under surface a glass plate, *a*, with a surface of 5 mm. square. Two threads supporting a paper scale-pan are attached to the glass disc (fig. 329). Arrange the glass plate, *a*, over the skin on the dorsal surface of the middle finger, just at the root of the nail. Add weights to the scale-pan until the skin becomes pale. Note the weight necessary to bring this about, but observe that the skin does not become pale all at once (N. V. Kries, *Arb. aus d. phys. Anstalt zu Leipzig*, 1875).

(*b*) Test how altering the position of the hand affects the pressure.

(*c*) The pressure is equal to the weight added plus weight of scale-pan. Estimate the size of the plate, *a*, in square centimetres. The pressure



Fig. 329.—Apparatus (V. Kries) for estimating the capillary blood-pressure.

expressed in cm. of a column of water is obtained by dividing the actual pressure weight by the pressure surface. From this the Hg equivalent is obtained. V. Kries found a pressure = 37 mm. Hg.

2. **Circulation of blood** (Microscope).—This may be studied in a lightly curarised frog in the interdigital membranes of the hind leg. The circulation in the capillaries was, however, first seen by Malpighi in the lung of a frog (*De pulmonibus*, 1661). One difficulty in connection with the frog's lung is that after it is exposed it is liable to collapse. Hence, the triton's lung is often used instead.

(*a*) *Holmgren's apparatus* (1874).—A lightly curarised frog is placed on its back on a frog-plate. A metallic tube is introduced into the glottis. It is provided with a rubber tube, and a stopcock. The tube (fig. 330) has on it two little rings with grooves, about 4 mm. apart. Over this is tied, like a muff,

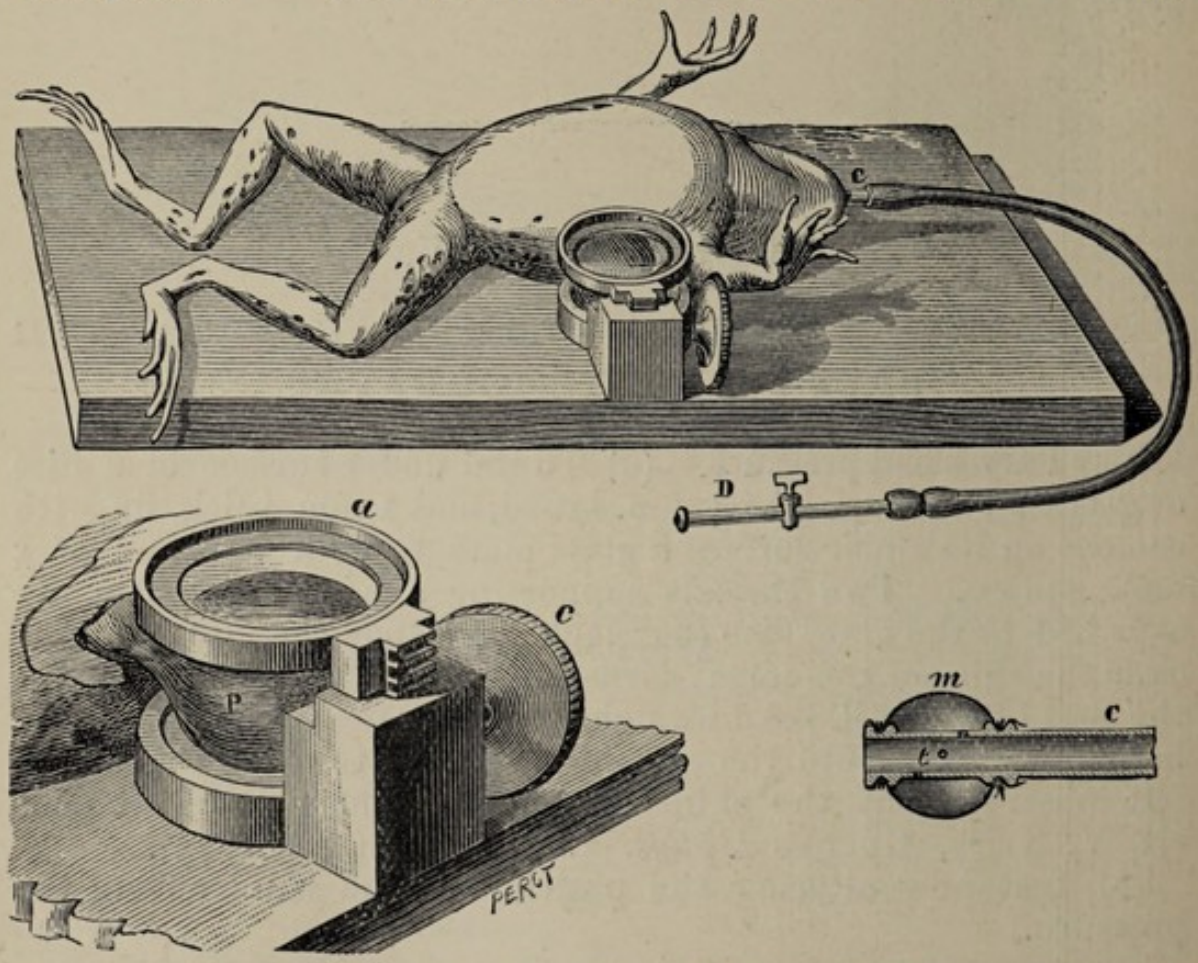


Fig. 330.—Holmgren's apparatus for lung of frog. *C*, Cannula in glottis; *m*, Membrane distended to obtain occlusion of glottis at moment of distending the lung; *D*, Cannula with tap; *a*, Metal ring carrying a cover-glass; *P*, Lung; *c*, Milled head.

part of the large intestine of a frog. There are three lateral holes in the tube between the two grooved rings. Fix the tube in the glottis; then blow into the tube to expand the lung, when, of course, the "muff" also expands, and blocks the glottis. Make an incision about 1½ cm. long on the lateral surface of the body below the axilla, taking care to injure no vessels, or expose the lung with a red-hot knife. This avoids bleeding. The distended lung is now gently pulled through the slip and placed in the apparatus. This gives a flat surface to be examined with the microscope instead of a concave one. With 100 diameters one can study the phenomena of capillary circulation.

(*b*) *Poiseuille's observations* (1835).—He used the mesentery of a pithed frog. Cohnheim's method or that of Ranvier is convenient. A slit is made in one side of the belly wall, a loop of intestine pulled out and pinned on to the apparatus of Ranvier (shown in fig. 331).

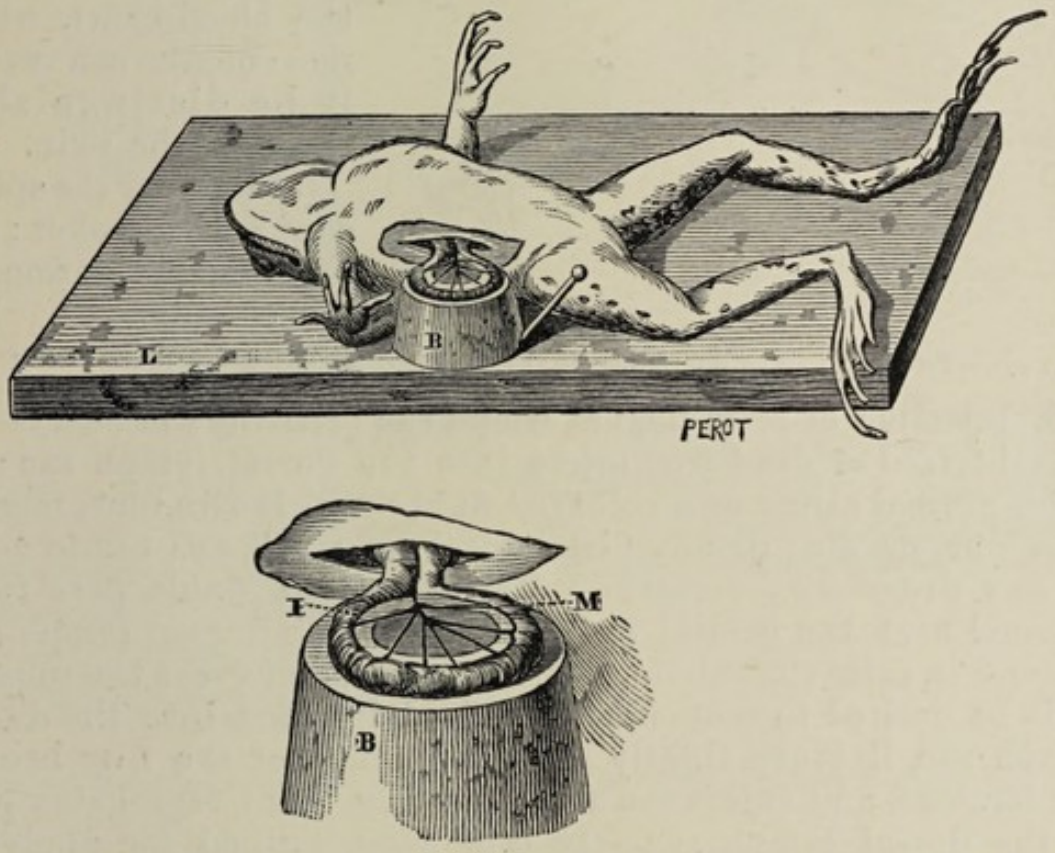


Fig. 331.—Apparatus for examining circulation in loop of intestine of frog. L, Frog-plate; B, Cork disc; I, Intestine; M, Mesentery.

3. **Inflammation.**—Destroy the brain of a frog. Curarise the frog very lightly. Examine microscopically the circulation in the web of its foot and in its mesenteric vessels. Apply a drop of croton oil or mustard for a minute or less. Observe the inflammation thereby produced, and the changes in the appearance of the blood-vessels and the blood-flow, until the latter is finally arrested in a condition of stasis, and exudation takes place.

4. **Posterior lymph-hearts.**—(*a*) Destroy the brain of a frog, place it on its belly, and watch the beating of the posterior pair of lymph-hearts, which are situated one on each side of the uro-

style in the triangle between coccygeo-iliacus, *ic*, gluteus, *gl*, origin of the vastus externus, *ve*, and pyramidalis, *p*, muscles (fig. 332).

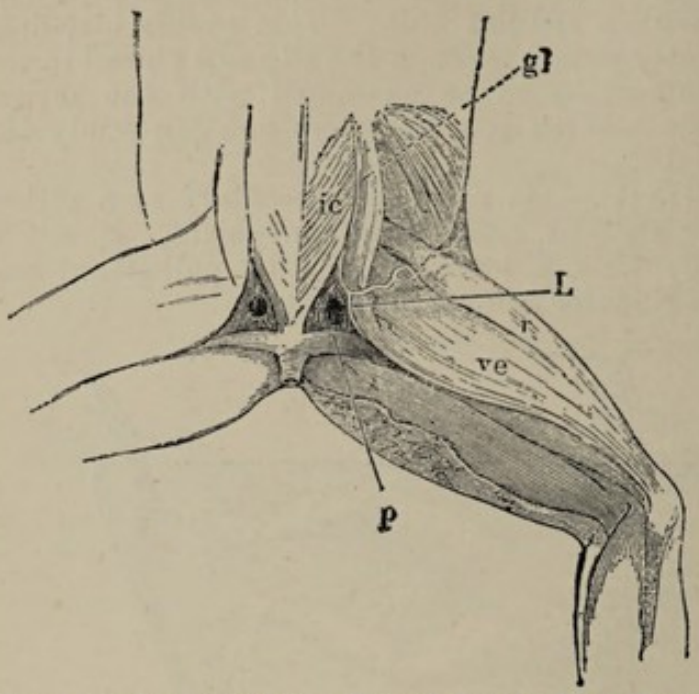


Fig. 332.—Posterior pair of lymph-hearts (*L*) of Frog.

(*b*) Remove the skin covering them, taking care not to cut too far outwards, to avoid injuring a cutaneous vein. Count the number of beats per minute, noting that the rhythm is not synchronous with the blood-heart, which movements can usually be distinguished through the skin.

(*c*) Destroy the spinal cord with a seeker; the rhythmical automatic movements of the lymph-hearts cease.

5. Injection of retro-lingual lymph sac (*Stirling's method*).—In an etherised or dead frog inject into the dorsal lymph sac any fluid, normal saline or a coloured fluid—*e.g.*, Berlin blue, methylene blue, or air; or any histological reagent—*e.g.*, osmic acid; or any drug—*e.g.*, veratrin. Very soon the fluids pass *viâ* a channel near the orbital cavity into the sub-lingual lymph sac, distend it, raise the sub-lingual membrane, and evert the tongue. If it be desired to economise the fluid injected into the dorsal lymph sac, ligature tightly the whole trunk of the frog behind the forelegs, and inject the fluid into the front segregated part of the dorsal lymph sac. It is a good method for applying drugs directly to the hyoglossus muscle still *in situ* and in normal condition. It is obviously also useful for applying various histological reagents to the retro-lingual membrane itself (Proc. Physiol. Soc., June, 1901, in *Journ. of Phys.*).

Plethysmography.—That the amount of blood in an organ or part thereof varies is well known, and that the condition of the blood-vessels and therefore the amount of blood in any given part varies with the state of the nervous system is equally well known—pallor and blushing. Nay, more, the volume of an organ may vary with each pulse-beat and every respiration. Thus if a means can be devised to measure the volume of an

organ, its variations under given conditions can also be recorded.

By means of volumetric instruments one can study variations in the volume of a part—variations which are not visible to the unaided eye. Such instruments are frequently called plethysmographs.

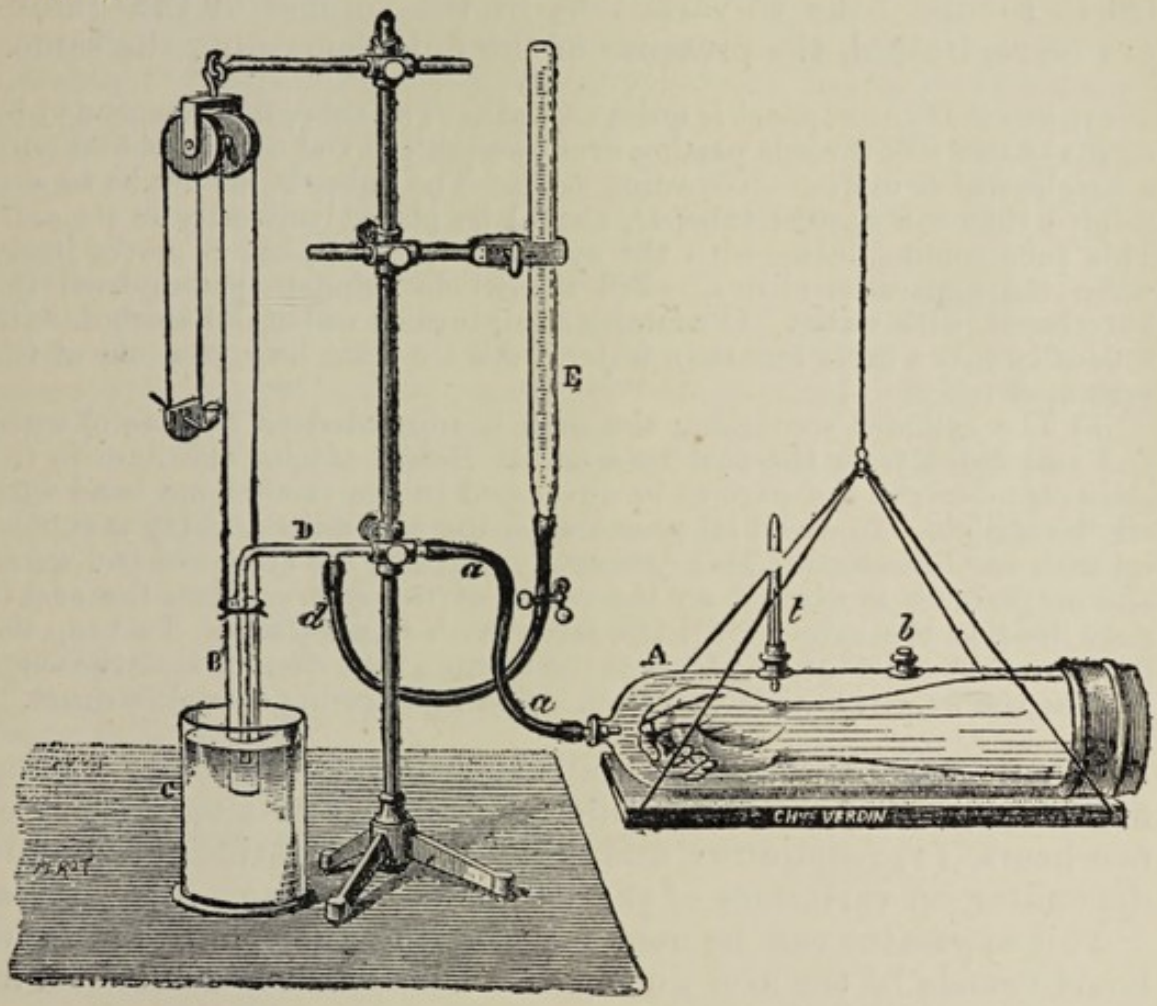


Fig. 333.—Plethysmograph of A. Mosso, arranged for human arm.

Other things being equal, the increase in volume of an organ depends on three factors—(1) Vascular dilation of the blood-vessels (blushing) produced either by paralysis of vaso-constrictor nerves, action of vaso-dilator nerves, or paralysis of the vessels themselves; (2) increase of arterial pressure with increased flow of blood through a part; (3) increase of venous pressure with slowing of blood flow. A diminution of volume is caused by the opposite conditions. These factors may act either singly or in combination.

Various forms of plethysmograph are in use with (i.) water or (ii.) air as a medium; (iii.) may record directly or (iv.) through the intervention of elastic walls.

The best known for measuring variations in the volume of a limb—*e.g.*, human arm—is the plethysmograph of Mosso (1875).

6. **Mosso's plethysmograph for arm** (fig. 333).—By means of this instrument the exact amount of fluid which passes to and fro in a tube—due to variations in the volume of the limb—can be registered, the pressure of the fluid remaining the same.

(*a*) See that the support is quite vertical. The tube, B, is suspended by means of two silk threads passing over two pulleys and counterpoised with a weight which carries a recording style. The tube, B, should be so suspended that the central tube, D, should be placed vertically in its axis. This tube communicates with the cylinder, A. See that B moves freely when the apparatus is dry. Fill the whole tubular system from the burette, E, with water. C contains a mixture of water and alcohol, with a density just a little less than water. All air must be driven out of the system of tubes.

(*b*) The cylinder containing the limb is supported on a plate of wood and suspended from the roof by a cord. Before placing the limb in the glass chamber rub the part to be enveloped in the caoutchouc band with oil or vaseline. To avoid all pressure on the arm a little putty is rubbed up with vaseline or oil. This completely prevents the escape of any water and no pressure is exerted on the vessels of the arm. When the arm is duly fixed in the cylinder fill the latter with tepid water. To keep the pressure in A constant, the level of the floating tube should be at the same level as the water in the large vessel. Record a tracing on a slow drum.

When the apparatus is carefully adjusted one obtains a tracing of the volume of the arm, which shows (1) the single beats of the heart, (2) respiratory undulations, (3) variations of volume depending on variations of the actions of the vaso-motor centre.

This apparatus can be used for studying the condition of the blood-vessels of the arm during the most varied conditions, as has been done by Mosso.

It is obvious that this method is applicable to the study of changes in volume in many organs—*e.g.*, kidney, spleen, and limbs in animals. Sometimes the surrounding medium is oil, sometimes air. The effects of stimulation of nerves, drugs, &c., on volume can be studied. In these cases it is important to determine whether the change of volume—*e.g.*, of spleen—is brought about actively or passively; *i.e.*, whether the organ dilates owing to rise of pressure in other parts of the vascular system. Hence in such plethysmographic experiments on animals, the arterial blood-pressure should be recorded simultaneously. A normal kidney or spleen plethysmograph curve shows cardiac and respiratory variations in its course corresponding exactly to those seen in a B.P. tracing.

7. **Volume pulse of arm** (Plethysmography).—Enclose forearm in a cylinder made of tin and corresponding to the size of the limb. Close the upper end with a muff or wide band of sheet rubber as before. Have a side tube attached to the cylinder wherewith to connect the interior of the air-filled cylinder with the recording tambour.

8. **Gravity on circulation** (Hill, *Int. Phys. Cong.*, 1898).

"An eel or grass-snake, the brain having been pithed, is affixed to a board in the extended position, and the heart exposed. On turning either animal into the vertical position (tail downwards) the heart, after a few beats, becomes emptied of blood. On pressing the body from the tail upwards, the heart immediately fills to repletion. On ceasing to compress the body, the heart once more as completely empties. So soon as the animal is placed head downwards the heart engorges. This engorgement is limited by the inextensile pericardium, which in the eel is extremely strong. On slitting the pericardium the engorgement becomes most marked.

"On opening the body of a snake held in the vertical position with the tail downward, the veins are found to contain blood in the lower two-thirds of its length only. It is evident that within these veins there cannot be more than half the quantity of blood required to fill to distension the whole vascular system of the snake.

"If a snake or eel be sunk vertically and tail downwards in a vessel of water, the heart does not empty.

"The hydrostatic pressure of the column of water exerted on the surface of the body tends to counterbalance the hydrostatic pressure of the column of blood within the body. The balance is not exact, for blood is heavier than water; moreover, the elastic wall of the body prevents the full compressing force of the water without from reaching the blood-vessels within" (Hill and Bernard, "Gravity on the Circulation," *Journ. of Phys.*, xviii., 1895; xxi., 1897).

9. **Position of arm.**—Extend arm and raise it vertically upwards. Observe the skin becomes paler and the veins empty. Let the hand hang down and observe the opposite effect (*Lister*).

LESSON LXVI.

BLOOD-PRESSURE IN ANIMALS—MERCURY KYMOGRAPH — MEMBRANE MANOMETERS — VAGUS—DEPRESSOR.

THE arterial blood-pressure is most frequently measured by means of the mercury kymograph of Ludwig. The carotid artery of an anæsthetised rabbit or dog is exposed, and its lumen connected with a manometer (1847).

To measure the blood-pressure one requires (1) a manometer of some kind connected with the interior of the artery, and (2) a

recording surface—*i.e.*, a surface on which to record the movements of the fluid in the manometer. Various forms of apparatus are used, according as one requires a longer or shorter continuous tracing, or as one wishes to record with ink on a white paper surface, or with a style on a smoked surface, as in Hering's form (Fig. 336). Fig. 334 shows a kymograph—Ludwig's pattern—where a long strip of paper is gradually unwound in front of the recording style of the manometer.

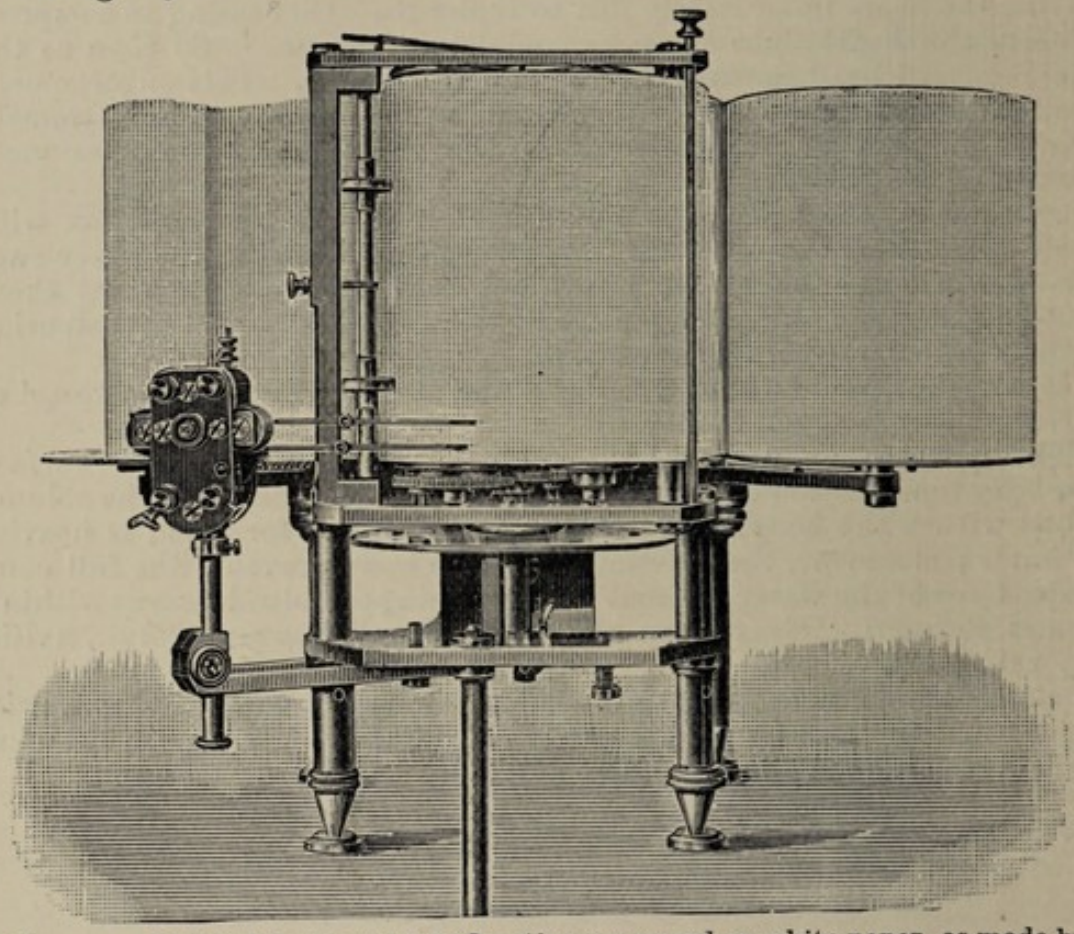


Fig. 334.—Ludwig's kymograph for continuous record on white paper, as made by Zimmerman of Leipzig.

Fig. 335 shows Hürthle's form, as made by Albrecht. In it a long strip of smoked paper is stretched over the two drums, as in Hering's pattern (Fig. 336). This is an excellent apparatus, and is capable of being adjusted in every direction.

The manometer used is the Hg-manometer of Ludwig, as shown in Fig. 337.

I. Rabbit.

1. Estimation of blood-pressure in rabbit by Ludwig's kymograph—Demonstration.—As the carotid artery of the rabbit is frequently used, the student should make himself familiar with the anatomy of this artery and its adjacent nerves (Fig. 338).

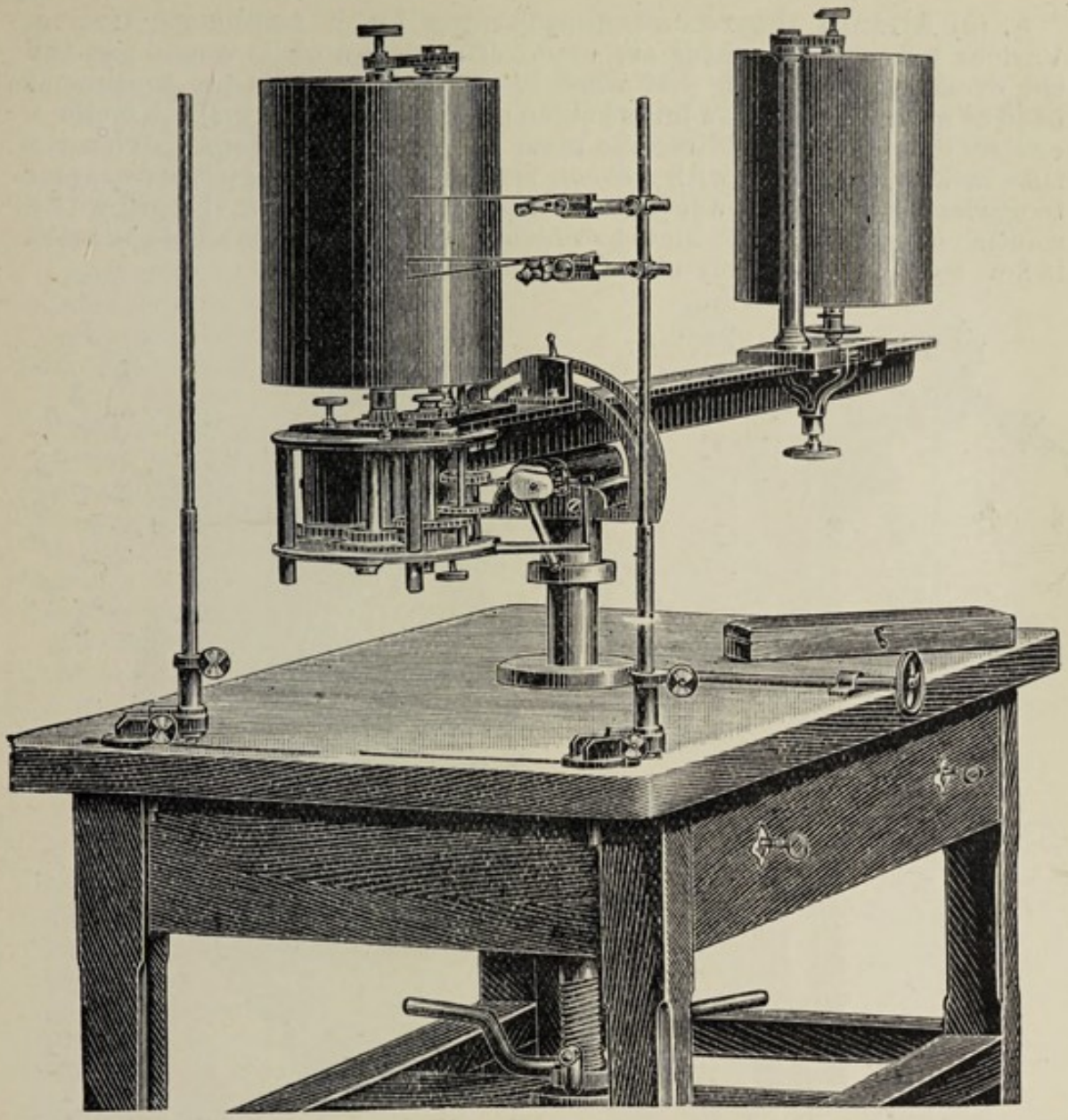


Fig. 335.—Hürthle's kymograph, as made by E. Albrecht of Tübingen.

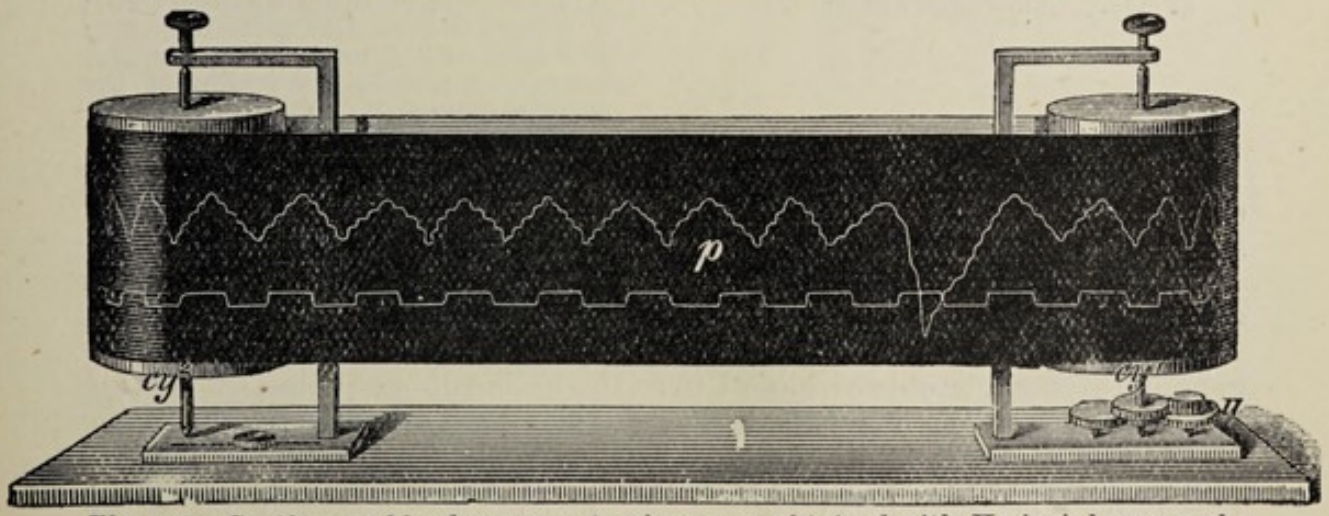


Fig. 336.—Continuous blood-pressure tracing, *p*, as obtained with Hering's kymograph; *cy* moved by *u*.

A. (*a*) Arrange the recording apparatus for a continuous tracing. Various forms of apparatus are used. The clockwork is wound up, and the drum is so adjusted that when it moves it unwinds the continuous band of white paper from a brass bobbin placed near it (fig. 334). A motor—electric or water—may be used to move the recording surface. Arrange a time-marker connected with a clock, provided with an electric interrupter to mark seconds at the lower part of the paper. A pen-writer charged with a solution of aniline (red or blue) to which a little glycerin is added to make it flow freely, is commonly used for non-smoked paper.

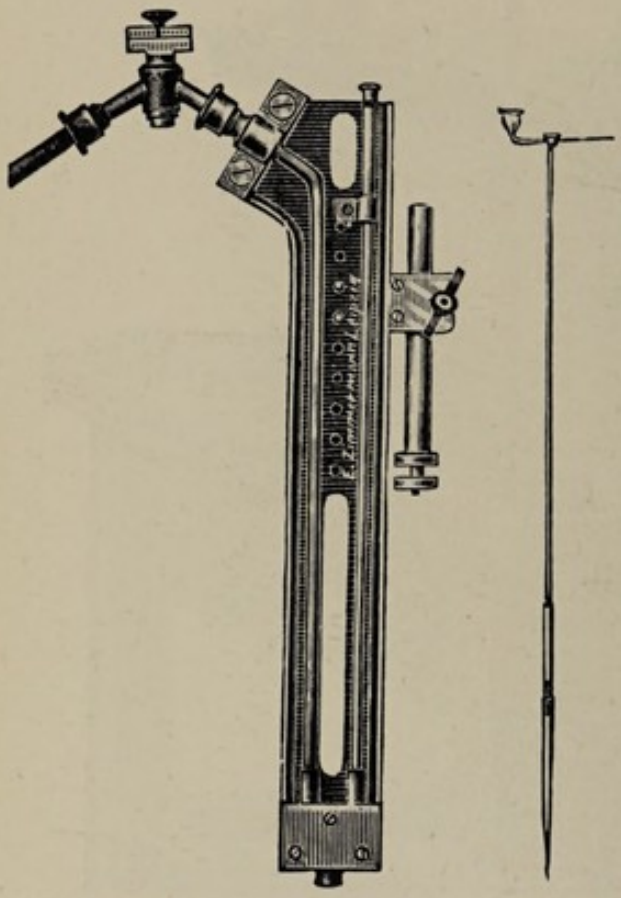


Fig. 337.—Ludwig's mercury manometer. Float and recording glass pen shown at side. Zimmerman's pattern.

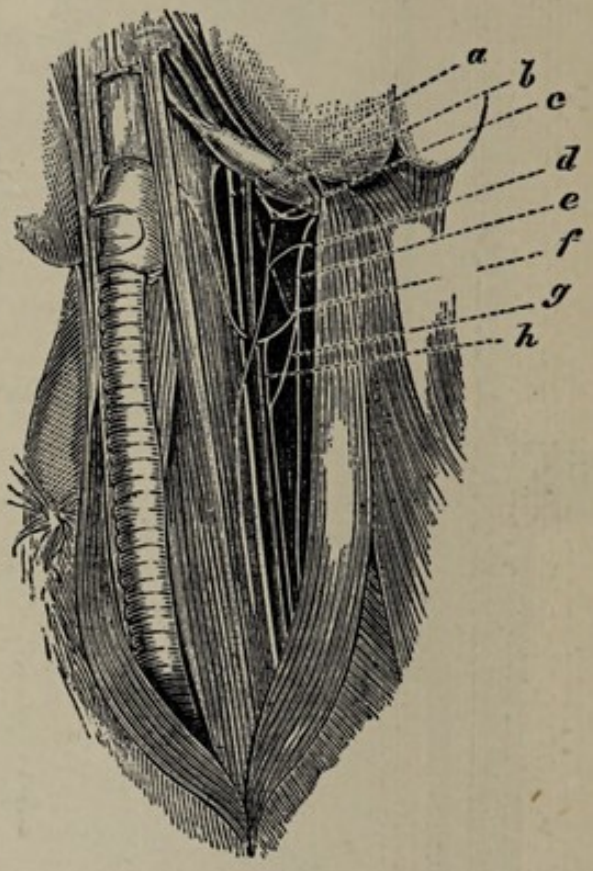


Fig. 338.—Nerves in the neck of the rabbit. *a*, Sympathetic; *b*, hypoglossal, with *c*, its descending branch (*descendens noni*); *d*, Branch of a cervical nerve joining *c*; *e*, Vagus, with *f*, its superior laryngeal branch; *g* and *h*, The origins of the superior cardiac or depressor nerve.

(*b*) Partially fill the manometer (fig. 337) with dry clean mercury, and in the open limb of the manometer place the float bearing a recording style. See that the float rests on the convex surface of the mercury (fig. 339).

(*c*) The closed or proximal side of the manometer at its upper part is like a T-tube, the stem of which is connected by thick india-rubber tubing to a piece of flexible lead tubing; on the free end of the latter is tied a glass cannula of considerable size, and over the india-rubber tubing connecting the cannula with the lead tube is placed a clamp. The proximal end of the manometer is filled by means of a syringe with a saturated solution of sodium carbonate as high as the stem of the T-piece. To it is attached a long india-rubber tube, which is connected with a pressure-bottle filled with

a saturated solution of sodium carbonate, and kept in position by a cord passing over a pulley fixed to the roof. A 25 p.c. solution of magnesium sulphate is perhaps better (50 parts of the salt to 150 water). Moreover, it does not make the cannula so slippery as the soda. Some use a solution of Witte's peptone—1 part to 12 parts 0·6 NaCl (*Tigerstedt*), others leech extract (extract of 10 heads to 100 c.c. of normal saline), and again, 1 to 2 p.c. solution of potassium oxalate (*Thunberg*). A clamp compresses the india-rubber tube just above the manometer. Open this clamp and also the one at the end of the lead pipe. The alkaline solution fills the whole system, and after it does so, and no air-bubbles are present, close the clamp at the end of the lead tube, and then the one on the pressure-bottle tube. It is well to have at least an inch of positive pressure in the manometer. See that the writing-style writes smoothly on the paper, and that it is kept in contact with the latter by a silk thread with a split lead shot attached to its lower end.

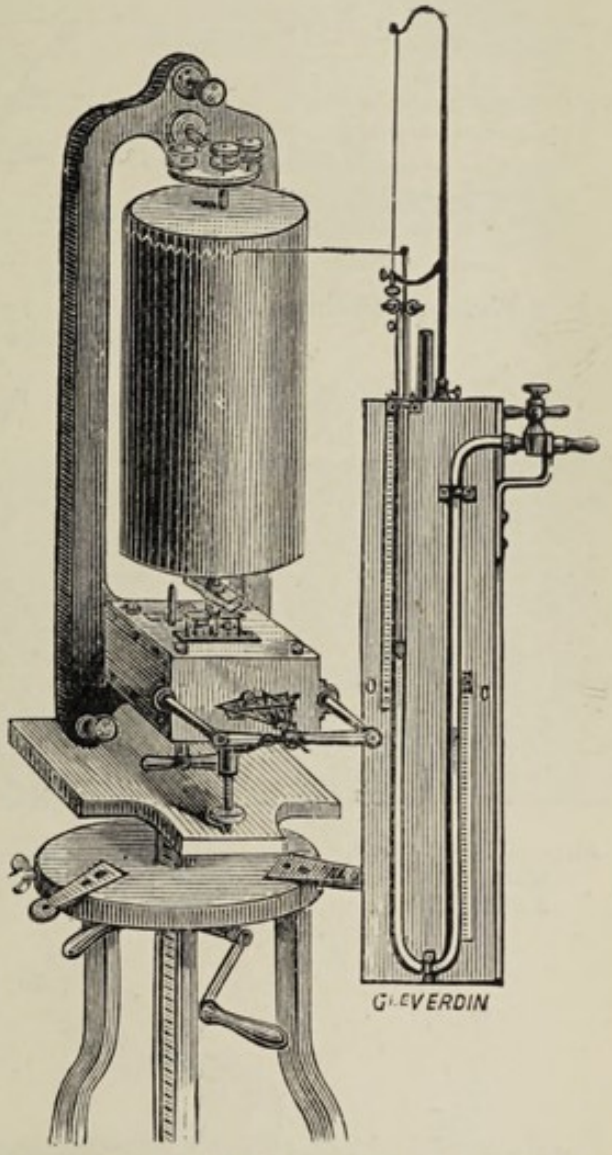


Fig. 339.—Modified kymograph. On the right the manometer, the float recording the movements of the mercury on a revolving cylinder.

B. Anæsthetise Rabbit. — Rabbit-Holder.

Anæsthetise a rabbit and fix it on a Czermak's or other rabbit-holder (fig. 340). The animal must be completely anæsthetised. Rabbits do not stand chloroform well. Use 0·6 to 0·7 grm. of chloral per kilo. of body weight dissolved in water and administered by a stomach-tube or by giving it per rectum. Urethane, 1 grm. per kilo., may be given instead of chloral, or, by means of a sound introduce into the stomach, 8 to 10 c.c. of alcohol diluted with twice its volume of water. This suffices to narcotise a rabbit weighing 2 to 2·5 kilos. (*Fredericq*).

C. As to the rabbit-holder to be used.

Czermak's holder is very convenient. Fig. 340 shows an improved form made by the Cambridge Scientific Instrument Company. It consists of a table with holes at the margins, to which the limbs of the animal can be tied by means of tapes. A special vice fixed in an adjustable clamp fixes the head. Malassez's form is both simple and convenient (fig. 342), and so is that of Cowl (fig. 341).

D. Expose nerves and insert cannula in carotid.—(*a*) *Arrange the necessary instruments* in order on a tray—scissors, scalpels, forceps (coarse and

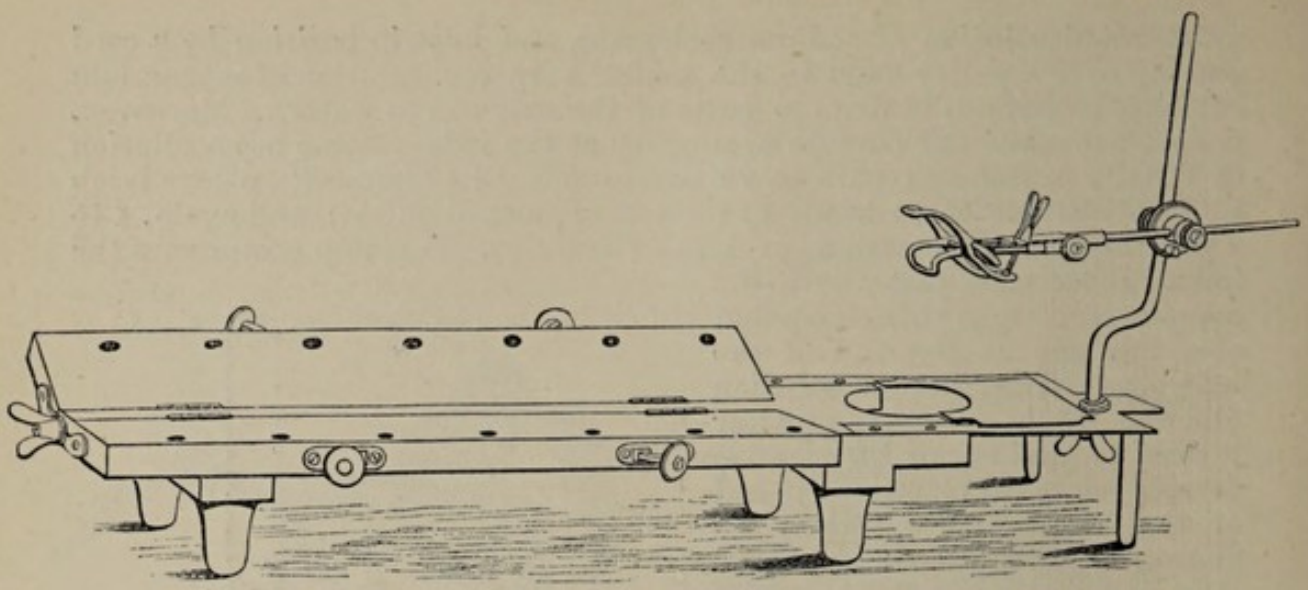


Fig. 340.—Czermak's rabbit-holder. Improved form by Cambridge Scientific Instrument Company.

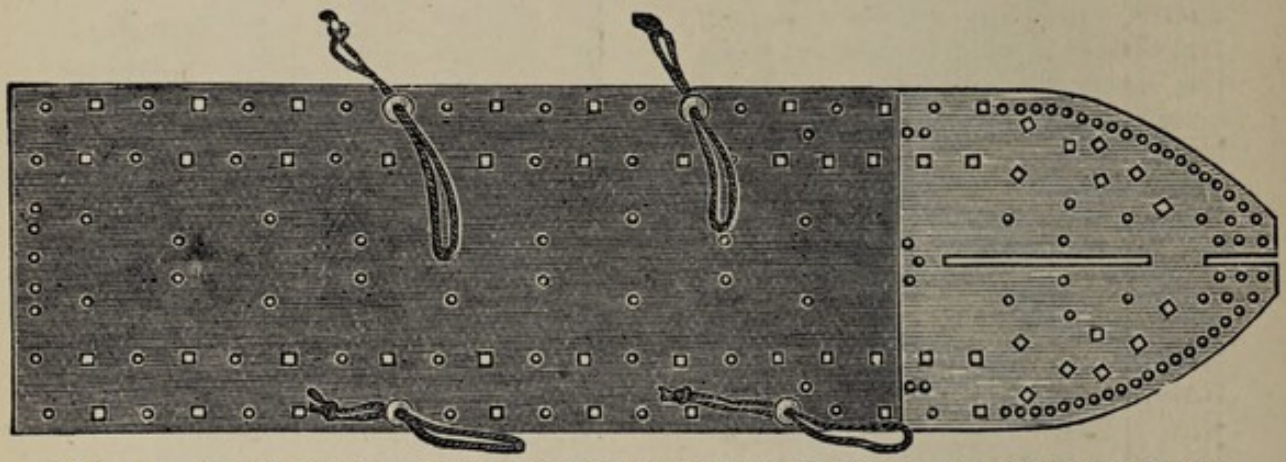


Fig. 341.—Cowl's animal holder, as made by Lautenschläger. It is supplied with numerous metallic pieces for fixing the animal in any position. It is suitable either for rabbit or dog, or smaller animal.

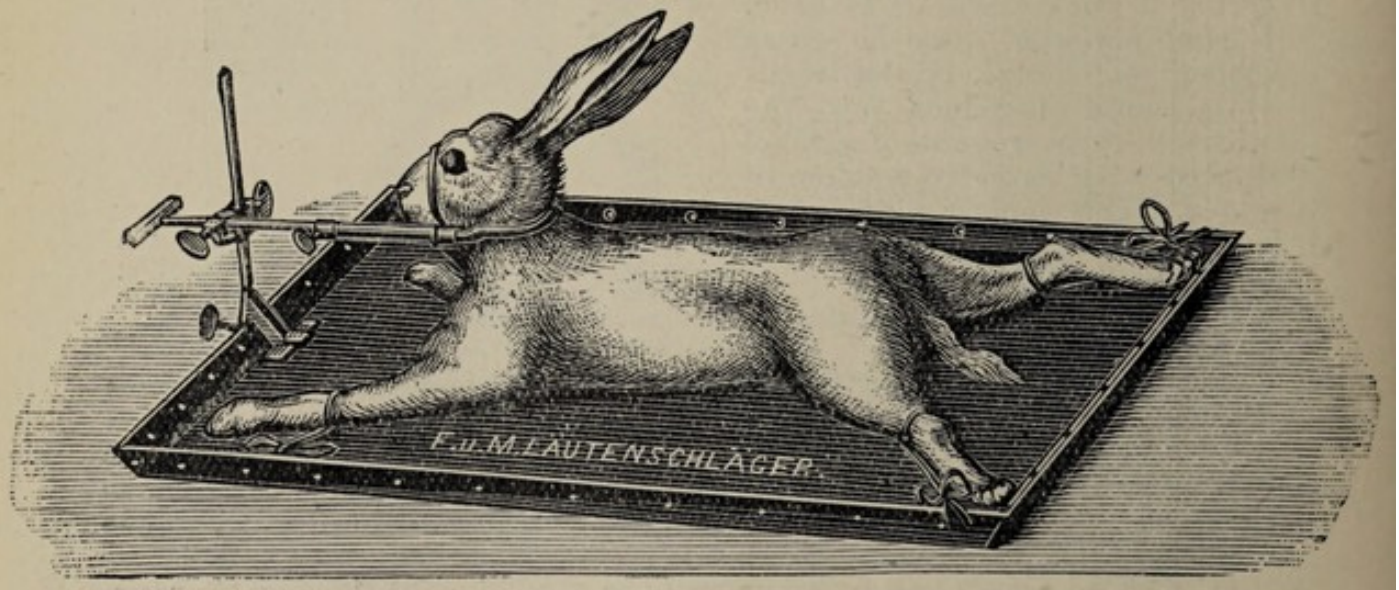


Fig. 342.—Malassez's rabbit-holder. The animal can be placed either on its back or its belly.

fine), seeker, well-waxed ligatures, small aneurism needle, bull-dog forceps, cannulæ, sponges.

(*b*) Clip away the hair over the neck, and with a wet sponge moisten the skin to prevent the hairs from flying about. Pinch up the skin on one side of the trachea, between the left thumb and forefinger, and divide it with a sharp scalpel. This exposes the fascia, which is then torn through with forceps; draw the sterno-mastoid aside, and gently separate the muscles with a "seeker" until the carotid, accompanied by the vagus, depressor, and sympathetic nerves, is seen. The dissection is made below the level of the larynx. Lying just external to the carotid is the vagus. After raising the carotid, under it, and internal to the vagus, are seen two fine nerves; the more internal or finer one is the depressor or superior cardiac branch of the vagus (fig. 338), the other is the sympathetic. Note that the smallest of the three nerves is the depressor, which is easily isolated from the sympathetic by means of a seeker. If in doubt, trace the sympathetic upwards until it merges into the large swelling of the superior cervical sympathetic ganglion. The depressor should be tied low down in the neck and divided below the ligature, as if for an experiment on its function. It is an afferent (centripetal) nerve, and therefore its central end must be stimulated.

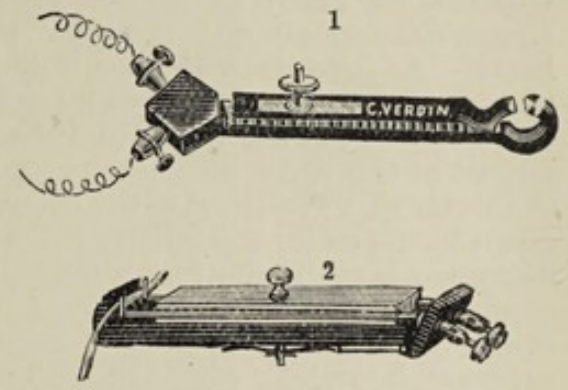


Fig. 343.—Shielded electrodes for stimulating the vagus or a deeply-seated nerve.

The vagus should also be isolated, ligatured, and divided between the ligature and the head. It is well to use shielded electrodes, such as are shown in fig. 343. The vagus is tied and divided, and if its peripheral end is to be stimulated, the peripheral end is drawn through the shielded electrodes, which are then connected with the secondary coil of an induction coil for interrupted shocks.

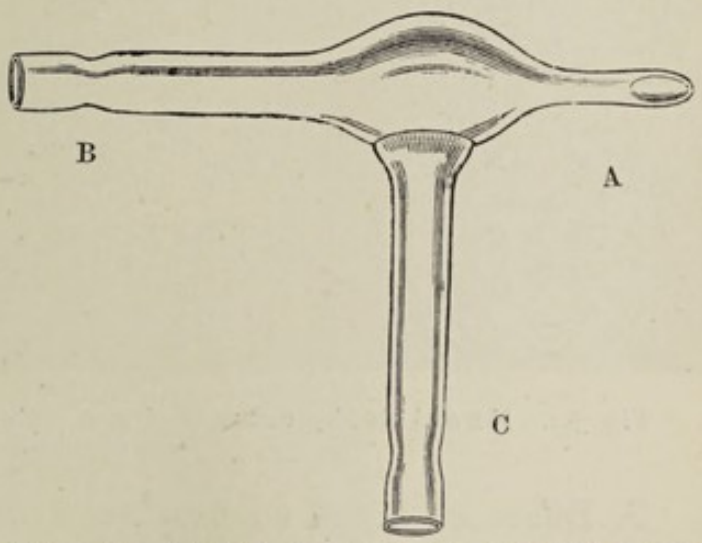


Fig. 344.—Arterial cannula of François-Frank. *A* is tied in the artery; *B* is attached to the lead tube of the manometer; and *C* is closed with an elastic (clamped) tube.

(*c*) Open the sheath, and with the seeker carefully isolate about an inch of the carotid. Pass a ligature under the artery by means of an aneurism needle, withdraw the needle, and ligature the artery. About an inch on the cardiac side of the ligature, clamp the artery with bull-dog forceps. Raising the artery slightly by the ligature, with a fine-pointed pair of scissors make an oblique V-shaped slit in the artery—the apex of the V towards the heart—and into the opening insert the shouldered nozzle of a

glass cannula with a short piece of rubber tubing tied on to it. Instead of a straight glass cannula with a shoulder, the form shown in fig. 344 may be used. It has a lateral tube, which is closed by means of a caoutchouc tube, and is useful in this respect, that the large bulb prevents clotting of the blood, while, if clotting does occur, the clot can readily be washed out by means of the pressure bottle through the lateral tube. Place another ligature round the artery, and tie it round the artery and over the shoulder of the cannula. The point of the cannula is, of course, directed towards the heart. Fill the cannula with the soda solution, and into the cannula slip the glass nozzle at the end of the lead pipe, tying it in securely. Unscrew the clamp at the end of the elastic tube. Set the drum going. Remove the clamp or forceps between the cannula in the artery and the heart. At once the swimmer would begin to move and record its oscillations on the paper moving in front of it (fig. 339).

(*d*) Before joining the lead tube to the cannula, isolate the **vagus**, the largest of the three nerves; put a ligature round it, and divide it above the ligature. Isolate also the **depressor** nerve, put a ligature round it low down in the neck, and divide it between the ligature and the heart. The depressor is easily distinguished from the sympathetic, as it is the smallest of the three nerves accompanying the carotid. Trace the depressor to its origin by two branches, one from the vagus, and the other from the superior laryngeal (fig. 338). Trace the sympathetic upwards till the superior cervical ganglion is reached.

(*e*) In every case a base line, abscissa, or line of no pressure (*i.e.*, atmospheric pressure), must be recorded to indicate when the mercury is at the same height in the two limbs of the manometer.

(*f*) Record a normal B.P. tracing (fig. 345).

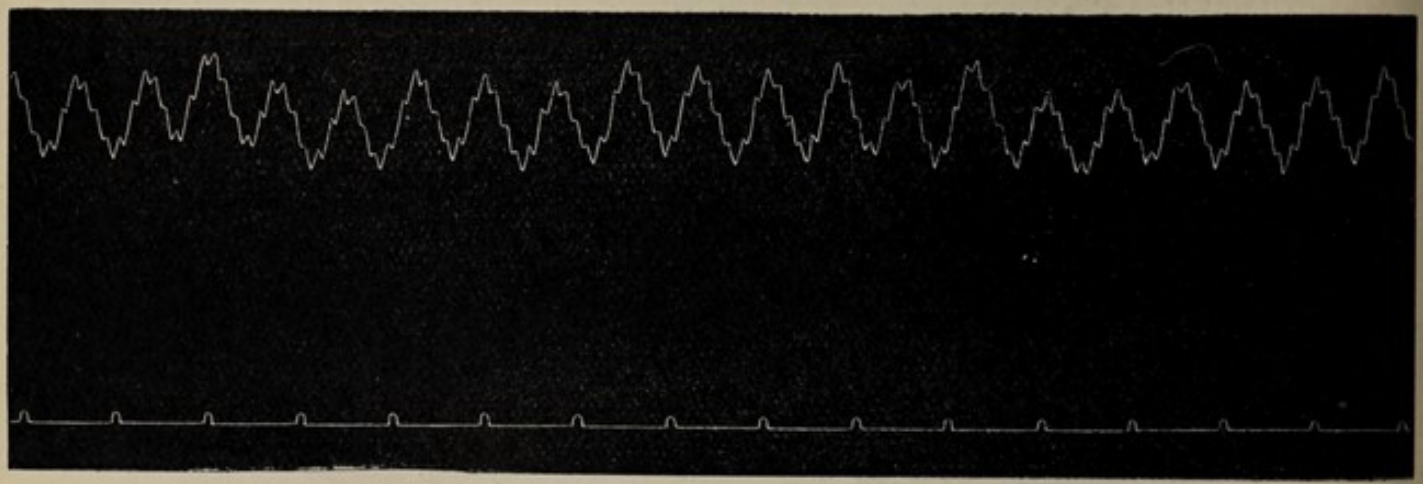


Fig. 345.—Normal blood-pressure tracing of rabbit. Lower line indicates intervals of 2 seconds.

2. **Effect of vagus on heart and B.P.**—Having arranged previously a coil to give interrupted shocks, apply the electrodes to the peripheral end—*i.e.*, the end of the vagus nerve connected with the heart—and stimulate it, regulating the strength of the stimuli according to the result desired to be obtained. If a not too strong stimulation be used, the heart is slowed and the arterial B.P. falls, but, if strong stimuli are applied, the heart is

arrested in diastole and there is a *sudden* fall of the pressure due to cardiac inhibition. In spite of continued stimulation, the heart will beat. Study tracing.

3. **Stimulation of depressor nerve.**—This is an afferent nerve conveying impulses chiefly from the endocardium of the heart to

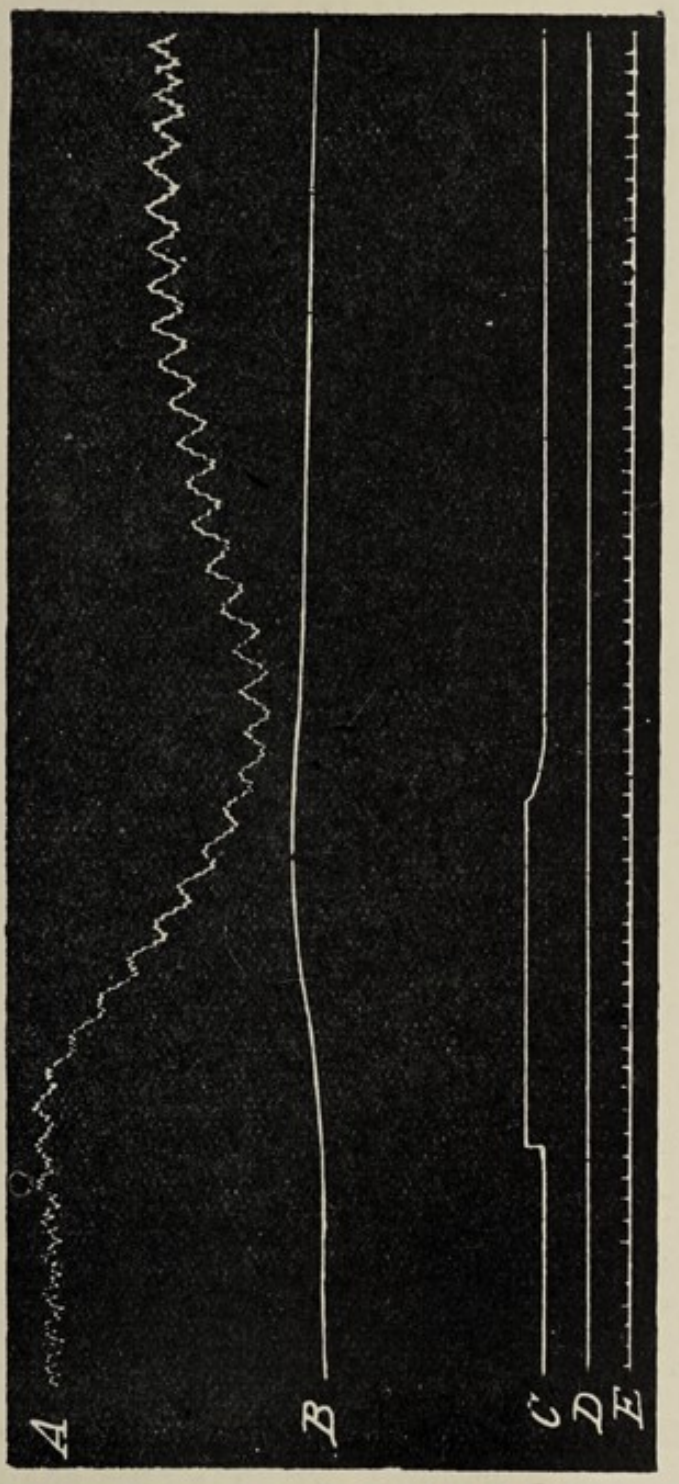


Fig. 346.—*A*, Blood-pressure tracing of carotid (rabbit); *B*, Volume of hind limb as recorded by plethysmograph; *C*, Stimulation of depressor at indent, and slow but steady fall of B.P.; *D*, Abscissa; *E*, Time in seconds (*Bayliss*).

the bulb, and as such is capable of influencing several so-called "centres" in that region. It can affect reflexly the cardio-

inhibitory heart centre, and *viâ* the vagus thus influence the heart. It also powerfully affects the vaso-motor centre. If, therefore, it be desired to obtain its pure "depressor" effect—*i.e.*, its power of lowering the arterial blood-pressure—then its reflex effect on the heart must be excluded. In the rabbit and some other animals the depressor nerve runs as a separate and distinct branch of, and alongside, the vagus. Divide both vagi in the middle of the neck; this excludes any cardiac effect.

(*a*) Expose the depressor nerve, put a ligature round it, as low down as possible, and divide it between the ligature and the heart. Lay it on electrodes and stimulate it with interrupted shocks. Take the usual precautions of recording time and moment of stimulation. Observe tracing.

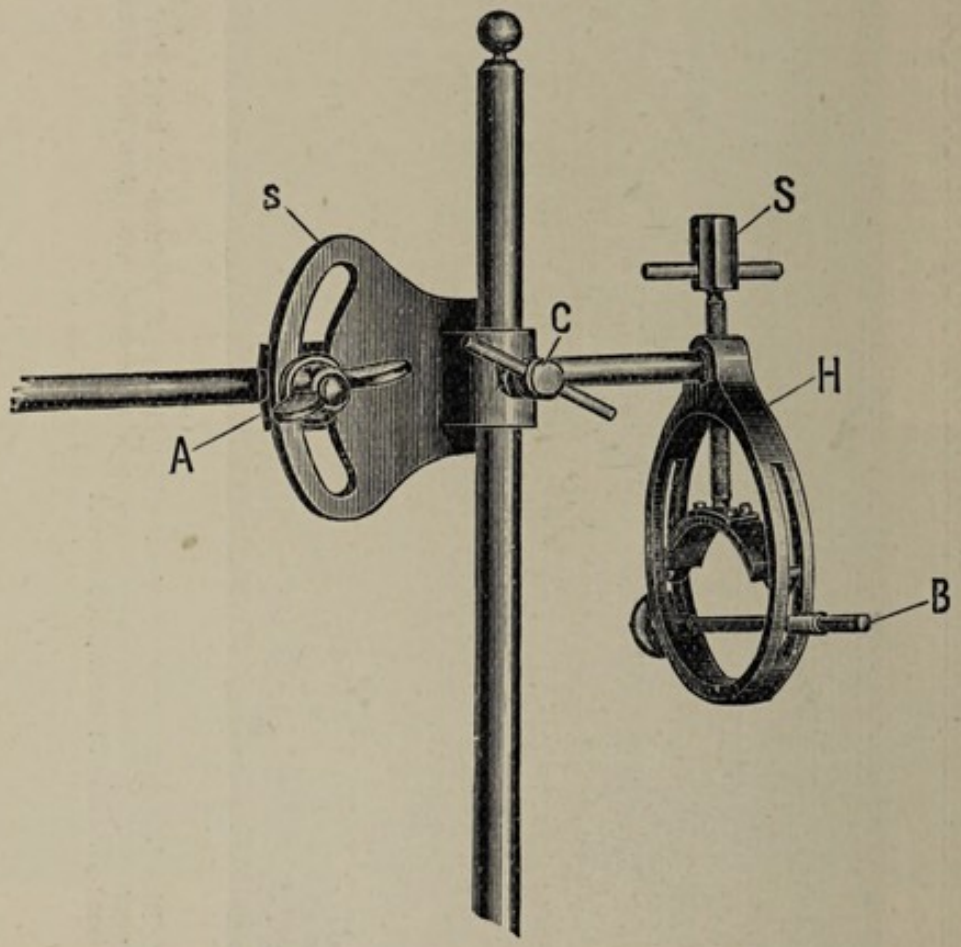


Fig. 347.—Apparatus for fixing mouth of dog. The bar, B, is placed in the mouth behind the incisor teeth, and the jaws are kept closed by S. There is an adjustment, C, for raising or lowering the head, and one for tilting it, A, S (*Lautenschläger*).

(i.) After a relatively long latent period of 2 to 3 seconds, the B.P. falls *gradually* and steadily without any change in the general character of the record either as regards the respiratory waves or individual heart-beats. This is its pure "depressor"

effect in lowering arterial blood-pressure. The fall may be 20 to 30 to 40 mm. Hg. Cease stimulation, the B.P. gradually becomes normal (fig. 346, A).

By its action on the general vasomotor centre in the bulb it causes dilation of the blood-vessels, especially in the abdominal area, and thus lowers the general arterial B.P.; hence the comparatively long latent period. As suggested by its discoverers, Ludwig and Cyon, it acts when the heart is over-distended, or when the aortic pressure is very high. It opens the flood-gates, especially of the splanchnic area (1866).

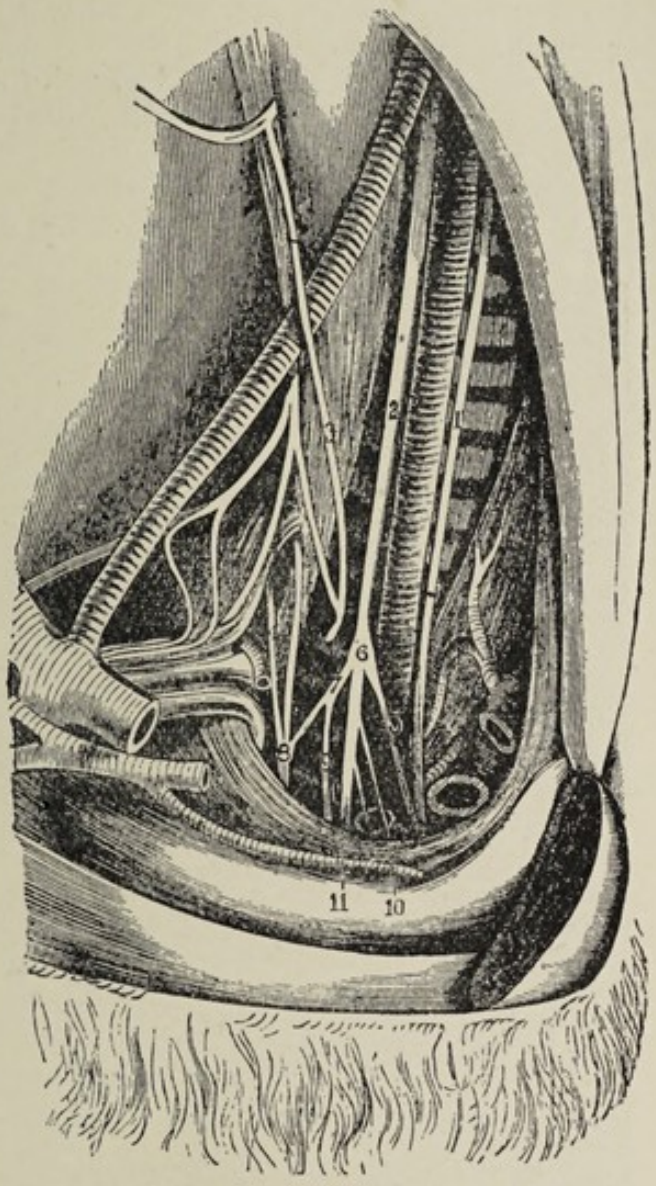


Fig. 348.—Dissection of supra-clavicular region in neck of dog, after O. Schmiedeberg's fig. in Ludwig's Arbeiten, vi., *Jahrgang*, 1871. 1, Recurrent laryngeal; 2, Vago-sympathetic; 3, Phrenic; 6, Inferior cervical ganglion; 7, Sympathetic trunk; 8, First thoracic ganglion; 9, Ramus cardiacus superior; 10 and 11, Trunk of vagus.

4. **Stimulation of afferent nerve — *e.g.*, sciatic.**—If a sensory nerve—*e.g.*, sciatic—be divided, and its central end be stimulated during a B.P. experiment — all else being intact—there is a rise of arterial B.P., which lasts for a certain time after cessation of stimulation. The increase may be 20 to 30 p.c. of original value of B.P. This is a *pressor* effect. But the respiratory movements are also affected, and express themselves in modifications of the respiratory waves of the B.P. tracing. Pure effects are obtained under curare with artificial respiration kept up.

II. Dog.—In the dog a combination of morphine and chloroform rapidly produces anæsthesia. Inject hypodermically about half an hour before the experiment 0·2 grm. per kilo. of morphine, and produce complete anæsthesia by the inhalation of chloroform. A different form of "holder" is required. A convenient form is that of Cl. Bernard (fig. 347).

The dissection of the parts of the neck involved in measuring the pressure in the carotid is shown in fig. 348. The vagus, as exposed in the dog, is really the vago-sympathetic nerve, as both vagus and sympathetic are enclosed in a common sheath. No separate depressor nerve exists as such in the neck.

It is well to record simultaneously the respiratory movements (Lesson LXVII.), and then the phases of the blood-pressure curve can be compared with the respiratory phases; and thus it can be determined how the B.P. is affected by the respiratory movements. Speaking broadly, in the dog the B.P. rises during inspiration and falls during expiration (fig. 364).

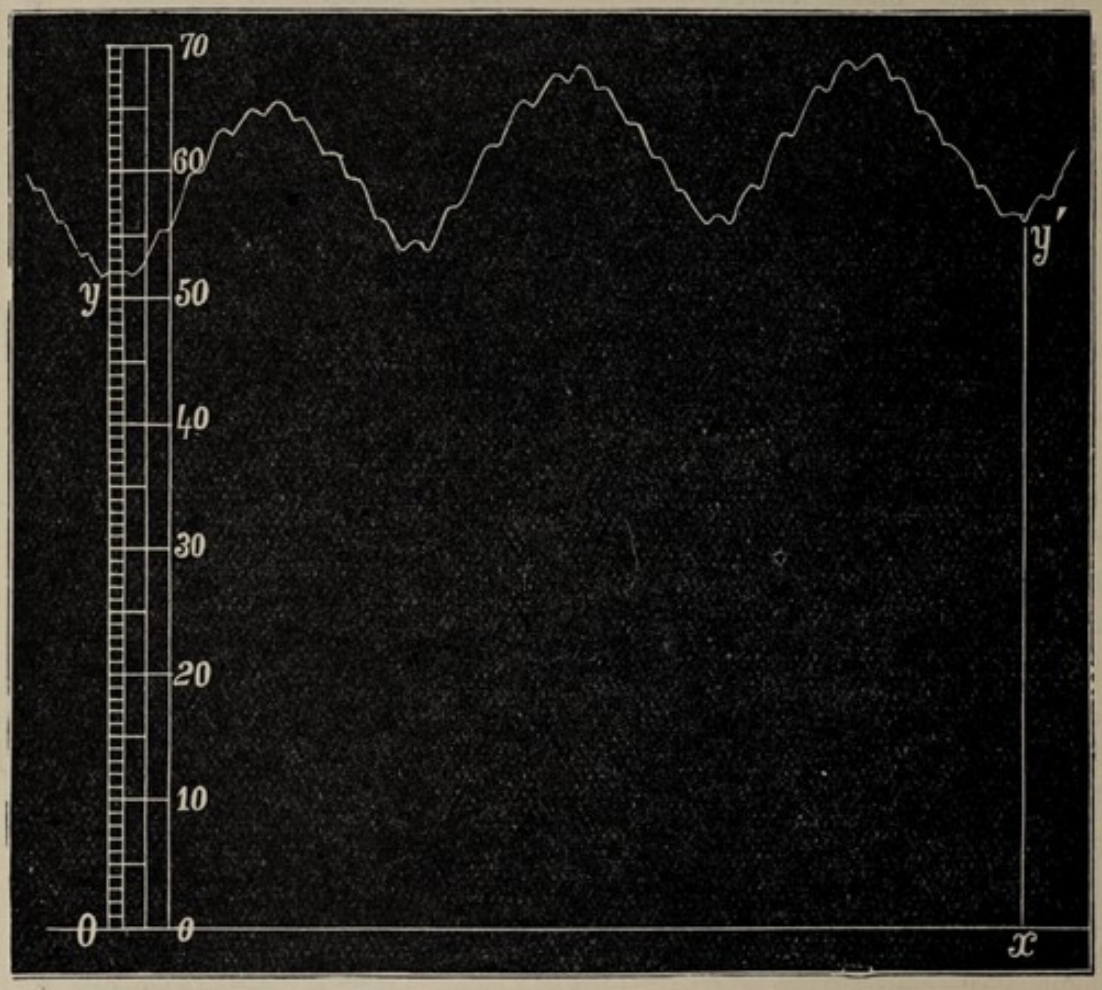


Fig. 349.—Blood-pressure tracing of the carotid of a dog, taken with Ludwig's mercurial manometer.

5. Measure blood-pressure tracing.—Lay the tracing on a table. Place a millimetre scale as in fig. 349. Read off the height in millimetres from the base line to the lowest point in the curve, and also to its highest point; take the mean of the two, and *multiply by two;* this will give the *mean arterial*

pressure. Instead of measuring only two ordinates, measure several, and take the mean of the number of measurements.

6. Study tracings.—(*a*) Notice the smaller undulations due to the beats of the heart, and the larger ones due to the respiratory movements (fig. 350). In a B.P. tracing taken from a dog with the vagi not divided, observe that the size of the heart-beats on the descent of the respiratory wave is greater, while the number of beats is less than on the ascent.

(*b*) Study blood-pressure tracings obtained during

(i.) Stimulation of peripheral end of vagus (figs. 350, 351).

(ii.) Stimulation of central end of depressor (fig. 346, *A*).

(iii.) Stimulation of central end of a sensory nerve (fig. 354).

(iv.) Stimulation of central end of one vagus (fig. 354).

(v.) Section of both vagi in neck (fig. 352).

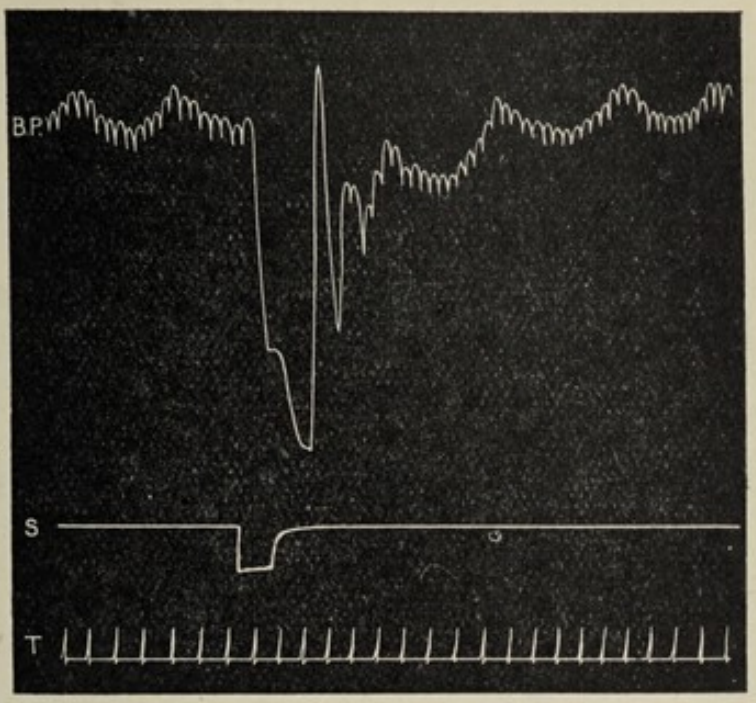


Fig. 350.—B.P., Blood-pressure tracing of dog's carotid —stimulation of vagus at the indent in S; T indicates time in seconds and abscissa (reduced).

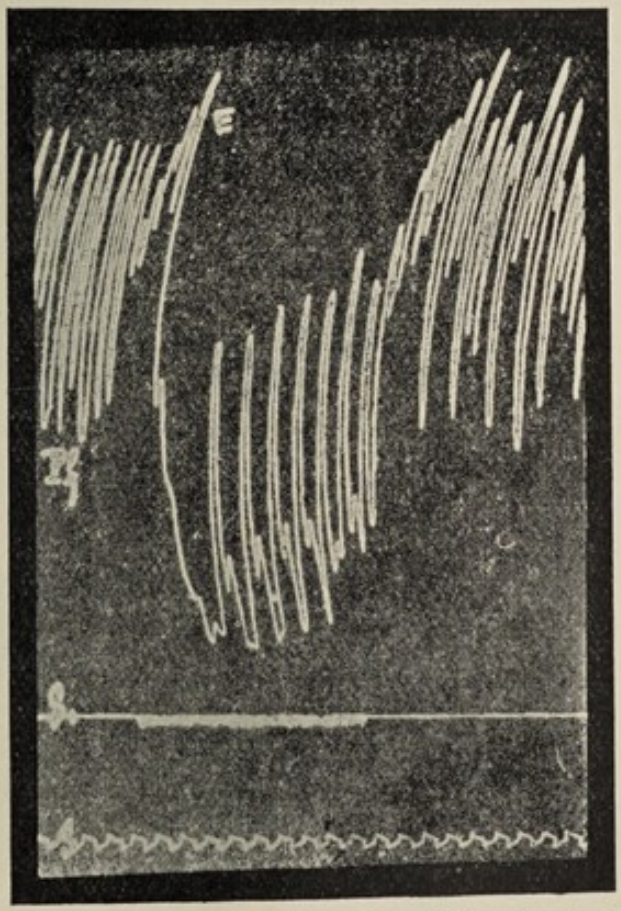


Fig. 351.—E, Stimulation of peripheral end of left vagus (dog). Heart-beats slowed. Fall of art. B.P. (*Laulanie*).

7. Effect of vagus on heart in animal just killed.—It can be shown on a rabbit or cat just killed. Expose the vagus rapidly, open the chest and observe the heart beating, or thrust a needle attached to a straw through the unopened chest into the heart, and observe the movements of the straw with each beat of the heart. Stimulate the peripheral end of the vagus with an interrupted current. The heart ceases to beat for a short time—the heart itself being in diastole. After cessation of stimulation, observe the slow but large excursions of the straw as the heart begins to beat.

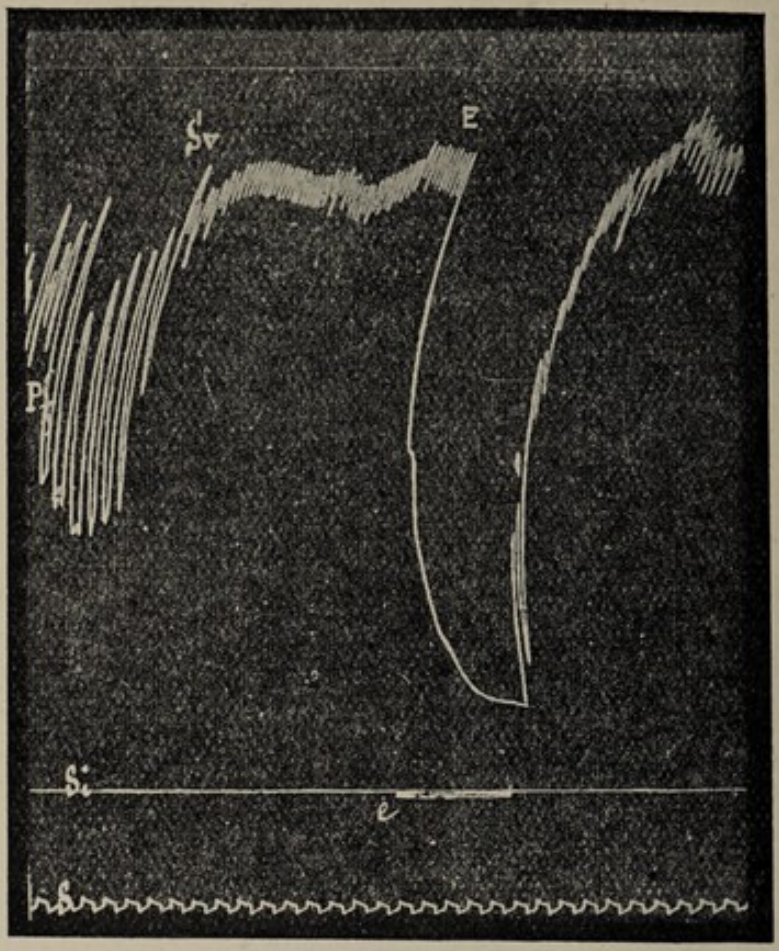


Fig. 352.—Art. (femoral) B.P. in dog increased by section of both vagi at S.V.—*i.e.*, by acceleration of cardiac rhythm. Decreased by stimulation of peripheral end of vagus (*Laulanie*).

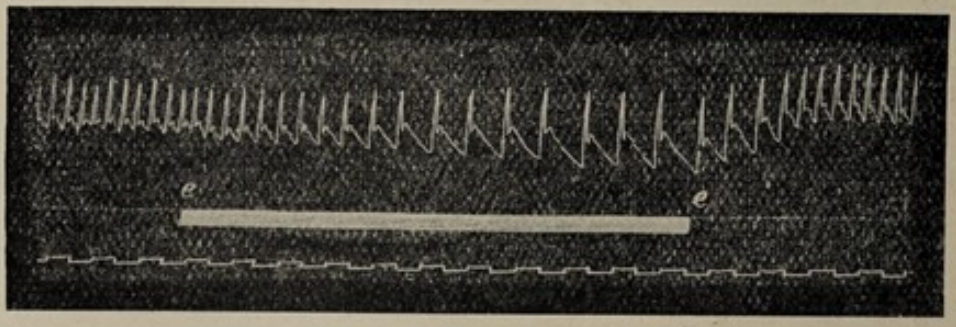


Fig. 353.—Increase in amplitude, as well as slowing, of pulse in femoral (dog) by stimulation of peripheral end of vagus in neck (*Laulanie*).

8. Effect of swallowing on the heart-beat (p. 416).

The mercury in Ludwig's kymograph, owing to its inertia, does not respond to rapid variations of pressure. Hence various forms of elastic manometers have been introduced, such as the "hollow-spring manometer," which, however, has been replaced by a flat spring.

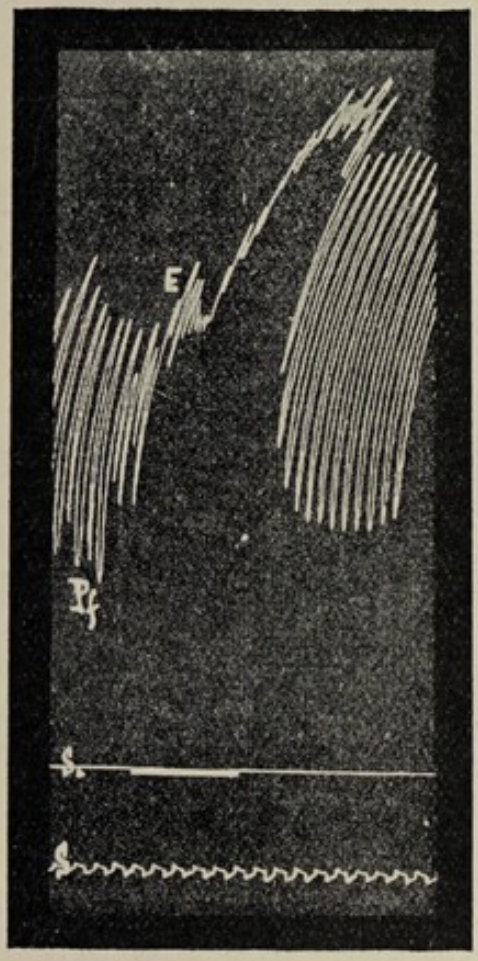


Fig. 354.—Art. B.P. (femoral) in dog raised by stimulation of central end of one vagus at E. In all cases Si is signal, and S, Seconds (*Laulanie*).

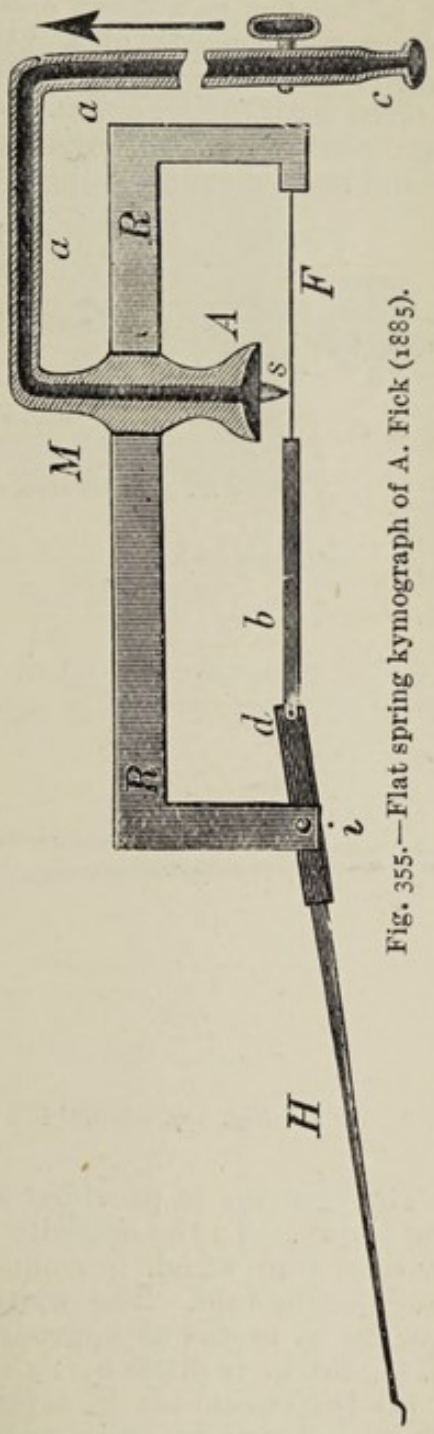


Fig. 355.—Flat spring kymograph of A. Fick (1885).

9. Flat spring kymograph of Fick (fig. 355), which consists of a metallic piece, A, perforated by a narrow canal, and terminating below in a very shallow saucer-like depression. This is covered with a rubber membrane, to which is attached a button, S, which presses against a steel spring, F, which moves the recording lever. There is attached to the framework, R, a second recording lever, which indicates when the pressure in the manometer is equal to the atmospheric pressure, and therefore records the abscissa. Originally Fick used air-transmission, but it is better to use a

fluid, one of those mentioned on p. 453—*e.g.*, magnesium sulphate. A cannula is inserted in the artery as for a kymograph experiment, the whole system is filled with the fluid to prevent coagulation, and then coupled up with the arterial cannula.

Hürthle's membrane manometer (fig. 357).—The mass of all the moving parts—the membrane and the fluid—is reduced to a minimum. The instrument consists of a very small tambour—something like that of Marey, but only 15 mm. in diameter, and of very small capacity. It is covered with thick rubber. A thin metal disc is fixed to the centre of the membrane, and is connected to the recording lever in the usual way. A second motionless recording style is attached to indicate the zero pressure.

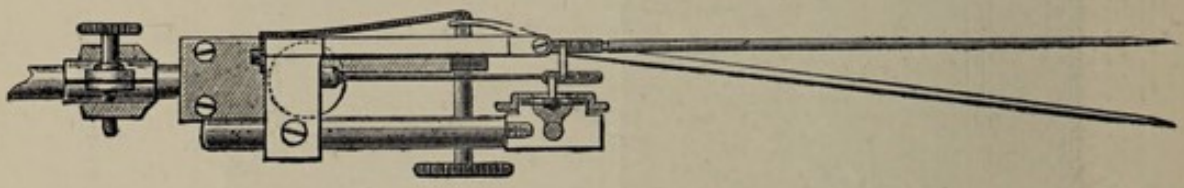


Fig. 356.—Hürthle's spring manometer, from the side.

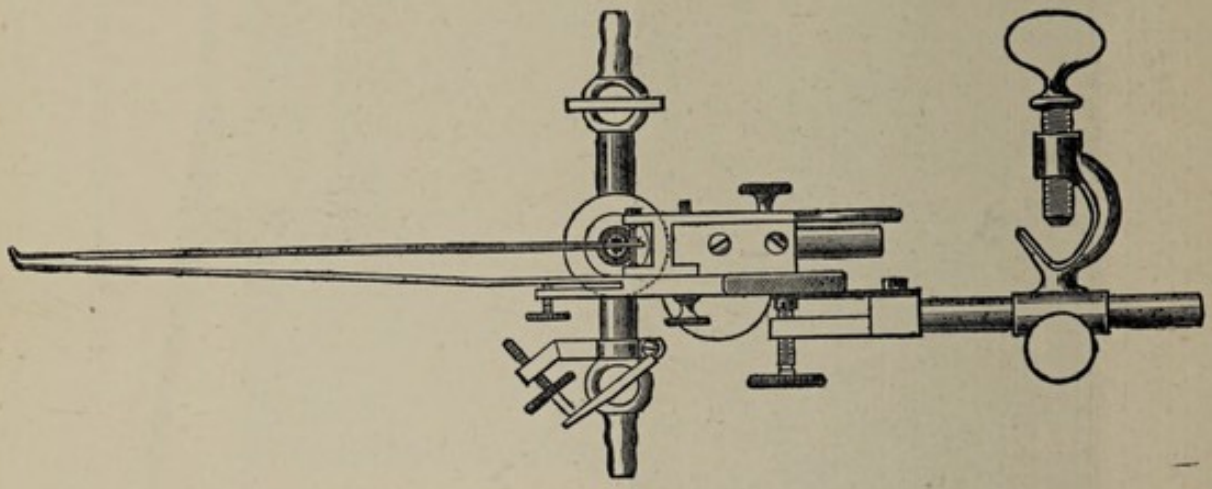


Fig. 357.—Hürthle's membrane manometer seen from above.

The tambour is provided with an inlet (with a tap) communicating with the artery. To the opposite end of the tambour is attached another tube (with a tap) which is connected to a pressure-bottle filled with the anti-coagulating fluid. The writing points can be adjusted to the blackened surface by means of appropriate screws. A modified form suggested by T. G. Brodie is made by Palmer.

(*a*) Insert cannula in artery. Fill the instrument with magnesium sulphate solution or half-saturated sodium sulphate from a pressure-bottle and connect by a short tube with the cannula. Close clip near cannula. Raise the pressure-bottle whereby the membrane and lever are raised. Bring the recording lever horizontal and clamp it in that position. Open the clip on the cannula, the lever falls and stands at the level of the zero pressure. Bring the control motionless lever to the same level. Close the cannula, raise the pressure-bottle to increase the pressure in the mano-

meter, close tap communicating with pressure-bottle. Finally, remove the clamp or bull-dog forceps on the artery, and the lever records its movements (fig. 358).

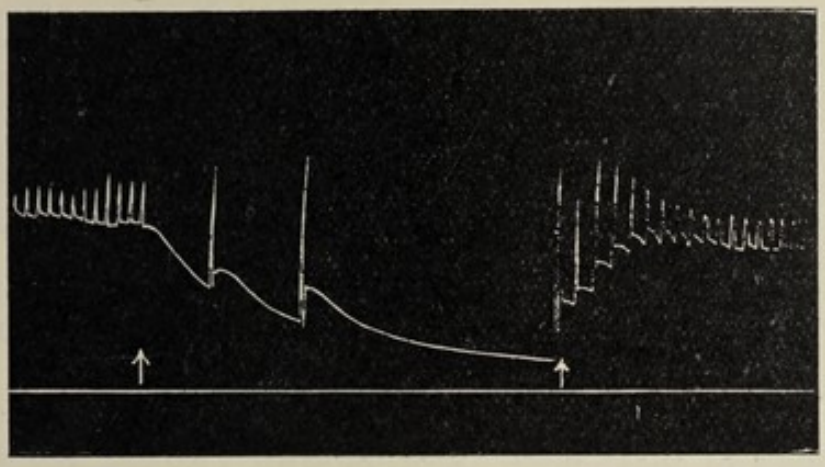


Fig. 358.—Arterial blood-pressure recorded with spring or membrane kymograph. Effect of stimulation of peripheral end of vagus (*Stirling*).

Of course the pressure cannot be read off directly. The instrument must be previously calibrated to determine the value of the pressure in terms of Hg.

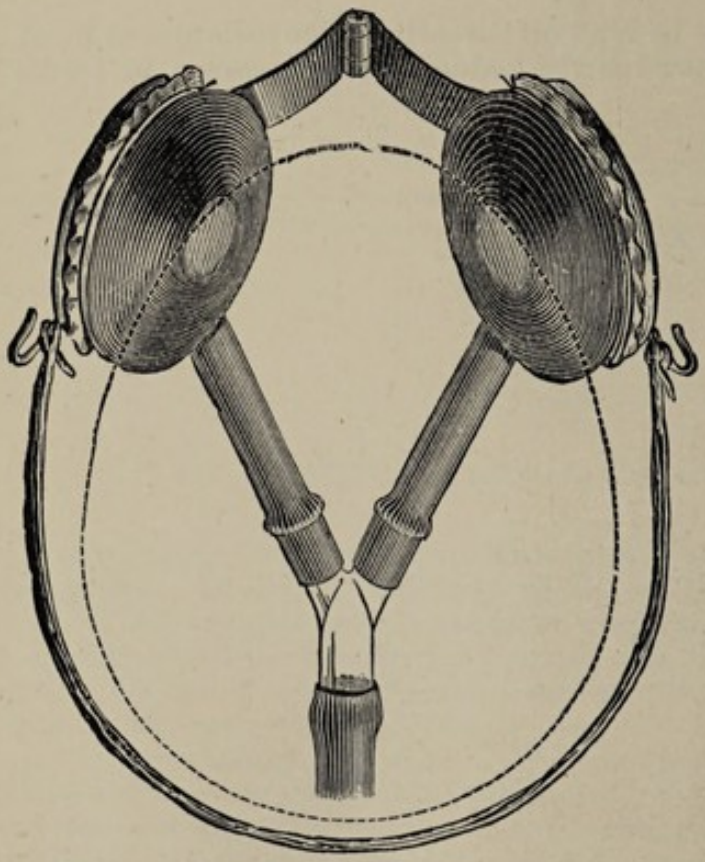
RESPIRATION.

LESSON LXVII.

MOVEMENTS OF CHEST WALL—ELASTICITY OF LUNGS—HYDROSTATIC TEST—STETHOGRAPH—APNŒA.

1. Movements of chest wall—Stethograph.

A. Rabbit.—(*a*) Arrange a drum and time-marker. Fix a rabbit on Czermak's rabbit-holder, or use the simpler form of Malassez or Steinach, and with tapes tie on its chest Marey's double tambour (fig. 359), connecting the latter with a recording tambour adjusted to write on the drum. Insert between the receiving and recording tambours either the valve usually supplied with Marey's apparatus or a T-tube with a screw clamp, whereby the pressure within the system of tubes can be regulated. Take a tracing. If one of the receiving tambours be placed over the cardiac impulse, the tracing will show also the heart-beats (fig. 360).



ig. 359.—Marey's double tambour, to be tied round the chest of a rabbit.

B. Man.—(*b*) Stethograph (*Marey's*).—Raise the screw, *g*, of the stethograph, and fix the plate, *f*, of the instrument on the

exposed chest, with tapes attached to *c* and *d*. Depress *g*, connect the tube, *a*, with a recording tambour, with the same precautions as in 1, A, and take a tracing (fig. 362). Examine the tracing, noting the relation between inspiration and expiration.

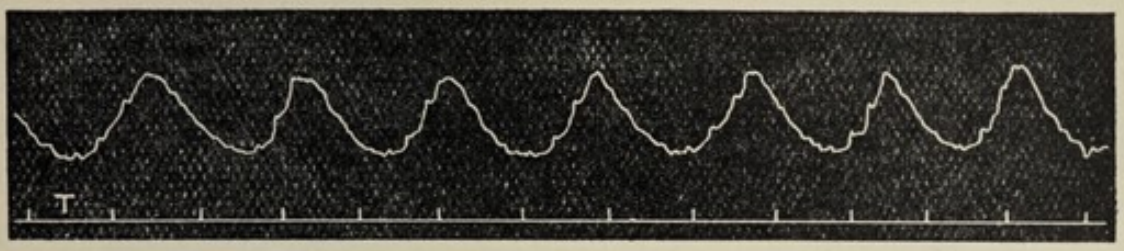


Fig. 360.—Stethograph tracing of a rabbit. The tracing shows undulations due to the beats of the heart. *T* indicates time in seconds.

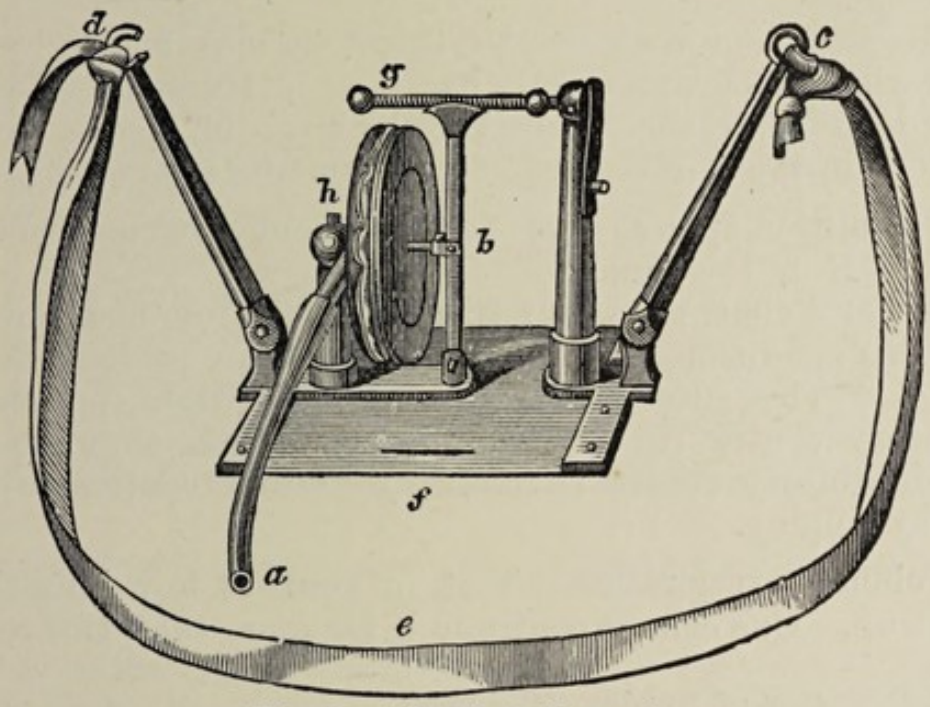


Fig. 361.—Marey's stethograph.

(*c*) **Polygraph** (*Rothe*).—Use this polygraph, record the respiratory movements by means of the bag (fig. 298, A), and study the tracing (fig. 362).

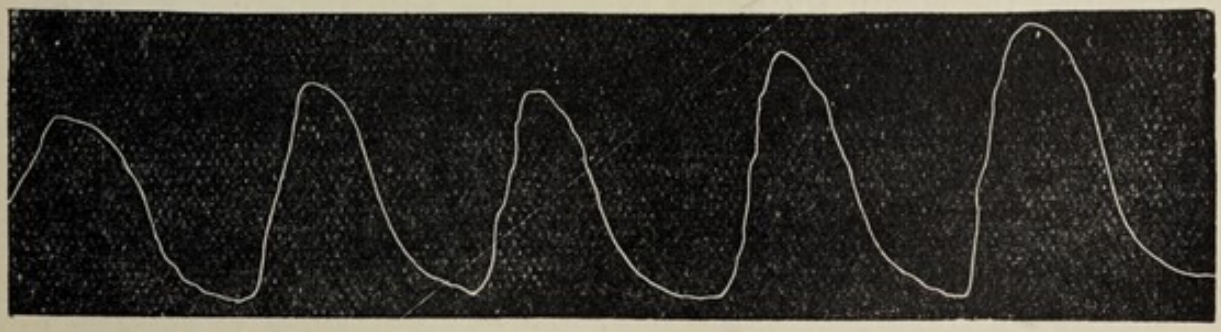


Fig. 362.—Stethograph tracing, taken with Rothe's polygraph.

2. Elasticity of lungs.—Remove the front of the chest in the rabbit already used. Observe the collapsed lungs. To the tracheal cannula attach the bellows of a spray-producer, and inflate the lungs. Cease pumping, and observe how the lungs collapse.

3. Hydrostatic test.—Cut out lungs and heart, and place them in water. They float, as the lungs contain so much air. Cut off a piece of lung, place it in water, it floats. This is the **hydrostatic test.** Compare a piece of pneumonic lung; it sinks.

4. Apnœa.—(*a*) Count the number of your own respirations per minute. Take a series of rapid respirations. Several seconds elapse before the next inspiration. This is the period of apnœa.

(*b*) Record your own chest respiratory movements by means of Marey's stethograph or other form of instrument. Take four or five deep inspirations; observe the stage of apnœa and compensatory increase of respiratory movements afterwards.

5. Deglutition apnœa.—(*a*) Test how long you can "hold your breath." Note the time.

(*b*) After a time, sip water without breathing, and note that, under this condition, the time the breath can be held is nearly doubled. The successive acts of deglutition influence the respiratory centre in the medulla oblongata, as well as the cardio-inhibitory centre (*Kronecker*). Other centres are influenced by sipping.

6. Voluntary respiration.—Test in yourself how long this can be kept up. One cannot continue it for more than two minutes.

7. Stethometer of Burdon-Sanderson.

(*a*) Prepare a drum and time-marker. Cause a person to expose his chest, and seat himself conveniently. The instrument is suspended by a broad band placed round the neck, the horizontal bar being behind the body.

(*b*) The most important diameters of the chest to measure are—"Those connecting the eighth rib in the axillary line with the same rib on the opposite side, the manubrium sterni with the third dorsal spine, the lower end of the sternum with the eighth dorsal spine, and the ensiform cartilage with the tenth dorsal spine." Measure only the first. Adjust the knob of the tambour on one side against the eighth rib, as above, while the movable bar with its knob is placed against the opposite corresponding rib. Connect the tambour with the recording tambour, introducing a T-piece, the stem of which is provided with an india-rubber bag and screw clamp to regulate the pressure within the air system.

8. Any particular part of the chest wall may be studied by applying a tambour directly over that part.

9. **Respiratory variations of pressure (rabbit).**—In the rabbit, which breathes almost entirely by its diaphragm, these methods are not sufficient. The respiratory variations of pressure can readily be recorded in an anæsthetised rabbit by tying in the exposed trachea one end of a T-shaped cannula; the other horizontal end, provided with rubber tubing, can be closed or opened at will. The vertical stem is connected to a recording tambour, but between the two is a 5-litre flask. This contains a great mass of air, and allows the animal to respire freely (fig. 363).

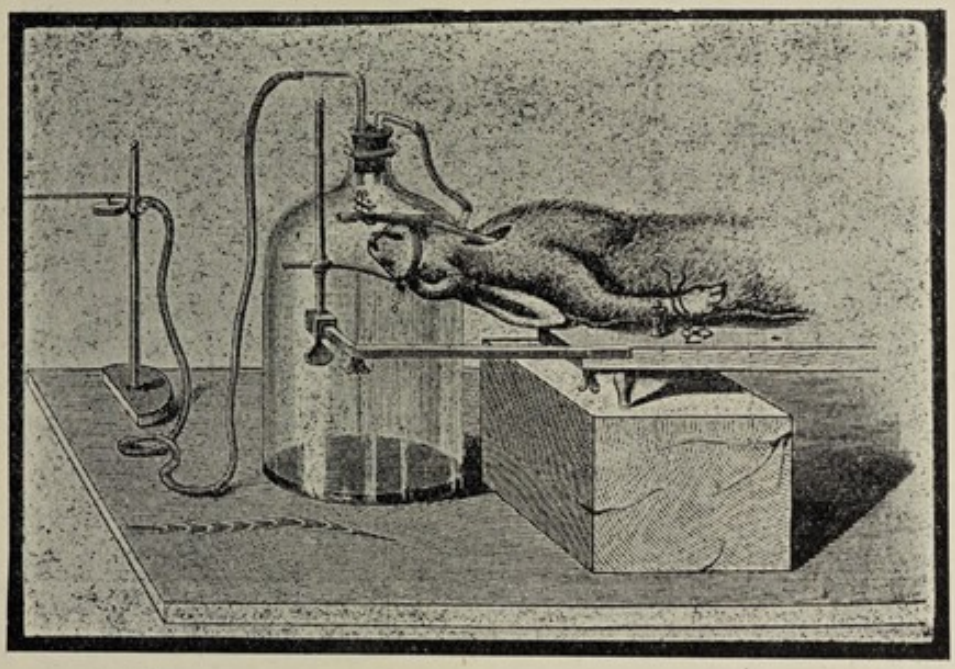


Fig. 363.—Mode of recording respiratory pressure, large bottle intervening between trachea and recording tambour.

(*a*) Expose the vagi in the neck. Raise one—left—on a thread and divide it about the middle of the neck. Probably there will be little alteration either in depth or rate of the respiratory movements. Divide the other vagus. At once the number of respirations diminishes, and each respiration is slower and deeper.

(*b*) Arrange a coil and electrodes. Stimulate the central end of one vagus, and observe the reflex arrest of the respiratory movements. Note phase of arrest.

(*c*) The respiratory movements in dyspnœa approaching asphyxia and their cessation in apnœa can all be readily studied by this method.

Many other methods are used—including Head's method of recording movements of a muscular slip of the diaphragm without opening the thorax—but they are omitted here.

10. **Relation of respiratory movements to B.P.**—This can readily be done in dog (fig. 364) or rabbit by recording simultaneously the B.P. and the respiratory movements. In the dog, on the whole the arterial B.P. rises during inspiration, and falls during expiration, although the two events are not exactly synchronous. This is not the case in all animals.

11. Reflex arrest of respiratory movements in rabbit.—Observe play of nostrils in rabbit. They dilate with inspiration and approximate during expiration. Bring near the rabbit's nostrils a sponge with chloroform; at once these movements of the nostrils and those of the diaphragm cease.

12. Pressure in the pleural cavity.—Fix one end of a caoutchouc tube to a water-manometer (water coloured red), and the other end to a trocar and cannula. Thrust the trocar obliquely through an intercostal space until the point of the trocar lies in the space between the two layers of the pleura. Observe how the level of the water rises in the proximal limb of the manometer, indicating the negative pressure in the pleural cavity.

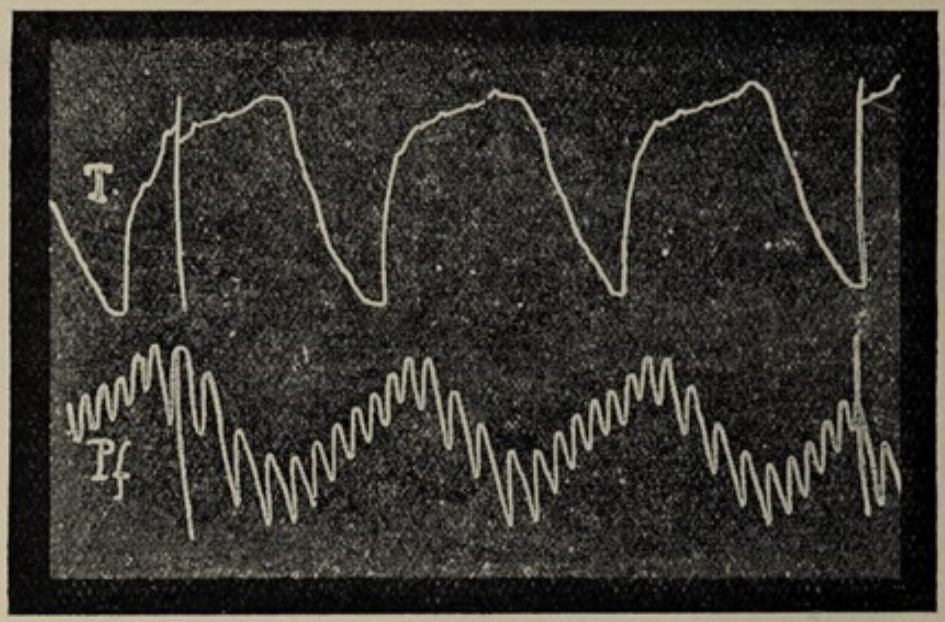


Fig. 364.—Variations of art. B.P. in dog due to respiratory movements, both vagi cut. *T*, Resp. movements (Insp. if descending); *Pf*, B.P. in femoral artery.

13. Intra-thoracic pressure.—This can be done on a dead rabbit.

(*a*) Fix the rabbit in Czermak's rabbit-holder. Expose the trachea, tie in it a knee-shaped glass cannula. Make a small water-manometer or bent U-tube with a millimetre scale attached, fill it about half full with coloured water, and to the proximal limb attach an india-rubber tube with a T-piece and screw clamp, as in other experiments. Connect the tracheal cannula with the manometer tube, tighten the screw clamp, and see that the water stands at the same level in both limbs of the manometer.

(*b*) Open both pleuræ without injuring the lungs. The lungs collapse, the water falls in the proximal side of the manometer, and rises in the open limb.

14. Respiratory movements of frog.—The air is forced into the lungs.

(*a*) Observe rhythmical movements of the muscles of the floor of the mouth and of the muscles attached to the hyoid bone, the cavity of the mouth is thus diminished. Coincident with these are—

(*b*) Movements resulting in closure of the external nares, and thus the air is forced into the lungs. At the same time the glottis is opened, but the mouth must be opened to see this.

(*c*) The act of expiration is performed by movements of the muscles of the flanks compressing the visceral contents.

15. Effect of respiratory movements (diaphragm) on entrance and exit of air and blood in thorax.—It is easy to arrange a simple model to show the action of inspiration represented by a descent of the diaphragm on (i.) entrance of air into lungs; (ii.) pressure on lungs; (iii.) blood-flow into and out of chest. Hering's apparatus (fig. 365), made by Rothe, shows these various points.

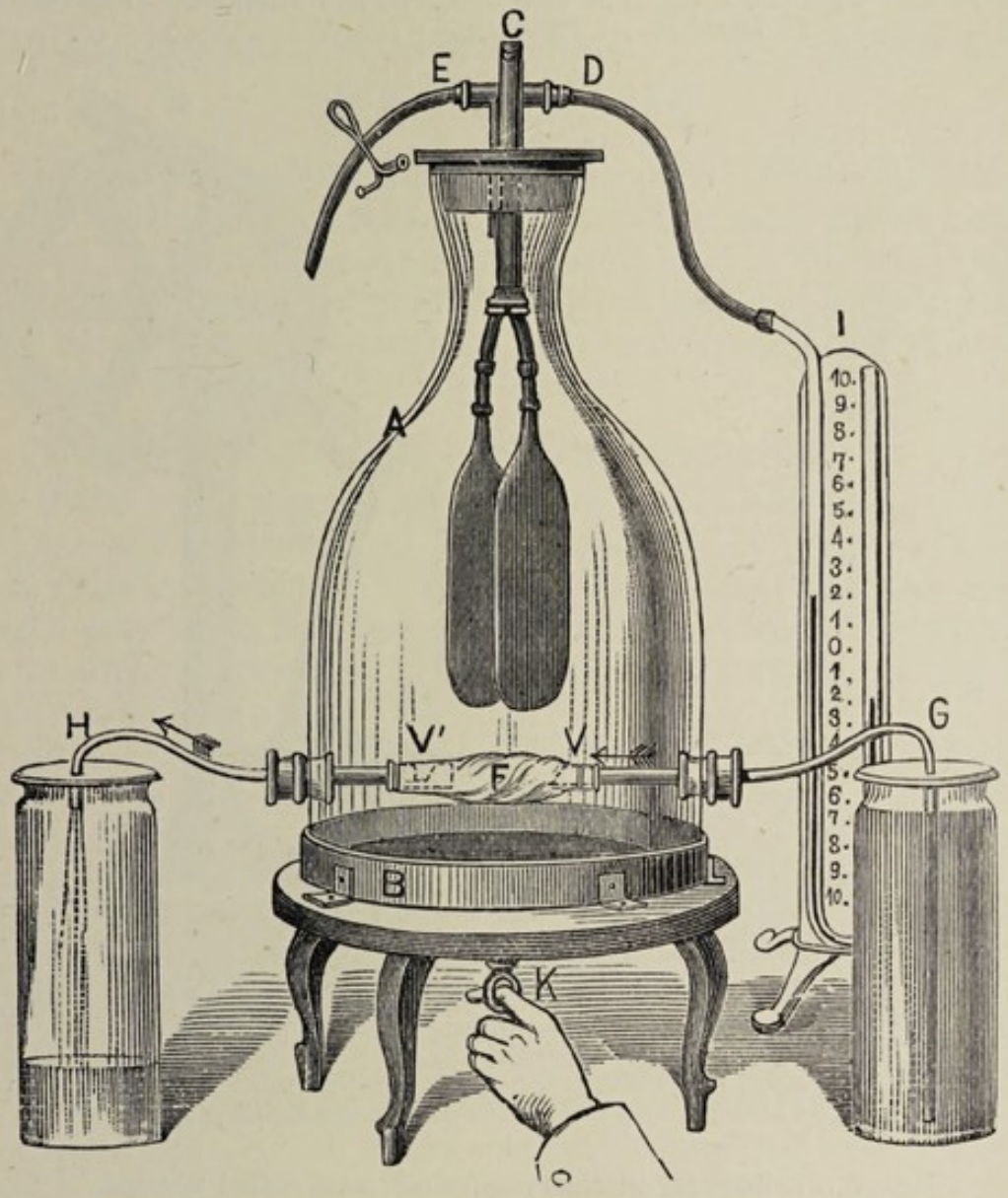


Fig. 365.—Hering's apparatus showing effects on entrance and exit of air and blood. *K* represents Diaphragm; *F*, Heart; *C*, Trachea; *D*, Manometer connected with air passages. Made by Rothe.

16. Artificial respiration in animals.—In some experiments—*e.g.*, where a curarised mammal is used—it is necessary to employ artificial respiration. This is done by fixing a metallic or glass respiration cannula in the trachea (fig. 367) and connecting this with a bellows (fig. 366). The rate and excursion of the latter can be regulated. In some cases the bellows is moved by hand, in others by means of an electric or other motor. If heated air be required, the tube leading to the trachea can easily be heated.

17. Permeability of lungs to gases.

(*a*) Expose the lungs of a recently pithed frog, ligature one bronchus, cut out the isolated lung and suspend it in an atmosphere of CO_2. It is rapidly distended by CO_2 (N. Zuntz, *Pflüg. Arch.*, xlii., 1888).

(*b*) Ligature the bronchus of a frog's lung, cut it out, attach a lead weight, and place the whole in soda-water in an open burette. The lung distends, and within a minute or two floats to the surface. Observe, wait —the lung sinks (L. Hill, *Proc. Phys. Soc.*, 1899).

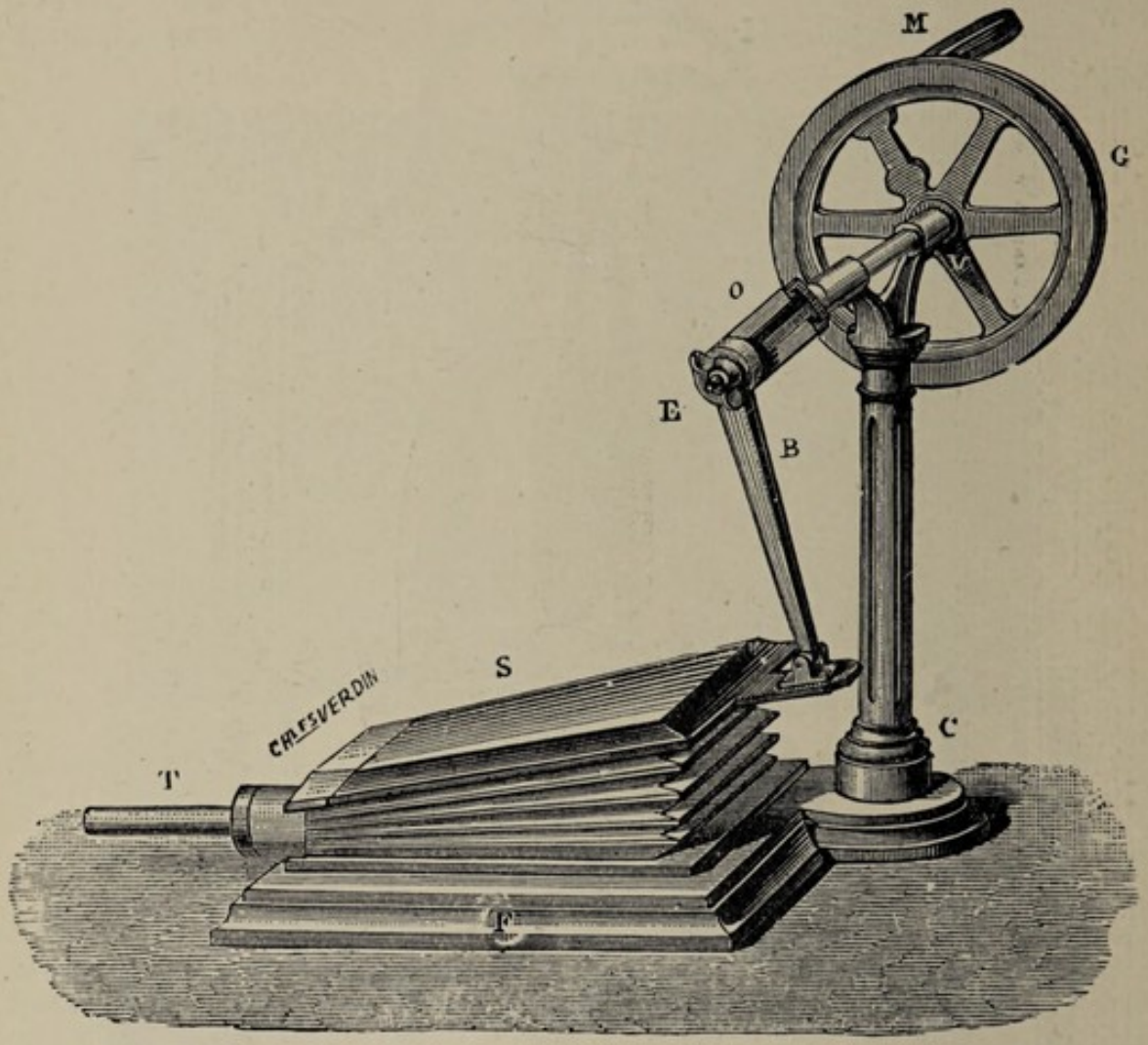


Fig. 366.—Bellows for artificial respiration, to be moved by hand.

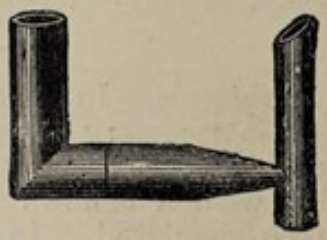


Fig. 367.—Respiration cannula for rabbit. The narrow tube is tied in the trachea, with open end towards the lungs.

LESSON LXVIII.

VITAL CAPACITY—EXPIRED AIR—GASES OF BLOOD AND AIR.

1. Vital capacity.—Use Hutchinson's spirometer—*i.e.*, take the deepest possible inspiration, and then make the deepest possible expiration, expiring into the mouthpiece of the spirometer. The average vital capacity is about 3700 c.c. (230 cubic inches), but it varies with age, height, sex, and practice in using the instrument, &c.

2. Changes in expired air—Black's experiment.

(*a*) Place equal quantities of lime-water in two vessels, A and B. Take a deep breath, close the nostrils, and expire through a bent glass tube into A. The lime-water soon becomes milky, owing to the large amount of CO_2 expired combining with the lime to form carbonate of lime. With the elastic pump of a spray-producer pump air through B. B remains clear. Therefore the CO_2 must have been added to the inspired air in the respiratory organs.

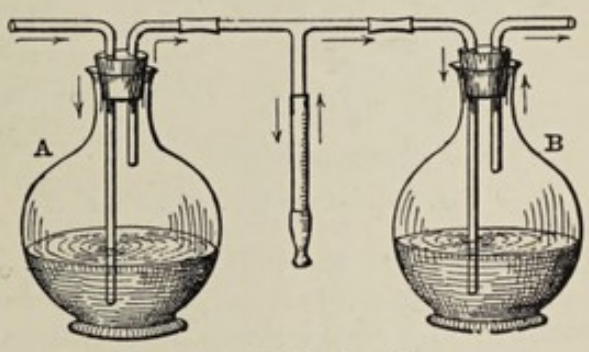


Fig. 368.—Müller's valves.

(*b*) **Müller's valves.**—Arrange two flasks, A and B, and tubes, as in fig. 368, with lime-water in both. Close the nostrils, apply the mouth to the tube, and inspire. The air passes in through A, and is freed of any CO_2 it may contain. Expire, and the air goes out through B, in which the lime-water becomes turbid.

(*c*) **Faraday's experiment.**—Place about two litres of water in a basin, and in it put erect a bell-jar. Ascertain that a lighted taper burns in the jar. Renew the air, place in the neck of the jar a glass tube with a piece of india-rubber tubing attached. Close the nostrils, apply the mouth to the tube, and inspire. The water rises in the bell-jar. Then expire, the water sinks, and the air which was originally present above the water has been taken into and expelled again from the respiratory passages. Remove the cork, and place a lighted taper in the expired air. The taper is extinguished (fig. 369).

3. Swallowing.—(*a*) Test on yourself how rapidly (few seconds) you can swallow a large glass of water. In swallowing

liquids, the liquid is projected through the pharynx and œsophagus right into the stomach, chiefly by the contraction of the mylohyoid muscles in the floor of the mouth (*Kronecker* and *Meltzer*).

(*b*) *Effect on pulse—i.e.*, heart (Lesson LXI., 3).

4. **Blood gases—Gas pumps.**—Blood yields about 60 volumes per cent. of gases to a vacuum. The gases in the blood—CO_2, O, and N—are extracted from it by means of a gas pump. Various forms have been constructed, including those of Ludwig, Pflüger, and Alvergniat. Study these and the principle of their construction. It requires a considerable amount of time to become thoroughly acquainted with the practical working of these instruments.

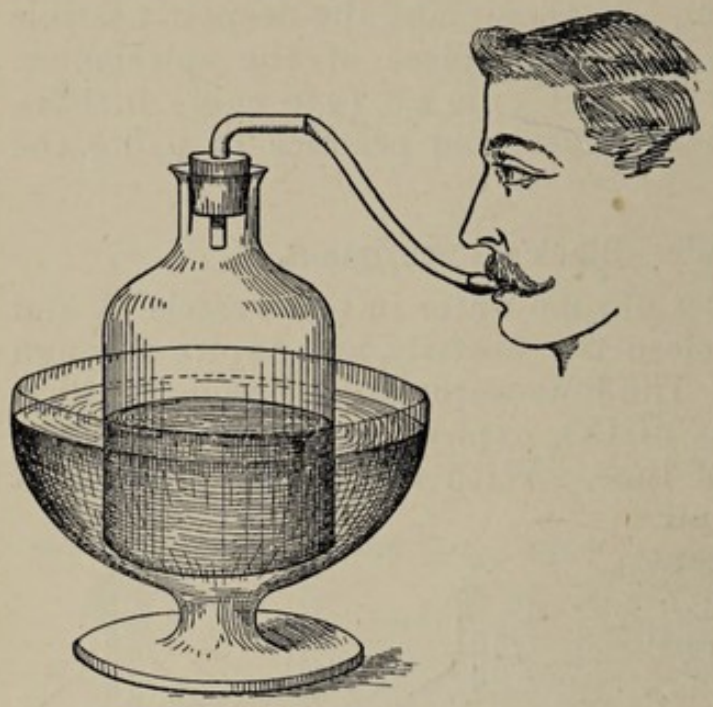


Fig. 369.—Faraday's experiment.

Extraction from blood.—The gases must first be extracted from the blood and collected over Hg. Then the composition of the mixture has to be ascertained.

L. Hill's gas pump (fig. 370) "consists of a mercury reservoir, A, about 300 c.c. in capacity, which is connected with a second reservoir, B, by means of 120 cm. of pressure tubing. The upper end of the reservoir B, is closed by a three-way tap, D. By means of this tap the reservoir, B, can be put in connection with either the tube, E, leading to the blood receiver, F, or with the tube, C, leading to the eudiometer, H. The tubes E and C are made of manometer tubing. The blood receiver, F, which also acts as the froth chamber, consists of three glass bulbs connected by short and wide junctions. To either end of the blood receiver is fixed a piece of pressure tubing, provided with a screw clip.

"In using the pump a blood receiver is affixed to the end of the tube, E, and the receiver is elevated into the position indicated by the dotted outline. The reservoir, B, is then put in connection with the tube, E, by means of the three-way tap, D, the reservoir, A, is raised above the pump, and the whole system is filled with mercury to the top of the blood receiver, F. The screw clip on the rubber tube at the upper end of F is then closed, and the reservoir, A, lowered until the blood receiver is exhausted, except for 2 or 3 c.c. of mercury, which are purposely left within. The screw clip on the lower end of F is next closed, and the blood receiver now clipped at either end, exhausted, detached from tube E, and weighed.

"Collect a sample of blood. The arterial or venous cannula is filled with blood, and immediately afterwards pushed into the rubber tube at the end

of the blood receiver, as far as the closed screw clip. Before the insertion of the cannula, the end of the rubber tube is compressed with the fingers to exclude the air within it. A sufficient quantity of blood is now withdrawn by opening at the same time the screw clip, and the clip placed on the vessel of the animal. The blood is defibrinated by shaking it with the mercury left within the blood receiver for that purpose, and the latter is then again weighed. The weight of the sample of blood is then obtained. The blood receiver is next affixed once more to the tube, E, in the dependent position shown in the figure, and the tube, E, is exhausted. Finally, the screw clip between E and the blood receiver is opened, and the gases are withdrawn and collected in the eudiometer. Since the blood receiver hangs freely from the tube, E, by means of a piece of rubber tubing, it can be both immersed in warm water and shaken to facilitate the complete escape of the gases. The bulbous form of the blood receiver prevents the blood from frothing over into the pump; and if the action becomes too violent, it can be immediately allayed by pouring a few drops of warm water on to the tube, E. The bubbles are thereby driven back into the receiver, and the pump is never fouled. The tap, D, is so manipulated that the gases only, and not the water which condenses in the reservoir, B, are driven over into the eudiometer. The water is returned back into the blood receiver. Three to four exhaustions are sufficient to extract all the gases from about 10 grms. of blood. The gases are estimated by the potash and pyrogallic acid method. The eudiometer tubes are surrounded with a water jacket to keep the temperature constant. In research work simul-

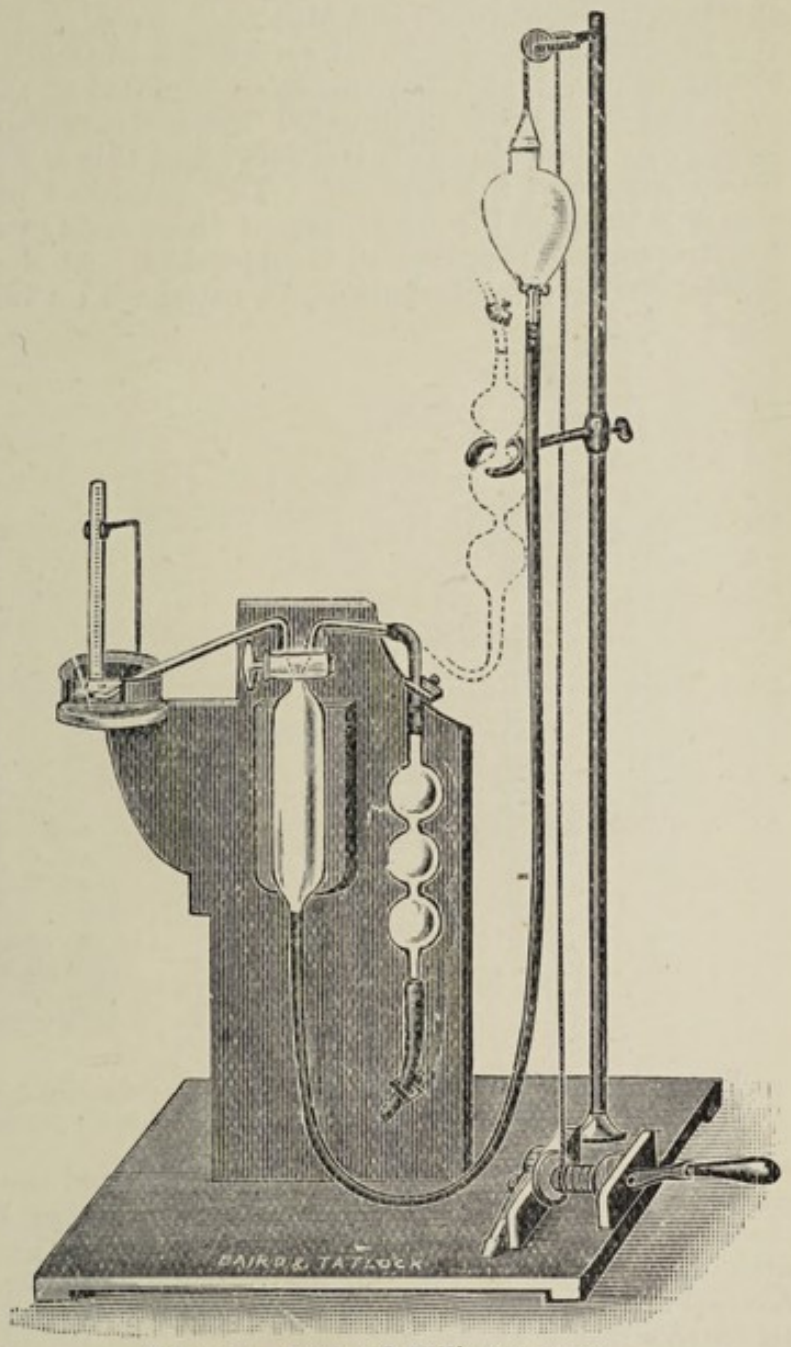


Fig. 370.—Leonard Hill's gas pump.

taneous samples are drawn from the arterial system, and from the vein leaving the organ, the metabolism of which is under investigation. The sample of blood can be drawn conveniently into small bulbs holding about 10 c.c., which have been previously washed out with oil; from thence the blood is rapidly transferred to the blood receiver. No loss of gas has been found to occur in using this method" (Hill & Nabarro on "The Exchange of Blood Gases in Brain and Muscle," *Journ. of Phys.*, xviii., 1895).

"In addition to the blood receiver, a muscle chamber is supplied with the pump (fig. 371). This chamber is closed at the upper end by a rubber cork, and the cork is surrounded by a mercury cup. A short piece of glass tubing, A, passes through the cork, and this is fitted with a piece of pressure tubing and a screw clip. Two insulated platinum wires, B and C, also pass through the cork—one of these ends in a hook. From this hook the frog muscle preparation is suspended. At the lower end of the muscle is attached a vulcanite bucket, D, fitted with a vertical rod, E. From the

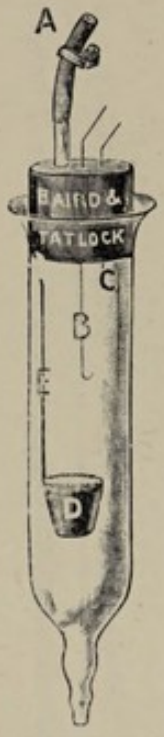


Fig. 371.—Muscle chamber for analysis of gases of muscle.

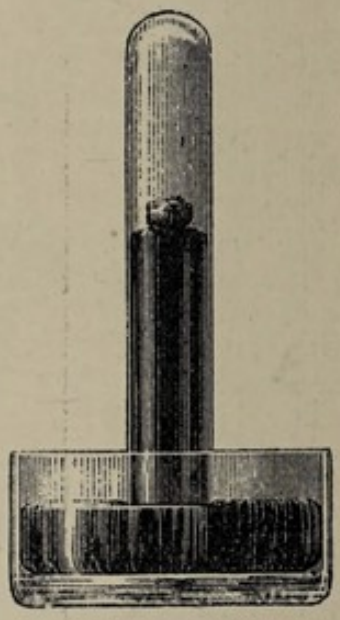


Fig. 372.—Gases collected over mercury. A ball of caustic potash absorbing the CO_2.

wire C passes a piece of fine wire, which is tied round the lower end of the muscle. After fitting the muscle preparation in position, the muscle chamber is first attached to the tube, E, of the gas pump, and then filled with mercury up to the top of the tube, A. During this operation the vertical rod fixed to the vulcanite bucket prevents the muscle from floating up on the top of the mercury. The screw clip on A is next closed, and a vacuum made in the muscle chamber. The bucket, D, is left full of mercury, and hangs as a weight on the muscle. The muscle can be excited to contract through means of the wires, B and C. By this apparatus the contraction of muscle *in vacuo* can be demonstrated, and the gas evolved by the muscle can be estimated. The gases of scalded and minced muscle (Hermann's method) can be extracted by the use of the blood receiver. The muscles, if desired, can be scalded and fixed while *in vacuo*, by allowing hot water to enter through the tube, A."

Analysis of blood-gases.—(*a*) Suppose the gases to be extracted and collected in a eudiometer over mercury (fig. 372). Or, for practice, and merely to grasp the principle how the relative proportion of the gases in a mixture is ascertained, the student may use air containing a small quantity of carbon dioxide.

(*b*) Fuse a ball of potash on the end of platinum wire (best done in a bullet mould). Introduce this under the mercury into the gases in the eudiometer. The caustic potash absorbs all the CO_2 (24 hours), and the diminution in volume represents the proportion of CO_2 in the mixture.

(*c*) With a curved pipette introduce a solution of pyrogallic acid into the eudiometer containing the remainder of the gases; this unites with the potash to form pyrogallate of potash, which rapidly absorbs the O. The decrease in volume represents the amount of O. The remainder represents N.

There are other methods of estimating the proportion of the gases, but this simple experiment is sufficient to explain the general principle on which such estimations are made. Of course there are corrections for temperature and pressure, and other precautions which require to be taken, but we do not enter into these here.

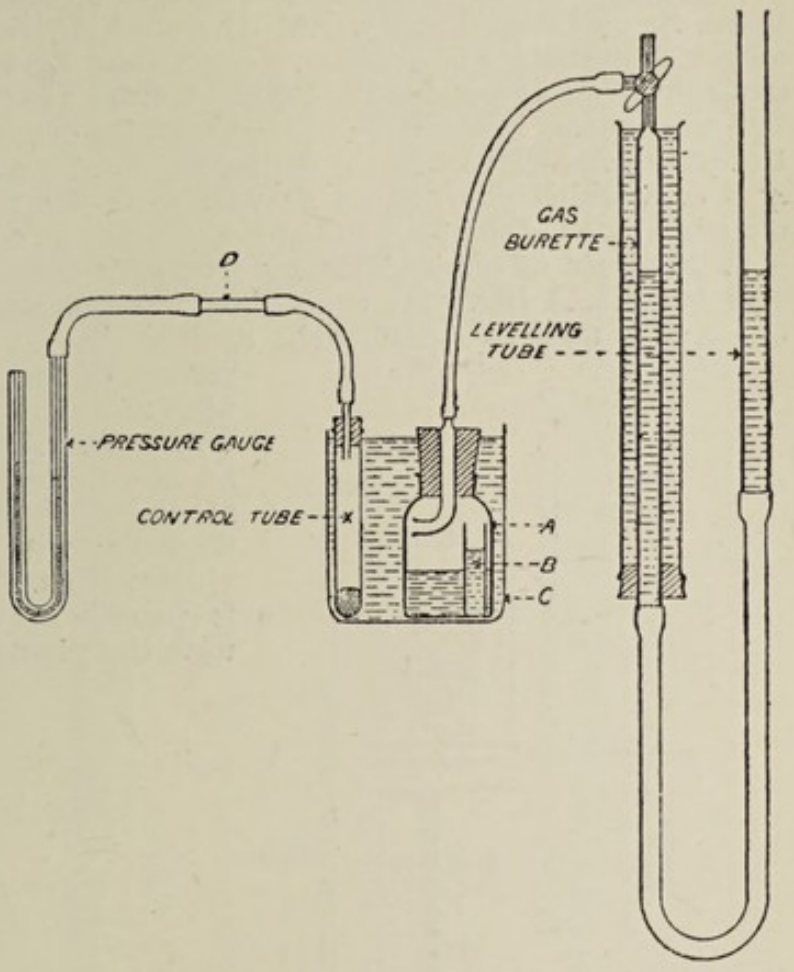


Fig. 373.—Haldane's apparatus for estimating O combined with Hb by ferricyanide method.

5. Oxygen capacity of blood (Ferricyanide method). — This method, devised independently by J. Haldane (*Journ. of Phys.*, xxii., 298; xxv., 295, 1900), and v. Zeynek and Hüfner (*Arch. f. Anat. u. Physiol.*, 1894 and 1899), depends on the fact that the combined O is liberated rapidly and completely on adding solution of potassium ferricyanide to laked blood. It may be measured by an apparatus similar to that of Dupré for estimating urea in urine (fig. 373).

The blood must be collected in a cleanly manner, and kept on ice unless the determination can be made at once, because bacteria may vitiate the results.

(*a*) Measure into the bottle, A, by means of a pipette, 20 c.c. of oxalated or defibrinated blood (Lesson V., 14), thoroughly mixed with O by swinging it round in a large flask.

(*b*) To it add 30 c.c. of ammonia solution, prepared by diluting ordinary

strong ammonia (S.G. 0·88) with distilled water to $\frac{1}{100}$. The ammonia prevents the CO_2 from being given off, and the distilled water "lakes" the corpuscles. Mix by shaking.

(*c*) Pour about 4 c.c. saturated solution of potassium ferricyanide into the tube, B, and place it upright in A.

(*d*) Connect A by stout rubber tubing (1 mm. bore) to the burette, and place A in the vessel of water, C, with T° as near as possible that of the room and blood. Open side tube of burette, and by means of levelling tube level water in burette at top. Close side tube, and note reading of burette.

(*e*) Adjust water-gauge (*i.e.*, for T° and P) (1 mm. bore) to a definite mark. It has some Hg in it to sink it.

(*f*) When the reading of the burette is constant, tilt the bottle to upset B. Mix thoroughly. All the combined oxygen is given off. Re-adjust temperature of water. Note T° and barometer. Measure volume of evolved gas and reduce to its dry volume at 0° and 760 mm. To calculate the amount in 100 c.c. of blood take the original 20 c.c. blood as equal to 19·6 c.c. Usually fresh defibrinated ox blood yields about 4·15 to 4·18 c.c., which gives about 24 vols. of O per 100 vols. of blood. With oxalated blood Haldane found the amount to be 20 to 22 vols. per 100.

6. Analysis of expired air by Hempel's method.*

A burette, A (fig. 374), containing 100 c.c., and graduated into tenths of a c.c., is used to measure the expired air. It communicates below by means of an india-rubber tube with the movable tube or reservoir for water, B. Above, A is connected to an absorption pipette by means of a short india-rubber tube of 1 to 2 mm. diameter with thick walls, and provided with a Mohr's clip. The tube, A, is placed in connection successively with the pipettes, *px*, which contain a

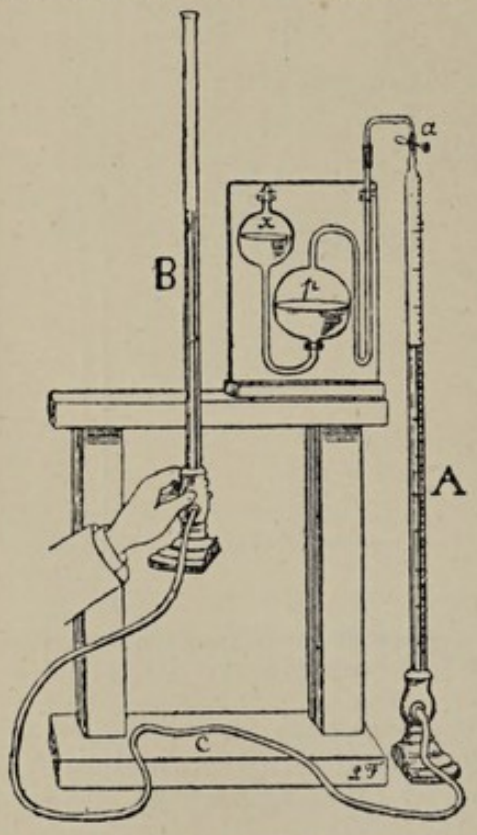


Fig. 374.—Hempel's burette connected with a potash pipette to absorb the CO_2.

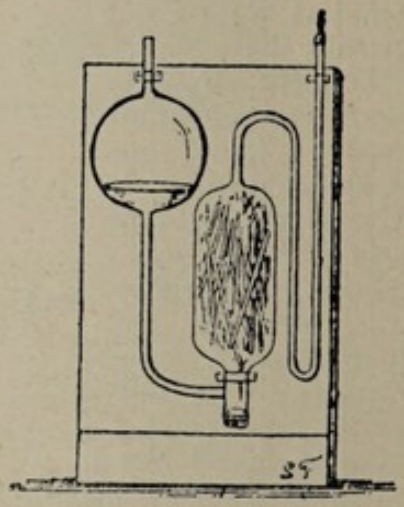


Fig. 375.—Pipette with phosphorus to absorb the oxygen.

solution of caustic potash to absorb the CO_2; and fig. 375, which contains sticks of red phosphorus in water to absorb the O.

---

* *Methods of Gas Analysis*, by W. Hempel. London, 1892.

Suppose the gas to be collected in A; measure its amount when B is so placed that the level of the acidulated water is equal in both.

Remove Mohr's clip from *a*, raise B, and force all the air into *p*. Then lower B, and withdraw unabsorbed air from *p*. Measure the volume of air.

Connect A with the phosphorus pipette and force the air into it by raising B. Lower B, and estimate the remaining volume of air. In each

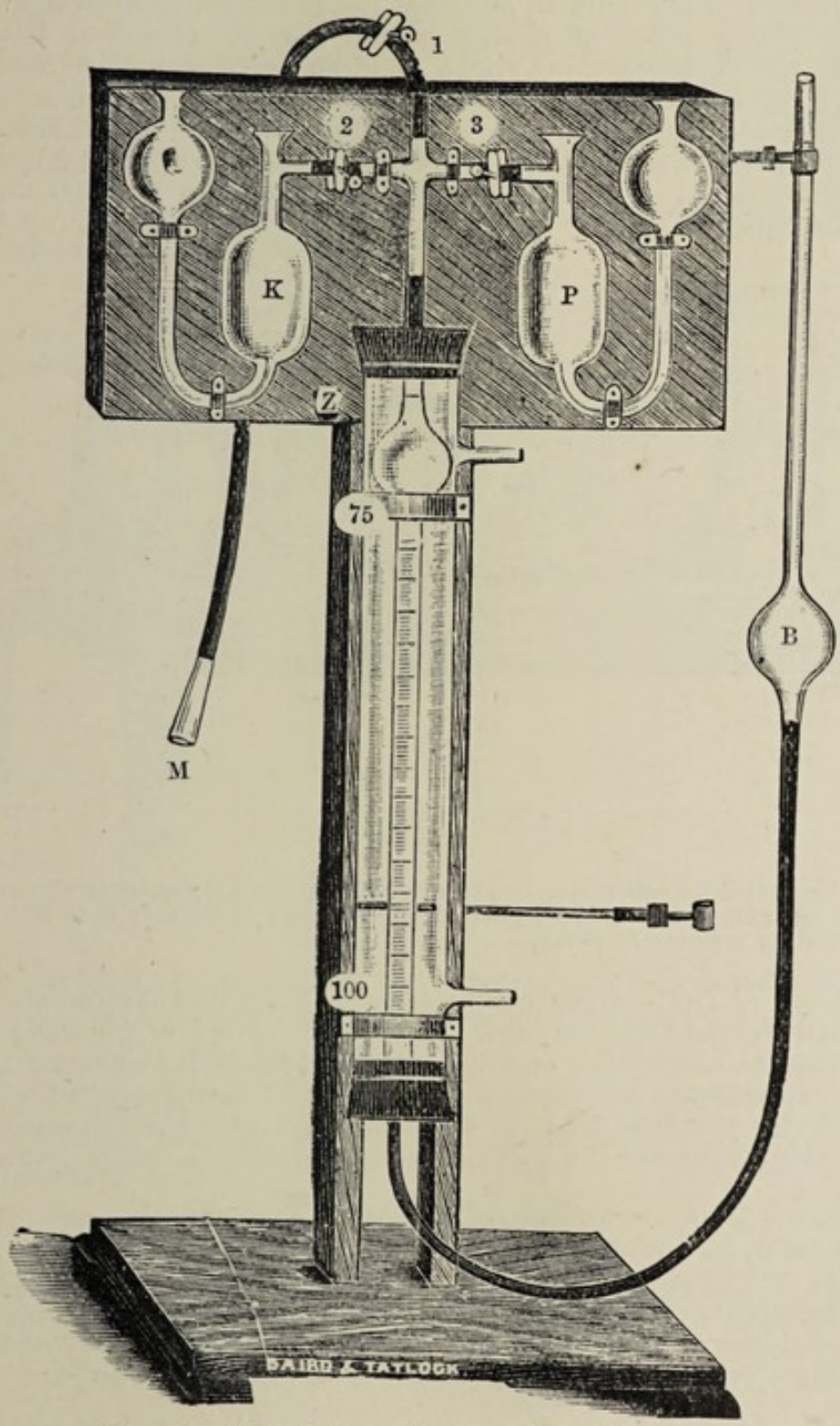


Fig. 376.—A. Waller's modification of Zuntz's gas pump.

case the difference of volume of air corresponds to the quantity of gas absorbed. The temperature of A can be kept constant by placing it in a wide tube through which water is kept circulating as in a Liebig's condenser.

7. Analysis of expired air, CO_2.—The modified apparatus by Waller (fig. 376) is used. It consists of a measuring tube (100 c.c.), graduated in tenths of a cubic centimetre between 75 and 100, and surrounded by a water jacket, a filling bulb, B, and two gas pipettes; that on the left, K, contains strong solution of caustic potash (for absorption of CO_2), the pipette on the right contains sticks of phosphorus in water (for absorption of O_2). When dealing with CO_2, clamp 3; with O_2, clamp 2.

(*a*) *Measurement of expired air.*—Open tap or clamp 1, fill B with acidulated water, raise B and fill the measuring tube to Z (zero). Through M breathe out 100 c.c. of expired air, at the same time lower B until the fluid in the bulb and burette is adjusted to 100. Close 1.

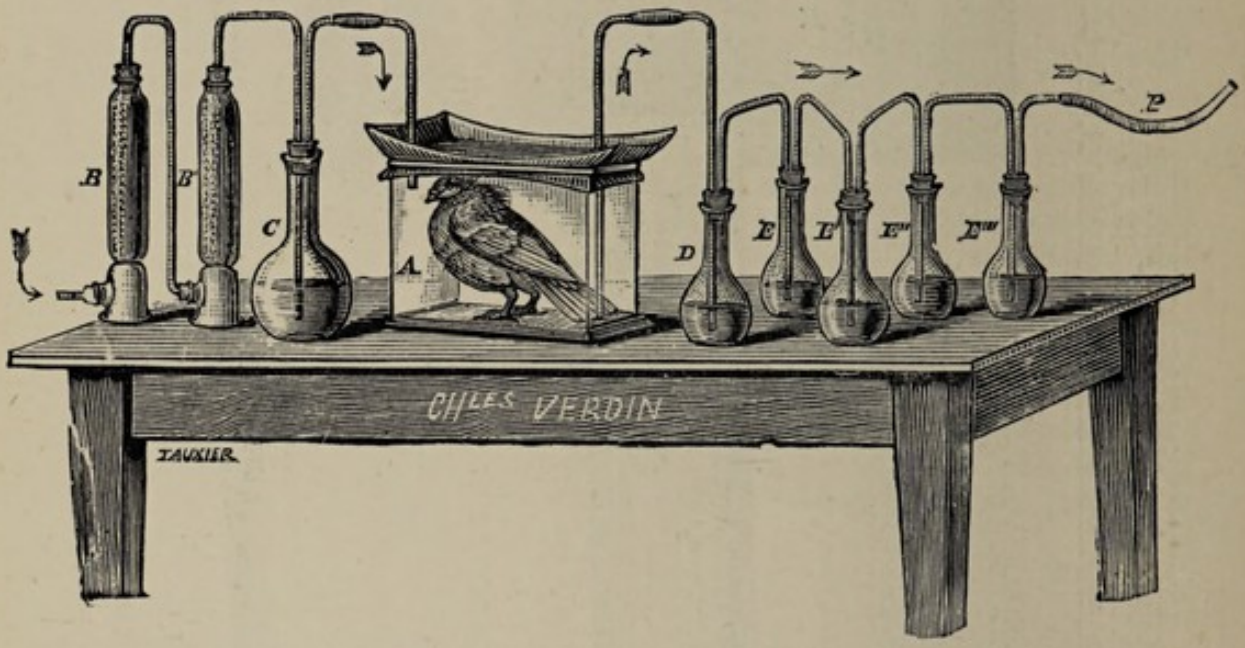


Fig. 377.—Apparatus for estimating CO_2 produced by a small mammal or pigeon. *B B'*, Vessels containing KHO; *C*, Baryta; *A*, For animal; *D*, Water; *E* to *E'''*, Baryta; *P*, Aspirating tube (*L. Fredericq*).

(*b*) *Amount of CO_2.*—Open clamp 2, raise B, propel expired air slowly into K until the fluid reaches zero, Z. Close 2 for a few minutes to allow of the complete absorption of CO_2. Open 2, lower B, whereby the unabsorbed constituents are sucked back into the measuring tube, and in doing so bring the fluid in B to the same level as in the measuring tube. Read off the diminution in volume, which represents the p.c. amount of CO_2 expired.

(*c*) *Amount of O_2.*—This is ascertained in a similar manner. Close 2, open 3, raise B, and propel the remaining air into P, pipette (5 to 10 mins.). Suck air back into measuring tube, and note loss of volume.

8. Estimation of CO_2 expired.—A small animal—*e.g.*, guinea-pig or pigeon—produces far more CO_2 per kilo. unit weight of body than does man. Air inspired contains ·03 to ·04 p.c. of CO_2, and expired air (man) 4 to 5 p.c.—*i.e.*, the CO_2 is increased one hundredfold.

(*a*) Place a pigeon or guinea-pig in A (fig. 377) hermetically sealed above

with a water valve. The apparatus is so arranged that a rapid current of air can be drawn through the whole system by means of an aspirator. B and B′ contain KHO, and C baryta water. The air, when aspirated, should not make C turbid. D contains water, and E, E′ E″ E‴ contain 500 c.c. of a titrated solution of baryta water. Aspirate air rapidly through the system—half an hour or more—as long as E‴ remains clear, and stop as soon as it begins to be turbid. Note duration of experiment—say 30 minutes.

(*b*) Collect and thoroughly mix the fluid in E′ E″ E‴. Let the carbonate of barium subside, and then remove with a pipette some of the clear supernatant liquid, and determine by titration the amount of barium still left—*i.e.*, uncombined with CO_2.

(*c*) Suppose the experiment lasted 30 mins., the weight of guinea-pig 527 grms., quantity of baryta 500 c.c. titrated *before* experiment (10 c.c. = 28·8 c.c. CO_2), and *after* (10 c.c. = 22·1 c.c. CO_2), then the quantity of CO_2 produced per hour will be (*L. Fredericq*)—

$$\frac{28{\cdot}8 - 22{\cdot}1 \times 50}{527} \times 2 \times 1000 = 1270 \text{ c.c. of } CO_2$$

—*i.e.*, several times the amount expired by man.

[See also a convenient apparatus for CO_2 and H_2O, by Haldane, where the CO_2 is absorbed by means of soda lime (*Journ. of Phys.*, xiii., 419, 1892).]

9. CO_2 in air.—A simple method for its estimation in air is given by J. Haldane, *Journ. of Hygiene*, i., 1901.

LESSON LXIX.

LARYNGOSCOPE—VOWELS.

1. **The laryngoscope** is used to investigate the condition of the pharynx, larynx, and trachea. Various forms are in use, but they all consist of (1) A small, usually circular, plane mirror fixed to a metallic rod at an angle of 120°; the metallic rod fits into a suitable handle, and is fixed by means of a screw. (2) A large concave mirror of about 20 cm. focus, perforated with a hole in the centre, and secured to the operator's forehead by means of a circular band passing round the head. The mirror itself is fixed in a ball-and-socket joint, so that it can be moved in every direction.

A. Practise first of all on a **model** of the head and larynx provided for the purpose.

B. On a living person.—(*a*) Place the patient upright in a chair. A good source of artificial light—*e.g.*, a suitable Argand or incandescent lamp—is placed near the side of the patient's head, a little above the level of his mouth. Mackenzie's rack-movement lamp is convenient. The observer seats himself opposite and close to the patient, places the large mirror on his forehead, and either looks through the central hole in it with one eye, or raises it so that he can just see under its lower edge.

(*b*) Seated in front of the patient, the observer directs a beam of light until the lips of the patient are brightly illuminated. The patient is then directed to incline his head slightly backwards, to open his mouth wide, and protrude his tongue. Place a clean handkerchief over the tongue, and give the patient the handkerchief to hold, which secures that the tongue is kept protruded and well forward. Move the large mirror until the uvula and back of the throat are brightly illuminated, the operator moving his head slightly to and from the patient until the greatest brightness is obtained.

(*c*) Take the small laryngeal mirror in the right hand, and warm it gently over the lamp to prevent the condensation of moisture on its surface. Test its temperature on the skin of the cheek or the back of the hand. Holding the handle of the mirror as one does a pen, rapidly carry it horizontally backwards, avoiding contact with any structures in the mouth, until its back rests against the base of the uvula. At the same time direct the beam of light upon the laryngeal mirror, when an inverted image of the larynx will be seen more or less perfectly.

(*d*) By moving the laryngeal mirror, not, however, pressing too much on the uvula, or continuing the observation for too long a time, one may explore the whole of the larynx. Perhaps only the posterior part of the *dorsum of the tongue* is seen at first; if so, slightly depress the handle of the mirror, when the curved fold of the slightly yellowish *epiglottis* and its cushion, with the *glosso-epiglottidean folds*, come into view. In the middle plane are the *true vocal cords*, which are pearly white and shining, and best seen when a high note is uttered, and between them the chink of the *glottis*. Above these are the *false vocal cords*, which are red or pink, the *ary-epiglottidean folds*, with on each side the *cartilages of Wrisberg* farthest out, the *cartilages of Santorini* internal to this, and the *arytenoid cartilages* near the middle plane (figs. 378, 379).

(*e*) Ask the patient to sing a deep or high note, or inspire feebly or deeply, and observe the change in the shape of the glottis. On uttering a deep note the tracheal rings may be seen.

N.B.—What is seen by the observer in the laryngeal mirror on his right or left corresponds to the *patient's* left and right. The lower part of the mirror gives an image of the more posterior structures, while the anterior structures are reflected in its upper part.

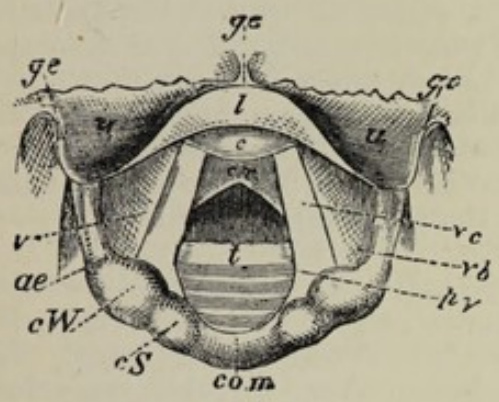


Fig. 378.—Larynx during a deep inspiration. *ge*, Glosso-epiglottidean fold; *lc*, Lip and cushion of epiglottis; *ae*, Ary-epiglottic fold; *cW*, *cS*, Cartilages of Wrisberg and Santorini; *vc*, Vocal cord; *vb*, Ventricular band; *pv*, Processus vocalis; *cr*, Cricoid cartilage; *t*, Rings of trachea.

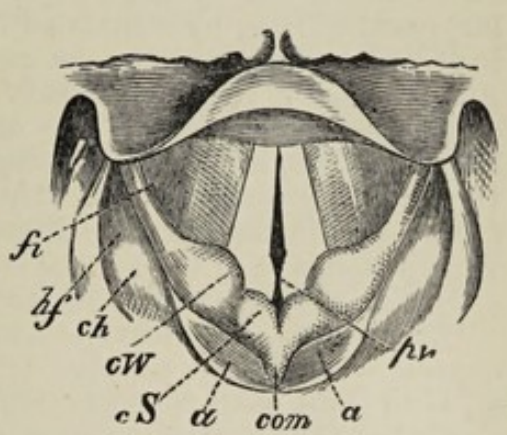


Fig. 379.—Larynx during vocalisation. *fi*, Fossa innominata; *hf*, Hyoid fossa; *com*, Arytenoid commissure.

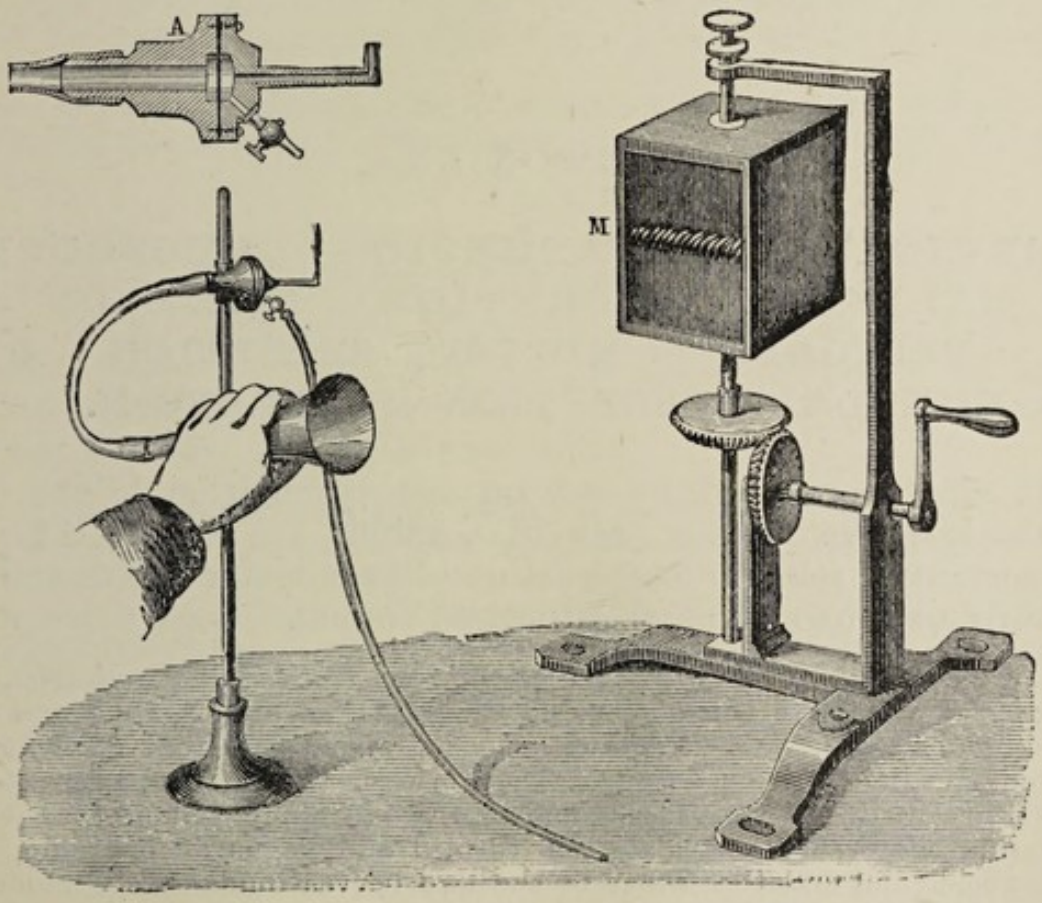


Fig. 380.—König s manometric flame apparatus.

2. Auto-laryngoscopy.—The student should learn to use the laryngoscope on himself. Sitting in a chair, he fixes the large reflecting mirror in a suitable holder about 18 inches in front of, and on a level with his mouth. Behind and to one side of this an ordinary plane mirror is placed vertically. On one side of his head he places the source of light. The light is reflected on to the uvula by the reflecting mirror, and, on introducing the small laryngeal mirror, by a little adjustment one sees the image of the larynx in the plane mirror. Or one may use in a similar way the apparatus of Foulis. In Dr. George Johnson's method the ordinary reflector is strapped on to the forehead, and the observer places himself in front of a toilet mirror. In a line with and slightly behind the mirror, and on one side of the observer place a lamp. By means of the reflector the image of the fauces seen in the mirror is illuminated. Introduce the laryngeal mirror, when the image of the larynx is seen in the toilet mirror.

3. Analysis of vowel sounds.

Use König's apparatus, as shown in fig. 380. Connect the tube of the capsule with the gas supply, light the gas-jet, and sing the vowels A, E, I, O, U in front of the open trumpet-shaped tube shown in the figure. With the other hand rotate the mirror, M, and observe the serrated reflection of the flame in the mirror, noticing how the image in the mirror varies with each vowel sounded. The analysis of vowels and other sounds, as carried out by M'Kendrick with the aid of the phonograph, and by Hermann, is omitted here.

LESSON LXX.

CERVICAL SYMPATHETIC — CRYOSCOPY — SALIVARY SECRETION — INJECTION OF "PEPTONES" — KIDNEY SECRETION — BILE FISTULA — ABSORPTION — EXCRETION.

THE effects produced by section and stimulation of the sympathetic nerve in the neck of a rabbit are among the most important in relation to the action of vaso-motor nerves and can readily be shown in an anæsthetised animal.

1. Cervical sympathetic nerve (*Action on blood-vessels and iris*).—(*a*) Arrange a coil for tetanising shock. Chloralise an albino rabbit and fix it on a holder back down. Cut away the hair from the front of the neck. Make a median incision through the skin over the trachea, pull asunder the skin, cut through the fascia, and separate in the mid-plane the muscles covering the trachea. Isolate the carotid and vagus on one side, turn these aside and find the larger of the two nerves immediately under the vagus—*i.e.*, the cervical sympathetic. (The other and smaller is the

depressor.) Isolate the nerve and place a ligature under it as low down in the neck as possible (fig. 338), but do not tie the ligature.

(*b*) Free the rabbit's head from the holder, observe the two ears, the central artery, and the laterally-placed veins. Note vascularity by looking through the ear from the inner surface; also the condition of the two pupils with equal illumination.

(*c*) Cut the sympathetic peripherally to the ligature. Within a few seconds the blood-vessels of the ear on that side become greatly distended with blood—hyperæmia—and red. The central artery is made wider, and running from it to the veins are many red vessels now visible, which were not so before. The marked congestion makes this ear much warmer. Feel the difference in T° of the two ears by putting the thumbs gently into each. There is a difference of several degrees. This difference in vascularity can readily be shown to an audience by placing small incandescent electric lamps in the ears, or even holding a lighted taper behind the ears. Observe also the effects on the palpebral fissure and amount of protrusion of eyeball.

(*d*) Stimulate with tetanising shocks the peripheral end of the nerve. After a considerable latent period note (i.) the condition of the *blood-vessels* of the ear on that side. The central artery gradually becomes constricted and narrower, and may be almost invisible, the vascularity of the whole ear is diminished, and it is colder—detectable readily by the hand. Anæmia.—The vessels remain in this constricted condition for some time after cessation of the stimulation. They suddenly return to the hyperæmic condition.

(ii.) *Eye effects.*—Keep the eyelids asunder and repeat the stimulation. The pupil becomes greatly dilated, only a small part of the iris being visible beyond the margin of the cornea. There may also be increased secretion of tears.

The cervical sympathetic nerve carries—besides other fibres—vaso-constrictor fibres to the blood-vessels of the ear and dilator fibres to the iris.

A short description of some fundamental demonstrations on the subjects of secretion, absorption, and excretion, are introduced here. The great importance attached to a study of osmotic pressures and cryoscopy, and their relation to these processes, leads me to give a short account of that process before dealing with the subject of secretion.

2. Osmotic pressure (*Semi-permeable membranes*).—If a parchment tube or closed bladder—permeable both to water and salts—containing a saline solution be suspended in distilled water, some of the salt diffuses out and water passes in by a process of osmosis. The bladder gradually swells and becomes tense owing to rise of pressure = osmotic pressure. The animal membrane, however, allows filtration to take place, and therefore the full osmotic pressure cannot be the reason. A membrane or vessel must be found which will allow the water to pass in, but prevent the salts from passing out, either by diffusion or filtration.

Wash out a porous cell—such as is used for a Daniell's battery—first with solution of 8 p.c. $CuSO_4$ and then 5 p.c. ferrocyanide of potassium. An insoluble precipitate of copper ferrocyanide, $K_4Fe(CN)_6 + 3H_2O$, is thrown down in the interstices of the porous cell. This vessel allows the

water to pass through, but prevents the salts from doing so. These "precipitation membranes" were first investigated by Traube (1867), and later by Pfeffer (1877).

If such a vessel be filled with a 1 p.c. NaCl solution, and connected up with a Hg-manometer, and then placed in distilled water, osmosis takes place. The actual osmotic pressure can be observed in terms of the Hg in the manometer. It will be about 5000 mm. Hg before equilibrium is established. But the osmotic pressure is not due to the liquid or to the nature of the dissolved substance, but merely to the *number of molecules of salt* present in solution (*van T'Hoff*). The osmotic pressure is equal to the pressure which the dissolved substance would exert, if it occupied the same space at the same T° in the form of a gas. The osmotic pressure is proportional to the concentration of the solution, or to the number of molecules and ions of the crystalloid in solution.

3. **Cryoscopy** (Raoult, 1885).—*Indirect determination of osmotic pressure by freezing point.*—This means the determination of the freezing point of fluids—either pure or containing other substances in solution. The presence of a substance lowers the freezing point of water, and the amount of lowering is proportional to the number of parts (molecules or ions) in the solution (Law of Blagden). Osmotic pressure is also proportional to the number of parts in solution. Instead of estimating the osmotic pressure directly, it may be done indirectly by taking the lowering of the freezing point as an index of the osmotic pressure.

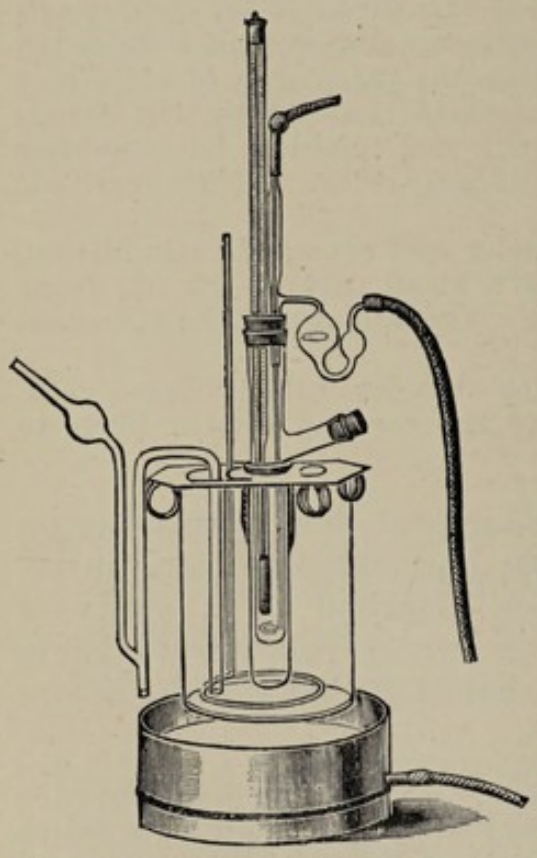


Fig. 381.—Beckmann's apparatus for determining freezing point of a liquid.

This is done by Beckmann's apparatus (fig. 381). It is provided with an adjustable differential thermometer. Pure water is placed in the inner tube, and the freezing point determined—ice and salt being placed in. Test the freezing point of mammalian *blood-serum*, and note the fractions of a degree by which its freezing point is lower than that of water. This is usually expressed as Δ. It will be found to be 0·56° C. By experiment with solutions of common salt of various strengths it is found that 0·95 p.c. NaCl gives the same Δ, hence the two solutions exert the same osmotic pressure and a 0·95 p.c. of NaCl is said to be isotonic—or isosmotic—with the mammalian serum.

As a matter of fact, experiments (by Winter) show that the Δ of blood-serum of most mammals is about 0·56° C. below that of pure water. It is

obvious that osmotic pressures must play an important part in the exchanges between the cell elements of the tissues and the lymph, and between the lymph and the blood, and *vice versâ*. Moreover, it has intimate relations to any theory of secretion.

Determine the osmotic pressure of *urine*. It will be found to have the highest isotonic coefficient of any fluid in the body. The Δ is equal to 1·85° C., while that of serum is only 0·56° C.

Physikalische Chemie in d. Medicin, by H. Koppe, Vienna, 1900. *Tonometrie* and *Cryoscopie*, by Marey, Paris, 1901.

Some fundamental facts regarding secretion may be learned from the following demonstration.

4. **Salivary secretion—Submaxillary gland.**—The operator should make himself acquainted with the anatomy of the parts by making a careful dissection (fig. 382).

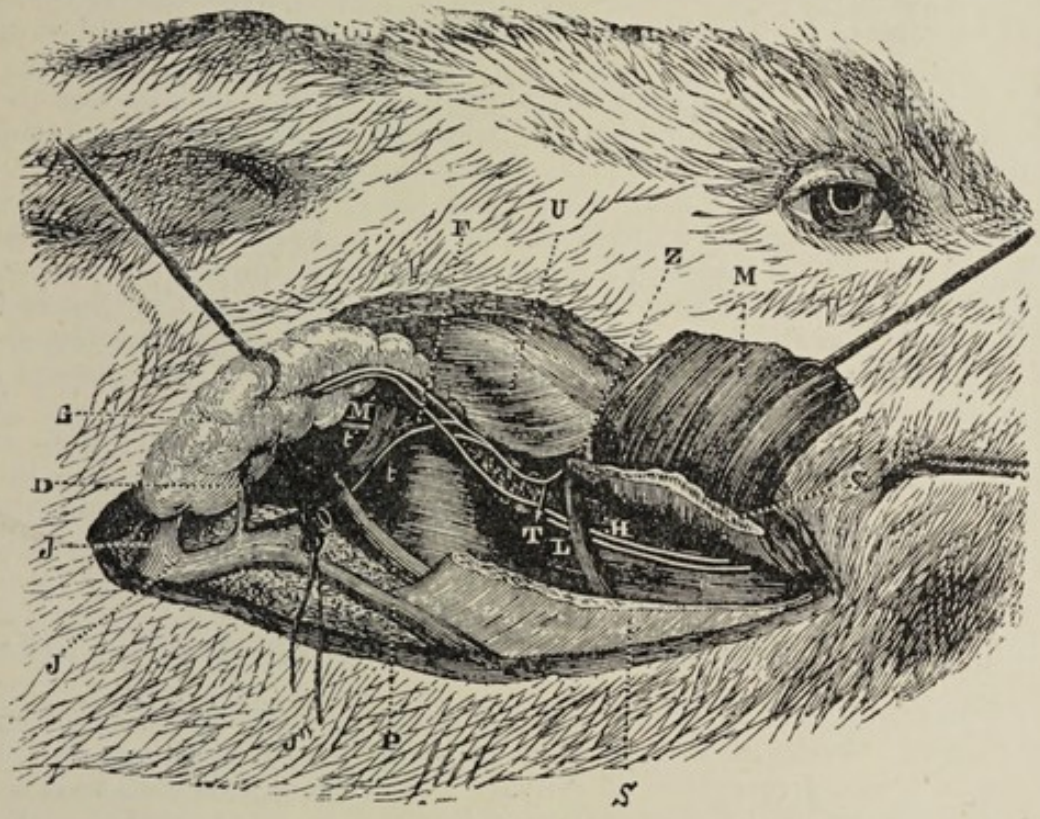


Fig. 382.—Dissection of the nerves to the submaxillary gland of dog. *G*, Submaxillary gland; *H*, Wharton's duct, and below it duct of Bartholinus—*i.e.*, sublingual duct; *L*, Lingual nerve; *T*, Chorda tympani; *J*, External jugular vein; *D*, Efferent vein of gland; *P*, Hypoglossal nerve; *M*, Anterior part of digastric muscle; *S*, Mylo-hyoid cut across to show course of the two ducts; U, Masseter; and *S' F'*, Carotid with *t t'* branches of the sympathetic nerve (*Cl. Bernard*).

(*a*) Arrange on a tray in order the necessary apparatus—scalpel, scissors, ligatures, forceps, cannula and tube (1 mm. bore), measuring glass, sponges, absorbent cotton wool, camel-hair brush, and 0·1 p.c. nicotin in normal saline. Arrange coil for repeated induction shocks.

(*b*) Fix a dog, back down, in a holder—*e.g.*, Bernard's—and etherise it,

or inject subcutaneously a 1 p.c. solution of morphin (0·01 grm. per kilo.), and then complete the anæsthesia with chloroform.

(*c*) Cut away the hair on one side in the intermaxillary region. Divide the skin parallel to and about 1 cm. from the inner border of the lower jaw, abut its middle third for 5 to 6 cm. Carry the incision deeper through the subcutaneous tissue and the platysma, ligaturing any vessels that may be divided. This exposes the digastric muscle, which is divided and raised, the divided parts being kept asunder by hooks with weight attached. The transverse fibres of the mylo-hyoid are now exposed. Insinuate a director under this muscle, and divide it in the direction of the original incision. Raise the divided parts of the muscle and keep them out of the way with hooks. There are now exposed two ducts running parallel to the original incision—the outer and larger the duct of Wharton from the submaxillary gland, and the smaller the sublingual duct—crossed by the much larger lingual nerve. Trace the lingual backwards, and note the fine chorda tympani passing from it at an acute angle, and running backwards towards the submaxillary gland. Isolate Wharton's duct; ligature it as near its orifice as possible. When it swells with saliva, make an oblique incision in it, insert in it the cannula towards the gland, and tie it in.

(*d*) Connect the cannula with a straight glass tube. Isolate the chorda tympani and put a ligature under it. Observe (1) the rate of secretion as the saliva advances in the tube; (2) apply acetic acid to the tongue. The flow is accelerated if the narcosis be not too deep. This is due to reflex stimulation of the secretory fibres of the chorda, the afferent nerve being the lingual branch of the fifth; (3) ligature and divide the chorda central to the ligature; the acid now has no effect; (4) stimulate the chorda with repeated induction shocks. After a latent period, observe how the saliva —"chorda saliva"—literally rushes forward in the tube. Its amount may be collected and measured in a small graduated cylinder.

(*e*) The vascular changes in the gland. It becomes red owing to vaso-dilation produced by the stimulation of the vaso-dilator fibres in the chorda. Thus there are two visible effects resulting from stimulation of the chorda.

(i.) Increase in amount of saliva,

(ii.) Vaso-dilation of the vessels of the gland,

(iii.) As a consequence of (ii.) greatly-increased outflow of blood from the vein.

(*f*) The sub-maxillary ganglion lies in the angle between the chorda and lingual nerve. With a camel-hair brush paint it with nicotin. Stimulate the chorda = rapid secretion of saliva. Paint the chorda just as it enters the hilum of the gland and again stimulate = no secretion. Why? Stimulate the hilum of the gland = secretion. Why?

If the secretion pressure in the duct be measured during continued stimulation of the chorda, and if, at the same time, the blood-pressure in the carotid be recorded, the secretory pressure will be found to be greater than the arterial blood-pressure. Therefore the secretion is not a mere filtrate from the blood (*C. Ludwig*, 1851).

By the subcutaneous injection of atropin it can be shown that stimulation of the chorda causes dilation of the blood-vessels of the gland, but no secretion of saliva. Therefore in (5), (i.) is not directly dependent on (ii.).

The nicotin experiments show that nerve cells intervene between the chorda at the place where it parts company with the lingual and the gland itself. Nicotin paralyses nerve cells or the terminations of nerve fibres

around nerve cells. In small doses it does not affect the nerve fibres. Clearly the chorda fibres in the dog do not come into relation with the nerve cells in the sub-maxillary ganglion. (This is really the "cell station" for the secretory nerve fibres of the sub-lingual gland.) The nerve cells for the chorda fibres to the sub-maxillary gland are placed near the hilum of the gland. The last experiment of direct stimulation of the gland—*i.e.*, stimulation of what Langley calls the post-ganglionic fibres—confirms this view (Langley, with Dickinson and Anderson, *Journ. of Phys.*, xi., xiii., xix., xxii.).

5. Kidney secretion.—In an anæsthetised dog, open the abdomen by an incision in the linea alba, turn aside and keep the intestines warm with thick pads of hot cotton wool, expose the ureters, scratch through the peritoneum, and tie into each a cannula.

Observe (1) the flow of urine, (2) the peristaltic contraction of the ureters, (3) peristaltic movements of intestines.

A cannula can be placed in the external jugular vein and directed towards the heart. Connect the cannula with a burette filled with normal saline fluid. Let it run slowly into the vein, and observe its diuretic effects and increase of urine.

A similar burette may be used to inject "peptones" into the blood stream (0·5 grm. per kilo.). Witte's or "Commercial" peptones, chiefly albumoses—when so injected—prevent the blood from coagulating when it is shed. Place a cannula in a femoral artery. Draw off some blood into a test tube and observe how soon the blood clots. Now, inject the peptones and draw another sample of blood. It does not coagulate. The same animal may be used to place a cannula in the thoracic duct—introduced from above the clavicle—to observe the effect of such lymphagogues as peptones in also preventing the coagulation of lymph. Note effect on secretion of urine.

If a burette filled with indigo-carmine be connected with the external jugular vein, the time that this fluid takes to appear in the urine can be determined.

6. Bile fistula.—(*a*) Anæsthetise an albino rabbit with chloral or urethan (2 grms.). Fix the animal on a rabbit-holder back down. Open the abdomen by a vertical slit in the linea alba, beginning at the xiphisternum. Pull the stomach aside to show the under surface of the liver, and isolate the common bile duct as it passes from the portal fissure to the duodenum. Prepare previously a glass tube with an internal diameter of 2·3 mm., 35 cm. long, and bend it at a right angle about 5 cm. from one end; on this end make a shoulder, just as one does for a cannula.

(*b*) Place a thread round the bile duct near the duodenum and ligature the duct; pull on this thread so as to raise the duct, and round it (between the ligature and the liver) place another thread. With sharp scissors make an oblique slit into the duct, introduce the cannula, tie it in the duct, fix the glass tube in a holder, and close the abdominal cavity with Péan's forceps.

(*c*) Observe (1) the colour of the bile and how it moves forward in the tube as it is secreted, especially if the tube be horizontal; (2) how the bile is pushed forward with every descent of the diaphragm in inspiration and recedes during expiration; (3) measure the amount of bile that flows out in a given time; (4) hold the tube vertical, and observe the oscillations of the bile with the respiratory movements.

Sulphindigotate of soda (indigo-carmine).—To the cannula attach a burette filled with solution of indigo-carmine which has a deep blue colour.

Adjust the pressure so that it slightly exceeds that in the portal vein. Open the stopcock and let the blue fluid run slowly into the duct; it passes backwards along the duct and soon reaches the liver. In about 5 minutes its *absorption* and presence in the blood is revealed by a blue tinge in the margins of the eyelids, lips, mucous membrane of the tongue, skin of the abdomen, &c.

Press on the belly to expel some urine. It is blue. Kill the animal and observe the blue colour in the liver, kidney, &c. None is excreted by the saliva.

This is a most important experiment, as it illustrates the low pressure under which absorption of bile or other fluid can take place in the liver, the rate of absorption, and the rapidity of secretion and excretion of this substance by the kidney. It obviously has important bearings on the question of absorption of bile in jaundice, where there is obstruction to the free outflow of bile into the duodenum.

7. Absorption from rectum.—That absorption can take place from the lower end of the alimentary mucous membrane is well known, and, indeed, is only too often necessitated in certain diseases where food or drugs cannot be given by the mouth. Gases are similarly absorbed. Meltzer has devised the following experiment on a rabbit to show this and to produce anæsthesia:—Half-fill a wide-mouthed bottle with ether. Through its cork pass a bent glass tube and connect the latter with another tube with a number of holes in its walls. Introduce the other end of this tube into the rectum of a rabbit keeping the ether bottle higher than the level of the rectum.

Place the ether bottle in water at 35° C.—the ether boils and the vapour, mixed with air, passes into the rectum, and anæsthesia is soon complete, the effect, of course, depending on the rate of absorption and the rate of excretion by the lungs. It appears that Pirogoff in 1840 used ether in this way for man, but the practice was discarded (S. J. Meltzer, *Amer. Journ. of Phys.*, i., 1900).

8. Subcutaneous absorption.—This method is made use of daily in medicine in the form of hypodermic injection. It can readily be shown on an anæsthetised rabbit. Empty the bladder of a rabbit by holding it vertically and compressing the lower part of the abdomen. Inject under the skin on one side sodium ferrocyanide (not K-salt), and on the other sodium iodide, with a hypodermic syringe.

After half an hour express the urine from the bladder. Test one portion with dilute starch mucilage either with nitric acid or potassium nitrite and dilute HCl. A blue colour indicates the presence of iodine. Test another portion with Fe_2Cl_6 and HCl; the Berlin blue colour shows the presence of the ferrocyanide. Control by testing urine before injection (*L. Hermann*).

9. "Witte's Peptones" injected into blood stream.—They consist of a variable proportion of proteoses with some peptone. Proteoses, when so injected in dogs, produce

(1) A rapid and marked fall of arterial blood-pressure, which slowly returns to normal, due to an effect on the neuro-muscular apparatus of the blood-vessels whereby they are dilated.

(2) Blood withdrawn from an artery fails to clot, or does so only after a long time. The effects are more marked with rapid injection.

(3) When the effects of the injection have passed off, a second injection fails to produce the characteristic action. A certain degree of "immunity" has been established.

(4) Increased flow, "lymphagogic" influence, and diminished coagulability of lymph.

(5) Changes in urine, cessation of flow, and respiration.

(6) If the "peptones" be added directly to blood, they have no clot-preventing power, so that their action is indirect. The power seems to belong to some substance formed in the organism, not to something formed from the peptone itself. It is suggested that the seat of formation of this anti-coagulating substance is the liver (*Contejean*, *Gley*, *Pachon*), and that the destruction of leucocytes plays an important part (*Delezenne*, 1898).

(*a*) Fasting dog, narcotised by subcutaneous injection of morphin and atropin, followed by inhalation of mixture of chloroform and ether. Arrange to measure arterial B.P. by cannula placed in carotid and Hg-kymograph. Connect a burette to a glass cannula fixed in a facial vein. Dissolve the "peptones" in 0·7 p.c. NaCl. Allow the peptones to flow into a vein, and observe the effects.

10. Supra-renal extract.—(*a*) Make a watery solution of the medulla of a supra-renal gland of an ox, or use the dried compressed tabloids made by Burroughs, Wellcome & Co. Inject a very small quantity into a vein of a rabbit, previously anæsthetised and arranged to record its arterial B.P. Observe the enormous rise of the B.P. The effect lasts a short time, and then passes off. The rise of B.P. can be obtained by again injecting the drug, which produces its effects largely by its action on the smooth muscle of the arteries. An excessively small amount of the substance—called epinephrin, and various other names—suffices to produce the result.

(*b*) Test the effect of a dilute solution of epinephrin or adrenalin on blood-vessels in a mucous membrane—*e.g.*, conjunctiva. What is its value as a hæmostatic?

CENTRAL NERVOUS SYSTEM.

LESSON LXXI.

REFLEX ACTION—ACTION OF POISONS—STRYCHNIN—KNEE-JERK.

1. **Reflex action.**—Destroy the brain of a frog without loss of blood. Place under a bell-jar a normal frog for comparison. Immediately the frog is pithed, on pinching one of its toes, very probably the leg will not be drawn up. After half an hour or more (by this time it has recovered from the shock of the operation), observe—

(*a*) Its *attitude:* its head lies on the plate on which it is placed, while in the intact frog the head is erect, the body and head forming an acute angle with the plate.

(*b*) Its eyes are closed, while those of the intact frog are open. The fore-limbs are either flexed and drawn under the chest, or spread out, so that the body is no longer supported on the nearly vertical fore-limbs, as in the intact frog, but lies flat upon the surface of support. The legs are pulled up towards the body.

(*c*) Absence of respiratory movements in the nostrils and throat, and of spontaneous movements, if left entirely to itself.

(*d*) Turn it on its back; it lies in any position it is placed. [Do this with a normal frog; the latter regains its equilibrium at once.] Extend one leg; it is drawn up towards the body. Pinch the flank with forceps; the leg of the same side is rapidly extended, then drawn up towards the spot stimulated. Pinch sharply the skin round the anus with forceps; both legs are pushed out and pulled up towards the body, as if to dislodge the offending body.

2. Suspend the frog vertically on a suitable support by means of a hook made from a stout pin. At first the legs may move, but they soon cease to do so, and hang motionless.

(*a*) *Mechanical stimuli.*—Pinch the tip of any toe of the right leg; the right leg is drawn up. If a toe of the left leg be pinched, the left leg is drawn up. These are *unilateral reflex* movements.

(*b*) Pinch the tip of one toe very feebly; perhaps only the foot will be flexed at the ankle-joint. Pinch more strongly, and a greater reflex movement will be obtained. It is evident, therefore, that the reflex movement varies not only with the part of the skin stimulated (**1**, *d*), but also with the intensity of the stimulus. Strong stimulation may cause reflex movements in all the limbs. This is due to irradiation of the excitatory state in the cord.

3. Latent period (*Türck's acid method*). **Summation of stimuli.**

(*a*) Dilutions of H_2SO_4 containing 1, 2, 3, and 4 c.c. per litre—*i.e.*, 0·1, 0·2, 0·3, and 0·4 p.c.—are provided. Place some of each in four glasses. Have ready a large beaker of water. Cause a metronome to beat 100 times per minute.

(*b*) Hold the frog in the left hand by means of the hook, and in the right take a glass rod to hold one leg aside. Dip the other leg up to the ankle into the 0·1 p.c. acid, and count the number of beats before it is withdrawn. Afterwards wash the leg in water to remove the acid. Note the time in hundredths of a minute—*i.e.*, the **latent period.** Allow the frog to rest at least five minutes, and repeat the experiment. Take the mean of the two observations—or, if you prefer it, of three or more observations—and this will give the mean "latent period," or "time of reflex action." This, however, does not indicate the minimum time of response between stimulation and reaction. In fact, the chemical method may give a response time—or latent period—of many seconds, whilst an electrical stimulus elicits a fraction of a second as the "time of reflex action."

(*c*) Repeat with suitable intervals of repose the same experiment with acid of 0·2, 0·3, and 0·4 p.c., noting that, as the strength of the acid increases, the latent period becomes shorter, but not in the ratio in which the acid is stronger.

(*d*) If only the longest toe is dipped into the acid, then the summation of stimuli takes place more slowly.

(*e*) *Record time of L.P.*—On a slow drum (1 c.m. in 2 secs.), arrange a time-marker and electric signal (with key in circuit) to write vertically over one another. Dip foot of frog up to ankle in the dilute acid. Note moment by closing signal, and keep it closed until the foot is withdrawn, then open the key of the signal. Count the time and tabulate results.

Strength of acid p.c.	Time before reflex response in seconds.

4. Chemical stimulation. (Purposive characters of reflex.)

(*a*) Either when the frog is lying on its back, or while it is suspended, apply a small piece of filter paper (3 mm. square) dipped in acetic acid to the skin on the inner side of the thigh. At once the leg on that side is violently drawn up, perhaps both legs are drawn up, and the foot of the leg first drawn up is swept over the spot stimulated, as if to remove the paper—*i.e.*, "purposive" co-ordinated movements are executed. Dip the frog in water to remove the acid; allow it to rest for some time. It is easier to obtain irradiation of the reflex movements by chemical than by mechanical stimuli.

(*b*) Repeat the experiment (after 5 mins.), but hold the leg to which the acid is applied. Probably the other leg will move, and the opposite foot remove the paper. Wash, and allow rest.

(*c*) Apply papers to the flank, skin over the gastrocnemius, &c.; in all cases characteristic but different reflex movements will be elicited, if sufficient interval for recovery (5 mins. at least) be allowed between the successive experiments.

(*d*) *Irradiation of reflex movements.*—Strong chemical stimuli applied to one leg usually give rise to movements in the opposite limb and other parts of the body—usually the "irradiation" is first seen to involve response on parts innervated from the same level of the cord on the opposite side; but with stronger stimuli irradiation spreads both up and down the cord, so that reflex response in a leg may be discharged from stimulation of an arm, or *vice versâ*. Strychnin greatly facilitates this result.

(*e*) Destroy the cord, all reflex action is abolished; nerves and muscles retain their excitability, and the heart continues to beat. Muscle and nerve respond to electrical and other stimuli.

5. Strychnin on cord (*Irradiation*).

(*a*) Using a frog with its brain destroyed, inject with a fine glass pipette or a hypodermic syringe into the dorsal lymph-sac a drop of dilute solution of acetate of strychnin (0·5 p.c.).

(*b*) When the poison is absorbed—*i.e.*, within a few minutes—

cutaneous stimulation of any part of the body, even tapping the table, excites general violent tetanic spasms, and not co-ordinated muscular responses, of the whole body. During the convulsive paroxysm the limbs are extended, hard, and rigid, while the trunk is similarly affected. The extensor muscles are more affected than the flexors. The tetanic paroxysm passes off, to be followed by another on the slightest stimulation. The excitability has been so increased that even the slightest stimulus applied to the skin discharges a reflex spasm—*i.e.*, provokes muscular responses which are maximal, so that a *minimal stimulus produces a maximal response.*

(*c*) Destroy the cord. At once the spasms cease. Strychnin, therefore, acts on the cord directly, and not on the muscles and nerves.

(*d*) In another frog, divide the cord below the bulb, the brain in front being destroyed, but the cord intact. Apply a crystal of sulphate of strychnin to the cord. Tetanic spasms soon set in, thus showing that strychnin affects the cord.

(*e*) *Strychnin tetanus.*—Record a tracing of a tetanic convulsion on a slow drum.

(*f*) *Time of reflex action.*—Expose the tendo Achillis and attach it to a recording lever, arrange an automatic break key to stimulate the skin or central end of the sciatic nerve of the opposite limb. Record and measure time of the reflex motor response.

(*g*) *Summation of stimuli.*—Before strychninisation, expose one sciatic nerve, divide it, excite its central end with (i.) single shocks, (ii.) series of interrupted induction shocks, and note minimum distance of primary from secondary coil at which response is obtained in opposite limb. Do the same after strychninisation, and note the result.

6. Latent period of reflex (frog).—As indicated, chemical stimulation does not give even approximately the true "time of reflex action" or latent period. This is best done by electrical stimulation. Arrange to record the contraction of a decerebrated frog's gastrocnemius on a drum. Stimulate the skin or central end of opposite sciatic nerve with strong induction shocks, using an automatic break key in the primary circuit.

7. Potassium chloride or bromide, or chloral.

Prepare a reflex frog as in Lesson LXXI., 1. Test the latent period with dilute sulphuric acid, 0·2 p.c., until constant results are obtained. Inject 2 minims of a 1 p.c. solution of KCl or KBr or C_2HCl_3O, and after ten minutes again test the latent period. Within a short time the latent period will be greatly prolonged. Plot a curve of the results, the abscissa to mark time and the ordinates the length of the latent period. (W. Stirling, "The Effect of Drugs on the Spinal Cord," *Journ. of Anat. and Phys.*, 1892.)

8. Electrical stimulation.—A. *Single induction shocks.*

(*a*) From the secondary coil (key interposed) apply two fine wire metallic electrodes, in the form of two loops, to the skin above the ankle, the electrodes being about ·5 to 1 cm. apart.

(*b*) Stimulate with different strengths of current. No reflex response. A single induction shock does not discharge a reflex movement.

B. *Repeated shocks.*

(*a*) Leave the electrodes *in situ*, but adjust the coil for repeated shocks. On applying a succession of feeble shocks, a reflex response is readily obtained. Make a table of the results obtained.

(*b*) Expose the sciatic nerve without injuring the adjacent parts; on stimulating the skin of the foot or leg as before, a reflex response is readily obtained, but on directly stimulating the sciatic nerve under the same conditions there may be no response until the current is made distinctly stronger. This result is explained (?) by stating that the peripheral terminals are more excitable than the nerve trunk, while others assume that in the sciatic nerve, besides excito-motor (reflex) fibres, there are nerve-fibres which inhibit the action of such fibres. It is said that very strong stimulation of cutaneous nerves also excites the reflex-inhibitory fibres.

(*c*) Isolate any one of the nerves traversing the dorsal lymph-sac of a frog, but leave a small square of skin attached corresponding to the terminals of the nerve. Apply repeated shocks directly to the nerve, in all probability there will be no reflex response, but if the skin be touched with dilute acetic acid, response will probably take place. If, however, strong sulphuric acid be applied to the skin, there will be no response.

9. **Knee-jerk in man** (*Tendon-reflex—so-called*).—(*a*) Sit on a chair and cross the right leg over the left. With the tips of the fingers (self), or a percussion-hammer, or the edge of a thin book strike the right ligamentum patellæ. The right leg will be raised and thrown forward with a jerk, owing to the contraction of the quadriceps muscle. An appreciable time elapses between the striking of the tendon and the jerk. The knee-jerk is almost invariably absent in cases of locomotor ataxia, while it is greatly exaggerated in some other nervous affections; its presence or absence is a most important clinical symptom.

(*b*) The knee-jerk is readily obtained in a *rabbit*, and can easily be demonstrated on an intact rabbit—fixed conveniently on its back—on tapping the patellar tendon with the back of a scalpel.

10. **Record knee-jerk** (man).—The person is seated conveniently on a table, with the legs hanging freely. On the front of the thigh strap a bag (fig. 298) and connect this up with a recording Marey's tambour to write on a rapid drum. Cause the drum to revolve. Record a time-tracing (100 D.V. per sec.). Strike the patellar tendon sharply as in LXXI., 9. The

curve will show two elevations—the first due to the mechanical shock of the blow—*i.e.*, moment of excitation—and the second due to the contraction of the extensor cruris. Measure the latent period or use the special hammer shown in fig. 383.

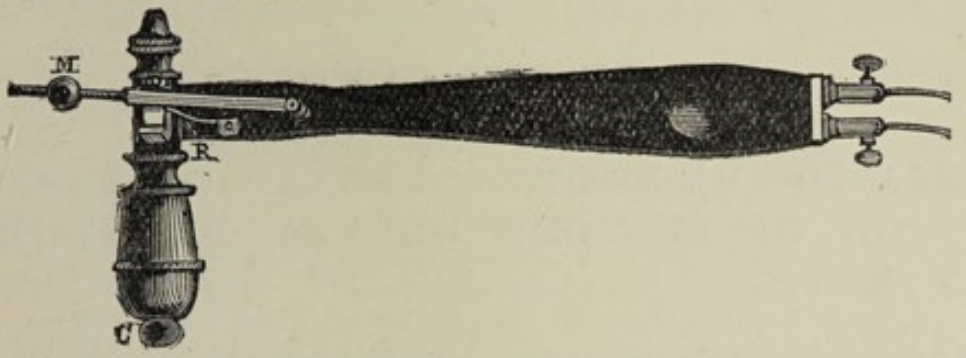


Fig. 383.—This hammer is so arranged as to record the moment the tendon is struck. The ebonite handle is connected at the right-hand end in circuit with an electric signal. When *C* strikes the tendon surface contact is made through *R* and *M*. Made by Verdin.

11. Record knee-jerk response (rabbit).

(*a*) Place a rabbit on its back in a shallow wooden angular trough. It will lie quiescent. Tap the table with a hammer, there is a true reflex response or start Connect one hind leg by means of a thread with a lever recording on a rapid drum. Record true reflex by tapping table. The tap and record will be seen in the tracing.

(*b*) Tap skin over tendo Achillis with paper knife or scalpel, and record the result. The moment of tapping and the mechanical muscular response of the quadriceps cruris will be visible in the tracing. Compare the time in the two cases. The latter is considerably shorter, and the so-called knee-jerk is not a true reflex or "tendon-reflex" (*Waller*).

12. Inhibition of reflex. See Lesson LXXII.

13. Stenson's experiment.—By means of the hand compress the abdominal aorta of a rabbit for a few minutes. There results temporary paralysis of both hind-legs or paraplegia. Soon after the circulation is restored in the cord and lower limbs, the paraplegia disappears. (Modified Stenson's experiment.)

14. Heat affects sensory more than motor nerves.—Sensory nerves (or terminals?) are paralysed by a temperature which may leave motor fibres still functional. Destroy the brain of a frog. Place one leg in water at 36° C. for 5 to 10 mins. Take it out and stimulate the toes of the heated leg—*e.g.*, with dilute acetic acid as for reflex action. There is no reflex-response. Stimulate the intact leg; it gives a reflex response, and so does the heated leg. The latter result shows that its motor nerves are not paralysed. The sensory cutaneous terminals are. Therefore, sensory nerves (or terminals?) are paralysed at a temperature which leaves the motor fibres intact.

15. Action of heat on intact frog.—In summer keep a frog in the hand for some time; it becomes as if it were dead. According to Bernard, it is "anæsthetised," for as soon as it is thrown into cold water it begins to swim.

LESSON LXXII.

SPINAL NERVE-ROOTS—OVERLAP OF DISTRIBUTION OF SENSORY NERVES—BRONDGEEST'S TONUS.

1. Functions of roots of spinal nerves (*Johannes Müller's experiment*, 1832). —To expose the roots destroy the brain of a frog. Lay it on its belly, and make a median incision in the skin of the back, from the neck to the upper end of the urostyle. Turn back the flaps of skin, and carry the incision down to the spines of the vertebræ. With a scraper or blunt knife remove the muscles along each side of the vertebral column, so as to lay bare the arches of the vertebræ. With scissors, or two saw-blades parallel to each other and fitted at a suitable distance into a handle, as devised by Ludwig, cut through the arches of the last vertebra, taking care not to injure the nerves in the spinal canal. Remove successively from below upwards the seventh, sixth, and fifth vertebral arches, when the tenth, ninth, and eighth spinal nerve-roots come into view. The posterior roots are larger, come first into view, and cover the anterior. The roots may be separated by a seeker. Select the largest posterior root—the ninth—and with an aneurism needle carefully place a fine silk thread (say a red one) under it.

(*a*) Tighten the ligature near the cord, and observe movement in some part of the body. Divide the nerve between the cord and the ligature, and observe further movements on division.

(*b*) With the thread gently lift up the *peripheral* or distal end of the nerve-root, place it on well-protected electrodes, and stimulate it with an interrupted current. No movement is observed in the muscles of the limb.

(*c*) Select the posterior root of the eighth nerve, ligature it at some distance from the cord, and divide it on the distal side of the ligature. There is neither contraction of the muscles of the leg nor movement of the body. Place the *central* stump—*i.e.*, the part still connected with the cord—on the electrodes, and stimulate it, when movements will take place in several parts of the body.

(*d*) Divide the posterior roots from the seventh to the tenth nerves. The leg on that side becomes insensible. Turn aside the roots of the divided nerves, and expose the anterior roots, which are very thin and slender. Repeat the preceding experiments on the anterior root of the ninth nerve—*i.e.*, place a ligature round it—tighten the ligature, and divide the nerve between the cord and the ligature. Stimulate the *distal* end with an interrupted current; this causes contraction of the muscles supplied by this root.

From the effects of section and stimulation of the nerve-roots, one concludes that the anterior are motor, and the posterior are sensory. (E. Steinach, "Motorische Functionen hint. Spinalnervenwurzeln," *Pflüger's Arch.*, Bd. 66, 593.)

Overlap of distribution of spinal nerves.—Türck showed in the dog that there is not an absolutely hard and fast limit of distribution of peripheral nerves. His pioneer work has been prosecuted and extended by Sherrington, who has shown conclusively that frontier lines do not exist either in things cutaneous or things sub-cutaneous (C. Sherrington, *Journ. of Phys.*, xiii., 1892).

2. Overlap of distribution of spinal nerves in skin and muscles—frog (C. Sherrington, *Journ. of Phys.*, xiii., 1892).

(*a*) Use a frog freshly killed, and destroy brain by pithing.

"Remove the coccyx with its surrounding tissues (by seizing the tip with forceps and somewhat lifting it, then cutting from behind forward with strong scissors each sciatic plexus), thus exposing seventh, eighth, and ninth spinal nerves. On one side cut through these nerves except the eighth, on the other except the ninth. Hang up the preparation by a thread, so that it is free from contact with anything except the thread.

"(*b*) With fine forceps pinch a toe of one foot. A movement is elicited. This is 'reflex,' and proves the persistence of an afferent channel between the toe and the spinal cord, although two nerves out of the three have been cut through.

"(*c*) Pinch the corresponding toe of the opposite foot. A reflex again ensues, proving similarly existence of an afferent path from it to the cord. The spinal nerve uncut on one side is not the one left uncut on the other; either the distributions of the spinal nerves right and left are asymmetrical, or the stimulated toe receives sensory fibres from two segmental nerves. As the segmental nerves are symmetrically distributed, the latter alternative must be the explanation.

"Repeat the observation on other toes and on the planta and dorsum of the feet, applying the stimuli to right and left alternately, and placing them as symmetrically as possible. It will be found that the skin of the foot contains sensory nerve-endings all over it from each of the two spinal nerves.

"Pith the preparation completely. Fix the lower end of each tibia with a pin to the frog-board in such a way as to let the foot hang over the edge of the board. Slit up the skin over the pre-tibial and post-tibial muscles. Place the preparation in a good light so that any twitching of the post- and pre-tibial muscles can be seen.

"Place a thick cotton thread round the uncut nerve, both right and left. Draw the ligature tight slowly whilst observing the exposed muscles. It will be found that all the muscles of the pre- and post-tibial groups twitch while the ligatures are tightened, both left and right.

"Each of the muscles of these groups is therefore supplied with motor nerve-fibres by each of the two roots excited.

"The contractions can, if desired, be studied, of course, graphically by the myograph" (communicated by *C. S. Sherrington*).

3. Brondgeest's "Tonus."—Expose the sciatic plexus in a pithed frog from behind. Suspend the frog by a hook placed through its nose. Divide the nerves of the sciatic plexus on one side. Observe the position of the corresponding limb—all joints more extended, and longest toe lower on that side. If only the posterior roots of the corresponding nerves be cut, the same result is obtained, showing that the gentle flexures of the limb—state of tonus—are reflex.

LESSON LXXIII.

REACTION-TIME—CEREBRAL HEMISPHERES—HYPNOSIS.

Reaction-time is the interval that elapses between the application of a stimulus to a sense-organ and the moment the stimulus is responded to by the individual, or "conscious reaction in response to a signal" (*Scripture*). For simple reaction-time, or sensori-motor reaction-time, all *discriminations* and *choice* are eliminated by repeating the same sensation and using the same response. Rutherford's results (*Proc. Roy. Soc. Edin.*, 1894) give rather longer periods than those of some German observers. He finds the pendulum-myograph advantageous in experiments on hearing and touch, as successive curves can be superimposed. The mean reaction-time he found to be, for sight, 0·20″ to 0·22″; hearing, 0·15″ to 0·16″; touch, 0·14″ to 0·15″ (cheek), 0·15″ to 0·18″ (skin of finger).

Reaction-time for touch in man. 1. Pendulum-myograph method (*W. Rutherford*).—Two persons are required (examiner A and examinee B), and the observed person should not see what the observer is doing.

(*a*) Arrange the apparatus (fig. 384). The stimulation is done always at the same moment when the pendulum in its swing breaks the primary circuit. It is convenient, as shown in the figure, to use an electro-magnet for releasing the pendulum.

(*b*) The electrodes from the secondary coil are applied to any part of the skin, and the observer, when he feels the shock, closes the "response key," whereby a mark is made on the glass plate. Record time on the plate beforehand (60 or 100 D.V. per sec.).

(*c*) If it is desired for sound, a telephone is placed in the secondary circuit and the observed person responds when he hears the click at the moment of breaking the primary circuit.

Fig. 385 shows the reaction-times result for touch by the pendulum-myograph (fig. 384), chronograph 60 D.V. per sec. The vertical line indicates the moment an induction shock was given to (1) skin of left cheek; (2) left side of neck; (3) left upper arm at insertion of deltoid; (4) left little finger; (5) dorsum of left foot at root of toes. Response signal was always given by

right forefinger. The vibrations following each signal of response result from the momentum of lever (*W. Rutherford*).

2. Recording on drum (also for sight and hearing).

(*a*) Another method is to cause two electro-magnets with writing styles to record on a rapid drum arranged as in fig. 386. One signal with a contact-key in the primary circuit of an induction coil. This is the "stimulating key."

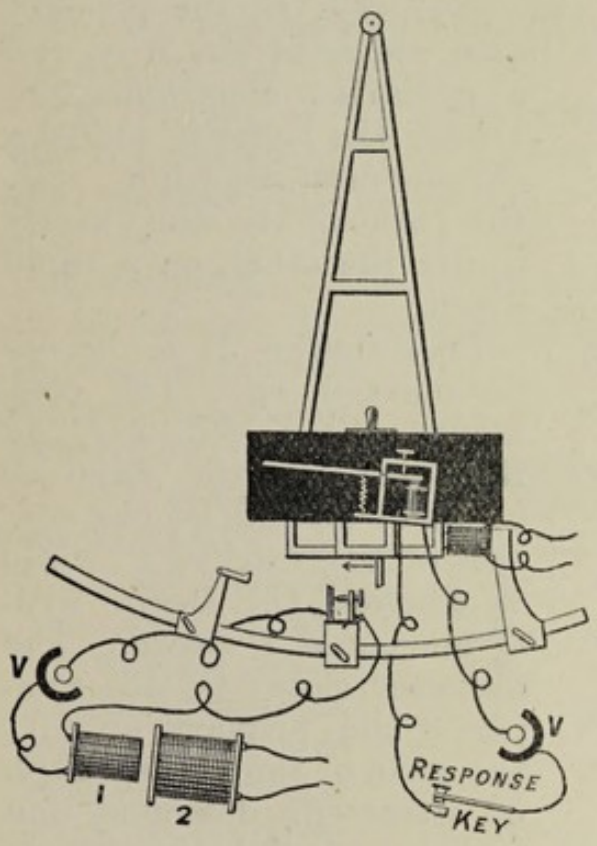


Fig. 384.—Rutherford's scheme of using pendulum-myograph for estimating simple reaction-time.

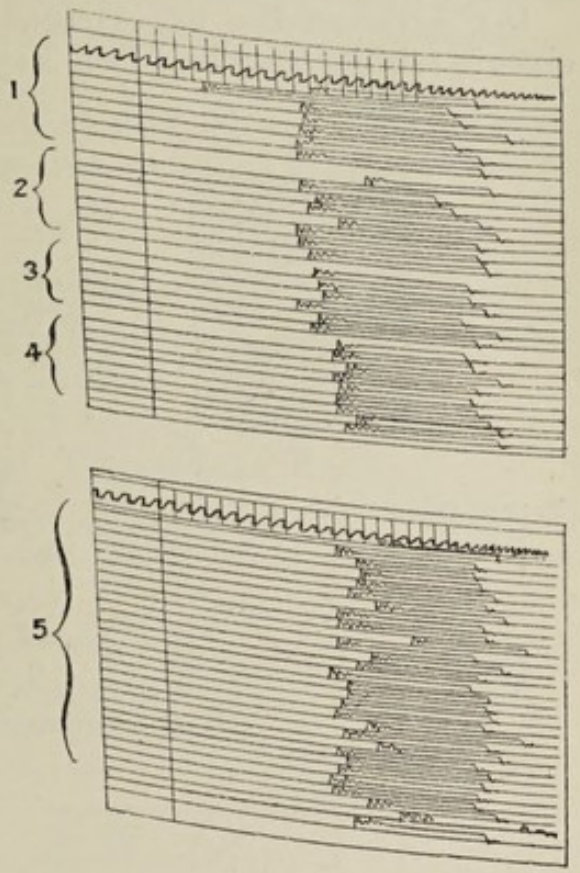


Fig. 385.—Result obtained for simple reaction-time with pendulum-myograph (*Rutherford*). Shock applied in 1, To skin of left cheek; 2, Left side of neck; 3, Left upper arm near deltoid; 4, Left little finger; 5, Dorsum of left foot.

(*b*) The other electro-magnet is in circuit with a battery, and a contact-key—the "response key." If this method be used for touch, the electrodes from the secondary coil are applied to some part of the skin, and the person marks response with the response key.

(*c*) If for sight, a white piece of paper is placed on the electro-magnet style in the primary circuit, and the person responds when he sees this move.

(*d*) If for **hearing**, place a telephone in the stimulating circuit. The observer puts the telephone to his ear, and responds when he hears the click of the induction shock due to closure of the primary circuit Record time.

3. Reaction-time for touch in man.—Two persons, A and B. Apparatus required: coil, batteries, wires, two du Bois keys, two electro-magnets to record, and tuning-fork (100 D.V. per second).

(*a*) Arrange the experiment (fig. 387)—*i.e.*, in the primary circuit (single shocks), two keys in the course of one wire, and a recording electro-magnet. Under the latter is placed a chronograph recording $\frac{1}{100}''$, the point of the one exactly under the other, on a rapid drum.

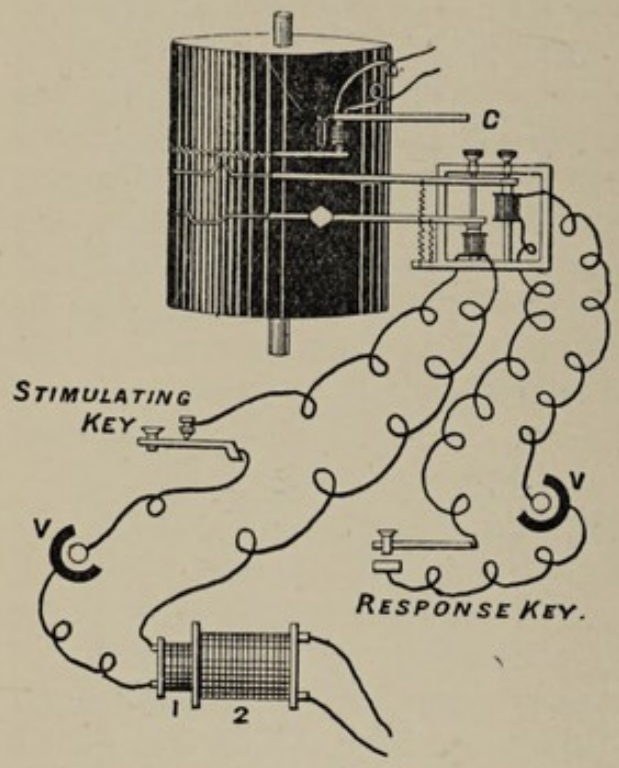


Fig. 386.—Arrangement for simple reaction time (*Rutherford*).

(*b*) Suppose B to be experimented on. The electrodes are placed, say, on the back of the hand or cheek of B, and he has control of key marked P, while A controls O. Begin with O open, and P closed. The observer closes O, this completes the primary circuit; the style of the chronograph is attracted, descends and makes a slightly oblique mark on the paper, which indicates the moment of stimulation. As soon as B feels this he opens key P, the primary current is broken, and the recording lever rises.

(*c*) Measure the time value between the down and up movements of the recording lever. The individual operated on knows the spot to be stimulated, but even with all his attention the results may not be constant. The time varies with the individual, his state of attention, fatigue, part stimulated, and many other factors.

4. Tambour method for reaction time.—(*a*) *Touch.*—Arrange a closed elastic tube in connection with a Marey's tambour, to record on a cylinder moving about 50 mm. per second. Control

with time tracing. Place across the tube a wooden lever. The person observed—blindfolded—places one finger lightly on the lever. The examiner or observer taps this finger, and the moment the examinee feels the tap he presses down the lever. Both the instant of the tap and the compression are marked on the cylinder, and the interval between them indicates the reaction time to a tactile stimulus (*Waller*). It is usually about $\frac{1}{5}$ second. Take an average of at least five observations. Or arrange two Marey's tambours—one to record, the other to receive. The lever of the latter the observed person places on his finger.

(*b*) *Hearing.*—The same apparatus may be used for hearing, but the examinee must not have his hand on the lever when the examiner taps the latter.

5. **Dilemma.**—When the individual has to make a deliberate choice between the parts of the body stimulated the reaction-time is considerably longer. Arrange experiment as in fig. 387, save that the wires from the S coil are made to pass to a Pohl's commutator *without cross-bars*, and provided with two pairs of electrodes. Thus at will the observer can pass the induced shock either through one or other pair of electrodes, the examinee not knowing when the reverser is changed.

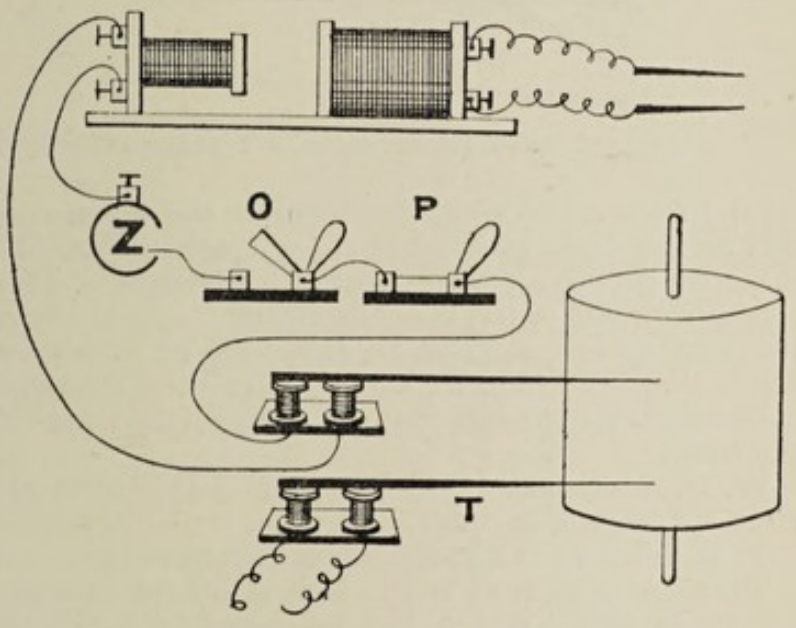


Fig. 387.—Reaction-time for touch in man. *T*, Time signal in circuit with a tuning-fork, vibrating 100 D.V. per second.

6. **Neuramoebimeter** (*Exner*), or **Psychodometer** (*Oberstein*), consists of two uprights, S, with a horizontal axis carrying a spring, F—which vibrates 100 D.V. per second—with a writing-style at its free end (fig. 388). A brass plate, B—*b*, moves in a slot, and carries a smoked glass plate, T, a catch, DG, and a handle, H. The handle, H, pushes up the glass plate and catch, G, until the latter meets the spring, F, and puts F on the

stretch. When the catch, G, is withdrawn, F vibrates, and if the style be arranged to touch the glass, a curve is obtained on the latter.

(*a*) It requires two persons. The observed person places a finger on the knob, K, while the catch, G, and glass plate are pushed up, the former to catch on F, and the style is arranged to write on the glass. The observed person must not look, but close his eyes and listen.

(*b*) The observer suddenly pulls on H, thus discharging the spring, F, which vibrates and produces a note. The moment the observed person hears the sound, he presses the knob, K, and raises the writing-style. Of course, a curve is recorded, and it is easy to calculate the time which has elapsed between the emission of the sound and the reaction by the observed person. Numerous observations must be made, and the mean taken.

(*c*) The instrument may also be used for vision—*i.e.*, when the slide, B—*b*, on being moved uncovers a coloured disc.

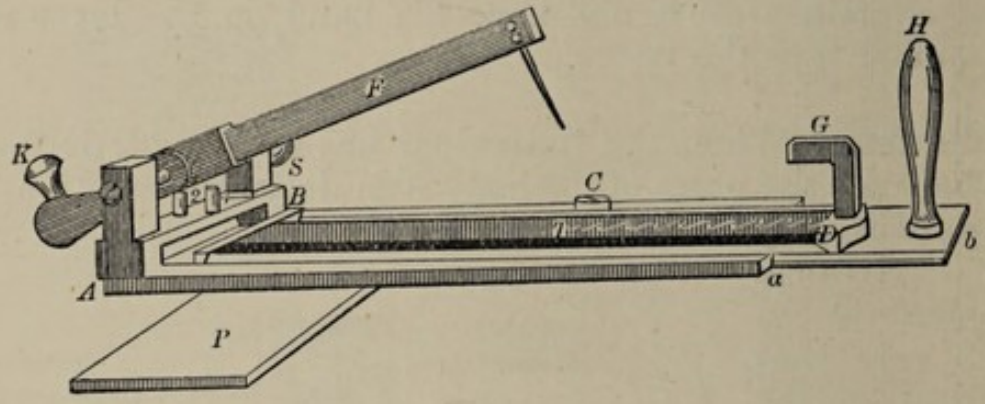


Fig. 388.—Neuramoebimeter of S. Exner, made by Castagna (Vienna).

(*d*) In the more complete form of the apparatus, a key is fixed on one side of the apparatus, so that an electrical current is made or broken at the moment the spring begins to vibrate. The key is placed in the primary circuit of the induction-machine, and the electrodes of the secondary battery are applied to any part of the skin, the observed person depressing the knob, K, when he feels the stimulus. One can thus make numerous experiments on the "Reaction-time" from different parts of the body.

W. G. Smith has devised another simple method (*Journ. of Phys.*, xvii.; *Proc. of Phys. Soc.*, Nov., 1894).

7. Inhibition of equilibration movements.

Take an uninjured frog, place it on its back, and observe that it will not lie in this position, but immediately rights itself. Tie pretty firmly a thick string round each upper arm. This in no way interferes with the movements of the frog; but on placing the animal on its back, it no longer rights itself, but continues to lie in this position for a long time. It may be moved or pulled by the legs, yet it does not regain its normal attitude. Notice the modification of the respiratory movements.

8. A. Kircher's "Experimentum mirabile" (fig. 389).

(*a*) Take a fowl and gently restrain its movements. Bring its bill in contact with a table. With a piece of white chalk draw a line directly outwards from its bill. Hold the animal steadily for a few seconds, and on removing the hands gently, it will be found that the hen lies quiescent and does not move for a considerable time. It may be rolled to one side or the other, yet it lies quiescent.

(*b*) Take a hen, gently restrain its movements, then lay a straw or white thread over the base of its bill. In a short time the animal becomes quiescent. Note the alteration of the heart-beat and the depth and number of the respirations.

That the cerebrum is not the active organ in connection with this so-called hypnotic phenomenon is shown by the fact that Verworn has obtained the same result after removal of the cerebrum (Verworn, *Die sogenannte Hypnose der Thiere*, Jena, 1898).

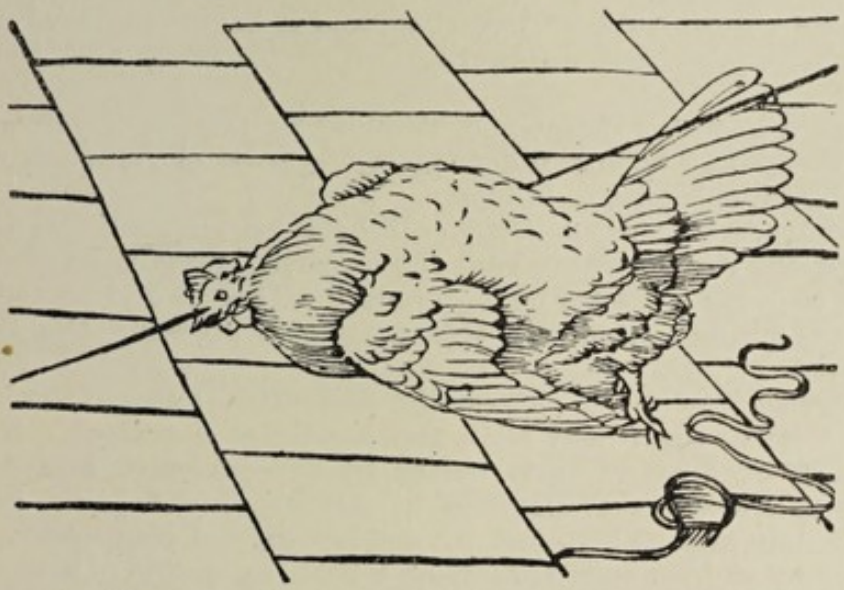


Fig. 389.—Kircher's experimentum mirabile, copied from the original figure.

9. Hypnosis (*crayfish*, *guinea-pig*, *rabbit*).—It is easy to produce this effect in the *crayfish*. If it be placed on its head and its movements restrained, it may remain often for half an hour in this position.

The *guinea-pig*, if seized rapidly and placed on its back, its movements restrained, and then one's hands be gently removed, it readily exhibits the phenomena of so-called hypnosis. It seems to me that the hypnosis is prolonged when an elastic ligature is tied round the animal's thorax. On tapping the table, the animal responds with a sudden start at each tap.

Frogs, if kept over the winter – especially in a dry place—readily become hypnotic if placed on their backs (*Stefanowska*).

10. Reactions of frog without cerebral hemispheres.

In the frog, as shown in fig. 390, the parts of the brain are arranged one behind the other. The guide on the surface of the skull to the posterior end of the cerebral hemispheres is a line connecting the front margins of the two exposed tympanic membranes. The brain may be exposed in a narcotised frog either by means of a small trepan or by severing the parts with a knife. After removal of the cerebral hemispheres, place a little cotton wool in the wound to prevent bleeding.

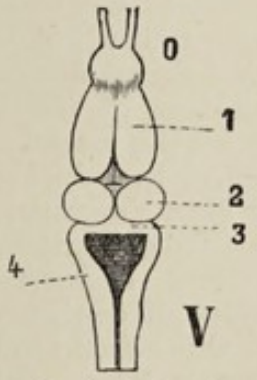


Fig. 390.—Brain of frog from above. 0, Olfactory bulb; 1, Cerebral hemispheres; 2, Optic lobes; 3, Cerebellum; 4, Medulla oblongata.

(*a*) Immediately after the operation the frog lies flat on any surface with its legs extended, but after the shock of the operation—*i.e.*, in about an hour—it draws up its legs and assumes the attitude and appearance of an intact frog, but it makes no spontaneous movements, although it responds readily to external stimulation. It does not feed itself spontaneously.

(*b*) Its eyes are open and its respiratory movements continue.

(*c*) If placed on its back, it immediately rights itself. If placed on the palm of the hand, or on a rough board held horizontally, it sits immovable, but if the board be tilted, or the hand rotated, then, when a certain angle is reached, its equilibrium is disturbed, and it begins to crawl up, until it comes to the top, where its equilibrium is restored, and there it sits motionless.

(*d*) If placed in water it makes continuous swimming movements.

(*e*) It will avoid an opaque object placed in front of it, when one causes it to jump by pinching its hind-legs.

(*f*) *Croaking experiment of Goltz.*—If held up between the thumb and forefinger of the right hand behind the forearm, and if it be pinched, then it responds to every pressure by a "croak." This is due to reflex excitation of the croaking centre. It also croaks on stroking the skin of the back or flanks.

"*Klopfversuch*" *of Goltz.*—Open the abdomen, expose the intestines, and tap the latter vigorously with the handle of a scalpel. Notice temporary arrest of heart. It is better to expose heart and observe its movements by a suspension movement. After several taps the heart ceases to beat. It may also be arrested by sudden digital compression over the abdomen, or by sudden constriction of a limb by means of a ligature; the bulbo-spinal centres are in that condition to which the name "shock" has been applied. These results do not happen if the vagi be cut or the bulb and cord be destroyed. It is a reflex effect, the afferent nerves being the splanchnic and the efferent the vagi (F. Goltz, *Beiträge z. Lehre v. d. Functionen der Nervencentren des Frosches*, Berlin, 1869).

11. Prolonged reflex tone.—A frog (*Rana temporaria*) deprived of its cerebrum (24 hours after operation) has the usual squatting attitude. If its skin along both sides of the vertebral column be gently stroked, the limb muscles will begin to contract and will raise the body into the grotesque attitude shown in fig. 391, an attitude which may be maintained for over half an hour. According to Verworn, this is due to a tonic state of excitation of the nerve cells at the base of the mid-brain, excited reflexly by the mechanical stimulation of the skin. Thus, the after-effects far outlast the duration of the stimulus (*Die sogenannte Hypnose der Thiere*, Max Verworn, Jena, 1898).

Such a frog, if placed on its back, not unfrequently does not regain its equilibrium. It remains so, with the muscles of its limbs extended in a tonic state of contraction (fig. 392). In *R. esculenta*, under the same conditions, this reflex-tonus is but slightly marked.

12. Optic lobes (Inhibition of reflexes).

(*a*) Expose the optic lobes in a frog, after removing the cerebral hemispheres. After recovery, determine the latent period of a reflex mechanical response of the legs by Türck's method (Lesson LXXI.).

(*b*) Apply a crystal of common salt to the optic lobes, and determine the latent period. It is greatly increased, or the reflex may be suppressed altogether (*Setschenow*).

13. Involuntary movements of skeletal muscles.

(*a*) *Ludwig's apparatus* (fig. 393).—Place the button of the apparatus

over any part of the closed fist at rest. Unknown to the person the recording lever will indicate involuntary movements of the hand or arm from time to time.

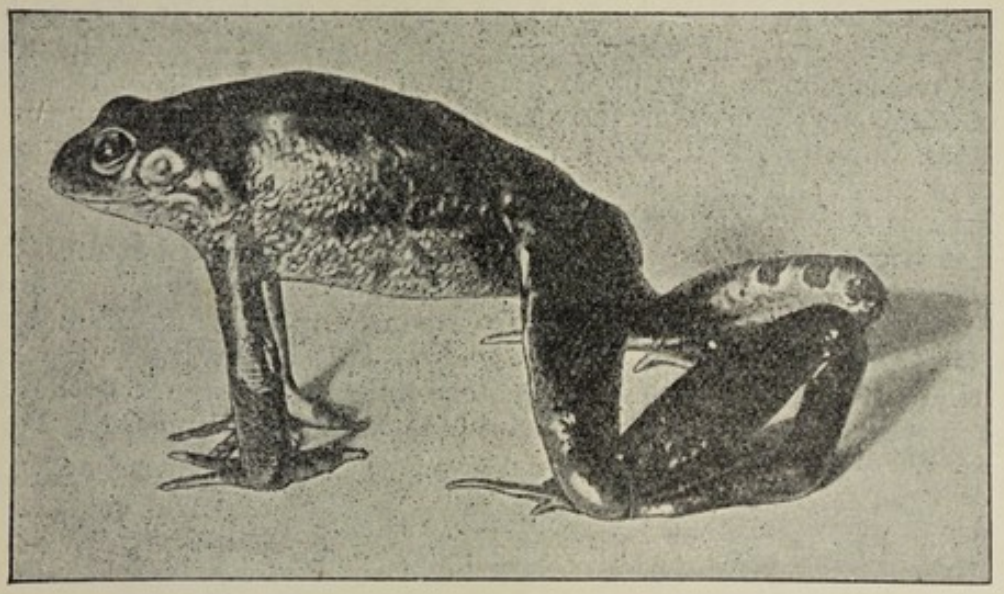


Fig. 391.—Frog deprived of its cerebrum. In the attitude of general reflex tone, the muscles of the limbs and back are in constant contraction. It stands motionless on its extended limbs (*Verworn*).

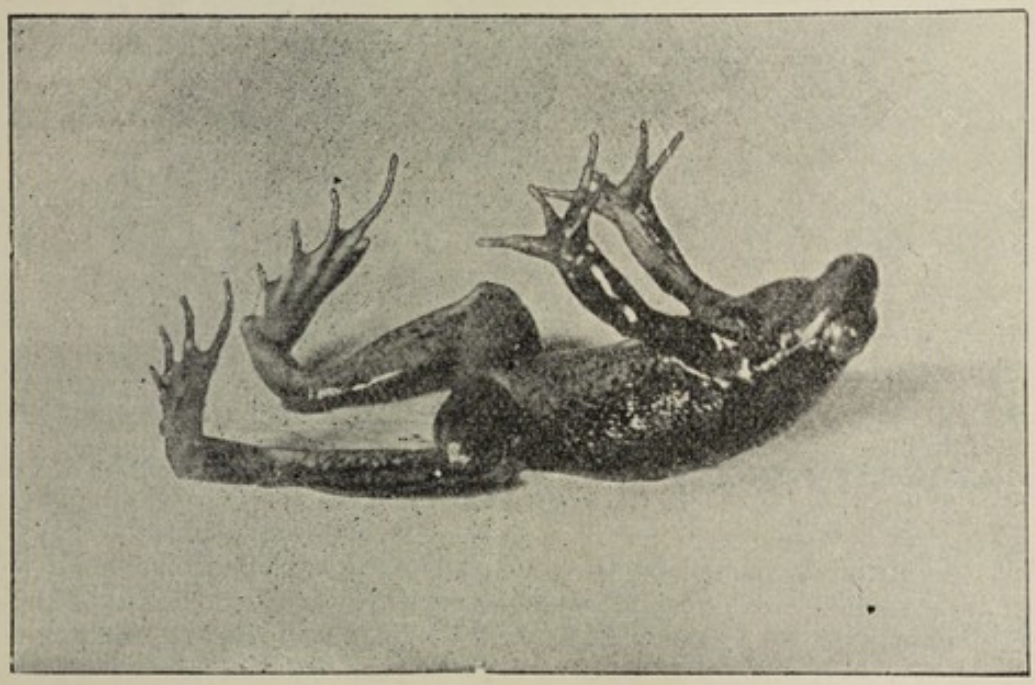


Fig. 392.—Frog deprived of its cerebrum and laid on its back (*Verworn*).

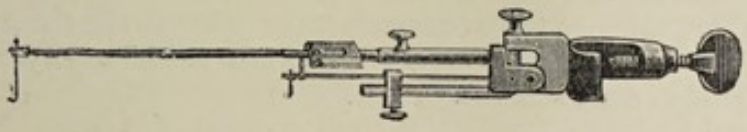


Fig. 393.—Ludwig's apparatus for involuntary movements of the hand. Made by Petzold.

(*b*) *Automatograph.*—This is a sort of planchette board. It consists of a light but strong wooden board (55 × 14 cm.), carrying at one end a block of wood hollowed out to receive the elbow, and at the other a circular hole in which the recording style is fixed. The board is slung by two loops of string and hooked together above where they are fixed over a looped end of a wire by which the whole apparatus is suspended from the ceiling. The style records on smoked paper placed on a sheet of glass. Record the normal indifferent tremors of the arm due to breathing and other involuntary motor impulses. It has been used by Titchener for studying the effects of pleasant and unpleasant sensations—*e.g.*, odours. (E. B. Titchener, *Experimental Psychology*, vol. i., 95.)

14. **Electrical stimulation of cerebral cortex.**—Electrical stimulation of certain regions of the cortex cerebri—the so-called "motor" or "sensori-motor" areas—usually causes muscular response in single muscles or groups of muscles on the opposite side of the body. If it be desired to record the contraction of certain muscles of the fore-limb of a dog under these conditions, provided its approximate cerebral area be stimulated, fig. 394

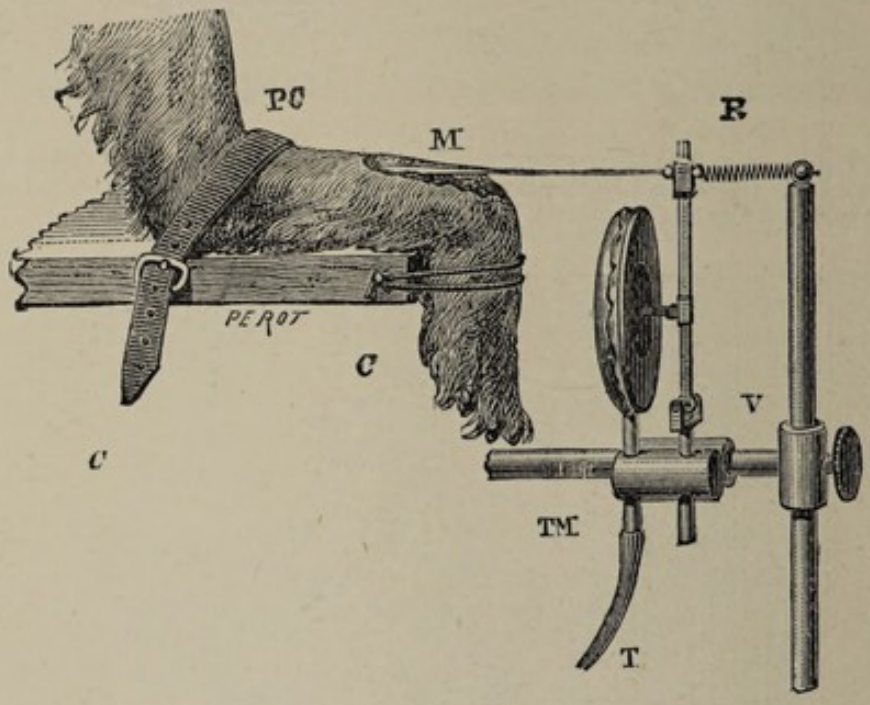


Fig. 394.—Method of recording contraction of a muscle, *M*, in the fore-limb of a dog by the tambour method.

shows the method of recording the contraction of a given muscle, M, whose tendon is attached by a thread to a receiving Marey's tambour. In this way the character of the resulting contraction and the latent period of response have been measured by François-Franck.

15. **Record fluctuations of a volition.**—Arrange a dynamometer to compress a Marey's tambour, or a glass cylinder with a guttapercha piston, and another tambour to record. Let the person choose any pressure, and try to maintain that pressure constant, or use Waller's dynamograph. What the person thinks is a constant pressure is shown by the tracing to exhibit considerable fluctuations.

PHYSIOLOGY OF THE SENSE ORGANS—VISION.

LESSON LXXIV.

FORMATION OF IMAGE — DIFFUSION — ABERRATION — ACCOMMODATION — SCHEINER'S EXPERIMENT — NEAR AND FAR POINTS — PURKINJE'S IMAGES—PHAKOSCOPE—ASTIGMATISM—PUPIL.

1 Formation of inverted image on retina.

(*a*) From a fresh excised ox-eye remove the sclerotic from that part of its posterior segment near the optic nerve. Roll up a piece of blackened paper in the form of a tube, black surface innermost, and place the eye in it with the cornea directed forwards. Look at an object—*e.g.*, a candle-flame—and observe the *inverted* image of the flame shining through the retina and choroid, and notice how the image moves when the candle is moved. Direct the eye towards a window, an inverted image of the window is seen distinctly at a distance of five or six yards, but the image becomes blurred as one approaches. Why? See (*c*).

(*b*) Focus a candle-flame or other object on the ground-glass plate of a photographic camera, and observe the small inverted image.

(*c*) Fix the fresh excised eye of an albino rabbit in du Bois-Reymond's apparatus provided for you, and observe the same phenomenon. The eye is fixed with moist modeller's clay. Observe the effect on the retinal image when a convex or concave lens is placed in front of the cornea. These lenses rotate in front of the cornea, and are attached to the instrument. Try the effect of corresponding glasses on your own eye, and note similarity of effect. The dead eye is adjusted for distant vision, so that the image of distant objects are clearer than near ones.

(*d*) Reverse the eye, and hold its retinal segment next a window. Observe the circular and radiating fibres of the iris.

(*e*) *Volkmann's method.*—Select a blonde, blue-eyed person, let him turn his eyes as far as possible to the right (40°). Hold a lighted candle at an angle of 85° on the right of the examinee. On standing before him one sees through the slightly translucent tissues of the eyeball, near the inner angle of the right eyelid, an inverted retinal image of the flame.

2. Diffusion.

(*a*) Close one eye, and bring a pencil gradually nearer to the other eye. When the needle is 5 to 6 inches distant, it ceases to be distinct, is blurred, dim, and larger. Owing to imperfect accommodation, there is diffusion and consequent dimness and apparent increase of size of the object.

(*b*) Prick a smooth pin-hole in a card, arrange a needle at the proper distance to obtain the previous diffusion effect. Introduce the card between the needle and the eye, bring the card near the eye, and look through the pin-hole. The needle appears distinct and larger—distinct because the diffusion circles are cut off, and larger because the object is nearer the eye.

(*c*) In a dark room place focus with a convex lens the image of a candle-flame on white paper. Introduce a blackened cardboard screen with a narrow hole in it between the light and the lens. A sharp image is obtained only at a certain distance from the lens. If the white screen be nearer or farther away, the image is blurred.

(*d*) Look through a small hole in a card at print placed nearer than the near point (3 to 4 mm.). The print appears sharper, though less bright. The diffusion circles are cut off.

3. Spherical aberration.—In good optical instruments this defect is corrected, and the lenses are "aplanatic."

Make a pin-hole in a blackened card, look at a light placed at a greater distance than the normal distance of accommodation. One sees a radiate figure, with four to eight radii. The figures obtained from opposite eyes will probably differ in shape. The spherical aberration of the eye is not very marked, though there are marked imperfections in the symmetrical disposition of the lenses in relation to the optic axis.

4. Chromatic aberration—Coloured fringes.—In the best optical instruments "achromatic" or "apochromatic" lenses are used—*i.e.*, they are corrected for chromatic aberration. In the eye this is not the case, for the focus for violet lies nearer the lens than that for red.

(*a*) With one eye fix steadily the limit between a white and black surface (*e.g.*, fig. 414), and while doing so bring an opaque card between this eye and the object, the other eye being closed.

Let the edge of the card be parallel to the limit between the white and black surfaces, so as to cover the larger part of the pupil. The margin next the black appears with a yellowish-red fringe when the part of the pupil which lies next the black surface is covered, while there is a bluish-violet fringe in the opposite condition.

(*b*) Make a pin-hole in a blackened card, and behind the hole place a cobalt glass, and look at a gas-flame through it. Cobalt glass allows only the red and violet rays to pass through. Accommodate for the violet rays or approach the light, the flame appears violet, surrounded with a reddish halo; on accommodating for the red, or on receding, the centre is reddish with a violet halo.

(*c*) Place a strip of red paper and one of blue on a black surface, the red appears nearer than the blue. Is it because one makes a greater effort to accommodate for the less refrangible red rays than for the more refrangible blue or violet? Chromatic aberration is increased with dilated pupil.

(*d*) **W. von Bezold's experiment** (b. 1837).—Make a series (5 to 6) of concentric circles, black and white alternately, each 1 mm. thick, the diameter of the whole being about 15 mm. (fig. 395). On looking at these circles when they are placed within the focal distance—*i.e.*, with imperfect accommodation—one sees the white become pink; to some eyes it appears yellow or greenish. One usually notes also at the same time the phenomena of irregular astigmatism in one's own eye. The same is seen on looking at concentric black and white circles, or parallel black and white lines from a distance outside the far point of vision; the white appears red and the black bluish.



Fig. 395.—W. von Bezold's experiment to show chromatic aberration of normal human eye.

(*e*) Bend a platinum wire into a ring half an inch in diameter, and heat it white hot in a Bunsen. Look at it through a pin-hole in a black card, so that the ring lies close to the edge of the field of vision of the pin-hole. Accommodate for centre of the ring, the inner edge of the ring appears blue or violet, and the outer red (*Sanford*).

(*f*) **Wheatstone's fluttering hearts.**—(i.) Make a drawing of a red-coloured heart on a bright blue ground. In a dark room lighted by a candle hold the picture below the level of the eyes, and give it a gentle to and fro motion. On continuing to look at the heart, it will appear to move or flutter over the blue background.

(ii.) On a bright blue ground make a square with black lines and subdivide it into smaller squares. On the same ground make a series of small squares—not coinciding with the previous ones—with red boundaries. On moving the figure to and fro in the shade below the level of one's eyes, one sees the red squares moving to and fro over the black ones. Some see the black moving behind the red. ("Zur Erklärung d. flatternden Herzen," A. Szili, *du Bois-Reymond's Archiv*, 1891, p. 157.)

5. **Accommodation.**—*The eye can accommodate itself for either near or far objects, but not for both at the same time.*

(*a*) Standing near a source of light, close one eye, hold up both forefingers not quite in a line, keeping one finger about 6 to 7 inches (15 cm.) or 16 to 18 inches (50 cm.) from the other eye, and the other forefinger about 16 to 18 inches from the eye. Look at the *near* finger; a distant image is obtained of it, while the far one is blurred or indistinct. Look at the *far* image: it becomes distinct, while the near one becomes blurred. Observe that in accommodating for the near object —positive accommodation—one is conscious of a distinct effort, and practically no sense of effort for a far object.

(*b*) Hold a thin wooden rod or pencil about a foot from the eyes, and look at a distant object. Note that the object appears double. Close the right eye; the left image disappears, and *vice versâ*.

(*c*) Ask some one to note the diameter of your pupil when you accommodate for the near and distant object respectively. In the former case the pupil contracts, in the latter it dilates. Ask a person to accommodate for a distant object, and look at his eye from the side and somewhat from behind; the half of the pupil projects beyond the margin of the cornea. When he looks at a near object in the same line, and without moving the eyeball, observe that the whole pupil and a part of the iris next the observer are projected forwards, owing to the increased curvature of the anterior surface of the lens.

(*d*) At a distance of 6 inches from the eyes hold a veil or thin gauze in front of some printed matter placed at a distance of 2 feet. Close one eye, and with the other one soon sees either the letters distinctly or the fine threads of the veil, but one cannot see both equally distinct at the same time. The eye, therefore, can form a distinct image of a near or distant object, but not of both at the same time; hence the necessity for accommodation.

6. **Scheiner's experiment** (Accommodation and inversion of retinal image) (fig. 396).—The Jesuit Rector, C. Scheiner (1579-1650), published this experiment in 1619. It shows the eye as an optical instrument furnished with lenses and screens, it illustrates the inversion of the retinal image, and it illustrates the laws of double images with a single eye.

(*a*) Prick two fine smooth holes in a card at a distance from each other (1 mm.) less than the diameter of the pupil. Fix two white pins or straws on corks. The person sits with his back to a window. One eye is bandaged. He has a black screen placed about 75 cm. in front of him. Fix the card in a piece of wood

with a groove made in it with a fine saw, and see that the holes are horizontal. Place the white pins in line with the holes—*i.e.*, in the line of vision—the one about 20 cm. and the other about 50 cm. from the card.

(*b*) Close one eye, if it is not already bandaged, and with the other look through the holes at the *near* pin, which will be seen distinctly, while the far pin will be double, but both images are somewhat dim. Look at either pin, it is seen single and sharply defined.

(*c*) With another card, while accommodating for the *near* pin, close the right-hand hole; the right-hand image disappears; and if the left-hand hole be closed, the left-hand image disappears.

(*d*) Accommodate for the *far* pin, it is seen single and distinct; the near pin appears double. Close the right-hand hole, and the left-hand image disappears; and on closing the left-hand hole, the right-hand image disappears.

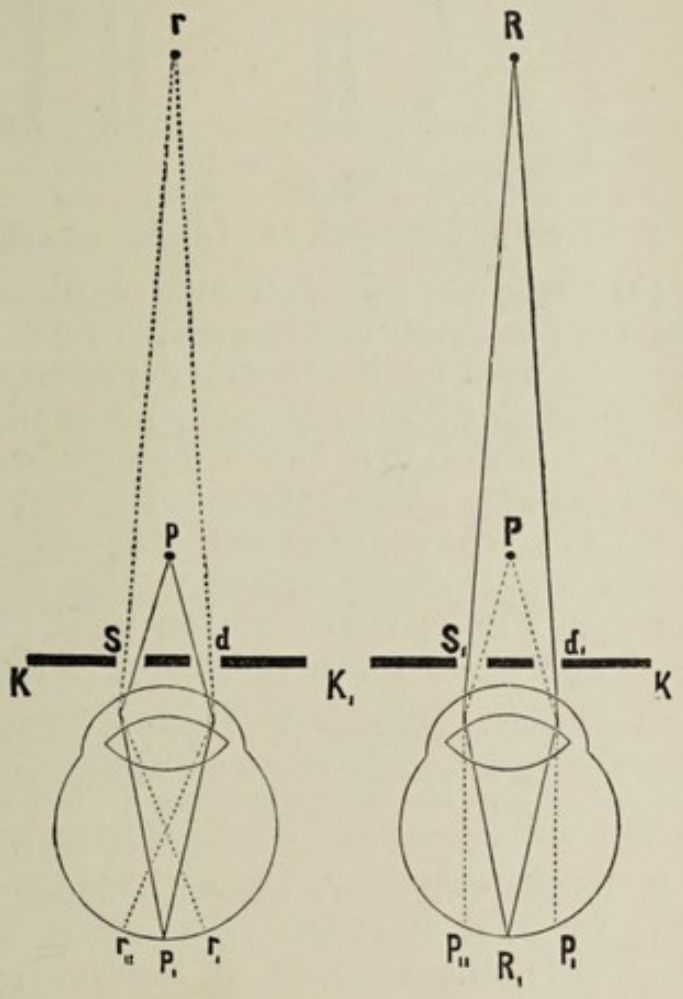


Fig. 396.—Scheiner's experiment.

(*e*) While either pin is fixated, on closing one hole there is no change in the pin fixated, but the whole field becomes darker, and the change in the double images of the non-fixated pin above described are seen.

(*f*) Instead of using a card perforated with two holes, use an apparatus so constructed that one hole is covered with a green and the other with a red glass or coloured gelatin. Repeat the previous observations, noting disappearance of the red or green image, as the case may be.

(*g*) The holes in the card may be made one above the other, but in this case the pin looked at must be horizontal.

(*h*) Make three holes in a piece of cardboard, as in fig. 397, *a*, so that they can be brought simultaneously before one eye, and look at a pin or needle. One sees three images of the needle. On looking at a near object,

the needles are in the position *b*, and at a distant object in that shown in *c*.

(*i*) Miles' experiment.

(i.) Look at a pin through a pin-hole in a card. Accommodate for the pin, move the card to and fro, and note that the pin appears immovable.

(ii.) Accommodate for a distant object beyond the pin, the pin appears to move in the opposite direction to that of the card.

(iii.) Accommodate for a nearer object, and note that the pin appears to move in the same direction as the card.

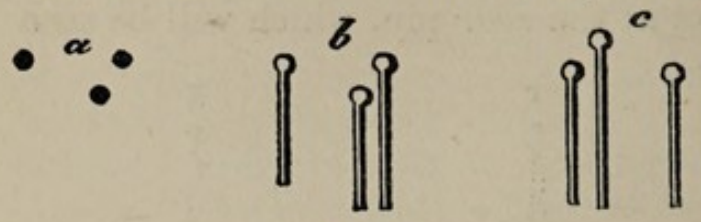


Fig. 397.

7. Near and far points (Limits of accommodation).

(*a*) Close one eye and hold a pin vertically about 10 inches (25 cm.) in front of the other. Look through the two holes in the card used for Scheiner's experiment, and when one distinct image of the needle is seen, gradually approximate the needle to the card; observe that it becomes double at a certain distance from the eye. This indicates the **near point of accommodation.**

(*b*) Hold the card in front of one eye, and gradually walk backwards while looking at the needle, observing when it becomes double. This indicates the **far point of accommodation.** *N.B.*—The experiment (*b*) succeeds best in short-sighted individuals.

(*c*) Determine the near point with a vertical needle and card with horizontal holes, and again with a horizontal needle and a card with the holes vertical. The two measurements do not usually coincide, because the curvature of the cornea is usually different in the two meridians.

8. Purkinje-Sanson's images.—These images were described in 1823 by J. E. Purkinje (1787-1869), and utilised in 1837 for diagnostic purposes by L. J. Sanson (1790-1841).

(*a*) The subject sits in a dark room with all light excluded. Hold a lighted candle to one side of the observed eye and on a level with it. The other eye is closed with a bandage. Ask the examinee to accommodate for a distant object (object at 300 cm.), and look into his eye from the side opposite to the candle, and three reflected images will be seen. At the margin of the pupil, and superficially, one sees a small very bright *erect* virtual image of the candle-flame reflected from the *anterior* (convex) *surface of the cornea.* In the middle of the pupil—but 8 to 12 mm. behind it—there is a second less brilliant, larger, and not sharply defined *erect* virtual image. It is reflected from the less convex *anterior surface of the lens.* The third image, which lies most posteriorly and towards the opposite margin of the pupil, at an apparent

depth of 1 mm., is the smallest of the three, and is a real *inverted* image reflected from the concave *posterior surface of the lens*. On the examinee accommodating for a near object (30 cm.), the pupil contracts, while the middle image—that from the anterior surface of the lens—becomes smaller and comes nearer to the corneal image. This shows that the anterior surface of the lens becomes more convex during accommodation. No change is observed in the corneal image, and hardly any in the third image.

(*b*) Instead of using a candle-flame, cut two small square holes (10 mm. square) in a card, and behind each place a gas-flame, and observe the three pairs of square reflected images. If two images are reflected from each surface, the observation is more easy, because the two middle images approach each other at the same time that they grow smaller.

(*c*) **Physical experiment.**—Standing in front of a large bi-convex lens supported on a stand, hold a watch-glass in the left hand in front of the lens and a few inches from it. Move a lighted candle at the side of this arrangement, and observe the three images described above. Use a convex lens of shorter focus, the images reflected from the lens become smaller.

9. The phakoscope (Helmholtz) is used to demonstrate the change in curvature of the lens, more especially of the anterior surface, during accommodation (fig. 398) [*Helmholtz*, 1821 to 1894].

(*a*) Use it in a dark room. Two persons are required. The observed eye (patient) looks through a hole in the box opposite to *c*, while the observer looks through the hole, *a*, at the side. Place a lighted lamp some distance from the two prisms, *b*, *b'*, so that its light is thrown clearly on the observed eye, and the observer sees two small bright square images of light, when the observed eye looks straight ahead at a distant object. These are the *corneal* images. He should also see in the observed eye two larger less distinct images, from the *anterior surface of the lens*, and two smaller much dimmer images, from the *posterior surface of the lens*. The last are seen with difficulty.

(*b*) Ask the patient to accommodate for a near object—viz., the pin above *c*, keeping the eye unmoved. The middle image becomes smaller and goes nearer to the corneal one, while the other two undergo no perceptible change. At the same time the pupil becomes smaller.

10. Principle of Helmholtz's ophthalmometer.—The principle of this instrument is illustrated by the apparatus of Auber (fig. 399). By means of the ophthalmometer Helmholtz measured the size of Sanson's images and the changes in size during accommodation. If one looks at an object through a plate of glass in a direction at right angles to the surface of the glass, the object is seen single and in its exact position. If one looks at it obliquely or displaces the glass, the image appears displaced to the right or left according to the angular rotation of the plate. In Helmholtz's instrument two glass plates (fig. 399) were placed one above the other, and could be rotated in opposite directions round a vertical axis. One looks through the glass plates at two black lines painted on a sheet of glass.

On looking at the two lines through the two glass plates, and on rotating the latter in opposite directions, one image is displaced to the right and the other to the left, and the object appears double. One rotates the plates until the inner edge of the one image coincides with the corresponding edge of the other, so that each image has been displaced exactly to the extent of the size of the object. The size of the image can be calculated, provided one knows the refractive index of the glass plates, their thickness, and the angle formed by them. In the ophthalmometer the extent of rotation is read off on a disc placed outside the box containing the glass plates.

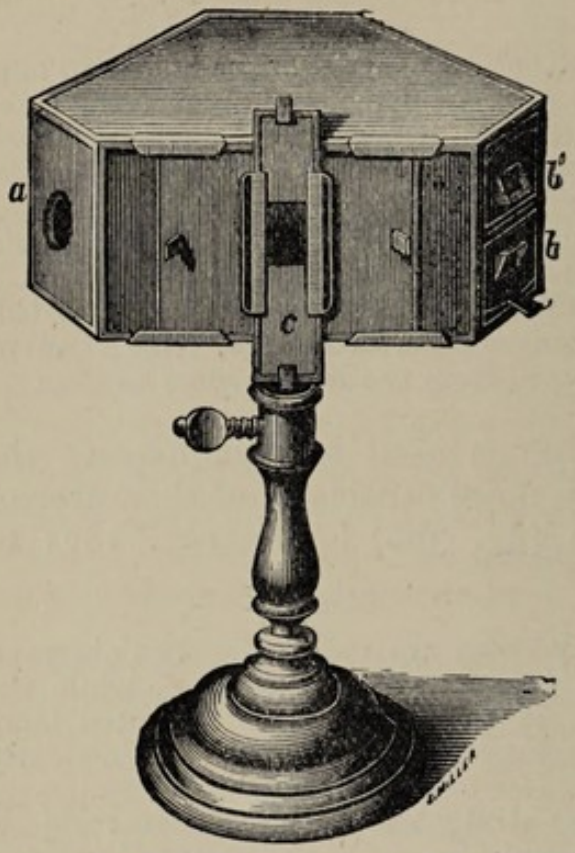


Fig. 398.—Phakoscope. *a*, Hole for observer's eye; *b*, *b'*, Prisms; *c*, Carries a pin for the observed eye to fix as its near point.

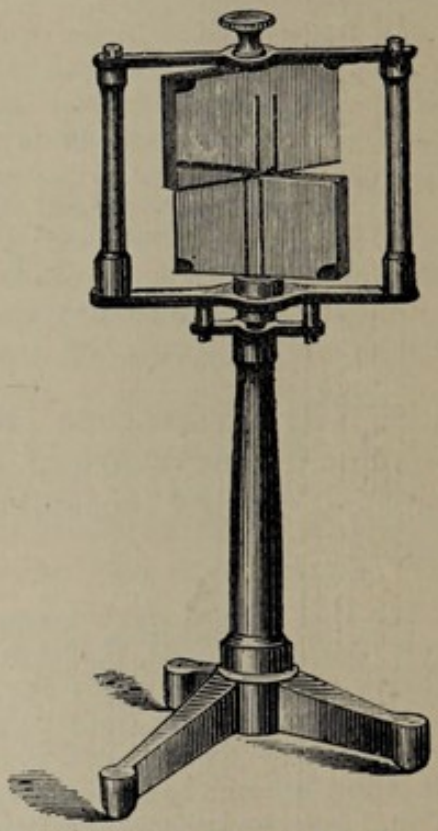


Fig. 399.—Auber's model to show principle of ophthalmometer of Helmholtz. Made by Petzold.

11. Line of accommodation—*i.e.*, the eye does not accommodate for a point, but for a series of points, all of which are equally sharply perceived with a certain accommodation.

(*a*) Stretch a white thread about a metre long on a black board. Through two narrow slits, about 2 mm. apart, in a blackened card, focus with one eye a particular part of the thread, which must be in the optic axis. A part of the thread on the far and near side of the point focussed is quite distinct and linear, but beyond or nearer than this the thread is double, and diverges from the point focussed.

(*b*) Make a small black spot with ink on a glass plate, and hold it in front of any printed matter. Bring the eye as close as possible to the glass plate without losing distinct definition of the point. At one and the same time only one of the objects can be seen; but not the point and

the print equally sharply defined. Remove the eye gradually from the glass plate, and ultimately at a certain distance both the point and print will be equally distinct; the point and print mark the extreme limits of the line of accommodation.

12. Astigmatism is usually due to unequal curvature of the cornea in different meridians—*i.e.*, the surface of the cornea is not part of a perfect sphere. Astigmatism is not uncommon, and usually the curvature of the cornea is greater in the vertical than in the horizontal meridian. This is "regular astigmatism." In such a "spoon-shaped" cornea a point of light is not focussed as a point—"pin focus," but is linear or "line focus."

(*a*) Draw on a card two black lines of equal thickness, intersecting each other at right angles. Fix it vertically at the far limit of accommodation and look at it. Probably either the vertical or horizontal line will be seen more distinctly. Test each eye separately. The line most distinct corresponds to the meridian of least curvature of the cornea.

(*b*) Instead of a cross, draw a star, the lines of equal thickness radiating at equal angles from the centre. Repeat the previous observations, observing in which meridian the lines are most distinct. Try effect of increasing and decreasing the distance.

(*c*) Repeat these observations with the "astigmatic clock" suspended on the wall, or with appropriate illustrations given in *Snellen's* "Test-types."

(*d*) Make a series of concentric circles of equal thickness and tint, about one-eighth of an inch apart, on a card. Make a small hole in the centre of the card. Look steadily at the centre of the card held at some distance. All the parts will not be equally distinct. Bring the card towards you, noting in which diameter the lines appear most distinct.

(*e*) This card may be used in another way. Hold the card in front of, and with the circles directed towards the eye of another person—especially one with astigmatism; place your own eye behind the hole in the card and look into the observed eye, noting the reflection of the circles to be seen in the eye. Observe in which meridian the circles are most distinct, and if there be any perceptible difference in the thickness and distinctness of the circles.

(*f*) Fix a fine wire or needle vertically in a piece of wood moving in a slot, and similarly fix another needle or wire horizontally. Move the needles until both can be seen distinctly at the same time, when it will be found that the needles are some distance apart; usually the horizontal one is the nearer.

13. Diplopia monophthalmica.

Make a small hole in a black card, hold it at some distance, and with one eye look through it at a luminous point, the eye being accommodated for a distant object. One sees either several objects (feeble light) or an irregular radiate figure with four to eight rays. Move the paper, and the long rays remain in the same position. Compare the figure obtained from the other eye. It will very likely be different.

14. Movements of iris.—(i.) Look at the white shade of an ordinary reading-lamp. Close the left eye and with the right look through a pin-hole. Note the size of the (slightly dull) circular visual field. Open the left eye, the field becomes brighter and *smaller* (contraction of pupil); close the left eye, after an appreciable time, the field (now slightly dull) is seen gradually to expand. One can thus observe the rate of movement of one's own iris.

(ii.) **Pupil-reflex.**

Place a person in front of a bright light opposite a window, and let him look at the light, or place oneself opposite a well-illuminated mirror. Close one eye and observe the diameter of the other pupil. Suddenly remove the hand from the closed eye, light falls upon it; the pupil of the other eye contracts.

15. Drugs on pupil.—On a rabbit or dog try the effect of atropin applied to the conjunctiva, and then physostigmin (1 p.c.) : 1 p.c. cocain (anæsthesia and pupil).

16. Drugs on excised eyeball.—These experiments should be done when frogs are used for other experiments. Place one eyeball in normal saline, the other in 1 p.c. *physostigmin*, in a dark place, and note size of pupil from time to time. Do likewise with 1 p.c. *cocain*, 1 p.c. atropin, 1 p.c. nicotin.

17. Light on excised eyeball.—Keep an excised eye of frog or eel in a dark box for some time. Expose it to light. The pupil contracts. It appears that this is not due to a reflex from the retina (*Steinach*). Indeed, if the excised eye of an eel be kept cool, the sphincter pupillæ still responds to light or to electric shocks, even after several days (E. Guth, *Pflüger's Arch.*, lxxxv., 119, 1901). The contraction of the pupil in these cases seems to be due to the direct action of the light on the muscular tissue of the iris.

[For Langley and Anderson's paper "On the mechanism of the movements of the iris," see *Journ. of Phys.*, xiii., 554, 1892.]

18. Pupillometry.—Changes in the size of the pupil are brought about normally by very varied conditions:—(i.) Amount of light, (ii.) accommodation and convergence, (iii.) stimulation of other parts of the body, (iv.) physical, but especially emotional, conditions. Its response to certain drugs and abnormal changes in the nervous system renders it an important index. The pupil contracts when light falls on the corresponding eye—*direct reaction.* Note, however, that the other pupil contracts also—*consensual reaction.* The contraction of the pupil accompanying convergence is an *associated movement.*

19. Pupil of albino rabbit.—The pupil in albinos appears red, although in other animals it is black. In the albino it is red owing to the absence of pigment in the choroid and iris, so that

light is admitted through the sclerotic and choroid and is reflected from the interior of the eyeball through the pupil to the eye of the observer.

Place in front of the eye of an albino rabbit a black screen with a hole in it of exactly the same size as the pupil. Let the hole and pupil correspond in position. The pupil appears black, as the card arrests the lateral rays that fall upon the eyeball.

20. Pupil appears larger than it really is.—The pupil is magnified by the refractive action of the cornea and aqueous humour.

To see the pupil at its exact size, an excised eyeball must be observed in water. If a glass model of a pupil be taken, and then be covered by another thick plano-concavo-convex glass in shape like the cornea, the pupil at once appears larger.

21. Ludwig's apparatus for vision of a point (fig. 400).

The black plate is fixed in the slot so that either a slit or a hole is just above the handle of the instrument. Remove from the instrument the carrier with the steel point, and on the bar of the instrument place the vertical slit of the black plate (visual) near the eye. There is a movable black plate with a small hole in it. On looking at this small hole through a vertical slit it appears oval from above downwards, while with a horizontal slit the round hole appears drawn out laterally. If there be two small holes near each other in the visual plate, then at a certain distance two are seen in the movable plate. If the movable plate be removed, and the steel point put in its place, on using the large hole in the visual plate, and bringing the steel point towards the eye, after a time one ceases to see it distinctly, or if seen it is blurred. On using the small hole in the visual plate, the rod appears distinct.

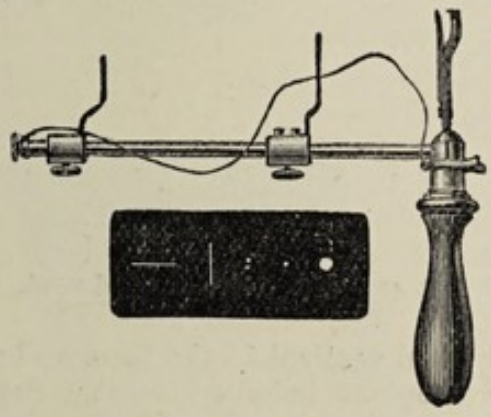


Fig. 400.—Ludwig's apparatus for vision of a point. Made by Petzold.

22. Listing's "Reduced Eye" (1853).—The various dioptric media of the eye may be considered as equal to a single substance with a refractive index of 1·35 and a single spherical surface of radius 5·1248 mm. The position of the nodal point is 5 mm. behind the refractive surface, and the principal focus 15 mm. behind this. This latter value is of special importance in enabling one to calculate the size of a retinal image—the size and distance of the object being known (J. B. Listing, 1808 to 1882).

LESSON LXXV.

BLIND SPOT—FOVEA CENTRALIS—DIRECT VISION—CLERK-MAXWELL'S EXPERIMENT—PHOSPHENES—RETINAL SHADOWS—ADAPTATION AFTER-IMAGES.

1. Blind spot.—The entrance of the optic nerve does not respond to light.

(*a*) Marriotte's experiment (fig. 401).—On a white card make a cross and a large dot, either black or coloured. Hold the card vertically about 10 inches from the right eye, the left being closed. Look steadily at the cross with the right eye, when both the cross and the circle will be seen. Gradually approach the card towards the eye, keeping the axis of vision fixed on the cross. At a certain distance the circle will disappear—*i.e.*, when its image falls on the entrance of the optic nerve. On bringing the card nearer, the circle reappears, the cross, of course, being visible all the time (*Marriotte,* † 1684).

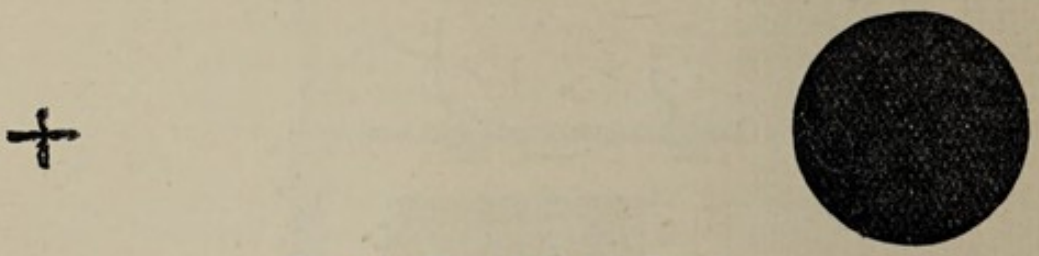


Fig. 401.—Marriotte's experiment.

(*b*) Or place the flat hand vertical to the face, and with its edge touching the nose so as to form a septum between the two fields of vision. Fix the cross in fig. 402, keep both eyes open, and on moving the paper to and fro at a certain distance both black dots will disappear.



Fig. 402.

(*c*) *Filling in the blind spot.*—Close left eye, fix the point *a* (fig. 403); on moving the paper a certain distance (about 16 cm.), one sees a complets cross, and to most observers the horizontal bar appears uppermost. Thue we have the blind spot filled out.

(*d*) *Shut right eye, and fix left on star.*

* Read this line slowly. Do you see the star all the time?

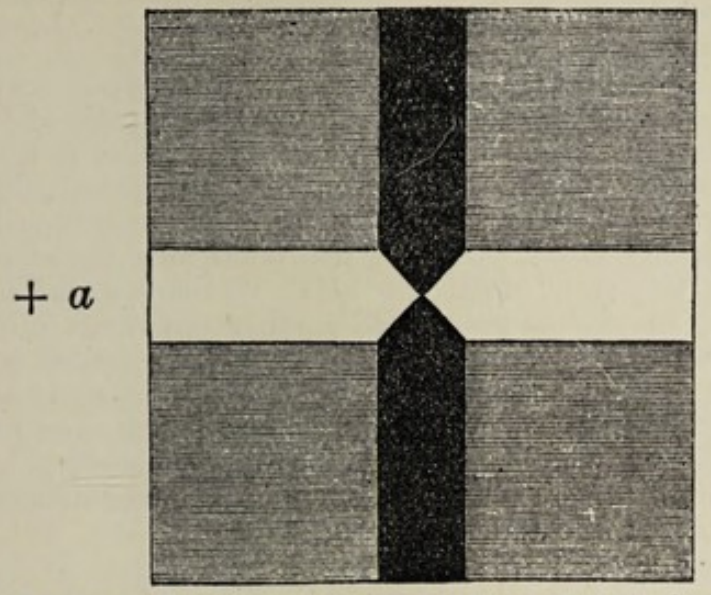


Fig. 403.—Experiment showing filling out of blind spot.

(*e*) Hold this page at seven inches (17 cm.), close left eye, and fixate the star. A and B will be seen, but the circle is not.

* A O B

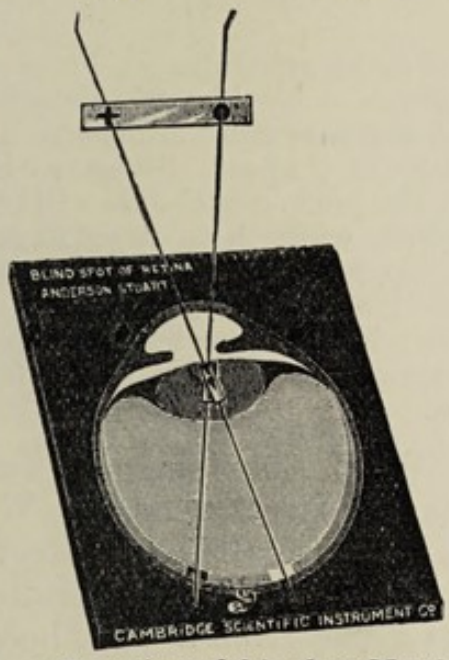


Fig. 404.—Anderson Stuart's model to show Marriotte's experiment.

(*f*) **Volkmann's experiment on blind spot.**

Look at the spot *a* (fig. 405) with one eye, the gap, *b*, *c*, disappears when

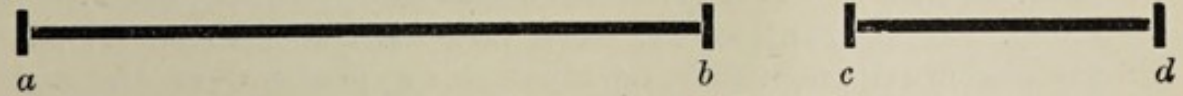


Fig. 405.—Volkmann's experiment on the blind spot.

it falls on the blind spot and the line looks continuous; the points *b* and *c* appear as if placed in the same point of the field of vision, so that the parts of the retina in the periphery of the blind spot behave as if two diametrically opposite points approached each other.

2. Map out blind spot.

Make a cross on the centre of a sheet of white paper, and place it on a table about 10 or 12 inches (30 cm.) from you. Close the left eye, and look steadily at the cross with the right. Wrap a penholder in white paper—or use quill—leaving only the tip of the pen-point projecting; dip the latter in ink, or dip the point of a white feather in ink, and keeping the head steady and the axis of vision fixed, place the pen-point near the cross, and gradually move it to the right until the black becomes invisible. Mark this spot. Carry the blackened point still farther outwards until it becomes visible again. Mark this outer limit. These two points give the outer and inner limits of the blind spot. Begin again, moving the pencil first in an upward and then in a downward direction, in each case marking where the pencil becomes invisible. If this be done in several diameters, an outline of the blind spot is obtained, even little prominences showing the retinal vessels being indicated.

3. Size of blind spot.

Helmholtz gives the following formula:—When f is the distance of the eye from the paper, F the distance of the second nodal point from the retina—usually 15 mm.—d the diameter of the sketch of the blind spot drawn on the paper, and D the corresponding size of the blind spot—

$$\frac{f}{F} = \frac{d}{D}.$$

4. Acuity of vision of fovea centralis.

(*a*) On a horizontal plane—a blackboard—describe a semicircle with a radius equal to that of the near point of vision, and fix in the semicircle pins at an angular distance of 5° apart. Close one eye, and with the other look at the central pin; the pins on each side will be seen distinctly; those at 10° begin to be indistinct, while those at 30° to 40° are not seen at all.

(*b*) *Minimum visible.*—At a distance of 5 feet look at a series of vertical parallel lines alternately black and white, each ·5 mm. wide. A normal eye will distinguish them; if not, approach the object until they are seen distinctly. The discriminative power for space diminishes rapidly in all directions from the fovea.

5. Direct vision.—When the image of an object falls on the fovea centralis, we have "direct vision." When it falls on any other part of the retina, it is called "indirect vision." Vision—spectral—is most acute at the fovea centralis of the yellow spot.

(*a*) Standing about 2 feet from a wall, hold up a pen at arm's length between you and the wall. Look steadily at a fixed spot on the wall, seeing the pen distinctly all the time. Move the pen gradually to one side; first one fails to see the hole in the nib, and as the pen is carried outwards one fails to recognise it as a pen. Hence, in looking at a large surface, to see it distinctly one must unconsciously move his eyeballs over the surface to get a distinct impression thereof.

(*b*) Make two black dots on a card quite close together, so that when looked at they are seen as two. Hold up the left index-finger, look steadily at it, and place the card with the dots beside the finger. Move the card outwards, inwards, upwards, and downwards successively, and note that as the dots are moved towards the periphery they appear as one, but not at equal distances from the fixed point in all meridians. For convenience, the card may be moved along a rod, movable on a vertical support.

6. Clerk-Maxwell's experiment—Yellow spot.

(*a*) A strong, watery, clear, filtered solution of chrome alum (pale slate) is placed in a clear glass bottle with flat sides. Close the eyes for a minute or so, open them, and, while holding the chrome alum solution between one eye and a white cloud, look through the solution. An elliptical spot, rosy in colour, will be seen in the otherwise greenish field of vision. The size of the rosy spot depends on the distance to which it is projected. The pigment in the yellow spot absorbs the blue-green rays between E and F of the spectrum, hence the remaining rays, red and greenish-blue, which pass through the chrome alum give a rose colour. The experiment may be made with purplish gelatine sheets (Hering, *Pflüger's Archiv*, liv., 1893).

(*b*) The yellow spot may be shown by looking through a blue glass (10 secs.) at a white surface. Then close one eye and keep the other open for a few seconds. A small dark patch—it may be rosy in tint—surrounded by a lighter larger area will be seen. The yellow absorbs some of the blue rays.

(*c*) Stimulation of one eye by intermittent light may show it—*e.g.*, moving the spread finger rapidly before it.

7. Bergmann's experiment.—Make a series of parallel vertical black lines, 2 mm. in diameter, on white paper, with equal white areas intervening between them. Look at them in a good light, at a distance of 2 to 3 metres. In a short time the lines will appear as in fig. 406, A. Why? Because of the manner in which the images of the lines fall on the cones in the yellow spot, as shown in B.

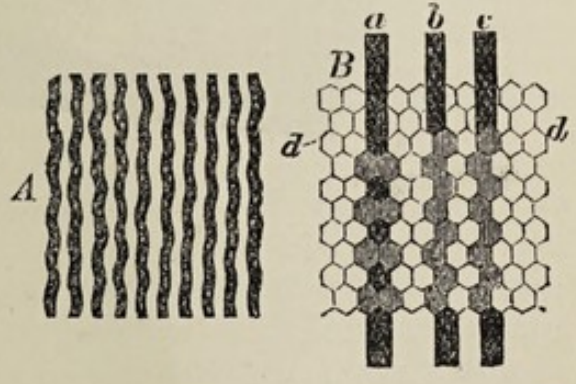


Fig. 406.—Bergmann's experiment.

8. Phosphenes.

Press the tip of one finger, or the end of a pencil, firmly against the inner corner of the closed eye, turned as far as possible towards the nose. A brilliant circular patch, with a steel-grey centre and yellow

circumference, is seen in the *field of vision* and on the opposite side. It has the same shape as the compressing body. Press any other part of the eyeball; the same spectrum is seen, and always on the opposite side. Impressions made on the terminations of the optic nerve are referred outside the eye—*i.e.*, beyond into space. The phosphene is seen in the upper half if the lower is pressed, and *vice versâ*.

9. Shadows of retinal blood-vessels on fovea.

Move, with a circular motion, a blackened card with a pin-hole in its centre in front of one eye, looking through the pin-hole at a white cloud. Soon a punctated field appears with the outlines of the capillaries of the retina. The oval shape of the yellow spot is also seen, and it will be noticed that the blood-vessels do not enter the fovea centralis. Move the card vertically, when the horizontal vessels are more distinct. On moving it horizontally, the vertical ones are more distinct. Some observers recommend that a slip of blue glass be held behind the hole in the opaque card; but this is unnecessary.

10. Corresponding points on retina—*i.e.*, points whose stimulation gives rise to single vision. They are to be regarded as

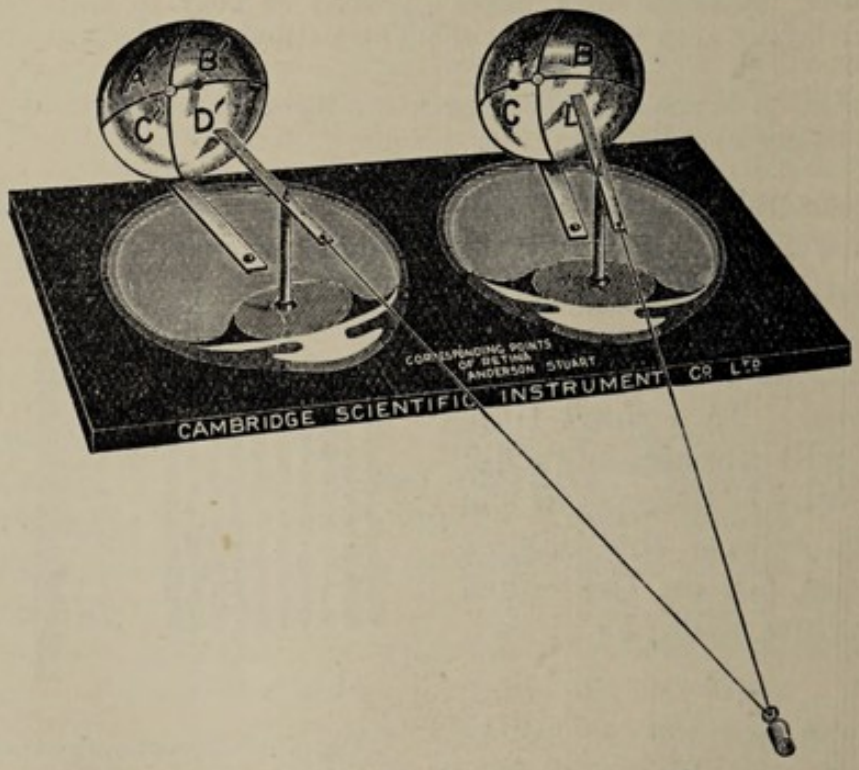


Fig. 407.—Anderson Stuart's model for showing corresponding points on retina.

physiological points acting in harmony rather than accurately located anatomical points. With binocular vision an object out-

side the body gives two images on the corresponding points of the retina. See the object single (fig. 407), but if the images fall on non-corresponding points, we have diplopia or double vision.

11. Outward reference of visual perceptions.

Physically considered, the image on the retina is inverted (Lesson LXXIV., 1). This is also shown by retinal shadows.

(*a*) *Retinal shadows* (Le Cat's experiment).—Hold a card with a pin-hole in front of the eye. Bring the head of a pin from below upwards between the eye and the pin-hole until it comes in exact line with the pin-hole. The pin in the centre of the circle of diffusion of the pin-hole casts a shadow on the retina, and appears as a shadow-inverted image of the head of the pin. The rays as they enter the eye are divergent, and cast a shadow. The shadow on the retina is erect, but is perceived as inverted (*N. E. Le Cat*, 1700 to 1768).

12. Purkinje's figures (Figures of retinal blood-vessels).

In a dark room light a candle, and stand in front of a monochromatic wall, or hang up a large white sheet, and while looking steadily with one eye towards the wall or sheet, accommodating the eye for a distant object, hold the candle close to the side of that eye, well out of the field of vision—downwards and laterally from the eye—and move the candle up and down. It is better to direct the eye outwards, keeping it accommodated for a distant object. Ere long, dark somewhat red-brown branching lines, shadows of the retinal vessels, will be seen on a red background, due to the shadows cast by the retinal vessels on the percipient parts of the retina. Therefore the parts of the retina stimulated by light must lie behind the retinal blood-vessels. If the candle be moved in a vertical plane, the shadows move upwards or downwards with the light. If the light be moved horizontally, the shadows move in an opposite direction.

Entoptical vision.—By this is meant the visual perception of objects situated within one's own eye.

13. Muscæ volitantes (Particles in media of eye or on its surface).

(*a*) Light a candle in a dark room; at a distance from it place a black screen with a pin-hole in it. Focus with a convex lens the image of the flame on the hole in the screen. Look through the hole with one eye. On the illuminated part of the lens images of dots and threads due to objects within the eyeball are seen.

(*b*) Rays of light proceeding from a point at or preferably within the anterior focus of the eye—*i.e.*, 13 mm. or less from the cornea—cast a

shadow of any object within the eyeball, because the rays fall parallel on the retina.

Look through a pin-hole in a card close to the eyeball at an illuminated surface—*e.g.*, a white lamp-shade, or white sky. The margins of the aperture become luminous—*i.e.*, they are the luminous body. One sees such floating objects as are present in the media of one's eye, the "muscæ volitantes."

14. Inversion of shadows on retina.

Make three pin-holes in a card, and arrange them in a triangle close to each other. Hold the card 4 or 5 inches from the right eye, and look through the holes at a bright sky or lamp. Close the left eye, and in front of the right hold a pin so that it just touches the eyelashes. An inverted image of the pin will be seen in each pin-hole. Retinal images, as we have seen, are inverted on the retina, shadows on the retina are erect, and therefore the latter, on being projected outwards into space, are seen inverted.

15. Duration of impressions on retina.

On a circular white disc, about half-way between the centre and circumference, fix a small black oblong disc, and rapidly rotate it by means of a rotating wheel. A grey ring appears, showing that impressions on the retina last a certain time.

16. Talbot (1834)—Plateau (1835) law.—When momentary stimuli—white, black—follow one another with sufficient rapidity, a grey once produced is not changed by increased rapidity of rotation of the disc exciting the sensation. The intensity of the light impression is quite independent of the absolute duration of the periods of illumination and shade.

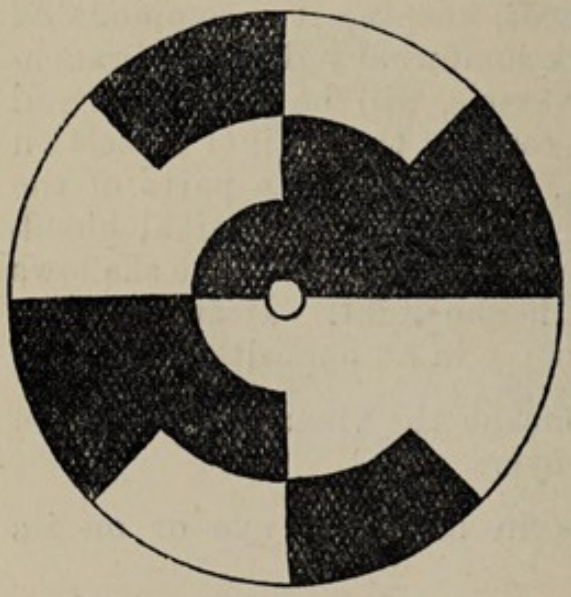


Fig. 408.

Rotate a disc like fig. 408 twenty-five times per second. The period in which illumination and shade alternately lasts for the inner zone is $\frac{1}{25}$ sec., for the middle $\frac{1}{50}$, and for the outer zone $\frac{1}{100}$ sec. In all three zones the period of illumination lasts exactly one-half of the period, and the three zones have exactly the same brightness. Rotate more quickly, and no further effect is produced. The number of rotations can be determined by Harding's improved counter.

17. Charpentier's experiments (slow-moving discs).

(i.) "*Black-band experiment.*"—Make a disc $\frac{1}{6}$ white, cause it to revolve (once in two seconds) in bright direct sunshine. On the white sector will be seen a narrow "black band" or sector near the black edge that has just passed in front of the eye, but

separated from that edge by a narrow white sector (fig. 409). The black band always appears at the same time from the moment the white sector appears in the field. The time is equal to $\frac{1}{60}$ to $\frac{1}{70}$ sec.—*i.e.*, 0·014″ to 0·016″. It is independent of the velocity of the disc. Sometimes there are two or three successive fainter bands, but they are difficult to make out.

The first effect is white, followed by an after-effect which is black even *during* the continued white stimulus. Thus there seems to be a slow oscillatory process in the retino-cerebral apparatus, showing a positive and a negative phase, each phase lasting 0·014″ to 0·016″.

The negative phase of oscillation takes place after the shortest possible illumination, and appears to be a general phenomenon. Charpentier suggests that it is possible that a single bright stimulus—*e.g.*, an electric spark—appears as a double or reduplicated bright sensation (*Archives de Physiologie*, 541, 1892). Another form of the experiment is given in a later paper, 629. *Nature*, xlviii., 380, 1893.

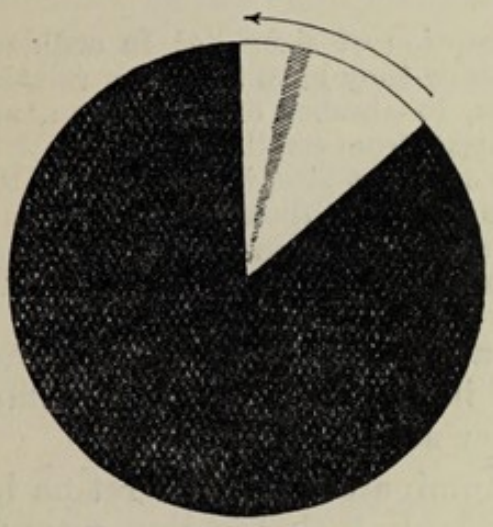


Fig. 409.—Charpentier's disc for "Black Band." The arrow shows the direction of rotation.

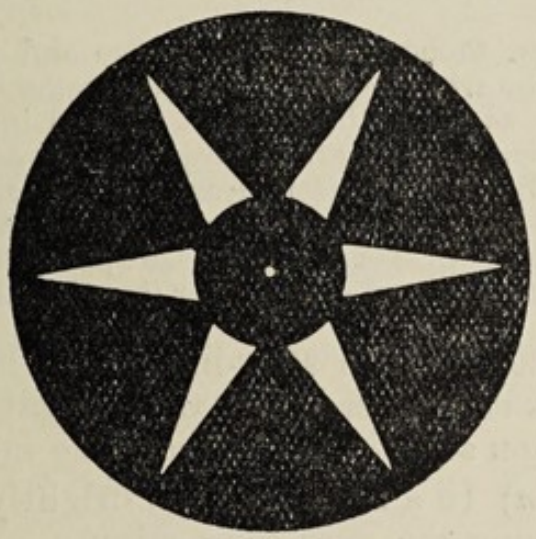


Fig. 410.—Charpentier's disc for vision of purple background.

(ii.) On a large black disc (40 cm. diameter) gum near its circumference a piece of white paper (1 cm. and angular deviation 1° to 2°), and cause the disc to revolve twice per second. The observer has a sensation of a white *ribbed* streak (about ¼ of the entire circle) on the black surface. There is not a uniform tint, and the ribbed appearance is due to an oscillatory process in the retino-cerebral apparatus.

(iii.) Arrange a black disc with narrow open equidistant sectors, to rotate opposite to a white surface illuminated by direct sunlight. The sectors have their apices towards the periphery and their bases at the centre (fig. 410). On rotating the disc before the eyes so that the retina is stimulated 40 to 60 times per second—*i.e.*, when each stimulus occurs

during the negative after-effect of the preceding stimulus, one gets a sensation of a purple-violet field, but the field is colourless at lower or higher rates of stimulation. Charpentier thinks that the coloured sensation is due to entoptical vision of the retinal purple.

18. **Adaptation to darkness, light, and colours.**—The eye adjusts itself to the intensity of the illumination partly by change in size of the pupil and partly by changes in the retina itself.

(*a*) Pass from ordinary daylight into a perfectly dark room and remain there. At first it is oppressively dark and black, but after two minutes it appears lighter, and after ten minutes a dull grey. Note if any colour sensations are felt.

(*b*) Pass into daylight. At first one is dazzled, the eyes are painful and one blinks, but in a few minutes adaptation is complete.

(*c*) Use spectacle frames with temple-frames provided with glasses of different colours—best from the yellow blue-green area of the spectrum—and look, say, through yellow at a landscape. After a few minutes the colour sensation almost vanishes.

19. Monocular retinal image and perception of size.—(*a*) In ordinary vision when objects are at the same distance they have the same relative size as the retinal image. Large images, in absence of other data, are taken as coming from large objects, and small from small.

(*b*) Known objects usually appear as of constant size, irrespective of the size of their retinal images. Look at one hand at a distance of 8, 16, and 24 inches. Its apparent size appears the same, although the retinal image is one-half and one-quarter its original length.

20. **Positive after-images.**—They are of shorter duration and less easily perceived than negative images. After brief stimulation shield the eye from the further action of light.

(*a*) In a room, not too brightly illuminated, rest the retina by closing the eyes for a minute. Suddenly look for two seconds at a gas jet surrounded with a white globe, then close the eyes. An image corresponding to that looked at will be seen.

(*b*) Rest the retina by closing the eyes, then look at a gas-flame surrounded with a coloured glass, or look at a gas-flame in which some substance is burned to give a characteristic flame —*e.g.*, common salt. Then look at a white surface, when a positive after-image of the same colour will be seen. In all these cases the image moves as the eye is moved, showing that we have to do with a condition within the eye.

(*c*) Turn off the gas suddenly in a dark room. The positive image of the flame and burner appears.

(*d*) Look at a window for one-third of a second and cover the eyes.

21. Negative after-images.—These are the after-effects of adaptation and may be regarded as a sign of retino-cerebral fatigue. —**Successive contrast.**

(*a*) Rest the retina, and then stare steadily for half a minute or less at a small white square or white cross on a black ground. To ensure fixation of the eyeballs, make a small mark in the centre of the white paper, and fix this steadily. Then suddenly slip a sheet of white paper over the whole, a *black* square or cross will appear on the white background. I find that the best black surface to use is the dull black of the "Tuch-papier," such as is used by opticians for lining optical apparatus. Notice also while staring at the white paper that its margins appear much brighter than the centre, owing to contrast.

(*b*) The black negative after-image may also be seen by closing the eyes.

(*c*) Look at a black square or cross on a white ground. Turn to a grey surface, when a white square or cross will appear. The apparent magnitude of the after-image varies with the distance of the background—best a grey background. The after-image enlarges as the distance increases. The after-image also moves to all parts of the room as the fixation point of the eye changes.

(*d*) Stare intensely at a bright red square on a black surface for twenty seconds, and then look at a white surface: a bluish-green patch on the white is seen. It waxes and wanes, and finally vanishes.

(*e*) A green stared at in the same way gives a red—*i.e.*, in each case the complementary colour is obtained as a "negative coloured after-image."

(*f*) Place a small red and a small green square side by side on a black background, stare at them, and quickly cover the whole with a sheet of white paper: a greenish-blue after-image will appear in place of the red, and a reddish-purple instead of the green.

These negative after-images are examples of so-called "Successive contrast" (Lesson LXXVII.).

N.B.—Note the "periodicity" of after-images. They come and go, wax and wane, relative brightness of its parts may change often with marked periodicity. Many conditions affect the after-image—change of position of eyeball, breathing.

22. Visual purple (Rhodopsin).—Keep a rabbit or frog for at least four hours in complete darkness, or in a perfectly dark room illuminated only by monochromatic light = sodium flame. The soda flame used for Laurent or Wild's polariscope is convenient, or a soda flame as used by photo-

graphers. Kill the animal and remove the eyes. In the case of the frog the best way is to cut away the head from the eyes. Cut across the eyeballs—best under normal saline—and remove the retina. For the rabbit's eye, after removing the corneal segment, Kühne removed the vitreous humour, and in order that the retina might be freed readily he used a small circular punch to cut round the optic nerve. The retina then separates readily and is floated on to a small porcelain capsule. One retina can be fixed with alum solution; the other covered with a black shade. Observe the purple colour and how it fades on exposure to light. One can follow the stages of bleaching (*Kühne*). Observe in the dark room that blood by sodium flame appears black.

23. Weber's law (*Fechner's experiment with grey glasses*). See also Lesson LXXIX.—(*a*) Select in a half covered sky two clouds with only the trace of a difference to the eye—*i.e.*, *just perceptible* difference. Place before both eyes a large sheet of grey or smoked glass which lets through about one-third, and then add a second sheet whereby the two together let through only about one-seventh of the light. The difference of intensity remains just as clear as before, and this although there has been such a considerable weakening of the total light.

(*b*) The same experiment can be done with photographic transparencies where faint differences of shading are present. Examine under different conditions of illumination, using various backgrounds.

(*c*) Demonstration with discs on rotating apparatus. Discs giving a geometrical increase of intensity from centre to circumference are constructed. On rotation the medium grey occurs halfway from the centre.

LESSON LXXVI.

PERIMETRY — IRRADIATION — GEOMETRICAL ILLUSIONS—IMPERFECT VISUAL JUDGMENTS.

1. Map out field of vision. Perimetry.

(*a*) A rough method is to place the person with his back to a window, ask him to close one eye, stand in front of him about 2 feet distant, hold up the forefingers of both hands in front of and in the plane of your own face. Ask the person to look steadily at your nose, and as he does so observe to what extent the fingers can be separated horizontally, vertically, and in oblique directions before they disappear from his field of vision.

(*b*) **Priestley Smith's Perimeter** (fig. 411*a*).—Let the observer seat himself near a table on which the perimeter is placed at a convenient height. Suppose the right eye is to be examined, fix a blank chart for the right eye behind the wooden circular disc. A mark on the hand-wheel shows which way the chart is to be placed.

(*c*) The patient rests his right cheek against the knob on the wooden pillar in such a position that the knob is about an inch directly under his right eye, the other eye is closed either voluntarily or with a shade, while the observer looks steadily with the right eye at the white spot on the end of the axis of the instrument.

(*d*) The observer turns the quadrant with his right hand by means of the wooden wheel, first to one and then to another meridian. With his left he moves the white mark along the quadrant, beginning at the periphery and gradually approaching centralwards until it is just seen by the right eye. A prick is then made in the chart corresponding to the angle read off on the quadrant, at which the observer can see the white spot.

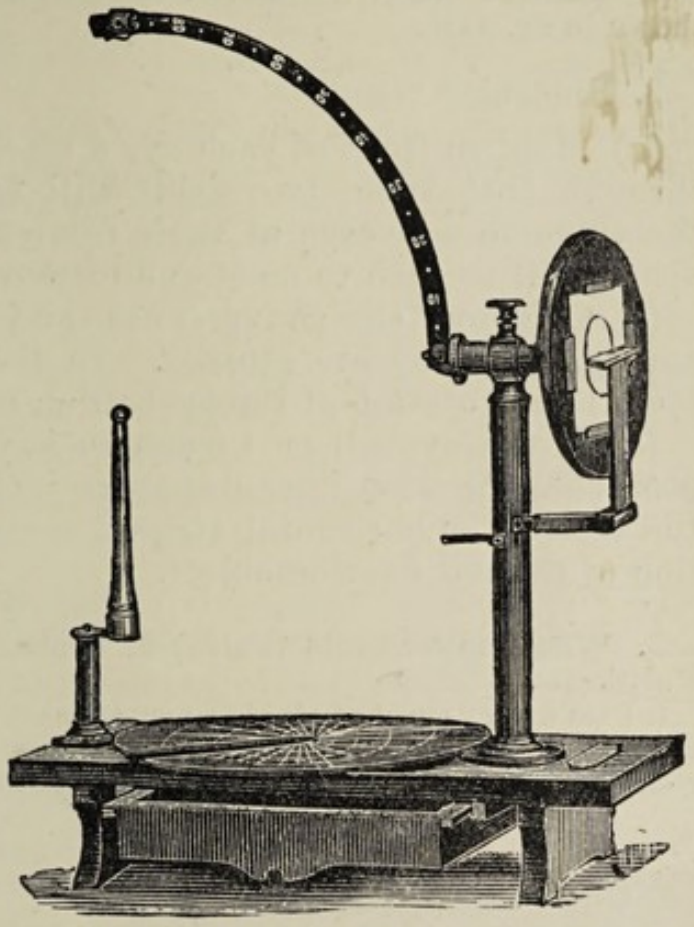


Fig 411*a*.—Priestley Smith's perimeter.

(*e*) Turn the quadrant to another meridian and determine the limit of the visual field as before. This is repeated for four or more meridians, and then the pricks on the chart are joined by a continuous line, when we obtain an oval field more extensive in the outer and lower portions. Test, if desired, the left eye, substituting a blank chart for that eye.

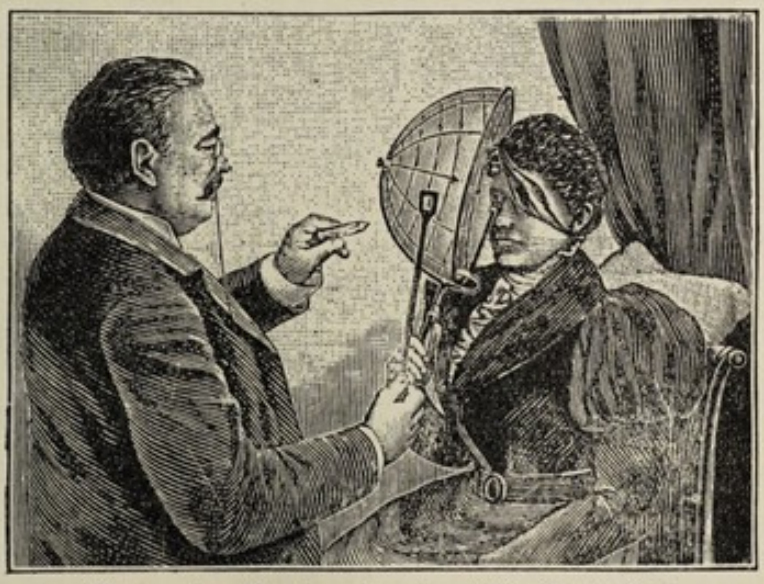


Fig. 411*b*.—Hand perimeter of Dr. L. Ascher. Made by R. Jung, Heidelberg.

(*f*) Test the field of vision for colours, substituting for the white travelling disc blue, red, and green. Mark each colour-field on the chart with a pencil of similar colour. Notice that the field for blue is nearly as large as the normal visual field. It is smallest for green, red being intermediate between green and blue (Lesson LXXVII.).

(*g*) With Ludwig's apparatus test when red, yellow, blue, and other coloured glasses cease to be distinguished as such in the field of vision.

(*h*) Ascher's hand perimeter.—A convenient and simple form is that shown in fig. 411*b*.

2. Binocular vision.

(*a*) Hold in front of each eye a blackened tube. On looking through both tubes two fields will be seen. Gradually cause the tubes to converge at their free ends, and the two fields of vision will be seen to meet and form a single field.

(*b*) Continue the convergence, and note that two fields reappear, but they are crossed. In these "**secondary positions**" there is no rotation of the eyeball on its antero-posterior axis.

(*c*) If the eyeball be turned in any other direction (**tertiary positions**) the after-image appears inclined, or at an angle with the vertical or horizontal stripes, according to the original position of the red fixation-object.

3. Wheel movements (False) of eyeballs (Secondary and Tertiary Positions).

(*a*) On a grey sheet of stout paper, at least 1 metre square, rule a number of vertical and horizontal faint black lines. Fix on the centre of the paper a strip of red paper on a level with the eyes, the eyes being in the primary position—*i.e.*, looking straight ahead. Gaze steadily at the latter, keeping the head fixed. After a time suddenly direct the eyeballs to another part of the grey surface; a green-blue after-image is seen which retains its same relative position with regard to the vertical and horizontal lines, provided the eyeballs be moved directly upwards, downwards, inwards, or outwards—*i.e.*, if the eyeball is moved up, along vertical or horizontal meridians, the after-image is still vertical. Turn the eyeball upwards and to the right, or downwards and to the left, the head being kept in the same position, the after-image appears tilted to the right; if the eyes are directed upwards and to the left, or downwards and to the right, the after-image appears tilted to the left. A similar result occurs with a horizontal strip of paper, but the after-images are inclined against the inclination of the vertical images.

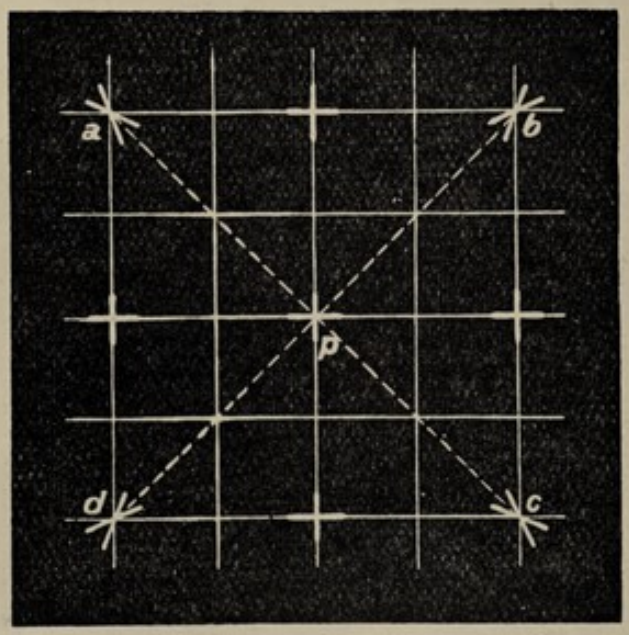


Fig. 412.—Appearance of a cross in false wheel movements of eyeballs.

Suppose we look at a rectangular red cross, *p*, under the same circum-

stances (fig. 412), on turning the eyes—*i.e.*, the visual line—to any vertical or horizontal line passing through *p*, the after-image is a rectangular cross, but it appears oblique, and its angles are neither horizontal nor vertical when the eyes look obliquely—*i.e.*, when the point of vision diverges considerably from the above-named lines. The *apparently* displaced crosses are shown in *a*, *b*, *c*, *d*.

These oblique after-images were formerly regarded as showing that the eyeball rotated on its antero-posterior axis—*i.e.*, *wheel movements*. This is not the case, the movements are only *apparent*. If they were real the after-images ought to move in the same direction with both vertical and horizontal strips, but they do not.

4. **Associated movements of eyes.**—Both eyes act together as a single visual apparatus. Up and down movements are performed simultaneously by both eyes.

Close one eye and rest the top of one finger on the upper eyelid, and feel how this eyeball moves as the other is moved from point to point in the field of vision.

5. **Reflex movements of eyes.**—Note how any bright object seen by indirect vision is at once brought into the field of clearest vision by a reflex movement of the eyeball.

6. **Involuntary movements of eyes.**—Fixate the edge of a small red patch on a blue ground. Soon the colour brightens, now on the red, now on the blue, owing to small unintentional, uncontrollable, involuntary movements of the eyes.

7. **Irradiation.**—By irradiation is meant the fact that, under certain circumstances, objects appear larger than they should be according to their absolute size and distance from the eye—larger than other objects of greater or less brightness of the same size and at the same distance.

(*a*) Cut out two circles as in fig. 413, of exactly the same size,

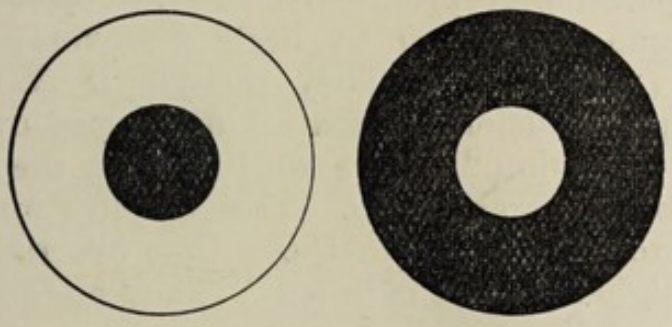


Fig. 413.—Positive irradiation.

of white and of black paper. Place the white patch on a black and the black on a white sheet of paper. Hold them some distance from the eye, and, especially if they be not distinctly focussed, the white circle will appear larger than the black one.

(*b*) Divide a square into four, as shown in fig. 414, two of the smaller squares being white and two black. Hold the figure at some distance from you. The two white squares appear larger, and they appear to run into each other and to be joined together by a white bridge.

(*c*) Look at fig. 415, placed at such a distance that the accommodation is imperfect. The white stripe, which is of equal breadth throughout, appears wedge-shaped, being wider below between the broad black patches, and narrower above. To me also the narrow black patches appear to be broader above and narrower below.



Fig. 414.

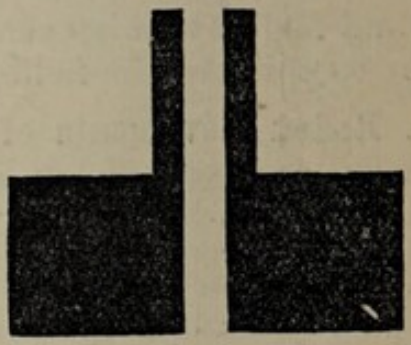


Fig. 415.

(*d*) Gum on a sheet of white paper two strips of black paper 5 mm. wide, and parallel to each other, leaving a white interspace of 8 mm. between them. Look at the object, and, especially if it be not sharply focussed, the smaller black strips will appear broader than the white one.

(*e*) Hold a pencil, ruler, or edge of cardboard in front of a flame. The flame seems to cut into the margins of the object, which look narrowed and constricted at that part.

8. **Visual spatial perception—Geometrical optical illusions.**—The latter name was given by their discoverer—in 1854-5—J. J. Oppel (1815-1874). They include in Wundt's phrase "erroneous apprehension of spatial distances, directions, and differences of direction." It is well to turn the diagrams and view them from different sides. This enables one to separate illusions due to position from those that are not. A perfect square looks higher than it is broad. They have been classified by Titchener as follows :—

A. **Illusions of reversible perspective** (Perspective interpretation of plane figures).—(*a*) Look with one eye at fig. 416; fixate *b*, and let the eye travel slowly from *b* to *c* and back again. Observe how the perspective changes; *b* may seem to be near

one or far away. It is like a folded calling card or half-opened book placed vertically on the table with the fold at one time like back of book, or next moment like an open book. Some refer to this as an "equivocal illusion." The stair-figure and the classical experiment on L. A. Necker's cube (1833) belong to the same order, and so does the illusion of the *fluctuating blocks* (fig. 417). The blocks appear to have their ends alternately above and below. The view changes against our will as we look at it.

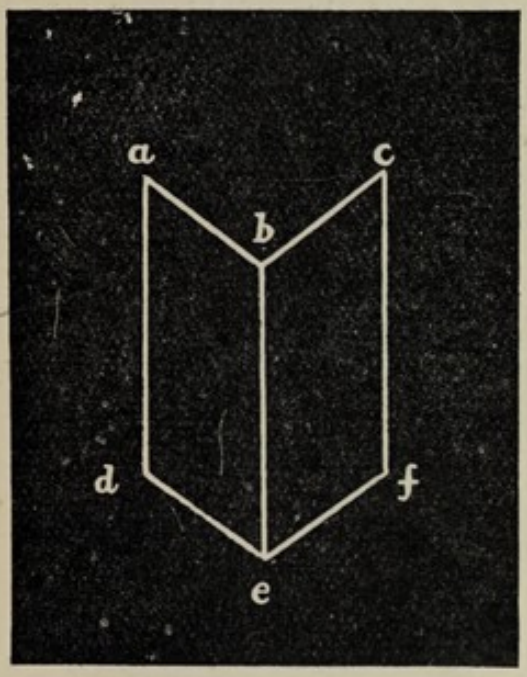


Fig. 416.—Folded card illusion.

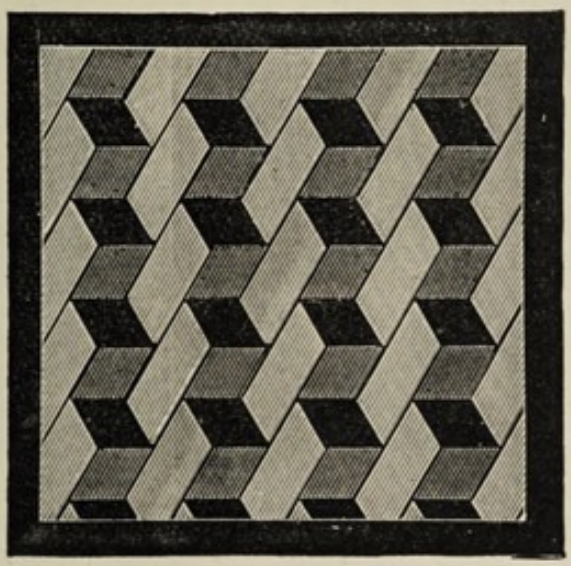


Fig. 417.—Illusion of fluctuating blocks.

(*b*) *Crossed lines.*—A short vertical or horizontal line (1 inch) cut at its mid-point obliquely by a similar line, if viewed with one eye, tend very readily to take on a tridimensional interpretation and appear in perspective. They are best seen when drawn on glass and observed against the sky or other uniform background (*Sanford*).

(*c*) *Contour illusions.*—Draw a circle with its circumference interrupted, say, in three places, leaving either equal or unequal arcs. The arcs appear too flat, and do not seem to belong to a circle of such radius.

B. Variable illusions of extent.—(*a*) *Oppel's lines.*—Make three round black dots, A, B, C, of the same size, in the same line, and let A and C be equidistant from B. Between A and B make several more dots of the same size. A and B will then appear to be farther apart than B and C. Or make two lines, A and B, equal length. Make a short vertical through B. It appears longer than A.

(*b*) *Helmholtz's squares.*—Make on a white card two squares of equal size, omitting the outlines. Across the one draw *horizontal*

lines at equal distances, and in the other make similar *vertical* lines. Hold them at some distance. The one with horizontal lines appears higher than it really is, while the one with vertical lines appears broader—*i.e.*, both appear oblong.

(*c*) The length of a line appears to vary according to the angle and direction of certain other lines in relation to it (fig. 418). The length of the two vertical lines is the same, yet one appears much longer than the other. Make similar figures, but vary the angle of the rays. (A large number of similar illusions will be found in *du Bois-Reymond's Archiv*, 1890, 91, by F. C. Müller-Lyer, and Láska, p. 326. For the explanations offered see Titchener, *Experimental Psychology* (Qualitative), 321, 1901.)

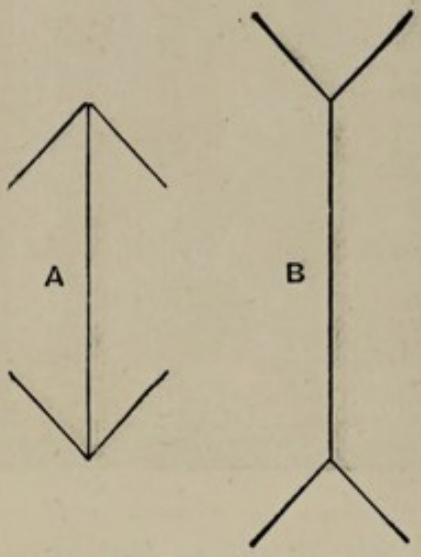


Fig. 418.—To show false estimate of size. Arrow-head and feather, or Müller-Lyer illusion.



Fig. 419.—Zöllner's lines.

C. Constant illusions of extent.

(*a*) Make a circle, and inside draw a square, with its angles touching the circle. Where they touch the circle appears as if it were pulled in (*Delbœuf*).

(*b*) Make a horizontal line, and from its middle draw a vertical line of exactly the same length. The latter looks much the longer of the two (*Kundt*).

D. Variable illusions of direction.

(*a*) Zöllner's lines (1860).—Make two parallel lines. Note that one can judge very accurately as to their parallelism. Draw short oblique lines through them. The lines now no longer appear to be parallel, but seem to slope inwards or outwards, according to the direction of the oblique lines. Rotate the figure on a vertical axis, and observe the effect. Or draw two parallel lines, and from the mid-point between them draw

radiating lines through both parallels. The parallel lines now appear to diverge (*Hering*).

(*b*) Look at fig. 419; the long lines do not appear to be parallel, although they are so.

(*c*) *Poggendorff's illusion.*—Draw two vertical parallel lines 2 to 4 mm. apart, and through them obliquely a line which does not traverse the interval between the lines. The two parts of the oblique line do not appear to have the same direction.

(*d*) Draw an outline of an arch, cover a small part vertically with a narrow strip of paper. One cannot convince one's self that the separated parts of the arc really were part of a complete arc.

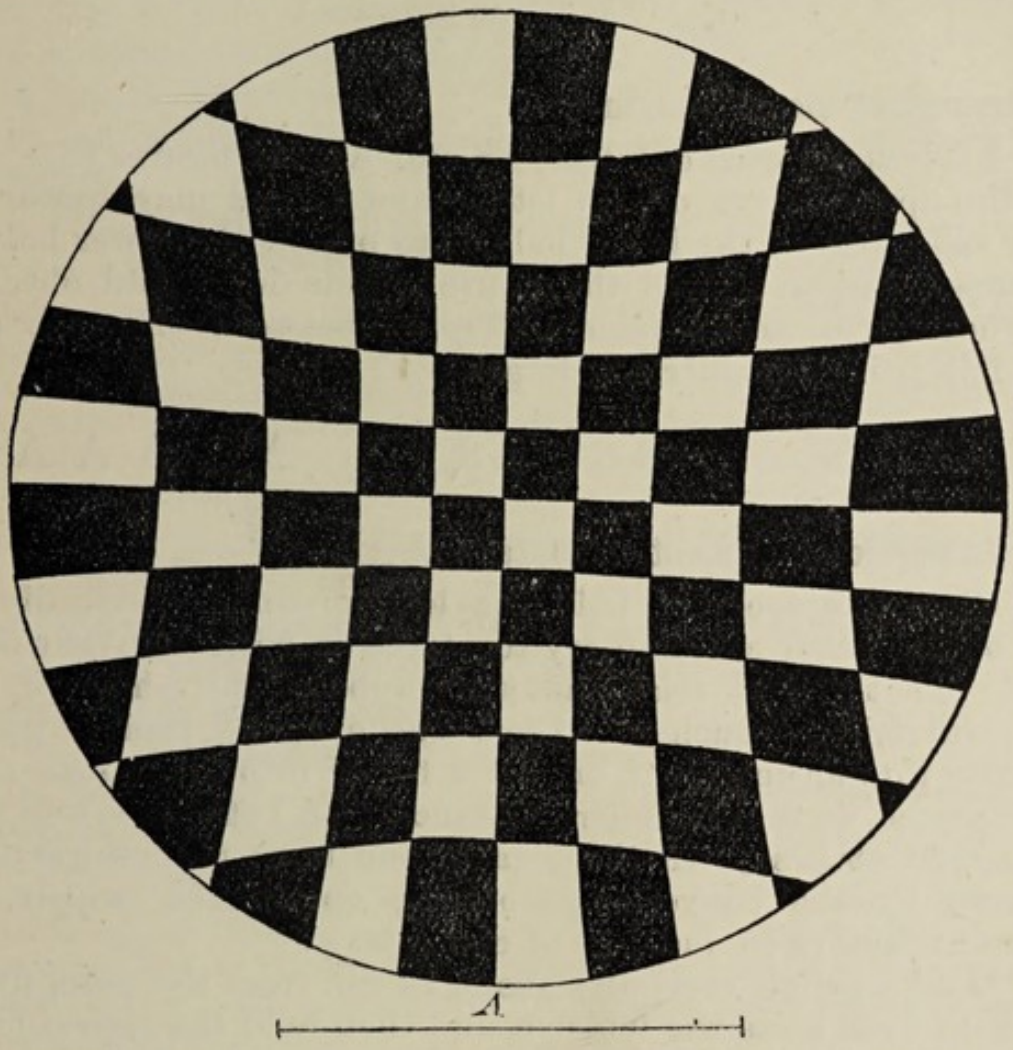


Fig. 420.—Helmholtz's chessboard; von Recklinghausen's illusion.

E. Constant illusions of direction (von Recklinghausen's illusion).—Curved lines that appear straight in indirect vision, Helmholtz's "circle of direction."

(*a*) *Monocular perception of direction.*—Fix the centre of fig. 420 at a distance of 3 to 4 cm. from the eye, when by indirect vision the broad white and black areas of the peripheral parts,

bounded by hyperbolic curves, will appear as small and the lines bounding them as straight as the smaller areas in the middle zone—*i.e.*, the lines are approximately straight and parallel.

F. The following illusion is from Sanford :—

In these two columns the type is of exactly the same size. On this side, however, it is set "solid," and looks smaller than on the other. According to Wundt, this is because the eye passes over the same number of letters in a shorter course.

Here the lines are "leaded"—*i.e.*, have greater space between them. Is it not possible that the effect is based on the general greater whiteness in this case and blackness in the other?

9. Imperfect visual judgment.

(*a*) Look at the row of letters (S and A) and figures (8). To some the upper halves of the letters and figures may appear to be the same size as the lower halves, to others the lower halves may appear larger. Hold the figure upside down, and observe that there is a considerable difference between the two, the lower half being considerably larger.

S S S S S 8 8 8 8 8 A A A A A

10. Imperfect judgment of distance.

(*a*) Close one eye, and hold the left forefinger vertically in front of the other eye, and try to strike it with the right forefinger. On the first trial one will probably fall short of the mark, and fail to touch it. Close one eye, and rapidly try to dip a pen into an inkstand, or put a finger into the mouth of a bottle placed at a convenient distance. In both cases one will not succeed at first. In these cases one loses the impressions produced by the convergence of the optic axes, which are important factors in judging of distance.

(*b*) Hold a pencil vertically about 15 cm. from the nose, fix it with both eyes, close the left eye, and then hold the right index-finger vertically, so as to cover the lower part of the pencil. With a sudden move try to strike the pencil with the finger. In every case one misses the pencil and sweeps to the right of it.

(*c*) Fix a wire ring about 3 inches in diameter into the end of a rod about 2 feet in length. Hold the rod at arm's-length, close one eye, try to put into the ring a vertical process attached to a rod of similar length held in the other hand.

(*d*) *Hering's experiment.*—The apparatus is shown in fig. 421. The observer has to determine whether marbles dropped fall in front of or behind the horizontal wire. This can easily be done by binocular vision, but the judgments are often very erroneous with monocular vision.



Fig. 421.—Apparatus for Hering's experiment with falling bodies.

11. Imperfect judgment of direction.

As the retina is spherical, a line beyond a certain length when looked at always shows an appreciable curvature.

(*a*) Hold a straight edge just below the level of the eyes. Its upper margin shows a slight concavity.

(*b*) In indirect vision the appreciation of direction is still more imperfect. While leaning on a large table fix a point on the table, and then try to arrange three small pieces of coloured paper in a straight line. Invariably the papers, being at a distance from the fixation-point, and being seen by indirect vision, are arranged not in a straight line, but in the arc of a circle with a long radius.

12. Convergence of visual axes influence conceptions of size and distance.

(*a*) Place a blackened paper tube before each eye, look at a fixed object, and then gradually converge the tubes; the object appears larger and nearer.

(*b*) Look at an object through two pieces of glass ($2\frac{1}{4} \times 2\frac{1}{2} \times \frac{1}{4}$ in.), held at first in the same plane, one in front of each eye. Let the adjoining edges of the two plates of glass be moved each on a vertical axis, so that they form either a more or less obtuse angle with each other. In order to see the object distinctly the axes of the eyeballs must converge to a greater or less extent, as the case may be, with the result that the object appears larger or smaller, or appears to approach or recede as the plates are rotated. Special forms of apparatus contrived by Rollett, and another by Landois, are used for this purpose.

13. Apparent movements depending on positive after-images.

(*a*) **Strobic discs** (S. P. Thompson).—Give the discs a somewhat circular but rapid movement and observe that the rings appear to move, each one on its own axis ("Optical illusions of motion," *Brain*, iii., 1880-81).

(*b*) **Radial movement.**—While another person rotates a disc with an Archimedean spiral drawn on it (fig. 422) on the rotating wheel, look steadily at the centre of the disc. One has the impression as if the disc were covered with circles which, arising in the centre and gradually becoming larger, disappear at the periphery. If the revolution is in the direction whereby the spiral approaches the centre of the disc, the entire

surface of the disc seems to expand during revolution and to contract after it has ceased, and *vice versâ* if the revolution is in the opposite direction. After long fixation look at printed matter or at a person's face; the letters appear to move towards the centre, while the person's face appears to become smaller and recede. If the disc be rotated in the opposite direction, the opposite results are obtained (Bowditch and Hall, *Journ. of Phys.*, iii., 299).



Fig. 422.

(*c*) Fix an object, turn the head rapidly, and note that the object appears to move in an opposite direction. When the eye does not move, we judge that a body is in motion when the image of that body falls successively on different points of the retina, and at the same time are conscious that the ocular muscles have not contracted (*Beaunis*).

(*d*) **Exner's illusion.**—A movement seen by indirect vision is over-estimated. Let A fixate one eye on some object. B moves a lighted taper to and fro (4 to 5 cm. excursion) in the outer part of the field of vision, and A is to indicate by a movement of the hand his conception of the extent of the movement of the taper. He will invariably greatly over-estimate it.

At the margin of the field of vision—*i.e.*, in indirect vision—the distance of objects from one another and, above all, the distance travelled by moving objects is greatly exaggerated. This is in sharp contrast to the diminished colour vision in this region.

(*e*) **Bidwell's illusion of motion.**—Hold fig. 423 vertically. Move it vertically slowly in front of the eyes. The thick black line will appear to travel over the thin one.

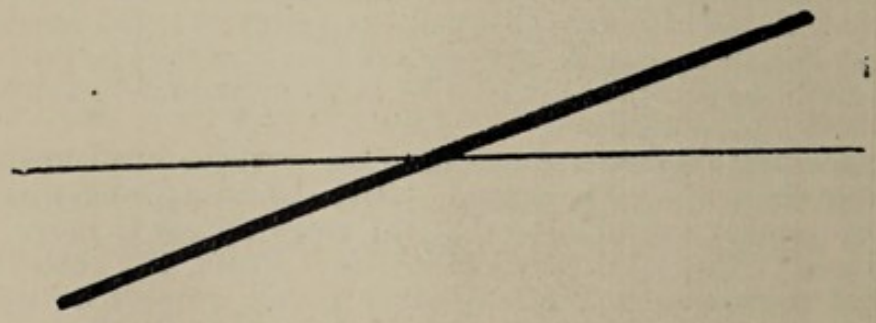


Fig. 423.—Bidwell's illusion of motion.

[*N.B.*—Many other figures and simple apparatus for most of the above experiments are supplied under the title of "Pseudoptics," by Milton-Bradley Co.]

LESSON LXXVII.

COMPLEMENTARY COLOURS—MIXING COLOUR-SENSATIONS—COLOUR-BLINDNESS—CONTRAST—FLICKER.

Our visual sensations are referable not only to *light* and *form*, but also to *colour*.

Any given colour-sensation may vary as regards

(i.) *Colour tone* or *hue—e.g.*, the colours of the spectrum.

(ii.) *Saturation* or *tint—i.e.*, changes in (i.) produced by addition or subtraction of white.

(iii.) *Intensity* or *shade*, or brightness of the colour.

1. Complementary colour—Colour mixture—Colour equation.—Given any colour it is possible to find another—the complementary or antagonistic colour—which, if mixed with it in proper proportion, will give white or grey (brightness quality).

Apparatus.—Colour-mixer (figs. 424, 425). Maxwell's coloured discs (10 cm. diam.) accurately centred and slit along one radius, and large black, white, and grey discs similarly slit. Grey screen. The colour discs are rotated on the colour-mixer. They must be accurately centred, and must not "wobble." The rotation speed should be about 40 to 50 times per second to get rid of flicker. The discs are best observed against a grey background, and must be uniformly illuminated.

Complementary colours are—

Red (carmine) and bluish-green.	Orange and greenish-blue.
Yellow and blue.	Green and purple.
Red and verdigris.	

(*a*) Select yellow and blue discs, or red and bluish-green. Slide one disc over the other, fix them on the spindle of the colour-mixer, and on rotating if the amounts of each colour be suitably adjusted they will fuse and give a grey or nearly white surface. The greys, however, by this method may be slightly coloured owing to the colours of the discs not being exactly complementary. This can be neutralised if inclined to red by adding a sector of a third disc—green; if greenish-grey add red. Put two small discs of black and white on the mixer over the

coloured discs, and adjust relative amounts of B and W until they give the same grey as is obtained with the colour discs. The match must be exact, both as regards colour and brightness.

The quantities of each colour can be read off on a scale of degrees. Find the amount in degrees of

$$\mathsf{Y} + \mathsf{B} + \mathsf{G} = \text{grey (white)}.$$

(*b*) Make an experiment to show that excess of either sector B or Y gives an unsaturated colour of the tone of the prevailing component.

N.B.—The blending of colours by rotation depends on the phenomenon of after-images.

(*c*) Repeat with overlapping discs of red and bluish-green.

(*d*) Blue and red discs. Adjust relative amounts to observe results.

2. **Mixing colour-sensations** (J. H. Lambert, *Photometria*, 1760).—Note that mixing of "colour-sensations" is a very different thing from the mixing of pigments.

(*a*) **Lambert's method.**—On a black background place a blue wafer or square of blue paper, and 6 or 7 inches behind it a yellow square or wafer. Hold a plate of clear class vertically about 10 inches above and midway between the two squares. Look obliquely through the glass, and get the reflected image of yellow to overlap the blue, seen directly through the glass; where they overlap appears *white*. Hering has arranged a large form of this apparatus suitable for class purposes.

(*b*) **Colour-mixer and Rotation apparatus** (fig. 424).—Arrange on the spindle of the rotation apparatus or colour-mixer, the disc with coloured sectors provided for you (fig. 425). On rotating the disc rapidly, observe that it appears grey or whitish. The disc is provided with sectors corresponding to the colours of the spectrum, and arranged in varying proportions.

(*c*) Arrange three of Clerk-Maxwell's colour discs—red, green, and violet—upon the spindle of the rotation apparatus. Adjust the relative amounts of these three colours, so that on rapidly rotating them they give rise to the sensation of grey or white. Each disc is of a special colour, and has a radial slit from the centre to the circumference. This slit enables a disc of a different colour to be slipped over the other, and thus many discs can be superposed, and the amount of each colour exposed regulated in any desired proportion.

(*d*) Combine chrome-yellow and blue discs in various proportions; the resultant colour is never green, but a yellowish- or reddish-grey.

(*e*) Arrange two coloured discs of vermilion and bluish-green in the proportion of 36 of the former to 64 of the latter. On the same spindle arrange a white and a black disc—with a diameter a little more than half that of

the former pair—the white being in the proportion of 21·3 to 78·7 of the black. On rotating, a grey colour is obtained from both sets of discs.

(*f*) *Double refraction.*—Look through a prism of Iceland spar at two coloured squares lying side by side. The fields of colour will partially overlap, but their brightness is diminished (*Hering*).

(*g*) *Mixture of spectral colours.*—Hold a prism horizontally and look at a white inch square lying on black. The prism refracts the different rays unequally, and thus produces multiple images which appear as coloured fringes. The horizontal edges of the paper will be seen as orange-yellow and blue.

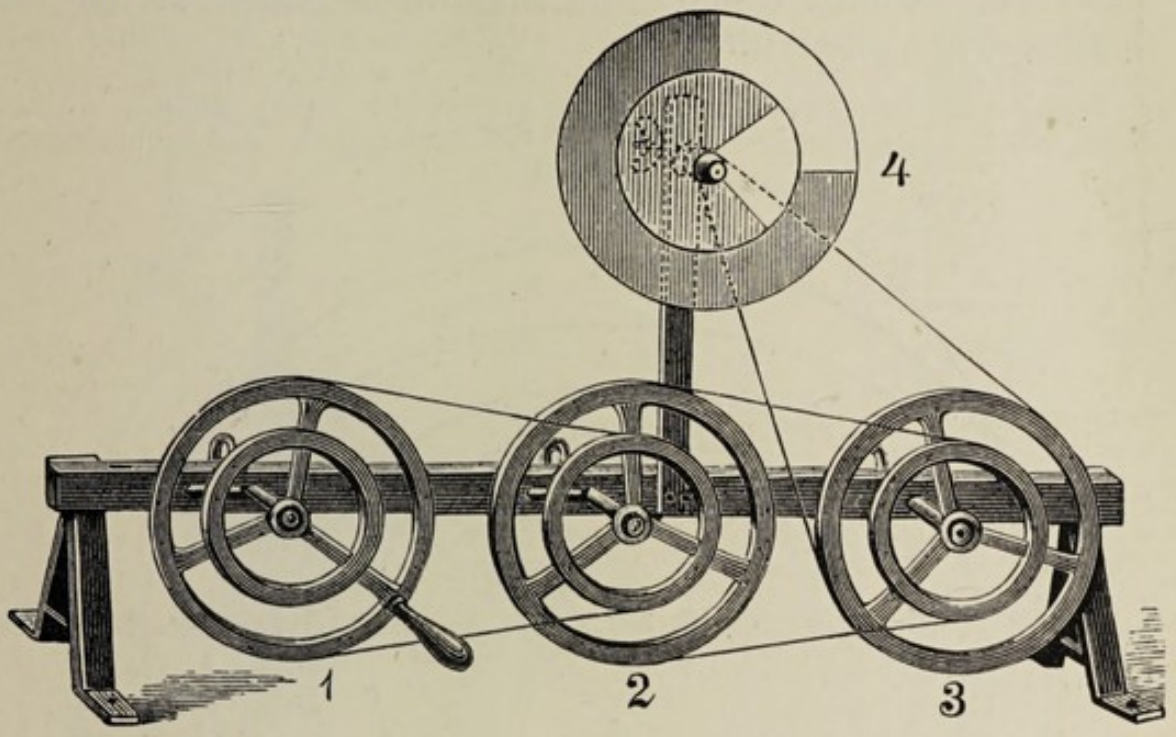


Fig. 424.—Rothe's rotatory apparatus for colour-discs. It is so arranged as to give various rates of rotation by combining the motions of 1, 2, and 3.

3. Colour vision in different parts of retina. Colour sensitivity. Campimetry.—Use a perimeter (Lesson LXXVI.). Make discs 0·75 cm. in diameter of white, red, green, yellow, and blue, and test the field of colour vision in different meridians, or simply let B stand over a table covered with a black surface, his forehead resting on a block 30 cm. high, and looking straight down on a table at a fixed object. A places the coloured discs on the cloth at various distances and uncovers them for a moment.

In using the perimeter B fixes a red disc, say, in the left quadrant, at 65°, covers it with a black screen, and then exposes for 2 seconds. A states if he can see it, and what colour—if any—it appears to him. Of course, A must fixate the mark or fixation point. Probably the red will appear greyish. Repeat the observation, diminishing the angle each time by 5°, until B sees the disc as red. Repeat with the other discs—blue, green,

yellow, and white. White and yellow are seen farthest out. Repeat for the other three quadrants.

These experiments show that the retina is not uniformly sensitive to colour over its entire surface. There are practically three zones of sensitivity—(i.) *inner zone*, where all the primary colours (Hering's red, green, blue, and yellow), blacks, greys, and whites are seen; (ii.) *middle zone*, partially colour-blind, where only blue, yellow, black, grey, and white are seen; (iii.) *outer zone*—totally colour-blind zone—over which we see only blacks, greys, and whites.

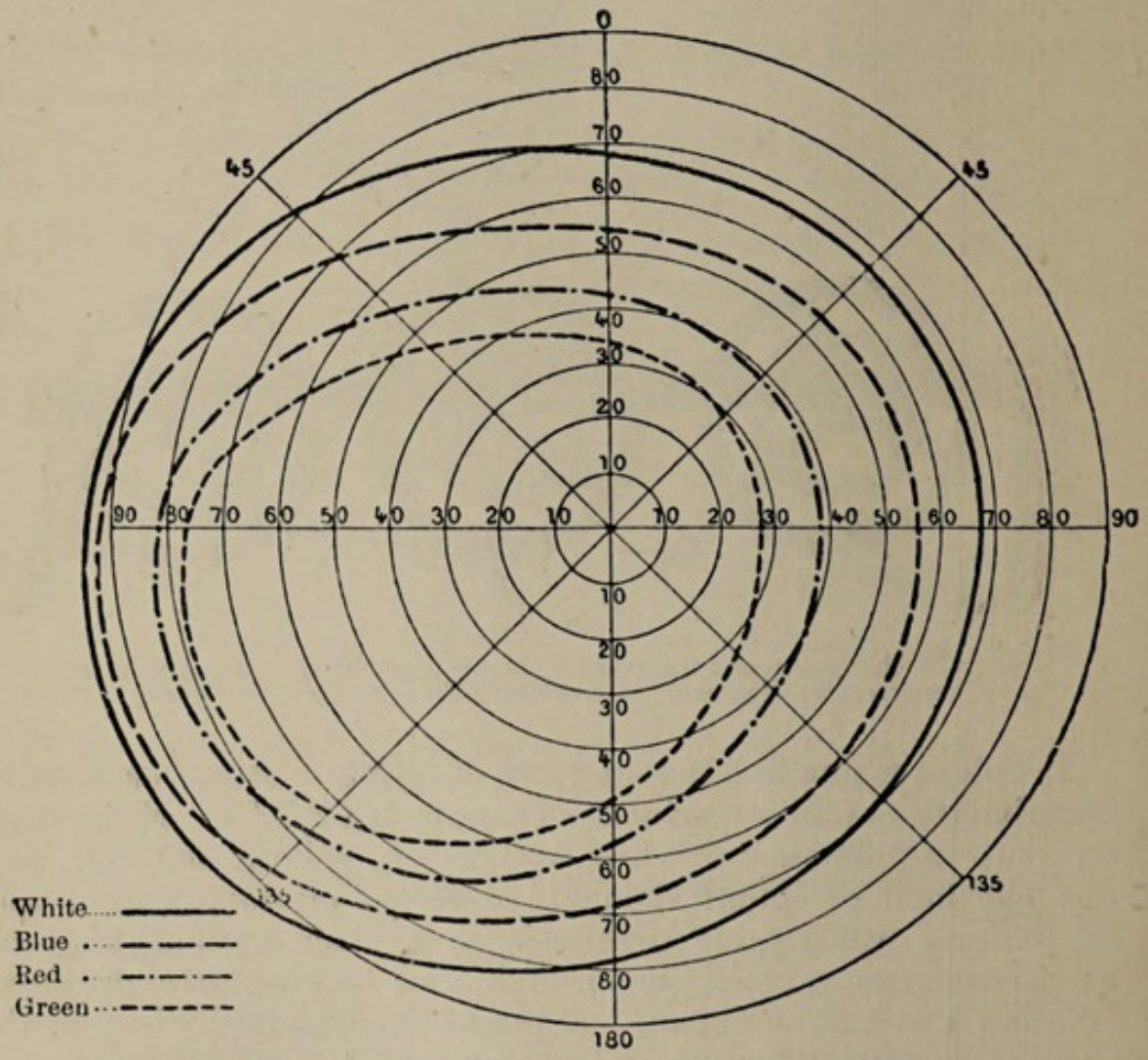


Fig. 425.—Visual field of left eye for different colours.

4. **Purkinje's Phenomenon.**—P. observed that a red and a blue which appeared equally bright with ordinary illumination, and further noted that, with diminished intensity of light, the blue became the brighter. It appears that this is referable to a more general phenomenon—viz., that, with decrease of the intensity

of the spectrum, the short wave end becomes relatively brighter (*W. H. R. Rivers*).

(*a*) Select, in moderate light, a piece of red and a piece of blue paper of about equal brightness and saturation. Observe, in bright sunlight, which is the brighter. Look at them now in a dark room and compare them. Or look at them through a fine pin-hole in a card.

(*b*) Lay a stick of red sealing-wax on an ultramarine ground distinctly darker than the red. Diminish the illumination. The red becomes black. The dark background, however, takes a bright bluish-grey tint.

5. **Hering's apparatus** for mixing colours of coloured glasses.

By mixing two primary colours (red, yellow, green, blue), one may obtain all intermediate hues, and by mixing three colours (red, green, and blue, or yellow, green, and violet), one can obtain white. The apparatus consists of a box, *k* (fig. 426), two pieces of mirror glass, *s* and s_1, each

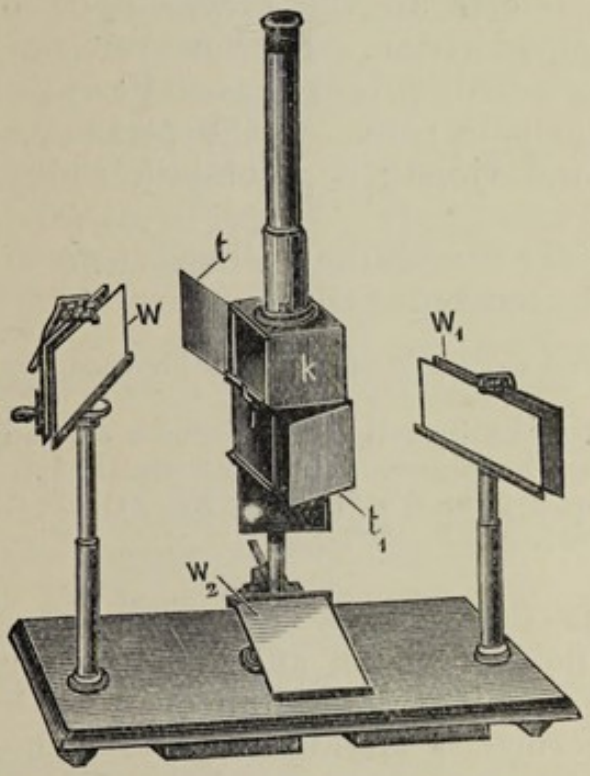


Fig. 426.—Hering's apparatus for mixing coloured light. *k*, Box; *t*, t_1, Lids; *W*, W_1, W_2, White reflecting surfaces.

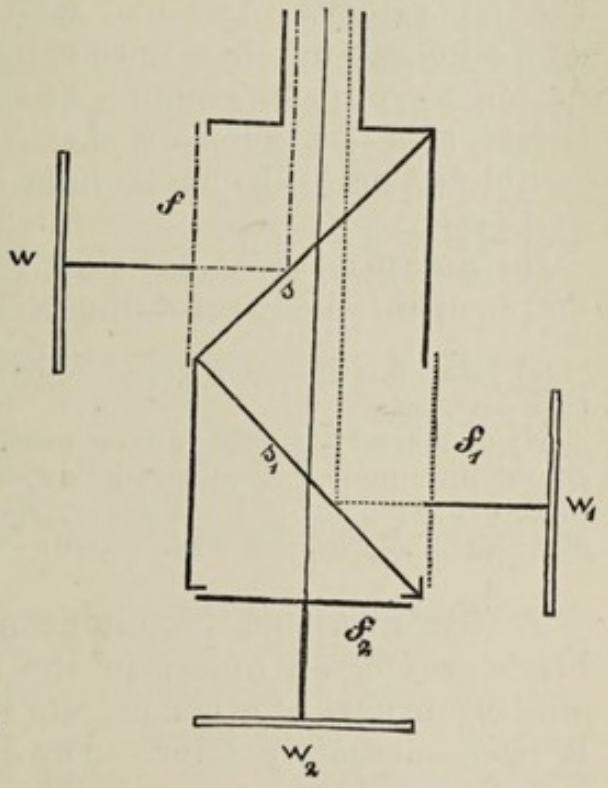


Fig. 427.—Scheme of fig. 426. *f*, f_1, f_2, Coloured glasses; *W*, W_1, W_2, White reflecting surfaces; *s*, s_1, Glass plates.

placed at an angle of 45° to the horizontal plane, as shown in fig. 427. The base of the box consists of a coloured glass, f_2, while the lower half of the right lateral wall is filled with the coloured glass plate, f_1, and the upper half of the left wall by the coloured glass plate, *f*. The white glass plates, W, W_1, W_2, reflect light through the coloured glasses (fig. 426). The light transmitted from below and that from the two sides is transmitted by a tube to the observer's eye. The brightness can be varied by adjusting the white reflecting surfaces, which are placed opposite a well-lighted window. By means of three small metallic doors, *t*, t_1, any one of the colours can be cut off. Thus any combination of coloured lights can be made, as the glasses are movable. The writer has found it best to put the violet or blue lowermost.

6. **Test colour-blindness.**—On no account is the person being tested to be asked to name a colour. In a large class of students one is pretty sure to find some who are more or less colour-blind. The common defects are for red and green.

(*a*) Place **Holmgren's worsteds** on a white background in a good daylight. Select, as a test colour, a skein of a light green colour, such as would be obtained by mixing a pure green with white. Ask the examinee to select and pick out from the heap all those skeins which appear to him to be of the same colour, whether of lighter or darker shades. A colour-blind person will select, amongst others, some of the confusion-colours—*e.g.*, pink, yellow. A coloured plate showing these should be hung up in the laboratory. Any one who selects all the greens and no confusion-colours has normal colour vision. If, however, one or more confusion-colours be selected, proceed as follows:—Select, as a test colour, a skein of pale rose. If the person be red-blind, he will choose blue and violet; if green-blind, grey and green.

(*b*) Select a bright red skein. The red-blind will select green and brown; the green-blind picks out reds or lighter brown.

(*c*) Daae has issued a set of coloured wools, fixed in a suitable case, for this purpose.

(*d*) **Coloured pencils** can be used. The investigator selects a certain colour and makes a coloured line on the paper. The examinee has to match that colour. (Hans Adler, *Wiener klin. Wochenschr.*, No. 21, 1891, and Görtz, *Münchener med. Wochenschr.*, No. 8, 1897.)

7. **Contrast and Simultaneous Contrast.** — The colour and brightness of an object in the field of vision affect the colour and brightness of other objects seen either simultaneously with it or immediately after. The former is simultaneous and the latter successive contrast. The colour causing the change is the *inducing* colour, the change effected is the *induced* colour. In simultaneous contrast the visual image of any object is modified by those sensations received simultaneously, more especially by those arising in adjacent parts of the retina. It passes into "simultaneous induction" if vision of a given object be kept up for some time. The best effects are obtained when the contrasting objects are close together, of nearly equal brightness and uniform texture, medium depth of colour, and not surrounded by shadows or black lines. The "psychological theory" of Helmholtz explains contrast as a "deception of judgment," and the "physiological theory" of Hering as due to physiological processes in the peripheral visual apparatus—*i.e.*, every physio-

logical process is correlated with a physical process in the nervous apparatus. Hering has devised many experiments in support of his contention. The former theory represents contrast as due to an error of judgment. On the physiological theory, Hering supposes that there are material chemical changes in a hypothetical retino-cerebral "vision-stuff" ("Seh-stoff"). These changes may be assimilative (anabolic), (black, blue, green), or dissimilative (katabolic), (white, yellow, red). A change in one area may influence the retino-cerebral apparatus outside the area directly affected by the stimulus. Physiological processes, and not defects or deception of judgment, are the true cause of colour contrast.

The following are examples of simultaneous contrast where stimulation of the retino-cerebral apparatus modifies the sensations excited by a different portion of the retina when the compared objects—light or colour—are looked at simultaneously. Contrast phenomena were carefully studied by Chevreul in relation to the effects produced by colours juxtaposed in tapestry in the Gobelin's factory of Paris. Contrast may apply to size, light, colour, and it may be simultaneous or successive.

(*a*) Place a small white square or oblong piece of paper or cross on a dull black surface. Stare steadily at the white square, and observe that the edges appear whiter than the centre; indeed, the centre by contrast may appear greyish. A white strip of paper placed between two black strips looks whiter at the margin near the black.

(*b*) Look with one eye at the sky through a 1-inch blackened tube, both eyes being open. The field of vision looks much brighter when seen through the tube than is the case with the other eye.

(*c*) Place side by side a white and black surface. Cut two oblong ($1\frac{1}{2}'' \times \frac{1}{2}''$) pieces of grey, yellow, or other coloured paper of exactly the same size, and lay one piece of the grey on the white background and the other on the black. Observe how much brighter the latter looks owing to contrast. Reverse the pieces, and notice that the same result occurs. Repeat with other colours.

(*d*) On the rotation machine cause a disc, as in fig. 428, to rotate with moderate rapidity, when several zones will be seen—the innermost black, while each one farther outwards is lighter in tint. Each zone, where it abuts against the inner darker zone, is lighter than the rest of the same zone, and shades off gradually to the outer part of the zone.

(*e*) Take two pieces of different coloured paper—say pale red and pale green—and place them side by side. Fix two similar

strips apart from each other and distant from the other two. The two slips juxtaposed differ in colour from the isolated pieces. In the juxtaposed slips the colour of the one influences the colour of the other—*i.e.*, each one looks as if it were mixed with a certain amount of the complementary colour of the juxtaposed slip (*Chevreul*).

(*f*) Place on a table a sheet (4″ × 4″) of red and one of green paper. Cut out of a sheet of red paper two pieces about 1 inch square, and place them on the two large squares. Observe that the small red square on the green ground appears far brighter and more saturated than the red square on the red ground.

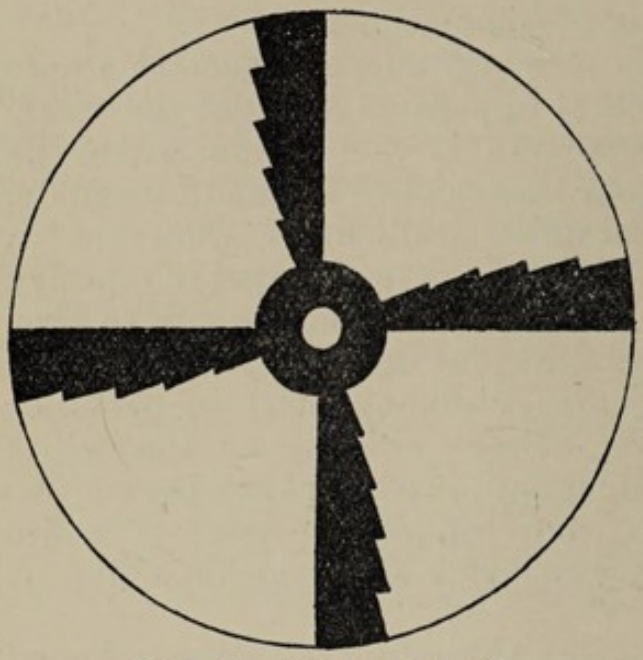


Fig. 428.—Disc for contrast.

(*g*) Cut a small hole (5 × 5 mm.) in coloured paper—*e.g.*, red—and look through the hole at a sheet of white paper; the hole appears greenish.

(*h*) On a mirror place a slip of transparent coloured glass—*e.g.*, red or green. Hold in front of the coloured glass a narrow strip of white paper; by adjusting the position of the glass in relation to the light we see two images reflected from the anterior and posterior surface of the mirror; one has the same colour as the coloured glass, while the other or posterior one has the complementary colour; if a red glass be used the latter is green, if a green glass it is red. Hold in front of the red glass a piece of white paper with black printed matter on it. The black print is seen green in the posterior image. Gum a few narrow strips of white paper (1 mm. in diameter) on black paper, and, on holding it up in front of the red glass as before, the anterior image appears in the complementary colour of the glass—viz., green.

(*i*) Place four lighted candles in a dark room before a white surface, and push between the candles and the screen towards the centre of the series an opaque screen—*e.g.*, cardboard, with a clean-cut vertical edge. A part of the white surface is illuminated by all four candles, then a vertical area illuminated by three, and so on, and finally a part not illuminated by any of the candles. Each of these areas is throughout its entire extent equally illuminated, yet on the side where each area abuts against a darker area it appears lighter, on the other side darker, and gradually shaded between its outer and inner limits. This is due to the fact that strong stimulation of one part of the retina diminishes the excitability in the other parts, and the parts most affected are those next the excited area. Thus a change in the excitability of one part of the retina is brought about by stimulation of an adjacent part.

(*j*) See also Waller, *Journ. of Phys.*, xii., 1891.

8. H. Meyer's experiments on simultaneous contrast.—Grey is tinged with the complementary colour, and the tissue paper eliminates contours (*Poggendorff's Annalen*, xcv., 1855).

(*a*) Cut out a small oblong of white, or preferably of grey, paper, and put it on a large piece of bright green paper (4 inches square); the grey suffers no change. Cover the whole with a thin semi-transparent sheet of tissue paper. The grey oblong appears *pink*.

(*b*) Instead of green paper place the grey slip on red, and cover it as before; a greenish-blue contrast colour is seen.

(*c*) Repeat (*a*), but place a red square on a grey ground; the red square will appear greenish.

(*d*) A grey square upon blue appears yellow; a yellow upon blue appears white, when covered with tissue paper. All the above are modifications of H. Meyer's experiment. The tissue paper is used, as contrast colours are far more readily excited by pale than by saturated colours, so that differences of sensation are much greater with weak than with strong stimulation.

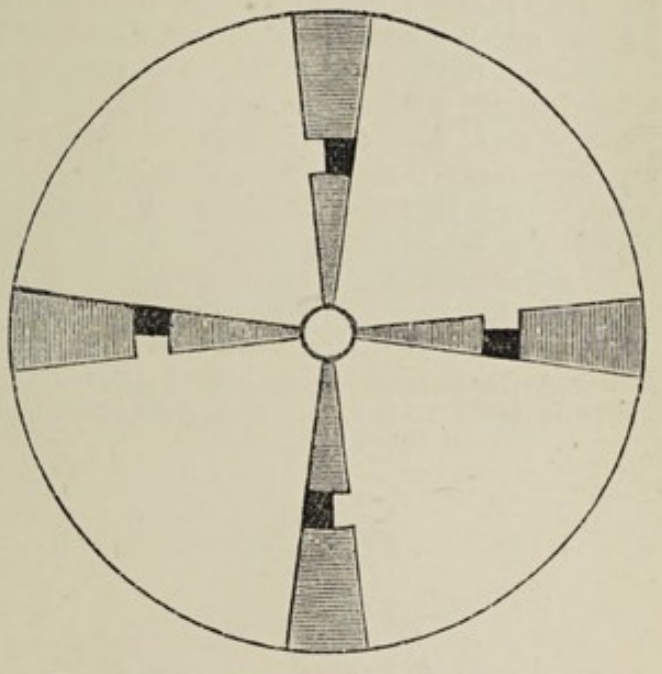


Fig. 429.—Disc for simultaneous contrast.

(*e*) Surround the small square with a broad black line, each square appears in its own colour. The effect of contrast is destroyed.

9. Other experiments.—(*a*) Make on one-half of a disc alternate rings of black and green, leaving the other half of the disc white. Rotate the disc. The black and white, instead of fusing to grey, give the contrast colour to the green—*i.e.*, we get pink rings alternating with unsaturated green.

(*b*) Place side by side two strips of paper, green and red (6″ × 3″). Over the line of junction place a strip of grey paper (¼″ × 6″), and cover the whole with tissue paper, as before. The grey appears pink on the green side, and greenish on the red. This contrast is also set aside by running a black margin round the grey strip. Do the same with yellow and blue.

(*c*) Arrange a disc like fig. 429 on the rotating wheel. On a white disc

fix four narrow coloured (*e.g.*, green) sectors, and interrupt each in the middle, as in the figure, with a black and white stripe. On rotating the disc, the ring, which one might expect to be grey from the black and white, appears reddish—*i.e.*, the complementary colour of the greenish ground.

(*d*) Place a strip of grey paper on a black background and a corresponding strip on a white ground. The former will appear much lighter, the grey on white much darker. Fix the eyes for a minute on a point midway between the strips ; close and cover the eyes. The after-images will show a great difference in luminosity.

(*e*) *Simultaneous contrast for toneless colours* (Brightness or light contrast as distinguished from colour contrast).

Place a small dark grey strip of paper, with a fixation mark at its middle, on the straight boundary line of a black-white card. The grey appears lighter on the dark than on the white surface.

Place a dark grey strip on a black velvet ground, and quickly push a white paper under the grey strip. The grey appears darker *suddenly*. *Hering* (*loc. cit.*, p. 23), regards this as an important evidence against Helmholtz's "psychological theory" of simultaneous contrast.

10 Ragona Scina's experiment (1859). —Two pieces of wood fixed at right angles to each other are covered by white paper, while a coloured sheet of glass is held at an angle of 45° between

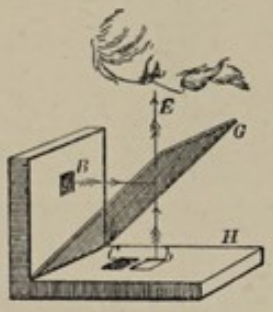


Fig. 430.—Ragona Scina's experiment.

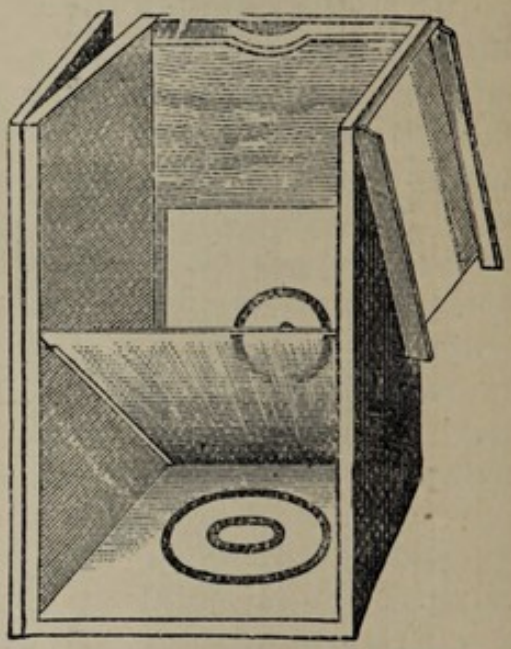


Fig. 431.—Hering's apparatus for contrast.

them (fig. 430), or the apparatus of Hering (fig. 431) may be used. Look vertically through the glass at the horizontal white paper, and observe a pale red tint. Attach a small black square to the centre of the vertical arm at B, the image of this square is seen at *b* as a deep red image. Place a similar black square on the horizontal board at C, it should appear grey ; but a grey on a red ground causes contrast, and so one sees a greenish-blue square alongside a red one.

11. Hering's apparatus for simultaneous contrast.

(*a*) By means of one or two doubly refractive prisms (fig. 432, P, P) a double image is obtained of narrow strips of coloured paper placed either on a white or a coloured background. If blue be placed on yellow, the double image is bluish ; and if yellow be placed on blue, the double image is yellowish (*Pflüger's Archiv*, vol. xlvii., 237).

(*b*) The apparatus of Hering (fig. 433) is also useful for simultaneous contrast. Coloured glasses—*e.g.*, blue and red—are placed in P and P′, and

light is reflected through them by the adjustable white surfaces, W, W'. On looking at a narrow black strip, S, on a white ground, one sees contrast phenomena according to the colours of the glass used.

The white surface in front of the red glass, when looked at with one eye, is red, just as that in front of the blue glass is blue under the same conditions. Focus the eyes for an object nearer than the black strip on the white ground. This is done by looking at a bead, *k*, fixed on the point of a rod (supplied with the instrument), the latter being held between the eyes and the white ground. The black strip seen under these conditions forms a double image — *i.e.*, its image is formed on two non-corresponding parts of the retina. The two images are in strong contrast, while the two surrounding areas scarcely contrast at all. (Hering's apparatus is made by Rothe, of Leipzig.)

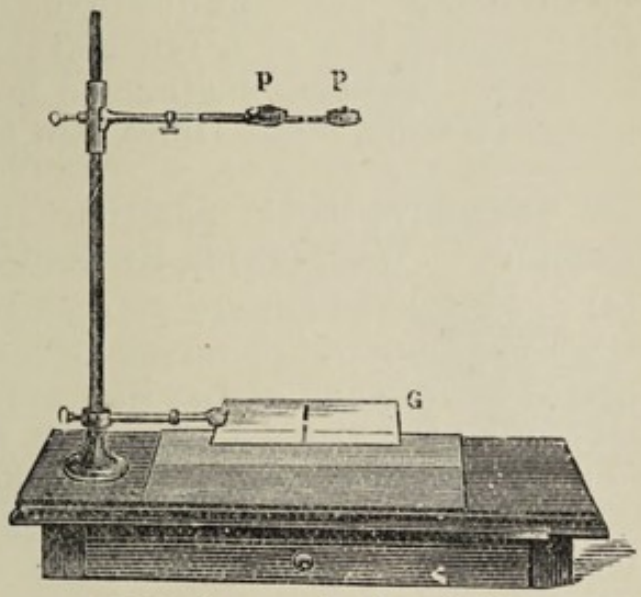


Fig. 432.—Hering's apparatus for simultaneous contrast with binocular vision by two doubly refractive prisms, *P*, *P*. *G*, Glass to avoid reflection.

12. Hering's experiments on simultaneous contrast.

Divide a large quadrilateral sheet of paper vertically into halves, and make one half deep black and the other white. Near the centre of the vertical division gum two (4 cm. × 0·5 cm.) V-shaped pieces of dark grey paper (one on the black and the other on the white half) with their apices 1 cm. apart. The V on the black looks lighter by contrast than that on the white. Close and cover the eyes, and the negative after-image appears, and as a rule the difference in brightness of the after-image is greater than in direct vision. Fix the V's for a minute, and then look at a uniform surface. Even after the after-image of

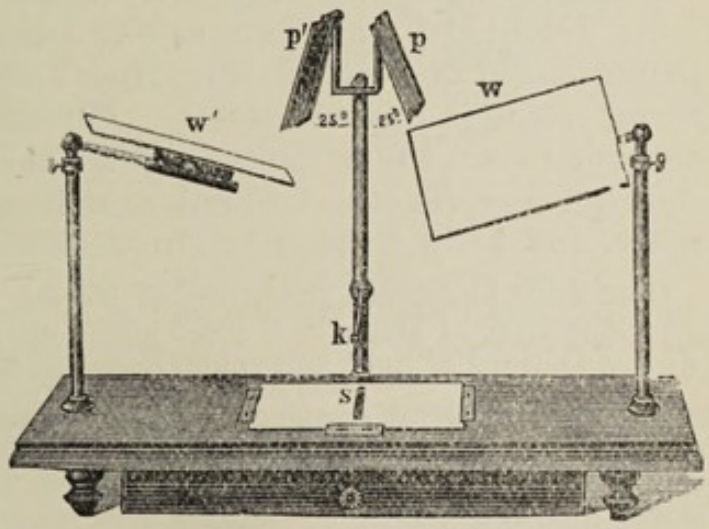


Fig. 433.—Hering's apparatus for simultaneous contrast. *P*, *P'*, Coloured glasses; *W*, *W'*, White reflectors; *S*, Black line on white surface.

the backgrounds has disappeared, the after-image of the V on the black ground looks darker than that of the V on the light ground. This, Hering contends, must be due to a material change taking place in a localised part of the retino-cerebral apparatus. It seems difficult to explain this result as dependent upon an error of judgment due to the influence of the background. Hering regards this as a fundamental experiment in support of his theory. Similar experiments may be made with coloured papers. (Hering, "Zur Lehre vom Lichtsinne.")

13. **Successive light induction** (Hering, "Zur Lehre vom Lichtsinne." Wien (1878), Erster Versuch, p. 5).

(*a*) Look for one minute at a small white circular disc on a black background—*e.g.*, velvet. Close and cover the eyes. A negative after-image of the disc appears, but it is darker and blacker than the visual area, and it has a peculiar light area or *halo* round it ("Lichthof"), brightest close to the disc, and fading away from it.

(*b*) Look at a black strip (4 mm. broad) on a white ground. On closing the eyes there is *no* partial darkening of the white ground, but only an intensely bright image of the strip. Therefore the light area or halo of (*a*) was not due to simultaneous marginal contrast (Hering, *loc. cit.*, pp. 11 and 12).

(*c*) Look with both eyes at two small white square patches of paper placed one-eighth of an inch apart on a black background, using a small fixation point. On closing the eyes, the black space between them looks brighter than the other three sides of the squares—*i.e.*, the two adjacent halos coincide in the narrow space, and yield a much brighter after-image.

[For some important criticisms of Hering's views and many new experiments, see W. M'Dougall, "Some new observations in support of Thomas Young's theory of light and colour-vision," *Mind*, vol. x., N.S., 1901.]

14. **Simultaneous contrast a physiological effect.**—On a disc two concentric rings are drawn ; each is half blue and half black. In one ring (*e.g.*, the outer) its blue half is surrounded by black, increasing the brightness by simultaneous contrast. The black half of the same ring and the blue half of the other are conversely surrounded by bright yellow, which by contrast diminishes their luminosity. On rotation (on the colour top) the outer ring shows highly-marked *flicker* when complete fusion has taken place in the inner ring. The rate at which successive stimuli must follow one another in order to fuse into a continuous sensation varies with the difference of brightness between the stimuli. But when the disc rotates perception of the difference of the background on which the rings lie is impossible. Yet the speed of fusion of the factors of the rings is influenced. The illusion of the contrast cannot therefore depend upon *judgment* of the difference between ring and background. Two colour equations, in

each of which the physical stimuli are exactly the same, behave markedly differently to the eye owing to a contrast from which the elements necessary for *judgment* are eliminated (*Sherrington*).

15. Successive contrast can be strikingly seen in a very simple manner by a black and white disc. A disc is prepared as in fig. 434. When the disc spins in the direction of clock hands, flicker in the middle ring band of the three persists at a higher angular speed of rotation than is necessary for extinction of flicker in the other two ring bands. When direction of spin is reversed, flicker persists longest in the outermost and innermost ring bands, disappearing at lower speeds in the middle one. The flicker is dependent on the difference of intensity of the white and black stimuli, and this depends for white on whether the most intense white—*i.e.*, that imbedded in black succeeds a period of exposure of the black or of the white sector of the disc.

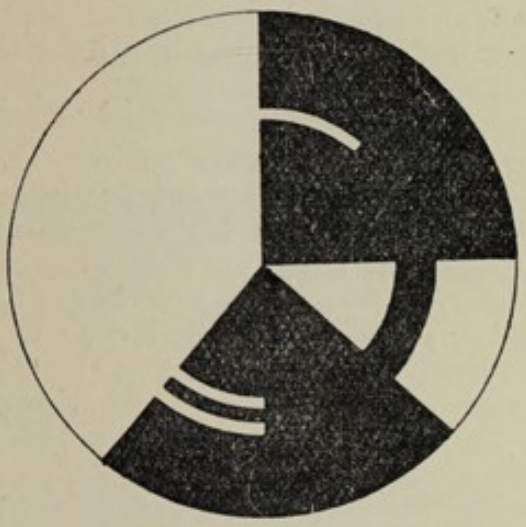


Fig. 434.—Disc for successive contrast (*Sherrington*).

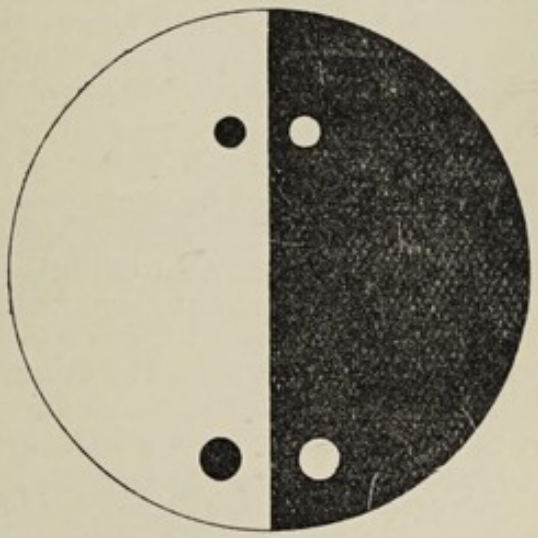


Fig. 435.—Disc for successive contrast (*Sherrington*).

In the above instance the successive contrast was studied as influencing the rate of fusion of intermittent stimuli. Its effect on tint can also be studied as follows :—A black and white disc is arranged as in fig. 435. On rotating the disc in the direction of clock hands the inner of the ring bands—that corresponding in the figure with the pair of smaller black and white circles—is lighter grey than the rest of the disc, and the outer of the ring bands is darker than the rest of the disc. Rotation in the opposite sense makes the outer ring-band lighter grey than the inner. The effect of the round white spot predominates or not over that of the round black spot according as the stimulation by the spot succeeds a longer or shorter exposure of the black sector (Sherrington, *Journal of Physiology*, "Simultaneous contrast and flicker," xx., 1896, and "Reciprocal action of retina," xxi., 33, 1897).

16. Coloured shadows.

(*a*) Place an opaque vertical rod (1 inch in diam.) in front of a white background. Admit not too bright daylight to cast a shadow of the rod. Place a lighted candle behind one side of the rod, the shadow caused by the yellow-red light of a candle, and illuminated by the daylight, appears blue—*i.e.*, a purely subjective blue, the complementary colour of the yellow-red light of the candle, which casts a yellow light. The effect is

more pronounced the darker both shadows are. To show that the blue is purely subjective, roll up a sheet of black paper—black surface innermost—in the form of a tube about ¼ inch or less in diameter. At a distance of 18 inches look at the centre of the blue shadow, and let an observer cut off the light from the candle by means of an opaque screen. On removing the screen no change is visible, but if the tube be directed to the line of junction of the blue shadow, with the illuminated background just beyond it, the blue appears.

(*b*) Hering's window experiment for contrast-effects.—In a window shutter of a dark room cut two square holes (10 cm.) on the same horizontal plane, and 2 feet apart. In one fix a piece of clear glass to admit ordinary white light, and into the other fit a red or green coloured glass. Both openings must be provided with a movable shutter to regulate the amount of light admitted. At 3 to 4 feet distance place a rod or flat piece of wood vertically against a white surface. Observe two shadows. Suppose the glass to be red, then the shadow due to the ordinary light is red, that of the red glass is greenish. Substitute for the red light that of a lighted candle. The shadow then appears blue.

17. Choroidal illumination.

(*a*) In a dark room light an ordinary lamp or fan-tailed gas-burner. Place the source of light at the right side, about 2 feet from an open book or sheet of paper. Partly separate the fingers of the left hand and place them over the face, so that different portions of the paper are seen by each eye. That half of the page seen with the right eye has a *greenish* tint, the other part seen with the left eye is red or *pinkish*. Change the source of light to the left side, the colours are reversed.

(*b*) With the conditions as in (*a*), hold a piece of paper (3 to 4 cm. wide), or a visiting-card, between the eyes with its flat surface towards the face, the same phenomena are seen.

(*c*) Cut in a piece of black cardboard two rectangular holes (4 × 10 mm.), separated by a distance about equal to that between the pupils, with the conditions as in (*a*). Hold the cardboard about 10 inches or more from you, and look through the holes at a white surface; four images of the two holes will be seen; the inner right and outer left images are impressions from the right eye; the inner left and outer right from the left eye. This is easily proved by closing either eye, when the images belonging to that eye disappear. If the source of light be on the right side, the former pair of images is greenish in colour, the latter is pale pink. Change the light to the left side and the colours are reversed (*H. Sewall*). The colour-phenomena occur without the aid of objective colour, and are due to light passing through the sclerotic and choroid coats.

18. Binocular contrast.

Place a white strip of paper on a black surface, look at the white paper and squint so as to get a double image. In front of the right eye hold a blue glass, and in front of the left one a grey (smoked) glass. The image of the right eye will be blue, that of the left yellow. Instead of the grey glass, a card with a small hole in it placed in front of the left eye does perfectly well. The yellow of the left eye is a contrast sensation.

19. Benham's spectrum top (Fechner's colours).

(*a*) A cardboard circular disc, about 4 inches in diameter, is made with one half black and the other half white. On the white are a number of arcs of concentric circles of different radius. On rotating this disc, coloured lines are seen whose order is reversed when the disc is made to

rotate in an opposite direction. The experiment is best performed by artificial light. (*Nature*, li., 1894-5.)

(*b*) Modification by Hurst.

On a circular disc, 4 or 5 inches in diameter, half white and half black, draw in black on the white half and in white on the black half arcs of various lengths and thicknesses, as, for instance, the arcs shown in fig. 436. Mount the disc on a peg and spin it. The arcs appear as circles of various colours, the colour of each depending on its position and length, on the velocity of rotation, and on the kind and intensity of illumination. The two outermost lines on the disc figured when the disc is turned to the left and seen in very bright lamp-light appear purple-grey, becoming, as the rotation becomes slower, brighter and redder, and then in succession bright crimson, scarlet, and orange-vermilion. By very bright direct sunlight the earlier shades are brighter than the later ones, the colour being at first usually a very pale blue. When the disc is turned to the right, the colours are in succession dark green, indigo fringed with pale blue, black, by lamplight ; while by bright sunlight the colour is first dull red, then brown, and finally dark blue. They appear, however, very different to different observers.

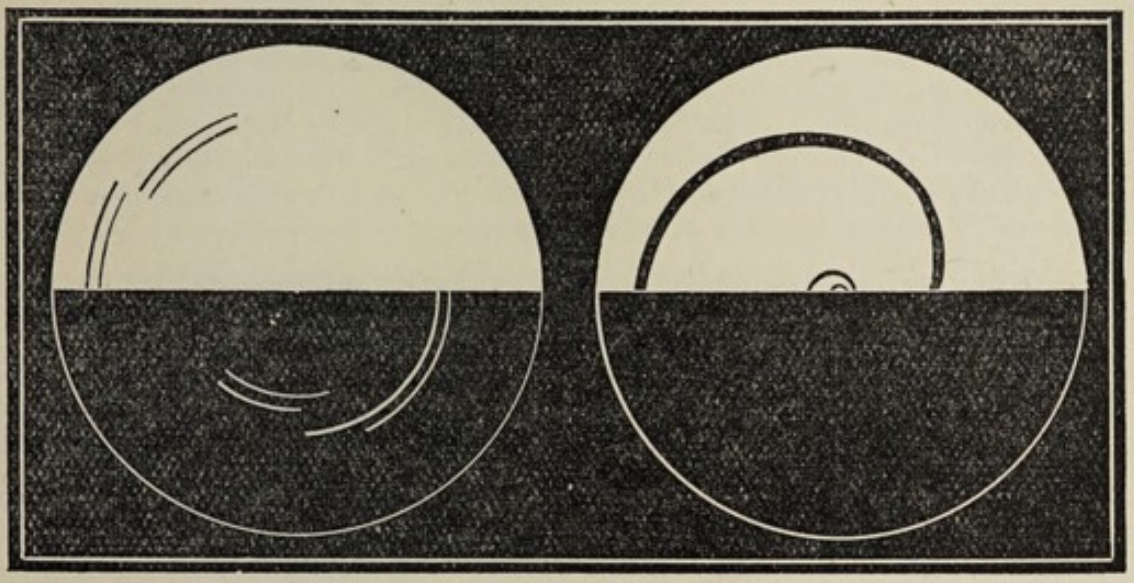


Fig. 436.—Modifications of discs for Benham's spectrum top (*Hurst*).

The colours of the white lines are almost entirely yellow, orange-pink, puce, and "electric blue."

If, instead of arcs of circles, a spiral-line is drawn as in fig. 436, the disc exhibits, when spun at a suitable speed, a broad band of colour consisting of a complete series of all the colours of the spectrum in their normal order, red being on the outer and violet on the inner side of the band when the disc is turned to the left, and in the reverse order when it is turned to the right. The purity of the colours seen depends very greatly on the light used. With bright daylight no trace of a spectrum is seen, but a series of colours ranging from purple through brown to green, or other series according to intensity of light and velocity of rotation. Even under the best conditions—namely, bright lamp-light, slow rotation, and the eyes too fatigued to follow the line round or sufficiently practised to remain motionless—the colours are not all brightest at the same moment. The violet has

merged into black before the rotation has become slow enough to give the brightest red and orange.

Beyond the limits of the spectrum-coloured band are two fringes, a purple or violet one beyond the red, and luminous pale blue on the violet side. These fringes, as well as the spectral band, change somewhat in colour as the speed of rotation changes.

The spiral is most easily drawn with a brush full of black paint by drawing it lightly across a rotating *white* disc while the disc is spinning. A suitable portion of the curve is chosen and the other half of the disc is blacked. Dull black paint, such as water-colour "lamp-black," is best.

A very different colour-band is produced by a similarly shaped spiral curve of white drawn on the black half of the disc. The colours are "electric" blue, pink, yellow, the blue being outermost when the disc is spun to the left. Spirals of various "pitches" may be used, the line itself being not more than one-fifth of the breadth of the space between two successive turns of the spiral.—(*Communicated by C. Herbert Hurst, Ph.D.*)

The appearances presented when the tops are viewed in monochromatic light are quite as surprising as those described above (see Abney, *Nature*, vol. li., p. 292, 1895).

20. Anaglyph.—For stereoscopic vision the pictures need not be completely separate, if they be so arranged that each eye gets its own figure only. This is done in the following way:—

The pictures of one object are printed on one card in different colours, say pale red and blue. The two pictures are slightly displaced relative to each other. On looking at the picture through a blue and red glass—*i.e.*, a blue glass in front of one eye, and a red one in front of the other—one sees a nearly colourless object, but the whole is stereoscopic.

21. Flicker.—Interleave two Maxwell's discs, one white and one black, so as to make a disc half white and half black. Rotate it on a whirling wheel, at first slowly, both sectors will be seen; increase the speed, more sectors will be seen, and then a marked flickering sensation will be felt—"coarse flicker," which gradually passes into a "fine flicker"—and when the speed is sufficiently great, the whole is fused into one continuous sensation of grey. If the experiment be done in diminished light, flicker appears with a lower rate of revolution; if in brighter light, a higher speed is required. Or use a black-white disc, in which the sectors are complete from centre to circumference. On rotating at a certain speed, one may find flicker is absent from the centre, but present at the periphery. Avoid too bright illumination.

(*a*) *Münsterberg-Jastrow phenomenon.*—Rotate a black-white disc to give a uniform grey. Move a thin rod rapidly in front of it. A number of shadowy images appear on the disc—largest where there are more white and black sectors.

(*b*) Repeat with disc of two colours. The images have the same colour as those on the disc.

LESSON LXXVIII.

STEREOSCOPE—HAPLOSCOPE—OPHTHALMOSCOPE—INTRAOCULAR PRESSURE—OPHTHALMOTONOMETER—KÜHNE'S ARTIFICIAL EYE.

1. Stereoscope (στερεός, solid; σκοπεῖν, to view).—Sir Charles Wheatstone's (1802-1875) reflecting stereoscope (1838-1849) is the earliest form. The form now used is the refracting prism stereoscope of Sir D. Brewster (1781-1868). Excellent forms of cheap hand stereoscopes are now in the market. The best set of stereoscopic slides for Brewster's stereoscope is the set of 36 issued by J. Martius-Matzdorff with a pamphlet, "Die interessantesten Erscheinungen der Stereoskopie," with Text, 2nd Ed. (1889), Berlin; and for lustre, "Zwölf Darstellungen des stereoskopischen Glanzes an Krystallformen." It does not take one long to "combine" binocular stereoscopic diagrams with free eyes and without the aid of a stereoscope.

(*a*) Examine a series of stereoscopic slides to show the combination of the images obtained by the right and left eyes respectively.

Retinal rivalry.—When the two retina are stimulated simultaneously by strong light of different colours or dissimilar objects—*i.e.*, where the two fields cannot be combined to give a single representation, or rather interpretation—there occurs a certain instability and alternation of the two fields. The rivalry is easily seen by looking at the skin through red glass placed close to one eye, and blue before the other.

(*a*) Place in a stereoscope a slide of glass with vertical lines ruled on one half of it and horizontal lines on the other half. Look at the two dissimilar images; note that they are not combined, but sometimes one sees it may be only the horizontal, at another only the vertical, lines. It may be done also with coloured slides.

(*b*) In the stereoscope place on one side some print, and in the other heavy horizontal lines. The print usually prevails.

(*c*) *Postage-stamp experiment* (*Stirling*).—Make a stereoscopic card by fixing to a stereoscopic card a green halfpenny stamp and the reddish halfpenny stamp that ceased to be issued a few months ago. This combination gives a *black*.

(*d*) **Lustre.**—Use a stereoscopic slide, preferably a geometrical pattern—*e.g.*, a crystal where the boundary-lines are white and the surfaces black. Besides rivalry such a slide shows a peculiar polish, glance, sheen, or lustre.

2. Lustre in coloured objects.

This may be shown by looking at a green patch (electric green) on a red ground through coloured glass—*e.g.*, a blue glass before one eye and a red one before the other eye. Other combinations may be made.

3. Stereoscopy dependent on differences of colour.

(*a*) Difference of colour may be the cause of an apparent difference in distance. If one looks from a distance of 3 metres at red and blue letters (8 × 4 cm.) on a black background, to most observers the red appears nearer than the blue. It is usual to explain this by difference of accommodation, more effort being required to focus for the red letters than for the blue; and hence the red is regarded as nearer. This is not a sufficient explanation, as many see the blue nearer than the red. The apparent difference disappears on closure of one eye, but on opening the other eye the difference of distance asserts itself. Is this due to stereoscopy? Einthoven supposes that it is. (Einthoven, "On the Production of Shadow and Perspective Effects by Difference of Colour," *Brain*, 1893, p. 191.)

(*b*) Brücke showed that the retinal images of differently coloured points are shifted with respect to one another. Fix on a black background a narrow vertical strip of paper, the upper and lower thirds being red and the middle third blue. On looking at the strip with one eye, the blue part deviates to one side and the red to the other side. "By covering either eye alternately a deviation of the red and blue parts in opposite directions will be observed; and on both eyes being used, the notion of a difference in distance is proved by the combination of the two images in such a way that the parts that deviate to the nasal side constitute the nearer image, the parts that deviate to the temporal side the further image." Einthoven finds that the stereoscopic effect is more marked with the coloured letters.

(*c*) The relative removal of the differently coloured images is due to the eccentricity of the pupil. The pupils may be made highly eccentric by covering them partially. With a nasal eccentric pupil—*i.e.*, covered on the temporal side—a shifting of the differently coloured images in one direction will be observed; with a temporal eccentric pupil—*i.e.*, nasal side covered—the shifting will be in the other direction.

Let any one who sees the red letters before the blue "cover his pupils symmetrically on the temporal side, the red letters retreat and soon appear to be behind the blue. On covering the pupils symmetrically on the nasal side, the red letters come forward more and more."

The bearing of these experiments is fully discussed by Einthoven in the paper already referred to.

4. Wheatstone's mirror stereoscope.—Place in the instrument the geometrical pictures marked R and L, and arrange to get a single solid stereoscopic picture. Reverse the L and R picture, and observe the "inverted stereoscopic picture."

Replace the pictures R and L and obtain a stereoscopic picture, as in (*a*). Slowly and equally draw them forwards, the stereoscopic figure gradually becomes *less*, until the single picture merges into two separate binocular flat pictures. Reverse the movement, pull them gradually and equally towards you, the picture becomes *larger*.

This experiment shows the effect of convergence of the eyeball on the

perception of distance, and of distance and visual angle on the size of the visual impression.

Pseudoscope (ψευδής, false) of Wheatstone (1852) and that of J. R. Ewald.

5. Tropostereoscope of Ludwig (fig. 437), made by Petzold (Stereoscopy with moving figures).—It consists of two metallic tubes (20 × 3 cm.) blackened within, attached to a handle and geared near the handle end. Coloured glass discs with incised diagrams are adjusted in the tubes at this end, while the opposite or open ends of the tubes are so arranged that they can be adjusted to the eyes of the observer. If red-coloured discs be adjusted to give a stereoscopic effect showing the apex of a truncated cone at one time towards and at another away from the observer, one can, by rotating the geared wheels, see the apex gradually approach or recede. The glass discs must be adjusted as they would be in a stereoscopic picture.

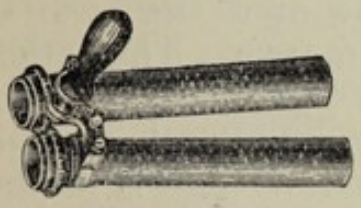


Fig. 437.—Tropostereoscope of Ludwig, as made by Petzold.

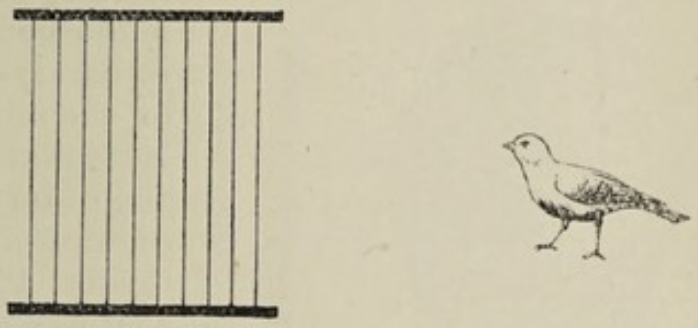


Fig. 438.—To illustrate haploscopic vision.

6. Haploscope (ἁπλοος, single).

Place the eyeballs in the primary position—*i.e.*, look straight ahead at a hypothetical object on a level with the eyes, but placed at the horizon. The visual axes are parallel, and we have two distinct and separate fields of vision. On looking through two parallel tubes, placed one in front of each eye, one obtains two different retinal pictures. Nevertheless, single vision is the result, and the two different pictures are combined to give an illusory sensation of one object. One gets approximately haploscopic vision with a stereoscope.

Haploscopic vision may be illustrated by vertical lines, parts of circles (Hering, *Hermann's Handbuch d. Physiologie*, iii., p. 355), or by the familiar bird and cage experiment (fig. 438). Hold the figure close to the eyes, separate the two fields of vision by a card held vertically in the mesial plane between the eyes, and look beyond the picture—*i.e.*, allow the eyeballs gradually to diverge from the point of convergence. On doing so, as the visual axes become less convergent, one has on the right visual field a bird, on the left a cage—the bird appears to move into the cage, and in consciousness we have the illusion as if the bird were in the cage.

7. Ophthalmoscope.—Two methods are employed, and the student must familiarise himself with both by examining the eye of another person, or that of a rabbit, or an artificial eye. A convenient form of this instrument is shown in fig. 439.

(1) **Direct**—giving an upright image.

(2) **Indirect**—giving an inverted image.

A. Human eye.—(1) Direct method.

(*a*) About twenty minutes before the examination is commenced, instil a drop of solution of sulphate of atropine (2 grains to the ounce of water) into, say, the *right* eye of a person with normal vision. The pupil is dilated, and accommodation for near objects is paralysed owing to the paralysis of the ciliary muscle. The patient is seated in a darkened room, and the observer seats himself in front of him and on a slightly higher level. Place a brilliant light, obscured everywhere except in front, on a level with the left eye of the patient.

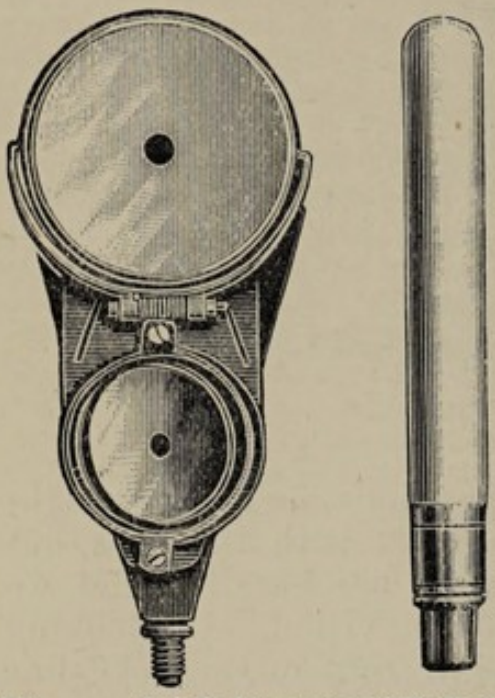


Fig. 439.—Ophthalmoscope of Walker Hall, made by Armstrong & Brother, Manchester.

(*b*) The observer takes the ophthalmoscope mirror in his right hand, resting its upper edge upon his eyebrow, holds it in front of his own eye, looking through the central hole in it, and directs a beam of light into the observed eye, when a red glare—the reflex—is observed. The patient is told to look upwards and inwards, which is conveniently accomplished by telling him to look to the little finger of the operator's right hand. The operator then moves the mirror, with his eye still behind it, and looks through the hole until the mirror is within *two to three inches* from the observed eye, taking care all the time that the beam of light is kept steadily thrown into the eye. If the eyes of the observer and patient be normal, the observer has simply to relax his accommodation—*i.e.*, look, as it were, at a distant object—when the retina comes into view as an erect or upright object.

(*c*) Observe the *retinal blood-vessels* running in different directions on a red ground. Move the mirror about to find the *optic*

disc, with the central artery emerging from it. Trace the course of the veins accompanying the arteries across the disc.

(2) **Indirect method**, giving an inverted image.

(*a*) The patient, the light, and the observer are as before. The observer places himself about 20 to 18 inches from the patient, and, holding the mirror in his right hand, by means of it throws a beam of light into the eye of the patient. When the eye is illuminated, he takes a small biconvex lens of 2 to 3 inches focus in his unemployed hand—the left in this case—holding it between his thumb and index-finger, placing it vertically 2 or 3 inches from the observed eye. To ensure that the lens is held steadily, rest the little finger upon the temple or forehead of the patient. Keep the lens steady, and move the mirror until the optic disc is seen, with the details already described.

In the direct method only a small part of the retina is seen at one time, but it is considerably magnified; while by the indirect method, although more of the retina is seen at once, it is magnified only slightly.

If the observed or observer's eye is abnormal, suitable glasses to be fixed behind the mirror are supplied with every ophthalmoscope. In some forms of ophthalmoscope, such as that of Gowers and others, these lenses (convex +, and concave −) are fixed to a rotating disc behind the mirror. As the disc is rotated, lens after lens can be brought to lie exactly behind the hole in the mirror, and thus correct any anomaly of refraction.

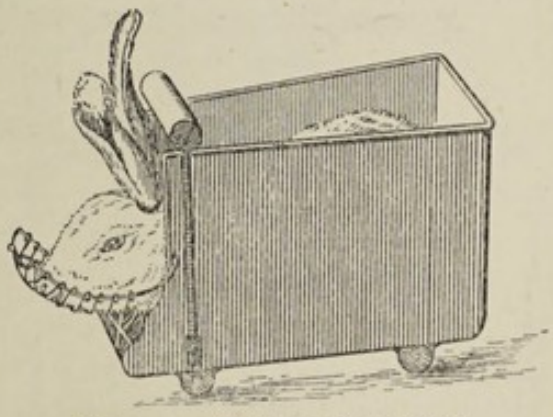


Fig. 440.—Carriage for rabbit.

8. Eye in living rabbit.

Instil atropine as before, or use an atropinised gelatine disc to effect the same result. Place the rabbit in a suitable cage to keep it from moving. A suitable one was devised by Michel; use it (fig. 440). Examine the eye by the direct and indirect methods. *N.B.*—If an albino rabbit be used, the observer sees the large choroidal vessels.

9. Perrin's artificial eye (*Lancet*, 1871).

Use this until a clear image of the fundus is obtained by both methods. In fact, it is well for the student to begin with this. In this model, eye-

caps to fit on to the eye are supplied, so as to render the eye-model either myopic or hypermetropic. Afterwards test these, and use the necessary lenses behind the mirror to correct these errors in the shape of the eyeball.

(*a*) Professor Grützner uses a little apparatus filled with normal saline and provided with a flat piece of glass to fit inside the eyelids. By this means one eliminates the effect of curvature of the cornea, and one can look directly to the fundus. It practically amounts to an orthoscope.

10. Kühne's method.—If an artificial eye is not at hand, a very suitable arrangement is that devised by Kühne. Paint a disc to resemble the normal fundus when it is seen with the ophthalmoscope. Remove the eye-piece—long one—from an ordinary microscope. Screw out the lower lens of the eye-piece, fix in the painted disc, and block up the lower aperture with a piece of cork. Fix the eye-piece in a suitable holder, and use it instead of an eye to be examined.

(*a*) A pill-box blackened inside, with a hole cut in the lid, does perfectly well.

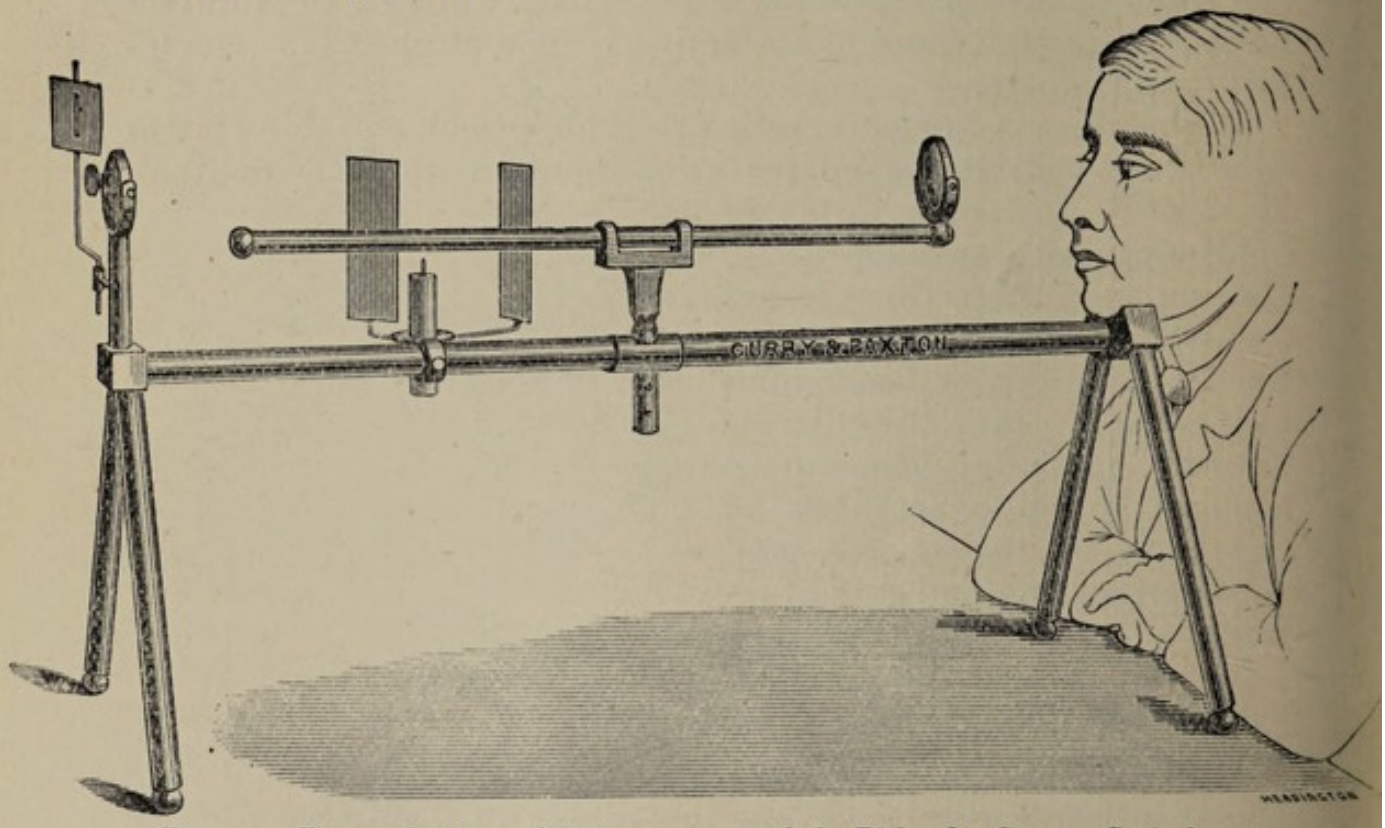


Fig. 441.—Demonstrating ophthalmoscope, made by Pickard & Curry. Cost, £3 10s.

11. Demonstrating ophthalmoscope (*Priestley Smith*).

The general arrangement of this instrument is shown in fig. 441. At one end of the horizontal bar is a chin support for the patient; at the other a perforated glass mirror, capable of steady adjustment to any position. The transverse arm near to the mirror carries a candle, provided with a light metal screen on either side of it; one of these hides the candle from the patient, the other hides it from the observer, and enables him at any

moment to cut off the light from the mirror, and thus to protect the patient's eye from unnecessary illumination without disturbing the adjustment of the instrument. A wire placed in the pillar of the mirror, and movable to either side, carries a piece of white paper, which serves as a fixation point for the patient's eye. At the middle point of the horizontal bar is a jointed support carrying a light rod, one end of which is held in the hand of the observer, while the other holds the lens. By means of this rod the observer can place the lens in any desired position in relation to the patient's eye.

(1) Arrange the instrument as in fig. 441.

(2) Adjust the patient's seat so as to bring his chin comfortably on the support; let him rest his arms upon the table.

(3) Place the rod quite horizontal, and then raise or lower the central support until the centre of the lens is on a level with the patient's pupil.

(4) Push the lens to one side and adjust the mirror so as to throw the light upon the patient's eye, telling him to look, *not at the mirror*, but at the paper placed upon the wire. The paper must be on the opposite side to the eye.

(5) Take the rod in one hand and adjust the position of the lens so as to bring the optic disc into view.

(6) In changing places with another observer, cut off the light from the mirror by means of the candle-screen.

12. Intraocular Pressure—Fick's Ophthalmotonometer.

This instrument is extensively used in German eye-hospitals, and consists of a small brass plate (6 mm. diameter), which is attached by means of a metallic spring to a base, which also carries a scale which indicates the amount of pressure applied. One presses the disc of the instrument against the eyeball until it flattens the part to which it is applied, when the pressure is read off in grammes. The experiment may be done first on a rabbit, as most of them remain quite passive. Place a person with his left shoulder next the window, ask him to turn his eyeballs to the right and open his eyelids, whereby sufficient of the eyeball is made visible for the application of the instrument (*Pflüger's Archiv.*, xlii., 1888).

13. Pupil.—Normally the pupil in man, rabbits, and other animals is black, but in albinos it is reddish. Why?

(1) Select an albino rabbit, and exactly in front of its pupil hold up a black card with a hole in it the size of the pupil. Direct the pupil to the light, and arrange the shade so that all light is kept from the eye except that which enters it by the pupil. The albino pupil then appears black.

This shows that the blackness of the pupils is not due to the light entering the eyeball being absorbed by the pigment of the fundus of the eye, but that light entering the eye can only emerge by the pupil when the iris and the neighbouring parts of the choroid, in virtue of their pigmentation, do not permit light to pass through them. The construction of the dioptric apparatus of the eye is such that light from the fundus of the eye must be reflected back to the source from which it came—*i.e.*, to the focus. As we emit no light from our eye, none can come to us from the observed eye, so we see the pupil black because we do not illuminate the fundus with our body (*Schenck*).

B. Kühne's artificial eye (fig. 442).

(*a*) Fill the instrument with water, and place it in a darkened room with the cornea directed to a hole in the shutter, through which sunlight is directed by means of a heliostat. If this is not available, use an oxy-

hydrogen lamp or electric light to throw parallel rays of light on the cornea. If these cannot be had, use a fan-tailed gas-burner, but in this case the illumination and images will be feeble. To enable one to observe the course of the rays of light, pour some eosin or fluorescein into the water in the instrument.

(*b*) Formation of an image on the retina. Observe the course of the rays of light which come to a focus behind the lens—the principal posterior focus. Move the ground glass representing the retina, and get a clear inverted image of the source of light. *N.B.*—In this instrument accommodation is effected not by altering the curvature of the lens, as in the normal eye, but by moving the retina.

(*c*) Place convex and concave lenses between the source of light and the cornea ; observe how each alters the course of the rays and their focus.

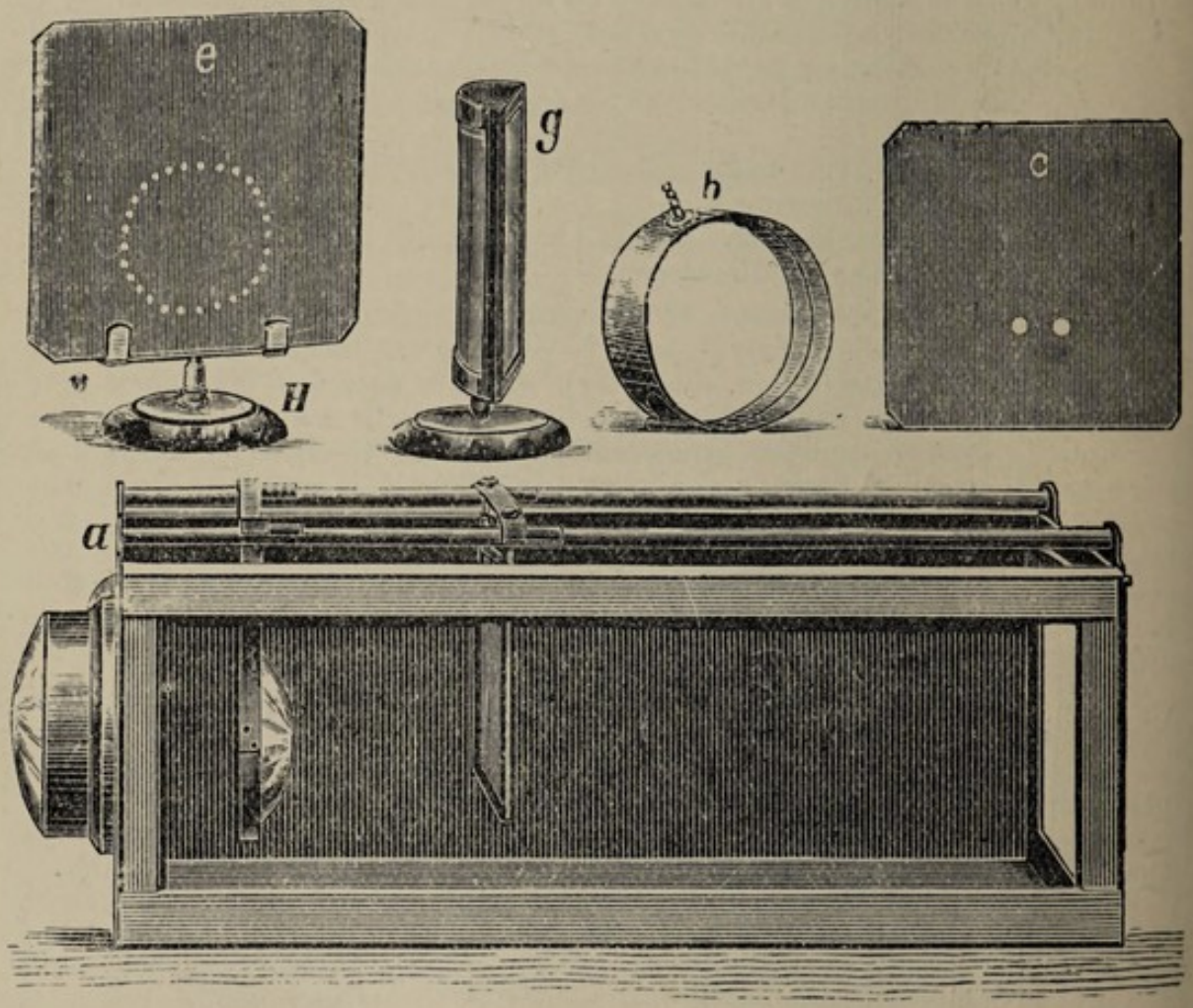


Fig. 442.—Kühne's artificial eye, as made by Jung, of Heidelberg.

(*d*) After having an image well focussed upon the retina, move the latter away from the lens, when the image becomes blurred owing to diffusion. If, however, a slip of zinc, with a hole cut in it to act as a diaphragm to cut off some of the marginal rays, be interposed, the image is somewhat improved.

(*e*) After seeing that the light is sharply focussed on the retina, remove the lens—to imitate the condition after removal of the lens for cataract—and observe that the rays are focussed quite behind the retina.

(*f*) Place the removed lens in front of the cornea, the principal focus is now much in front of the retina, so that a much weaker lens than the one removed has to be used after removal of the lens for cataract.

(*g*) **Astigmatism.**—Fill the plano-convex glass, *g*,—to imitate a cylindrical lens—with water, and place it in front of the cornea. Between the cornea and the cylindrical lens place a sheet of zinc with a cross cut out in it, or with a number of holes in a horizontal line. One cannot obtain a distinct image of the cross or the holes as the case may be.

(*h*) **Scheiner's experiment.**—With the light properly adjusted, place in front of the cornea a piece of zinc perforated with two holes, *c*, 1 cm. in diameter, in a horizontal line, the distance between the holes being less than the diameter of the pupil. Find the position of the retina—and there is only one position—in which the two beams of light are brought to a focus. Move the retina towards the cornea, and observe two images; close the right-hand hole, and the right-hand image disappears. Bring the retina posterior to the principal focus, and again there are two images. On closing the right-hand hole the left-hand image disappears, and *vice versâ*.

LESSON LXXIX.

CUTANEOUS SENSATIONS—COLD, HOT, PRESSURE, AND PAIN SPOTS—MUSCULAR SENSE—RESISTANCE.

In many of these experiments two students work together—a kind of "laboratory partnership" where A is the person experimented on and B the experimenter who makes the experiment on A. B arranges the instruments and records the results. At the end they change.

In studying the phenomena of touch, A closes his eyes or is prevented from seeing what is going on.

From the skin we obtain at least four different qualities of sensation—pressure, cold, warmth, and pain. The last is associated with organs in the epidermis, and the other three come from the cutis. There are separate localised cutaneous terminal organs for cold, warmth, and pressure, so that the skin is a mosaic of organs administering to several sensations of different quality, and does not give us a series of graduated qualities such as we get from the eye or ear.

How accurately can the skin by itself, and without the aid of vision, localise an impression made on it? Our tactual sensa-

tions differ in locality and in intensity. Under the latter is included contact and pressure.

1. Touch—Localisation of single point on skin.—(*a*) Let A sit at a table and place his left hand flat on the table, with digits outstretched and dorsal surface uppermost, right hand resting on the right knee. Eyes shut. B tells him to touch with his index (or middle) finger of the right hand the tip of a finger named by B. Record result and number and extent of failures.

(*b*) With the head of a pin dipped in a pigment (soot used by Weber) B makes several marks on A's forearm, then B touches and asks A to put his finger on the spot touched by B.

(*c*) Using the volar edge of the arm of A, supported and arranged as in (*a*), with a pencil point B makes an impression on A, and A is asked to touch this spot with the point of another pencil. Make a map and record the result.

2. Tactile spatial discrimination—Threshold for two points.—How far apart must two cutaneous impressions lie to be

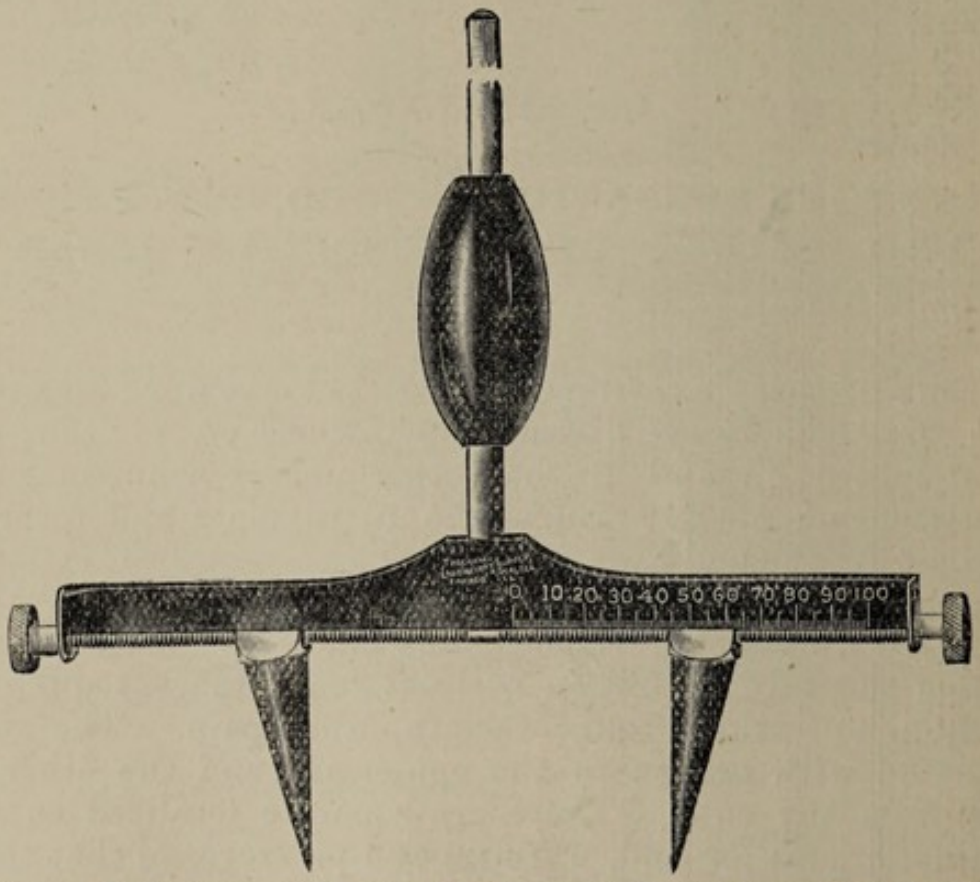


Fig. 443.—Æsthesiometer of Jastrow, made by the Chicago Supply and Scale Co.

perceived separately—*i.e.*, localised at different places (Weber's method, 1834). The instruments used are called **Æsthesiometers.** B uses a pair of dividers with their points

guarded by small pieces of cork, or Sieveking's or Jastrow's æsthesiometer (fig. 443), or Griesbach's dynamometrical æsthesiometer (fig. 444). The latter are already graduated. The limbs of the instrument should be applied vertically to the cutaneous surface. B applies lightly the points of the compasses simultaneously to different parts of A's skin, and ascertains at what distance apart the points are felt as two. The following is the order of sensibility :—Tip of tongue, 1·1 mm. ; tip of the middle finger, 2·3; palm, 8 to 9; forehead, 22; back of hand, 31·6; back, 66.

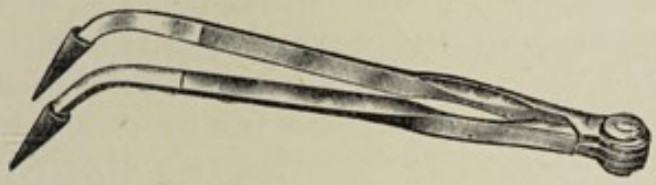


Fig. 444.—Æsthesiometer with guarded points.

(*a*) Let A expose his forearm and seat himself near a table [eyes closed]. B applies both points of the compasses, gently, equably, and simultaneously to A's forearm, say, in the longitudinal axis—peripheral-central—of the arm, the points being 35 mm. apart, and kept applied for 2″. A says if he feels one point or two points, or if he is in doubt. B records result, distance of points, and A's statement. It is well also to test from time to time by applying only one point. Make the distance 30 mm. and try again, and so on until A can distinguish two points in a given area on forearm. The minimum distance is the threshold. A is then shown the result.

(*b*) Test as before other regions—*e.g.*, tip of forefinger and tip of middle finger on palmar surface. The spatial sense is more acute on the latter. Work also cross-wise on the arm. Avoid temperature spots. Record result—*e.g.*, example from Titchener.

Left Forearm, Volar Surface ← →.

Mm. ↓	Judgment.	Mm. ↓	Judgment.
24	2 points.	20	1 point.
23	2 ,,	19	1 ,,
22	2 ,,	18	1 ,,
21	2 ,,		

(*c*) Test as in (*b*) the skin of the arm, beginning at the shoulder and passing downwards. Observe that the sensibility is greater

as one tests towards the fingers, and also in the transverse than in the long axis of the limb.

(*d*) The tactual sensations may be *successive*. The threshold for distance is less—*i.e.*, they are more acutely judged (*Weber*)—when the points are applied successively instead of simultaneously. The direction in which the points are applied can also be studied; also the effect of differences of the temperature of the two points and of exercise.

(*e*) By means of a spray-producer spray the back of the hand with ether, and observe how the sensibility is abolished.

3. Localisation—Aristotle's experiment.—Stimulation of parts whose relative position can be changed—*i.e.*, how localisation is affected by change in the normal position of two stimulated surfaces (*Titchener*), or the localisation of two impressions which lie in two different areas.

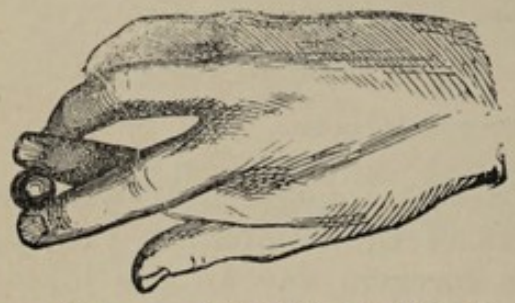


Fig. 445.—Aristotle's experiment.

(*a*) Cross the middle over the index-finger (fig. 445), roll a small ball or pea between the fingers; one has a distinct impression of two balls. Or, cross the fingers in the same way, and rub them against the point of the nose. The same illusion is experienced. Touch first with the forefinger and then with the second finger, the contacts seem to occur in different points of space, the forefinger touch being higher.

(*b*) A lays his hand flat, volar surface uppermost, and closes his eyes. B crosses middle under third finger, and places in the fork a pencil so as to touch both phalanges on the radial side of the third finger and ulnar side of the middle one. A has the impression of two distinct objects.

4. Illusions of tactual judgments of space, locality, and motion.—(*a*) Touch two points of the skin, *a* and *b*, simultaneously with the compass-tips. The distance appears greater than if one compass-tip travels from *a* to *b*. The distance between *a* and *b* seems shorter the quicker the movement, and longer if the compass-tip is moved slowly. If the edge of a card be used, the distance seems shorter than that between the compass-tips.

(*b*) Close the eyes, and touch the circumference of a ball or any circular disc with a rod, follow the periphery with the rod, the ball appears larger the shorter the rod.

(*c*) Press the cross-section of a tube on the skin of the forearm. It feels transversely oval.

(*d*) Estimation of the distance of two neighbouring parts depends on the size of the sensory circles. If the points of a pair of compasses—blunt points—about 2 cm. apart, are placed on the skin in front of the ear and moved across the face towards the lips, so that the lips fall between them, the person feels as if the points diverged as they pass towards the lips, and include it in an ellipse (*E. H. Weber*).

(*e*) Similarly draw the compass-points, 1 to 2 cm. apart, down over the forearm, wrist, palm, and fingers. It feels like a single line opening up

into two lines, which seem to separate more widely below the wrist, to become narrower in the palm, and to diverge rapidly towards the adjacent finger-tips (*E. H. Weber*).

(*f*) Place 1, 2, 3, 4, or 5 fingers at different distances apart and on different parts of the scalp, and ask the person touched to indicate how many fingers are applied.

(*g*) *Illusion of length.*—Take a coarse thread, 2 feet long, and make a knot in the middle. Let A hold and gently press the knot between finger and thumb. B moves the thread at first slowly and then quickly, and asks A (eyes closed) to estimate the length.

(*h*) Without his seeing it, let A draw his hand over a cooled dry and highly-polished nickel-plated iron bar. He will feel a sensation as if the bar is *wet*.

(*i*) Let B show A a clothes brush. Let A close his eyes and turn his back to B, who runs his hand over A's back, at the same time and in the same tempo rubs the brush over his own clothes. Probably A will form the impression that the brush is actually being passed over his own back.

5. Over-estimation of tactual space.—Apply to A's hand part of the toothed edge of a card, and then ask him to pass his finger over the toothed edge to indicate the length applied to his hand. A will most probably indicate the distance as too short. Pass the actual length over A's finger, it appears longer than the distance felt during the first mere passive contact. In combination with movement the length is over-estimated.

6. Dresslar's card.—Draw diagonals across a card, perforate these with holes at short intervals by means of a thick pin, so as to produce a series of elevations on the opposite side of the card. Run a finger along one diagonal row of these elevations. One obtains the impression that this diagonal does not cut the other at one point, but rather as if it were met by two elevations, one in each section. (*American Journ. of Psychology*, vi., 1893-95.)

7. Sense of temperature.

(*a*) Ask A to close his eyes. Use two test-tubes or spoons (hot, cold). Apply one or other to different parts of the surface and mucous membranes, and ask A to say whether the touching body is hot or cold.

(*b*) Touch fur, wood, and metal. The metal feels coldest, although all the objects are at the same temperature. Water at the same T° feels cooler than air.

(*c*) Cold spots.—With a blunt metallic point touch different parts of the skin. Certain points excite the sensation of warmth, others of cold, although the temperature of the skin and the instrument remain constant.

(*d*) Draw a blunt pencil-point slowly across the back of the hand, several cold spots will flash out. The hot spots are more difficult to localise.

8. Study of "temperature spots."—*Apparatus.*—Two of Goldscheider's metal rods (1 × 9 cm.), ending below in a round point (1 mm.), and above in a disc 0·5 cm. in diameter. They may be fixed in an adjustable clip with a handle, or merely covered near the middle with rubber tubing.

Weak alcoholic solutions of red, brown, and green aniline dyes. Camel-hair brushes. Transparent millimetre tracing paper. Vessels to hold ice and salt, and a Bunsen burner.

Instead of Goldscheider's rods, the *Kronecker-Miescher thermo-æsthesiometer* may be used. It consists of a conical tube 45 cm. long, divided by a septum incomplete near the apex like a perfusion cannula. One compartment is connected with the inlet for the water, and the other with the outlet. The apex terminates in a platinum circular surface 1 mm. in diameter. Water of any desired temperature can be circulated, and its exact temperature ascertained, as there is a thermometer bulb in the outlet compartment. The instrument is held like a pen.

9. Cold spots.—The cold spots are not all equally sensitive. In some areas—*e.g.*, forehead—the intense cold spots are most abundant. Some excite only a feeble sensation of cold. There are areas where neither cold nor heat are experienced; these areas are totally thermo-anæsthetic. It is to be noted that the cold and hot spots are locally quite distinct. Moreover, the areas of greatest tactual sensibility do not necessarily coincide with those of greatest temperature sense. The tips of the fingers are relatively poor in temperature spots, while the spots are abundant on the dorsal surface of the hand. Tactual sensation is absent from these spots.

(*a*) Let A lay his left hand lightly clenched on the table, dorsal surface uppermost, and with forearm exposed.

(*b*) B, with a camel-hair pencil dipped in alcoholic solution of Bismarck brown, maps out on the skin over the dorsal surface of the second and third metacarpal bones, or on the dorsal surface of the forearm of A, a parallelogram—width 2·5 cm. (peripheral-central direction) and 1·5 cm. (right-left direction). Cool the rod in iced water or mixture of ice and salt and dry it.

(*c*) B applies lightly the rounded tip of the cooled rod for 2 seconds at one corner of the parallelogram. If it is said to be felt by A as cold, mark the spot with a green dot. Proceed in this way, by moving the point of the rod slowly and steadily—using not too great pressure—along one side of the parallelogram, marking each cold spot green. When a cold spot is found, A says "There." Repeat the process, beginning a new line about 1 mm. from the first and parallel to it. Go on until the whole area is mapped out.

This gives the distribution of cold spots in the skin area chosen. A will be able to state that at some of the points the cold was sharp and felt at once, while in other spots it was just felt, and in still others no sensation of cold was felt. The same process may be repeated in the transverse direction.

(*d*) If desired, after an interval re-investigate the "cold

spots" and mark those most sensitive (and with ready and prompt response) with some other distinctive colour. Thus one gets a mosaic of cold spots of different intensity. For a permanent record, make a tracing on tissue paper placed over the parallelogram.

N.B.—The temperature spots are easily fatigued.

10. **Warm spots.**—Proceed as above, but heat the rod to about 70° C. either in warm water or with a Bunsen. Let the instrument travel over the skin rather more quickly than for determining the cold spots. Mark the hot spots with a red dot. They will probably be less defined than the cold spots.

The cold spots are more numerous than the warm, and arranged either in small clusters or in chains, often radiating from near the exit of a hair. The cold sensation has a short latent period, like touch, and is localised more superficially than the warm spots, which are less easily located and have a longer and slower-reached latent period. There are no cold or hot spots on the cornea. Spots are not all equally sensitive, some are "tuned," "adapted," to respond by sensations of varying intensity to a given stimulus.

11. **Mechanical stimulation of a cold spot.**—Make skin of spot tense, tap the cold spot, and a sensation of cold may be obtained. Cold spots are analgesic. Thrust a fine needle, slightly warmed, through a cold spot. It is usually not felt as a pricking pain, but as cold.

12. **Chemical stimulation of a cold spot—Menthol.**—Rub the skin of the back of the hand or temple with menthol. A sensation of cold is felt, because the cold spots are made hyperæsthetic, so that they respond more readily to stimuli. The T° of the skin is not lowered (*Goldscheider*). Later all the cutaneous senses become more or less anæsthetic.

13. **Mechanical pressure excites cold spots.**—Shape an ordinary cork at one end to terminate in a conical point, or use elder pith. Press with the cork tip here and there on the back of the hand—first, one has a feeling of contact, and on deeper pressure, if in a region where cold spots are abundant, out flashes a sharp deep-seated sensation of cold. It is more difficult to localise the hot spots in this way. Personally, I find good areas for cold over the veins on the dorsum of the hand and at the metacarpo-phalangeal joints. The after-sensation seems to last for a considerable time.

14. **Electrical stimulation of thermal spots.**—Locate a cold spot. Begin with weak induced currents, and apply one pole or needle over this point, and let the other electrode be large and applied to the hand, and resting on a piece of moistened blotting-paper (*Blix*).

15. **Cold spots are analgesic.**—Test with pin (§ 11).

Herzen's Observation.—In a limb which is "sleeping"—*e.g.*, by compression of sciatic on the edge of a chair—the cold spots are paralysed, and the warm not. Compress the radial, ulnar, or sciatic nerve, and it will be found that pressure disappears first, then cold, hot, and, lastly,

pain. Herzen concluded that there are different paths for the conduction of cold and heat impulses (*Pflüger's Arch.*, xxxix., 1886).

16. Temperature sensitivity.—(*a*) Heat to about 70° C. a metallic knob with a flat surface (0·5 cm. diameter), or use the broad end of a Goldscheider's rod. Apply it to forearm, forehead, cheek, palm of hand, back of hand, &c., and compare intensity of sensation obtained.

(*b*) *Continuous stimulation over a larger area—i.e.*, stimulus, hot or cold, over a mosaic of temperature spots. Map out a parallelogram (5 cm. × 3 cm.) on the radio-dorsal surface of the skin of the forearm just below the elbow joint. For cold stimuli use rods at 12° to 15° C., and for warm 37° to 40° C. Apply broad end of cylinder to one corner of the parallelogram for 1 second. Let A call out "Intensely cold," "Cold," "Just cold," "Nothing." Record result on arm or on tissue paper of same size as skin area. Mark the areas in accordance with these results, varying depth of tint of green, or use dots or other distinctive mark. Map out whole area.

(*c*) Make a similar experiment with the warm cylinders (*Experimental Psychology*, by E. B. Titchener).

17. Skin *v.* mucous membrane for temperature.—The temperature sense is very unequally distributed over the surfaces of the body. The mucous membranes are very insensitive. Sip hot coffee, it does not feel uncomfortably hot so long as it is in contact with the mucous membrane of the mouth or red part of the lips. Let it touch the skin of the lip or dip a finger into it, it feels burning hot.

18. Some experiments on temperature sense.

(*a*) *Successive temperature contrast* (alteration of *physiological zero*).—For 1 minute hold one hand, R, in water at 40° C., and the other, L, in ice-cold water. Place both in water at 20° C. The water will feel cold to R and warm to L. Original experiment was suggested by John Locke (1632 to 1704).

(*b*) Hold one hand in water at 10° C., and afterwards place it in water at 20° C., at first the latter causes a sensation of heat, which soon gives place to that of cold.

(*c*) *Effect of area-extension.*—Place a finger in water at 48° C. It can be kept in for a long time. Hold the whole hand in, it soon becomes painful.

(*d*) Plunge the hand into water at 36° C. One experiences a feeling of heat. Then plunge it into water at 30° C., at first it feels cold, because heat is abstracted from the hand. Plunge the other hand direct into water at 30° C. without previously placing it in water at 36° C., it will feel pleasantly warm.

(*e*) *Discrimination of T°.*—Test with the finger the acuteness of the sense of temperature—*i.e.*, in two given fluids of different temperature, what fraction of a degree C. can be distinguished. One can usually distinguish $\frac{2}{5}$°, although the acuteness is greater when the fluids are about 30° C.

(*f*) The median line is less sensitive to temperature than parts right and left. Touch the tip of the nose or middle line of forehead with a piece of hot or cold metal, and then several points right and left.

(*g*) *T° after-images.*—Press cold metal on the forehead for 30 seconds. The cold feeling persists, although the T° is rising. The after-images are always positive.

19. Illusions relating to temperature.

(*a*) *Effect of temperature on estimation of weight.*—Place a thin disc of *cold* lead the size of a florin on the forehead of a person whose eyes are

closed, remove the disc, and on the same spot place two warm discs of equal size. The person will judge the latter to be about the same weight, or lighter, than the single cold disc.

(*b*) Compare two similar wooden discs, and let the diameter of one be slightly greater than that of the other. Heat the smaller one to over 50° C. and it will be judged heavier than the larger cold one.

(*c*) Lay on different parts of the skin a small square piece of paper with a small central hole in it. Let the person close his eyes while another person gently touches the uncovered piece of skin with cotton wool, or brings near it a hot body. In each case, ask the observed person to distinguish between them. He will always succeed on the volar side of the hand, but occasionally fail on the dorsal surface of the hand, the extensor surface of the arm, and very frequently on the skin of the back (*Fick and Wunderli*, 1860).

Pressure.—The pressure spots, sometimes called "touch spots," are intimately related to the hair-follicles, though they occur elsewhere. Every hair-follicle has its pressure spot, which "lies to the windward of the hair itself"—*i.e.*, it lies directly above the hair-follicle on the side from which the hair slopes. It would thus appear that the hairs play other offices besides those of mere mechanical and thermal functions. Touch the tip of a hair and notice the exquisitely clear and well-defined sensation. It is greatly more sensitive (3 to 12 times) than the pressure spots. There are about fifteen such spots in a square centimetre of skin of the calf of the leg. A fine needle thrust into one of these spots gives rise to a sharply localised feeling of pressure and is unaccompanied by any painful sensation. The spots are usually arranged in short lines near the mouth of the hair-follicles. Goldscheider counted seven to eight such spots in an area containing fifteen hairs on the back of the forearm and a greater number on the flexor aspect. They are absent from the cornea and glans penis.

20. Pressure spots (Touch Contact).—Use a rounded point of hardwood, 1 mm. in diameter, fixed in a suitable rod. Map out a square on the dorsal surface of the hand between the fourth and fifth metacarpal bones. Cut away the hairs and make a map of them (blue dots) on tissue paper. Make separate pressures along the sides of the square, as for warm and cold spots. These spots are easily fatigued.

(*a*) **Von Frey's method.**—A hair of the head or beard or a horse hair (20 to 40 mm. long) is fixed to a wooden match. On pressing the point of the hair against the skin it may or may not be felt as a tactile sensation. This depends on the pressure exerted on the hair, and this in turn on the sectional area and stiffness of the hair itself. One can measure the pressure exerted by pressing the hair on a balance, and from the sectional area of the hair deduce the pressure per sq. mm. Fig. 446*b* shows another

form of the instrument. According to v. Frey the sensibility of the cornea and conjunctiva is distributed in a punctiform manner, insensible areas existing between ; pain alone, according to v. Frey, being experienced from stimulation of the cornea with the exception of the margin, and the teeth, or rather the dentine and pulp. (V. Frey, "Beiträge z. Physiologie d. Schmerzsinns," and "Beit. z. Sinnesphysiologie d. Haut," *Berich. a. d. math.-phys. Classe d. Königl. Sächs. Gesell. d. Wissen.* Leipzig, Dec., 1894, and March, 1895. Criticism by Nagel, *Pflüger's Archiv*, Bd. 59, p. 563, 1895.)

(*b*) *Adaptation to pressure.*—Weight a large cork with sheet lead. Place the cork on the exposed arm of A, whose eyes are closed. In a short time the pressure sensation disappears.

(*c*) Dip the hand or a finger into mercury. The greatest sensation is felt at the plane of the fluid in the form of a ring, but even this is best felt on moving the hand up and down.

(*d*) Select a number of similar corks and cover their under surfaces with substances of varying roughness—plush, velvet, flannel, buckram, hard and soft wood. Let A try to identify or describe the pressing surfaces. To do this requires active touch as well as pressure.

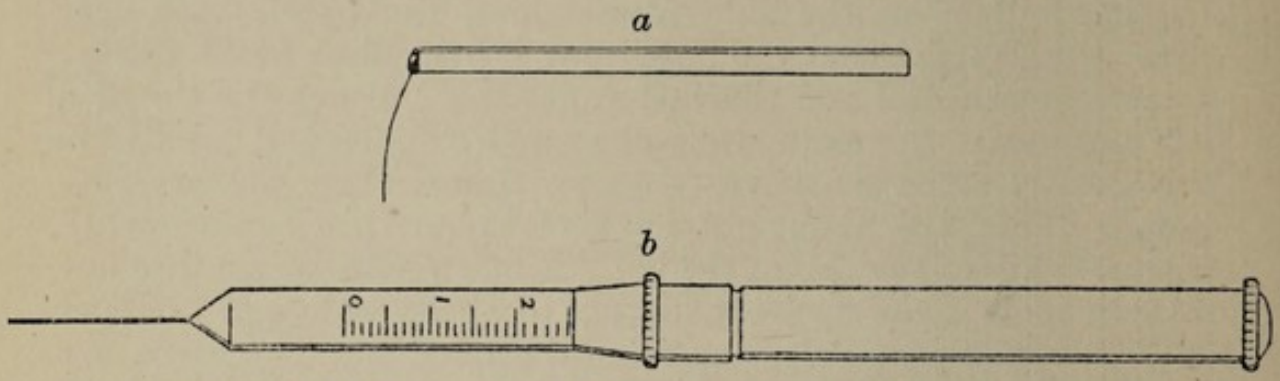


Fig. 446.—Von Frey's hair æsthesiometer. *a*, Original form made with a lucifer match. *b*, Latest form.

(*e*) *Feeling of double contact* (eyes closed).—Usually the part that moves feels the one that does not, but it is not always so. Feel the palm of the right hand with the ball of the left thumb. Feel the forehead with the hand ; one usually feels the forehead. Feel the forehead with knuckle of the forefinger sharply bent, and move the forehead. One seems to feel that it is the finger that is in motion.

(*f*) Touch your forehead with your forefinger, the finger appears to feel the contact ; but on rubbing the forefinger, or any other digit, rapidly over the forehead, it is the latter which is interpreted as "feeling" the finger.

(*g*) *Judgment of motion and direction.*—Let B move a pin-head slowly up and down on A's forearm (eyes closed). A will perceive motion before discovering its direction.

(*h*) *Weber's circles.*—Cut short lengths from glass tubing of various sizes, varying from a quarter of an inch to 2 inches or more in diameter, and provide glass vessels of similar size, each with a glass base. Press the small circles and corresponding size of vessel on the cheek and forehead, and the larger ones on the thorax or abdomen. It is impossible when the eyes are shut to determine whether a closed or open vessel is pressed on the skin. The size of the vessel to obtain this result varies with the cutaneous surface experimented on.

21. **Time discrimination of contact.**—Strike a tuning-fork vibrating 50 to 200 or more D.V. per second, and touch it lightly for a second, and remove the finger. The impacts of the fork are not summated into a smooth sensation, but one feels a succession of rapid successive taps.

22. **Unequal distribution of pressure.**—(*a*) Feel radial pulse with palmar surfaces of fingers, back of the fingers, side of hand.

(*b*) Press as equally as possible with a pencil-point on the skin near to nail, and on the nail itself. Note great difference.

23. **Active touch and active pressure.**—(*a*) Feel any surface by mere contact, and then move the finger over it. When touch is combined with motion, one obtains spatial and other relations.

(*b*) Place a hair on a glass plate, cover it with a dozen sheets of writing-paper. The position of the hair will be readily felt by active pressure.

24. **Weights for minimal pressure** (*Contact*).—Make rectangular prisms of elder pith or cork, 3 mm. base of side = 9 mm. square, the original size used by Weber. Provide them with bristle or hair handles, arranged like the handle of a bucket. Make a series of weights from 2 to 20 mgrms.

(*a*) Test volar side of forearm and tips of fingers. Apply weights successively to the same spot until the weight is just perceived.

(*b*) Test with bits of paper or elder pith allowed to fall from a constant height, say, 3 inches. Note differences in different areas. One is astonished to find how relatively large pieces of pith may be allowed to fall on the skin without being perceived.

25 **Pressure sense.**

(*a*) Rest the back of the hand comfortably on a table, cover a small area of the palm with a non-conducting material—*e.g.*, a soft blotting-paper. On the latter place different weights. Estimate the smallest difference of weight which can be appreciated. Use weights of 10, 11, 12, 13, 14, 15 grms., all of equal size of base, or 100, 110, 120, 130, &c. B places, say, 10 grms. on A's hand, and allows it to remain until B is able to estimate its weight. Remove the 10-grm. weight and apply 13 grms., allowing it to remain as long as the first. If A cannot detect a difference, try with 14. This is the method of "just observable difference." If 10 and 12 can just be distinguished, then with the heavier weights 100 and 120 will be distinguished. [If the weights be placed upon the unsupported hand, and the person be allowed to bring into action his muscles to, as it were, weigh the objects, then less differences are perceived.]

Weber's law.—The ability to distinguish stimuli, visual, tactual, or otherwise, depends not on the absolute difference,

but on their *relative* differences. It is purely empirical, and holds best for medium stimuli.

26. Traction or negative pressure.—Pass a thread through a small piece of court plaster, knotting it on the gummed side and sticking the plaster to the skin. Traction on the thread excites the sensation (*Sanford*).

27. Peripheral projection.

(*a*) Press the ulnar nerve at the elbow, the prickling feeling is referred to the skin on the ulnar side of the hand.

(*b*) Dip the elbow in ice-cold water; at first one feels the sensation of cold owing to the effect on the cutaneous nerve-endings. Afterwards, when the trunk of the ulnar nerve is affected, the pain is felt in the skin of the ulnar side of the hand where the nerve terminates.

28. Reference of tactile impressions to exterior.—Usually the sensation of touch is referred to our cutaneous surfaces. In certain cases, however, it is referred even beyond this.

(*a*) Holding firmly in one hand a cane or a pencil, touch an object therewith; the sensation is referred to the extremity of the cane or pencil.

(*b*) If, however, the cane or pencil be held loosely in one's hand, one experiences two sensations—one corresponding to the object touched, and the other due to the contact of the rod with the skin. The process of mastication affords a good example of the reference of sensations to and beyond the periphery of the body.

29. Tickling.—Tickle yourself near the angles of the mouth or eyes, or near auditory meatus. Notice the tickle sensations, their long durations and after-effects. Press same parts with a pen-holder, and observe difference of effects.

30. Pain spots.—Strong horse-hairs, pointed with a razor, are fixed in a light wooden rod. Map out a small parallelogram in the area of the skin on the back of the hand or forearm already studied. Proceed as before. Soften the skin with a drop of water, and keep it moist. Make firm pressure *dot by dot*, but do not pierce the epidermis. Mark the pain spots when A calls out "There."

I have often shown these spots both on the back of my own forearm and on students by means of a sewing needle.

31. Algesometer or Algometer.—Fig. 448 consists of a strong spring, by means of which a point is pressed against the skin. Use Aly's form (fig. 448) for the skin of the forearm. A needle is kept projected by means

of a spring. Apply the needle to the skin and press until pain is felt. Read off the pressure in grms. on the graduated scale.

If the needle be tipped with cork or rubber disc, it can be used for the pressure sense. It takes a pressure of 4 to 6 kilogrms. to excite pain produced by pressure applied to the skin. Great variation in individuals.



Fig. 447.—Algometer, made by Chicago Supply and Scale Company.

Some areas are devoid of pain spots—*e.g.*, the buccal mucous membrane opposite the second molar tooth of the lower jaw (*Kiesow*), although it is provided with touch spots. The cornea is anæsthetic, but it is acutely sensitive to pain.

The liminal or threshold sensibility of mechanical stimuli is higher for pain than for pressure—

$$\frac{\text{Pressure limen}}{\text{Pain limen}} \quad \tfrac{1}{3}\ (\text{arm})\ \left(\tfrac{1}{100}\right)\ \text{finger tip};$$

hence if finger (palm) and forearm be equally squeezed, the former causes less pain than the latter. The lowest limina for pain are over the temple and skin of finger joints, the highest in the pelvic area.

32. **Pain—Slow conduction.**—Strike the sole of the foot smartly with a flat rod. One feels contact first, and appreciably later that of pain.

33. **Threshold for pressure and pain.**—Set up a coil, with two dry cells and simple break-key in primary circuit. Make one electrode—large—either to be strapped to leg or held in the hand; and apply the other—small—to any part of the skin. Use either interrupted shocks or

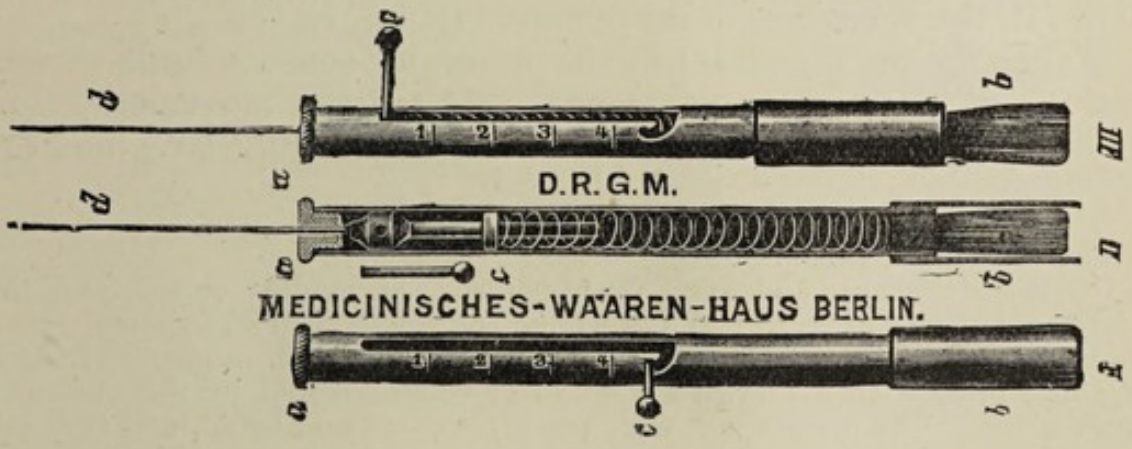


Fig. 448.—Aly's Algesometer.

single break shocks with the kathode of small electrode at break on the skin. Find the distance of secondary from primary coil when

(i.) B first feels a sensation from the electrode.
(ii.) When pressure is felt.
(iii.) When pain is felt. Record result.

Muscular sense (*Sir C. Bell*, 1830).—The primary acceptation of this term has undergone much alteration.

Sherrington includes "all reactions on sense arising in motor organs and their accessories." Most of the writers on psychology draw a sharp distinction between the afferent impressions received from muscle and those from articular surfaces or tendons. On the whole it is convenient, with Sherrington, to regard it as based on a "specific set of sensations obtained by specific sense organs in muscles, joints, and all the accessory organs of movements." The brilliant experimental researches of Sherrington on the sensory apparatus of muscle must remain, whatever view one takes of the nomenclature.

This group of senses—muscular-sense, tendon-sense, joint-sense—yield important data regarding the position and movements of our limbs and of the body as a whole which play a great part in our perception of space.

In the attempt to disentangle how far sensory impressions are received from muscles, those from skin, tendons, and joints are respectively involved. Goldscheider (*Gesammelte Abhandlungen* ii., Muskelsinn, Leipzig, 1898) has made use of the fact that when an induced current is passed through the first joint of a finger—by means of sponge electrodes—both this and the other parts of the finger become hypæsthetic. By a series of ingenious experiments he has shown that the sensations derived from joints are of the utmost importance in relation to movements; in fact, that "the sensibility of the joint takes the lion's share in the sensation of movement" (p. 28, *loc. cit.*).

Taken in its broad sense the muscular sense affords us sensations of (i.) active movement, (ii.) passive movement, (iii.) feeling of weight and resistance to movement, (iv.) posture (*Goldscheider*).

34. Muscular sense.

(*a*) With the arm and hand unsupported, the eyelids closed, and the same precautions as in 25 (*a*), determine the smallest difference which can be perceived between two weights. It will be less than in gun cartridges (*Galton*) filled with a known weight of shot, and tested by the pressure-sense alone. The cartridges—*e.g.*, 100 grms.—are numbered, and they are so made as to have a small increasing increment of weight. They are alike in external appearance.

(*b*) Take two equal iron or lead weights; heat one and leave the other cold. The cold one will feel the heavier.

(*c*) Lift a weight slowly and then quickly. It seems heavier when lifted slowly.

[See Waller, "Sense of Effort," *Brain*, xiv., and *Journ. of Phys.*, xiii.]

35. Lifting weights (Weber's law, 1851).—(*a*) Weber found in lifting weights that the just perceptible difference in the weights was not a constant amount, but was a constant fraction of the weight lifted. Thus he found that for a weight of 32 drachms the average difference was 3 drachms, and for a weight of 32 ozs. the just perceptible difference was 3 ozs.

(*b*) *False judgment of weight.*—Three cylinders of equal weight but unequal volume are placed before a person, who with eyes open is to lift each in turn and estimate their relative weights. As a rule, the largest is estimated to be lightest, and the smallest to be the heaviest. The illusion disappears when one does the same with the eyes averted.

36. Sternberg's experiment (Illusory movement in an immovable digit).—(*a*) Press the tips of the thumb and three outer fingers on the edge of a table, or on a book, so that they are fully extended, leaving the index unsupported and free. Fully flex the index finger except at the end joint. The last joint is still movable—test with other hand—although it is impossible for anatomical reasons to move it voluntarily. Close the eyes and try to move the end joint; one has a sensation of motion, although no actual motion takes place—*i.e.*, one believes that the movement intended is actually carried out. Of course the tendons of the wrist and many muscles are active. Formerly this experiment was regarded as indicating a "sense of innervation." It would appear, however, that the feeling of effort depends far more on peripheral than central changes.

(*b*) *Exner's illusion.*—Bite an unyielding plate—*e.g.*, ebonite—and observe feeling of penetration, although no actual penetration or movement is effected.

37. Sensation of motion of joints.—(*a*) *Passive motion at elbow.*—The arm of A rests on an arm-board exactly counterpoised. A closes his eyes and B gives the arm a slow passive movement by pulling on the counterpoise. A has to state when he first feels the motion of the forearm. Read off the angular movement necessary to get the first noticeable sensation of movement—best felt in the hand.

(*b*) *Active movement of ungual phalanx.*—Fix with court plaster a straw to the nail of the ungual phalanx of the middle finger. The straw and third phalanx = 115 mm. "Rest the hand on a thick book, letting the last joint of the finger extend beyond the edge. Set up a millimetre scale at right angles with the straw. Close the eyes and make the least possible flexion of the finger at the last joint, while someone notes its extent on the scale" (2 mm. = 1° angular measure) (*Sanford*).

38. Resistance.—Touch an object with a pencil held in the hand. A peculiar sensation referred to the extreme end of the pencil is felt—viz., the resistance to the movement of the pencil. It is not due to the complex of sensations derived from contact with the skin and sensations from muscles.

The skin sensations are not the essential factors, for let the pressure on the skin be increased to the utmost the sense of resistance is undisturbed.

The resistance is felt equally whether the pencil be grasped lightly or as tightly as possible.

Place a rubber air cushion over the end joint of a finger, and over this a string carrying a weight. The air pad reduces the pressure to an extremely small amount, yet on lifting the weight the resistance is plainly felt.

Even with the cutaneous sensibility almost entirely lost in the tip of the finger, a touch of the finger on the table is felt with little loss of intensity, so that the sensation of resistance is independent of pressure (Goldscheider, *Gesammelte Abhandlungen*, ii., 1898).

39. Resistance paradoxes.—(*a*) Tie a heavy weight to a string (length of string unknown to B; eyes closed). Hold the weight by the string, lower it rather rapidly till it rests on a cushion or bag of sand. On impact B feels a distinct resistance to the movement as if the hand were supported by a rod. It is due to the sudden release of muscular strain whereby the muscles themselves produce pressure on the articular surfaces of the joints.

(*b*) *Du Bois-Reymond's experiment.*—Hold an elastic cord or rubber band between finger and thumb of the hand; then stretch and relax it three or four times. At sudden relaxation, a sudden blow is felt.

LESSON LXXX.

TASTE—SMELL—HEARING—COMPENSATORY MOVEMENTS—STEREOGNOSTIC SENSE.

TASTE.

Taste and smell usually act together and have been called "chemical senses." Touch and taste are closely allied. The four qualities of true taste are—sweet, sour or acid, bitter, and salt—all quite distinct and distributed in a punctiform manner over the dorsum of the tongue. Though the papillæ are anatomically distinct, one papilla may respond to more than one of these stimuli. Besides these specific tastes, the tongue responds to touch or pressure, temperature, and pain. What is sometimes called taste is in reality more frequently due to smell.

1. Close the nostrils, shut the eyes, and attempt to distinguish by taste alone between an apple and a potato, or between water and weak solution of essence of cloves in water.

2. Distribution of taste.—Prepare a strong solution of sulphate of quinine, with the aid of a little sulphuric acid to dissolve it (*bitter*), a 5 p.c. solution of sugar (*sweet*), a 10 p.c. solution of common salt (*saline*),

and a 1 p.c. solution of acetic acid (*acid*). Use camel-hair brushes to apply these solutions to the tongue.

(*a*) Wipe the tongue dry, lay on its tip a crystal of sugar. It is not tasted until it is dissolved.

(*b*) Apply a crystal of sugar to the tip and another to the back of the tongue. The sweet taste is more pronounced at the tip.

(*c*) Repeat the process with sulphate of quinine in solution. It is scarcely tasted on the tip, but is tasted immediately on the back part of the tongue.

(*d*) Test where salines and acids are tasted most acutely.

3. **Taste reaction of single papilla.**—Prepare strong and weak solutions of sugar (20 and 40 p.c.), common salt (2 p.c. and sat. sol.), tartaric acid (0·5 and 8 p.c.), quinine (·002 and 2 p.c.). Five camel-hair brushes, and distilled water for washing these. Water to rinse mouth. Hand lens fixed in support.

(*a*) The person to be observed (A) protrudes his tongue, and B selects a fungiform papilla on the tip or sides of the tongue. Let B use the lens to magnify the selected papillæ. B applies for two seconds to a papilla, in any order, with the point of a camel-hair brush moistened with one of the solutions, and A indicates the taste. One papilla may respond to several. The mouth must be rinsed with distilled water before another stimulus is applied. In this way several papillæ may be investigated. Record results.

(*b*) If a papilla is found that responds to bitter, paint it with cocaine. This abolishes the bitter taste, while the others remain.

(*c*) If one be found to be very sensitive to sweetness besides others, paint it with saturated alcoholic solution of gymnemic acid. The sweet taste alone is abolished.

4. **Electrical taste.**—(*a*) Fill one vessel with saturated solution of NaCl and another with ordinary water. In each place a platinum electrode connected with a battery through a commutator and key.

Fold a piece of blotting-paper, dip it in the water vessel, and place a finger in the salt solution. Apply the tongue to the paper. During the flow of the current there is an acid taste when the tongue electrode is the anode.

(*b*) Connect two zinc terminals with a large Grove's battery, apply them to the upper and under surface of the tongue, and pass a constant current through the tongue. An acid taste will be perceived at the positive, and an alkaline one at the negative pole.

5. **Drugs.**—(*a*) *Cocaine on pain sense.*—Press a needle on the tongue and note the pressure at which a sensation of pain is felt. With a camel-hair pencil paint the part with cocaine. Find the stage at which the pain sense is lost, but touch (pressure) is still present.

Gymnema sylvestris.—Use a 5 p.c. decoction of the leaves and apply it to limited areas of the tongue by means of a camel-hair pencil. In 20 to 30 seconds wash out the mouth and then test the action of glycerin (5 to 10 p.c.), quinine (1 p.c. with ·01 p.c. of H_2SO_4), H_2SO_4 (·5 p.c.), NaCl (·5 p.c.).

The sweet and bitter tastes are readily prevented in all regions, but

acid and saline tastes are not influenced (L. E. Shore, "A Contribution to our Knowledge of Taste Sensations," *Journ. of Phys.*, xiii., 191). It has no effect on tactile sensations.

SMELL.

Olfactory sensations.—Most of our food is really tasteless, and what we call "flavour" depends far more on smell than taste, so that many tastes are really "taste-smell complexes."

6. Field of smell.—Breathe through the nostrils on a cold metallic mirror, or on a cold bright tin plate, and observe the form of the two distinct breathing fields. Each is soon seen to be divided obliquely. Mark shape of field.

7. Course of odours in nose.—(*a*) Hold a paper funnel over some olfactory substance and insert one end of the funnel into the posterior half of a nostril. One smells nothing. Place it in anterior half; at once one smells the substance (*A. Fick*). The respiratory passage is below, the olfactory above.

(*b*) A piece of camphor held under the nostrils is not smelled until one inspires.

8. Exhaustion of sense of smell.—Smell tincture of iodine or spirits of camphor by one nostril until it ceases to excite smell;

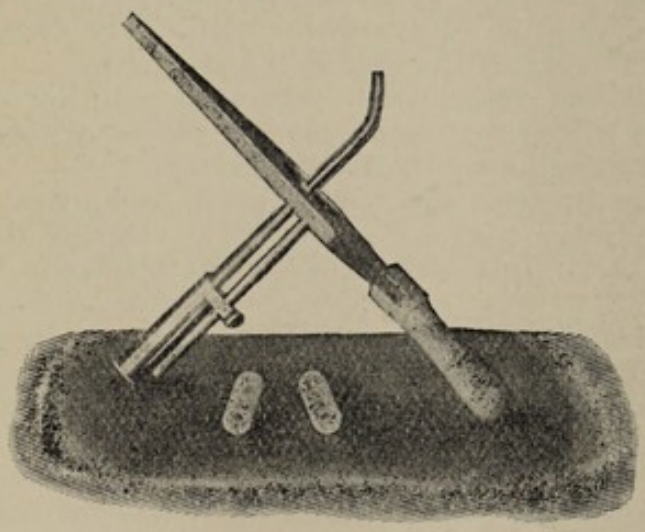


Fig. 449.—Single olfactometer of Zwaardemaker. Made by Zimmermann.

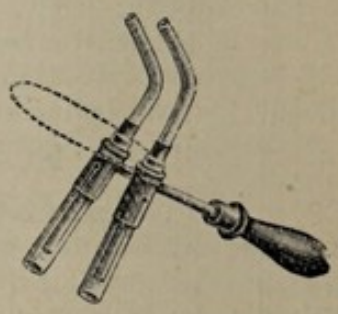


Fig. 450.—Double olfactometer of Z. Made by Petzold.

plug other. Note time. Test now if clove oil can be smelled. The smell sense is easily and rapidly fatigued. Or is it adaptation? How soon does the sense return? Try.

9. Change in quality before exhaustion.—Breathe in through one nostril and expire by the mouth. (*a*) Nitrobenzole. One smells first heliotrope, then bitter almonds, then that of rubber tubing or benzine.

10. Olfactometer of Zwaardemaker—single or double (figs. 449, 450).—The curved end of the tube—protected by a cherry-wood shade—is inserted into the nostril, while the other end of the tube is covered with a new rubber tube protected by an outer glass tube or by a cylinder, impregnated with some olfactory substance. The rubber tube or cylinder can be pushed over the tube from zero, and thus a longer or shorter length of the cylinder has to be traversed by the inspired air.

Place tube in front part of nostril and inhale steadily, altering position of cylinder until the limen of smell is obtained. The tube is graduated in "olfacties" (Zwaardemaker, *Die Physiologie des Geruchs*, Leipzig, 1895). Breathe out by mouth. Use a new tube of india-rubber as a cylinder.

EAR AND SOUND.

11. Dissection of middle ear.—It is most important for the student to do this. Use the head of a sheep. Remove the lower jaw, expose the temporal bulla. Open this and thus reach the tympanic cavity, when the various structures situated in the middle ear are readily brought into view.

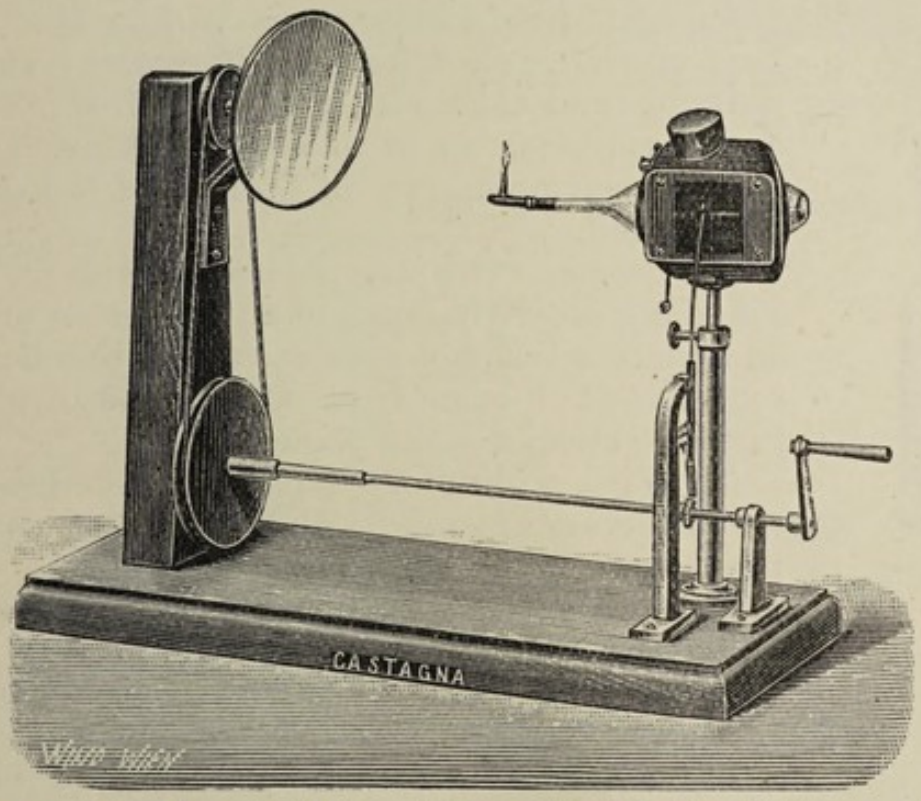


Fig. 451.—Exner's model of the middle ear adjusted with a König's capsule.

12. Exner's model of middle ear (fig. 451) consists of a drum; a box—provided with a window—represents the tympanic cavity, with a membrane at one end to represent the tympanic membrane. The inner surface of this is connected by means of a rod—representing the chain of ossicles

—with another membrane at the other end to represent the fenestra ovalis. This second membrane acts like the plate in a König's capsule, which is placed behind the wooden box. The gas in the König's capsule represents the perilymph of the inner ear. The flame of the capsule is reflected in a revolving mirror, and thus its vibrations are made visible. One can show diminution of hearing during contraction of the Tensor tympani (a cord is attached to the part representing the malleus), a similar diminution on opening the Eustachian tube, hearing through the cranial bones, increase of the sounds transmitted through the cranial bones when the outer auditory meatus is closed, and diminution when the Eustachian tube is opened. The apparatus is made by Castagna of Vienna.

13. Hearing.

(*a*) Hold a ticking watch between your teeth, or touch the upper incisors with a vibrating tuning-fork, close both ears, and observe that the ticking is heard louder. Unstop one ear, and observe that the ticking is heard louder in the stopped ear.



Fig. 452.
Galton's whistle.

(*b*) Hold a vibrating tuning-fork on the incisor teeth until you cannot hear it sounding. Close one or both ears and you will hear it.

(*c*) Listen to a ticking watch or a tuning-fork kept vibrating electrically. Close the mouth and nostrils, and take either a deep inspiration or deep expiration so as to alter the tension of the air in the tympanum ; in both cases the sound is diminished.

(*d*) Connect two telephones in circuit with a vibrating Neef's hammer of an induction machine, and place a telephone to each ear ; one hears the sound as if it came from within one own's head in the vertical median plane.

(*e*) With a blindfolded person test his sense of the direction of sound—*e.g.*, by clicking two coins together. It is very imperfect. Let a person press both auricles against the side of the head, and hold the hands vertically in front of each meatus. On a person making a sound in front, the observed person usually refers it to a position behind him.

(*f*) Test the highest audible sound by means of Galton's whistle (fig. 452).

(*g*) Insert in the ears the openings of an elastic tube, 1 cm. in diameter. Strike a tuning-fork and arrange it on the tube so that the sound appears equally intense in the two ears. It will be located about midway between the ears. Pinch the tube on one side—say left—of the vibrating fork, and

wait till the sound becomes faint. Open the left tube, the sound seems louder on the left than the right, and is referred to the left ear.

(*h*) Place a watch at such distance from the ear that one can just hear it ticking. Listen, one does not hear the ticking uninterruptedly, but there are regular periods when one fails to hear it at all, due to variations and fatigue of one's attention (?)—*i.e.*, marked intermittences of sound on the limit of audibility.

(*i*) *Fechner's experiment on unconscious* (unnoticed) *psychical processes.* — Bring a vibrating tuning-fork near a person's ear and ask him to indicate, by a movement of the hand, when he ceases to hear its sound. At once then remove the tuning-fork. The person notices that all appears quieter. He will hear the still vibrating tuning-fork if it be brought into its original position.

14. Semicircular canals.— The following models, devised by Exner and made by Castagna, are very useful :—

(*a*) On rotating the model (fig. 453) on a vertical axis, one sees how the hairs are dragged through the fluid, indicating the extent of the rotation.

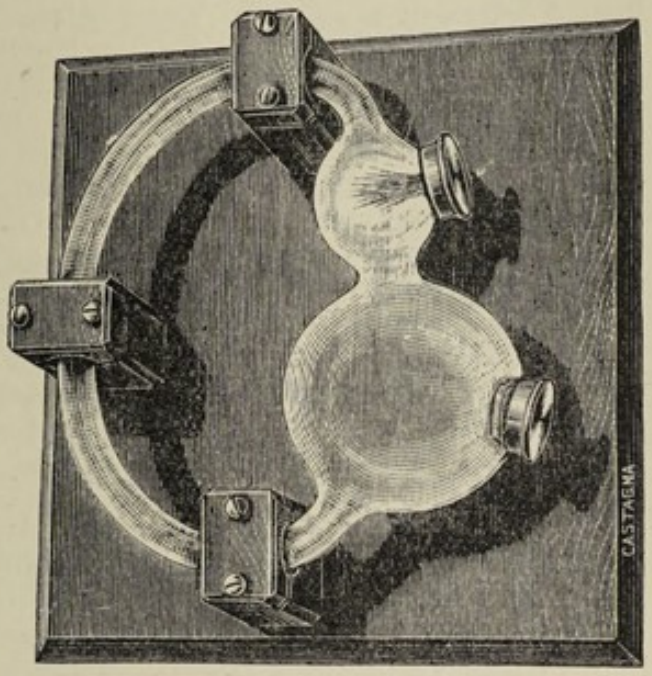


Fig. 453.—Exner's model of the hairs in ampullæ of the semicircular canals.

(*b*) *Exner's model for otoliths* (fig. 454) to indicate the acceleration of movement (*Das Räthsel d. Brieftauben*, Vienna, 1892).

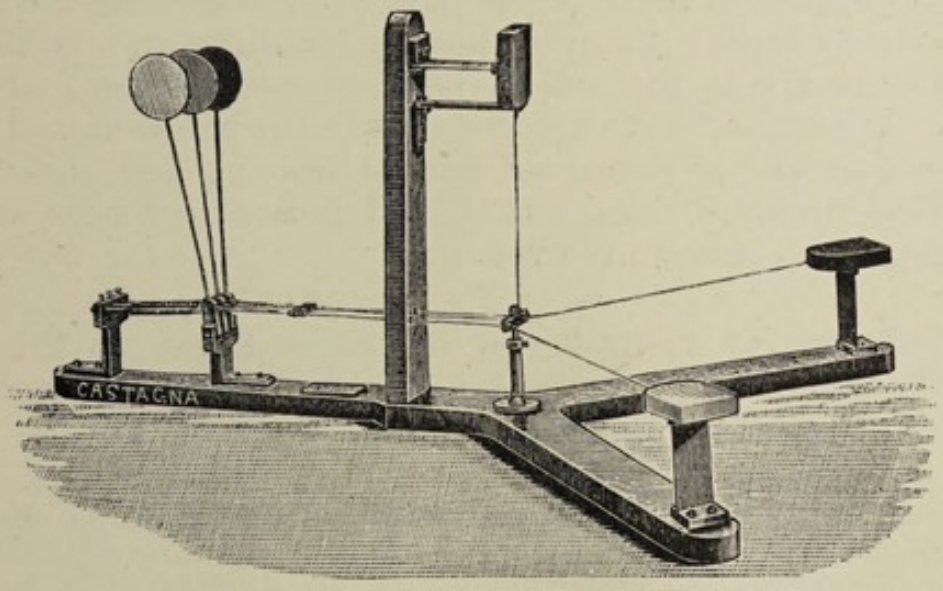


Fig. 454.—Exner's model to show movement of otoliths in three planes.

15. Compensatory movements.—(*a*) Place a frog on a horizontal turn-table, and revolve it, not too rapidly. The frog turns its head to one side

in the direction opposite to that of the revolution, and this side-inclined position is retained as long as the rotation is kept up. Suddenly stop the revolution, the frog moves its head back not only beyond the mid-plane, but now in the direction of rotation.

"After-effect."—In a few seconds the head returns to its normal position. If "hooded," it makes no compensatory movements (*Cyon*). Or, float a flat piece of cork in water, and on the cork place a frog. Gently turn the cork by hand, and observe the movements of the frog.

(*b*) Pigeons show the same bending of head as in frog. Breuer has called it a "reaction movement," but the head is not retained at an angle with the trunk, it jerks its head back suddenly in the direction of the rotation of the table—"nystagmus jerk." Immediately the head moves again slowly and steadily oppositely to direction of body revolution. This is called "reaction phase." Head is again jerked back, and so on, each phase being followed by a nystagmus phase. The result is a series of pendulous movements in a horizontal plane—quick convulsive reflex in one direction, slow compensatory in the opposite. Gradually the movements cease. Stop rotation. The pigeon turns its head in direction of rotation past the mid-plane, and then shows marked rapid after-nystagmus. If the head be held, the eyes show corresponding movements.

(*c*) Mouse.—Revolve it in horizontal plane; it runs in circles opposite to direction of revolution. A horse placed on a horizontal moving plate, in a radial direction, does likewise with its forefeet—a phenomenon that is sometimes exhibited in a circus. Compensation is by locomotion. Insects behave like vertebrates (*Loeb*). They have neither semi-circular canals nor otocysts (E. P. Lyon, "Compensatory Motions," *Amer. Journ. of Phys.*, iii., 86, 1899).

[The rotation experiments of Mach, Breuer, and Crum Brown (*Journ. of Anat. and Phys.*, viii., 1874) are omitted here.]

16. Stereognostic sense or the power of recognising the nature of a body by feeling it, or rather by handling it. It is, however, the result of the action, joint or combination, of many senses. The sensations are correlated in the cerebrum. Its loss may be due to peripheral or central lesions, and these cases show that pressure, localisation, and muscular senses are the most important senses involved.

17. Influence of excitation of one Sense-Organ on the other Sense-Organs.—Urbantschitsch has made a large number of experiments on this subject (*Pflüger's Archiv*, xlii., 154).

(*a*) Small coloured patches whose shape and colour are not distinctly visible may become so when a tuning-fork is kept vibrating near the ears. In other individuals the visual impressions are diminished by the same process.

(*b*) On listening to the ticking of a watch, the ticking sounds feebler or stronger on looking at a source of light through glasses of different colours.

(*c*) If the finger be placed in cold or warm water, the temperature appears to rise when a red glass is held in front of the eyes.

(*d*) Sounds on the limit of audibility became audible when lights of various colours were admitted to the eye.

It would seem that the cerebral processes associated with the first sensation are affected—reinforced or enfeebled—by new incoming currents. The explanation seems to be best effected on physiological grounds.

APPENDIX.

A.—WORKS OF REFERENCE.

I. USEFUL IN THE LABORATORY.

R. Wagner, Handwörterbuch d. Physiologie, 1842-53.—Todd and Bowman, Cyclopedia of Anatomy and Physiology.—R. Gscheidlen, Physiologische Methodik, 1876 (incomplete).—E. Cyon, Methodik der physiologischen Experimente u. Vivisektionen, with Atlas, 1876 (only one part issued).—Ott, The Actions of Medicines, Phil., 1878.—Claude Bernard and Huette, Précis iconographique de médecine operatoire et d'anatomie chirurgicale, with 113 plates, 1873; also Leçons de physiologie operatoire (edited by Duval), Paris, 1879.—Sanderson, Foster, Klein, and Brunton, Handbook for the Physiological Laboratory (Text and Atlas). The French edition contains additional matter.—Meade-Smith, Trans. of Hermann's Toxicol.—J. Burdon-Sanderson, Practical Exercises in Physiology, London, 1882.—Foster and Langley, Pract. Phys., 7th edition, London, 1899.—B. Stewart and Gee, Pract. Physics.—Vierordt, Anat. Physiol. u. Physik. Daten u. Tabellen, Jena, 1888.—Müller-Pouillett, Lehrb. d. Physik., 8th ed., Braunschweig.—Wüllner, Lehrb. d. exp. Physik., 5th ed., 1894-97.—Livon, Manuel de Vivisect., Paris, 1882.—Harris and Power, Manual for the Phys. Lab., 5th ed., 1892.—Straus-Durckheim, Anat. descrip. comp. du chat, Paris, 1845.—W. Krause, Die Anatomie des Kaninchen, Leipzig, 2nd ed., 1833.—A. Ecker and R. Wiedersheim, new edition by E. Gaupp, Die Anatomie des Frosches, 1882, 2nd ed.; 3rd ed., 1896-99; English translation of 2nd ed. (G. Haslam).—On the Cat.—Mivart (1881).—Wilder and Gage (1882).—Levy-Dorn, Centralbl. f. Physiol., 1895.—Ellenberger and Baum, Die Anatomie des Hundes.—Biolog. Memoirs, edited by Burdon-Sanderson.—Helmholtz, Physiol. Optik, 2nd ed.—Hermann, Handbuch der Physiologie (by various authors).—Gad, Real-Lexikon d. Med. Propädeutik, Wien, 1893.—L. Fredericq, Manipulations de Physiologie, 1892.—Langendorff, Physiol. Graphik.—Gotch, Practical Exercises in Physiology.—Halliburton, Syllabus of Experimental Lessons, 1893.—Schenck, Physiologisches Practicum, Stuttgart, 1895.—W. Biedermann, Elektrophysiologie, Jena, 1895 (translated by Miss Welby).—Richet, Dictionnaire de Physiologie, vol. i., Paris, 1895 (not yet completed).—L. Hermann, Physiologisches Practicum, Leipzig, 1898.—Goldscheider, Gesammelte Abhandlungen, 2 vols., Leipzig, 1898. Deals chiefly with physiology of cutaneous nerves and muscular sense.—Aubert, Grundzüge de phys. Optik, Leipzig, 1876.—Waller,

Exercises in Practical Physiology, 1877.—Brodie, Essentials of Experimental Physiology, 1898.—De Burgh Birch, Practical Physiology, 1899.—Sollmann, Laboratory Exercises, Cleveland, Ohio, 1900.—W. T. Porter, Introduction to Physiology, Cambridge, Mass., 1901.—W. S. Hall, Laboratory Guide in Physiology, Chicago, 1898.—J. G. M'Kendrick, Life in Motion (Muscle and Nerve), 1892.—Raphaël Dubois, Leçons de Physiologie, Paris, 1900.—Marey, La methode graphique, Paris, 1878, and last ed., 1885; also, Traité de physique biologique (by various authors), 1901.

The above list takes no account of the various Physiological Journals.

II. ON CHEMICAL PHYSIOLOGY.

Tiedemann and Gmelin, Die Verdauung nach Versuchen, 1831.—Bidder and Schmidt, Die Verdauungssäfte u. d. Stoffwechsel, 1852.—Kühne's Lehrbuch d. physiol. Chem., 1864.—Hoppe-Seyler, Physiologische Chemie, Berlin, 1892-94.—Hoppe-Seyler and Thierfelder, Handbuch d. physiol. Chemie, 1895.—Lehmann, Lehrb. d. phys. Chem., 3rd ed., Leipzig, 1853; and Handbuch, 1859.—Leo. Liebermann, Grundzüge der Chemie des Menschen, Stuttgart, 1880.—Robin and Verdeil, Traité de chem. anat. et phys. (with Atlas), Paris, 1853. This Atlas contains beautiful plates with figures, many coloured, of all the most important organic crystalline bodies.—A. Wynter Blyth, Foods, 1887.—Gorup-Besanez, Anleitung zur Zoo-chemischen Analyse, 1871.—Gautier, Chimie appliqué à la Physiologie, 1874.—Lehmann's Phys. Chem. (translated by Cavendish Soc., 1851-1854), with Atlas of O. Funke's plates. These plates contain some histological figures, and many coloured plates of blood-crystals, deposits in urine, &c.—Kingzett, Animal Chem., 1878.—Thudichum, Ann. of Chem. Med., 1879.—A. Gamgee, Physiological Chemistry of the Animal Body, vol. i., 1880; vol. ii., Chemistry of Digestion, 1893.—Munk and Uffelmann, Die Ernährung d. gesunden Menschen, 3rd ed., 1895.—Hoppe-Seyler, Medicinische Chemische Untersuchungen, Berlin. This contains a number of special memoirs by the pupils of the author.—Hoppe-Seyler, Zeitschrift für physiologische Chemie, Strassburg, 1877, and continued by Kossel.—Watts' Dictionary of Chemistry, second supplement, London, 1875.—Ralfe, Clinical Chemistry, London, 1880; and Clinical Chem., 1883.—Wurtz, Traité de chim. biol., Paris, 1880.—Parkes' Hygiene.—T. C. Charles, Physiological and Pathological Chemistry, London, 1884.—Maly's Jahresb. ü. Thierchemie. This gives a *résumé* of the most important memoirs published during the year.—Articles in Hermann's Handbuch d. Physiologie, 1879-1884, and the various Text-books on Organic Chemistry.—Roscoe and Schorlemmer, (Organic) Chem., 1884-1889.—Beilstein, Handb. d. organ. Chem., 3rd ed., 1892-1900.—Krukenberg, Grundriss d. med. chem. Analyse, 1884.—MacMunn, The Spectroscope in Medicine, 1880; Clinical Chemistry, 1890.—Drechsel, Anleit. z. Darstell. phys. Chem. Präparate, 1889.—Ladenburg, Handwörterbuch d. Chemie. This consists of special articles, and is on the plan of Watts' Dictionary of Chemistry.—Kossel, Leitfaden für med. chem. Curse, 4th ed., 1898.—Röhmann, Anleitung z. chem. Arbeiten, 1890.—Landolt, Das optisches Drehungsvermögen is the standard work on polariscopic methods.—MacMunn, On the Spectroscope. This work has good lithographed and coloured spectra.—Tollens, Handbuch d. Kohlenhydrate, Breslau, 2nd ed., 1898. This is the best work on the Carbohydrates.—

Sutton, Volumetric Analysis.—Neubauer and J. Vogel, Anleit z. quan. u. qual. Analyse d. Harns, 9th ed., by Huppert and Thomas, 1898. This is a standard work on urinary analysis. This work gives all the most important methods for this process.—Bunge, Phys. and Path. Chem., trans. by Wooldridge, 1890, 4th German ed., 1898.—V. Jaksch, Clinical Diagnosis, trans. by Cagney, 4th ed.—Simon, Clinical Methods, 2nd ed.; Clinical Diagnosis, 3rd ed., London, 1901.—E. Fischer, Einleitung d. Darstellung organ. Präparate, 5th ed., 1896.—F. G. Novy, Physiological Chemistry, Ann Arbor, 1898.—John A. Mandel, Handbuch f. d. chem.-phys. Lab., German ed., Berlin, 1897.—Fr. Krüger, Lehrbuch d. physiol. Chemie, 1898.—Lenhartz, Mikroskopie u. Chemie am Krankenbett, 3rd ed., 1901.—Halliburton, Chemical Phys. and Path., 1891, and Essentials of Chemical Physiology, 3rd ed., 1899.—Hammarsten, Lehrb. d. Physiol. Chem., 4th ed., Wiesbaden, 1899 (translated by John A. Mandel), 3rd ed., New York, 1900.—S. Lea, The Chemical Basis of the Animal Body, 1892.—Salkowski, Practicum d. phys. u path. Chemie, Berlin, 2nd ed., 1900.—Armand Gautier, Cours de Chemie; Chimie Biologique, vol. iii., Paris, 1892.—Hempel, Gas Analyse, translated by L. M. Dennis.—Neumeister, Lehrb. d. phys. Chem., 2nd ed., 1897.—Chittenden, Digestive Proteolysis, New Haven, Conn., 1895.—Arthus, Elements de Chemie Physiologique, 2nd ed., 1897.—Cohnheim, Chemie der Eiweisskorper, 1900, translated by G. Mann, 1901.—G. Ruppel, Die Proteïne, Marburg, 1900.—Franz Hofmeister, Beiträge z. Chem. physiologie u. Path., Braunschweig, 1901.—Remy, Encyclop. Chimique, vol. ix., 1895.—A. Slosse, Chemie physiologique, Brussels, 1896.—Robertson, Clinical Diagnosis, 1896.—Engel and Moitessier, Chimie biologique, Paris, 1897.—A. D. Waller and W. L. Symes, Physiological Chemistry, Part I., 1897.—Tappeiner, Chemical Methods of Clinical Diagnosis (translated by E. J. M'Weeney), London, 1898.—Bottazzi, Chimica fisiologica, Milan, 1898-99.—Fr. N. Schulz, Prakticum d. phys. Chemie, Jena, 1901.

The literature on the "Urine" is necessarily very large, and may readily be obtained on consulting any of the standard works on that subject.

III. ON THE SENSES.

In connection with the physiology and psychology of the sense organs, the author is largely indebted to the following works, besides others mentioned in the text:—

(A) Vision.—H. Aubert, Grundzüge d. physiol. Optik., Leipzig, 1876; and Physiologie d. Netzhaut, Breslau, 1865—G. T. Fechner, Elemente d. Psychophysik, Leipzig, 1889.—von Helmholtz, Handbuch d. physiol. Optik., 2nd ed., 1896.—E. Hering, Zur Lehre v. Lichtsinne, Wien, 1878.—A. Höfler and S. Witasek, Physiologische Schulversuch, mit Angaben d. Apparate, Leipzig, 1900. (A small pamphlet but a useful guide. All the apparatus is made by W. J. Rohrbeck's Nachfolger, Kärtnerstrasse, 59 Wien, i.).—W. James, Principles of Psychology, 1890.—E. Mach, Contributions to the analysis of the Sensations, Wien, 1900, trans. by C. M. Williams, Chicago, 1897. There is a later German ed. Contains many important observations besides those related to vision.—J. Jeffries, Colour-blindness, its dangers and detection, Boston, 1879.—A. Fick, Anat. u. Physiologie d. Sinnesorgane, Lahr, 1864.—Ed. C. Sanford, A course in experimental psychology, Pt. I.; Sensation and Perception, published in Heath's Pedagogical Library. Two small, compact, tersely-written

volumes, that thoroughly bear out the motto on the cover, "Thou that teachest another, teachest thou not thyself," 1897-98.—**E. W. Scripture**, The new psychology, Contemp. Sc. Series, London, 1897 (deals, of course, with the other senses).—**C. S. Sherrington**, Articles on Cutaneous Sensations and Muscular Sense in Text-book of Physiology, edited by E. A. Schäfer, vol. ii., 1900.—**E. B. Titchener**, Experimental Psychology: Qualitative (Students' Manual), i., Quantitative (Instructors' Manual), ii., 1901. The author is greatly indebted to this work as well as to that of Sanford. It deals with all the senses.—**W. Wundt**, Lectures on human and animal psychology, translated by Creighton and Titchener, 1892; and Geomet. Opt. Tauschungen, Leipzig, 1898.

(B) CUTANEOUS AND ALLIED SENSATIONS.—**E. H. Weber**, Tastsinn u. Gemeingefuhl, Wagner's Handwörterbuch d. Physiologie (a classic), 1846.—**Blix**, Specif. Energie d. Hautnerven, Zeit. f. Biol., xx., 1884.—**Max von Frey**, Sinnesfunction d. mensch. Haut, Leipzig, 1898.—**Goldscheider**, Gesammelte Abhandlungen, Leipzig, 1898.

(C) SMELL, TASTE, AND HEARING.—**M. von Vintschgau**, Hermann's Handbuch d. Phys., iii., 1880.—**A. Zwaardemaker**, Die Physiologie d. Geruchs, Leipzig, 1895. The standard work on the subject. The old work of Cloquet is worthy of attention.—**Helmholtz**, Sensations of tone, translated by A. J. Ellis, 1895. Standard work. A fairly complete series of references is given by Sanford.

IV. CHIEF JOURNALS AND PERIODICALS.

Proceedings and Transactions of the Royal Societies (Lond., Edin.), and others.

Journal of Anatomy and Physiology (Turner and others) from 1868.

The Journal of Physiology (Foster, and presently Langley) since 1878.

Archiv für Anatomie und Physiologie (Müller 1834 to 1858, du Bois-Reymond from 1859). As Archiv für Physiologie by du B.-R., from 1877-96, presently by Engelmann.

Zeitschrift für Biologie (Kühne & Voit from 1865, and presently by Voit).

Archiv für die gesammte Physiologie (Pflüger from 1868).

Archiv für path. Anat. und Physiologie (Virchow from 1847).

Archiv für exp. Path. und Pharmacologie (Naunyn & Schmiedeberg from 1873).

Skandinavisches Archiv für Physiologie (Holmgren in 1889—presently R. Tigerstedt).

Zeitschrift für Physiol. Chemie (Hoppe-Seyler from 1877, presently by Kossel).

Zeitschrift für Psychologie und Physiologie der Sinnesorgane (H. Ebbinghaus and A. König from 1890).

Sitzungsberichte der Acad. d. Wissenschaften (of Berlin from 1836, of Vienna from 1848).

Ludwig's Arbeiten Leipzig from 1866 to 1877 (continued in du Bois-Reymond Archiv from 1877).

Comptes rendus de l' Acad. des Sciences from 1835.

Comptes rendus de la Société de Biologie from 1850.

Berichte d. deutsch. chem. Gesellschaft.

Journal de la Physiologie (Brown-Séquard from 1858 to 1863).

Archives de Physiologie (formerly Brown-Séquard, now Bouchard, Chau-

veaux & Marey) from 1868. Modified and continued as Journal de Phys. et de Path. générale by Bouchard and Chauveau.

Journal de l'Anat. et de la Physiol. (Robin, Pouchet, now Mathias Duval) from 1864.

Archives Italiennes de Biologie (Mosso from 1882).

Journal of Experimental Medicine, Baltimore.

American Journal of Physiology, Boston (since 1895).

V. GENERAL REFERENCES AND ABSTRACTS.

Schmidt's Jahrbucher (from 1834). Canstatt's Jahresbericht; Hoffmann & Schwalbe's Jahresbericht (from 1873, now by Hermann). Maly's Jahresbericht ü. d. Fortschritte d. Thier-Chemie (chiefly physiological chemistry). Hayem, Revue des Sciences medicales. Reports of the Chemical Society. Centralblatt f. d. med. Wissenchaften (from 1863). Biologisches Centralblatt (from 1868). Chemisches Centralblatt. Centralblatt f. Physiologie (from 1887). Ch. Richet, Bibliographia physiologica, 1895-96. Le physiologiste russe, Moscow (from 1898).

B.—CHEMICAL APPENDIX.

Nucleo-proteids and their derivatives. Purin bodies.

Nucleo-proteid

Albumin Nuclein

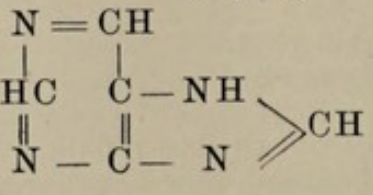
Albumin Nucleinic acid

Nuclein bases: Xanthin, Hypoxanthin, Guanin, Adenin Phosphoric acid

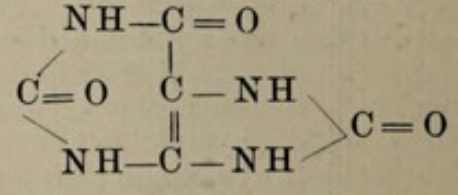
The nuclein bases, sometimes called alloxuric bodies, are very important. They are derivatives of Purin, and are also called purin bodies.

Purin bodies.

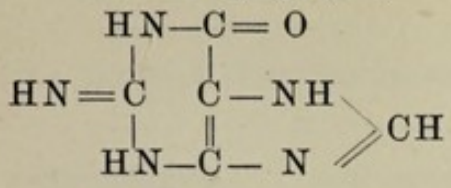
Purin $C_5H_4N_4$;



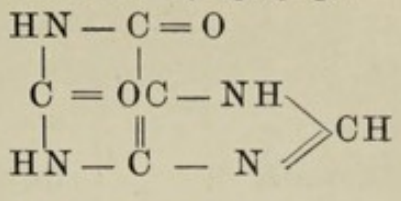
Uric acid $C_5H_4N_4O_3$;



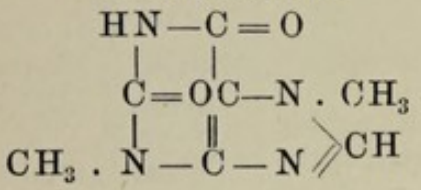
Guanin $C_5H_5N_5O$;



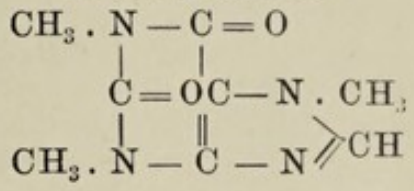
Xanthin $C_5H_4N_4O_2$;



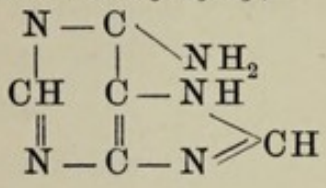
Theobromin $C_7H_8N_4O_2$; Dimethylxanthin



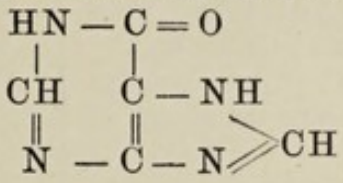
Caffein $C_8H_{10}N_4O_2$; Trimethylxanthin



Adenin $C_5H_5N_5$;



Hypoxanthin $C_5H_4N_4O$;



Oxidases.

(*a*) Fresh organs do not, as a rule, exhibit an oxidase reaction. If the blood-vessels of the animal be perfused with water to wash away all blood, then tincture of guaiacum gives the blue reaction with lung, spleen, fibrin, but not with liver, brain, pancreas, muscle. Macerate for 24 hours fresh lung, spleen, or fibrin in pure water, adding a little chloroform to preserve it. On filtration no oxidising reaction is obtained. Proceed as before, but extract with KNO_3 (8 to 10 p.c.), NaCl (8 to 10 p.c.), NaFl (1 to 3 p.c.). At 40° C. a clear filtrate with strong oxidising action is obtained. The residue also gives the reaction. The substance which causes the reaction is precipitated by CO_2, or saturation with $MgSO_4$. It seems to be of the nature of a globulin (Abelous and Biarnes, *Arch. de Phys.*, 1898).

(*b*) Extract the skin of frog with 1 p.c. NaCl and expose the extract to air. It becomes brownish-black. This does not occur if the fluid be boiled (*Physalix*).

(*c*) I have myself often observed the changes in colour that occur when a potato is grated in water. The fluid rapidly runs through a series of colour changes.

(*d*) *Action of drugs.*—Test the effect of any one of the following reagents:—10 p.c. carbolic acid, 1 p.c. caffein, 1 p.c. quinin sulphate, and $HgCl_2$ ·02 p.c. Put in each test-tube 1 volume reagent, 5 drops tincture guaiacum, and 10 drops H_2O_2. Note time until a distinct blue colour appears, and depth of colour at end of an hour. Arrange them in order of extent of oxidation (*Sollmann*).

[*Les Oxydases*, by P. Portier, Paris, Thèse, 1897.]

BODIES OF THE

The Aromatic Compounds of the Urine and their

Tyrosin.	Oxyphenyloxypropionic Acid.	Oxyphenylpropionic Acid.
$C_6H_4{}^{OH}_{CH_2 . CH . NH_2 . COOH}$.	[Oxyhydroparacumaric acid.]	[Hydroparacumaric acid.]
From albumin—	$C_6H_4{}^{OH}_{CH_2 . CH . OH . COOH}$.	$C_6H_4{}^{OH}_{CH_2 . CH_2COOH}$.
By trypsin.	In the urine of the rabbit after feeding with tyrosin.	Normal constituent of urine, decomposition product of tyrosin. When given to an animal, part is excreted unchanged, part is oxidised to
By putrefaction.	In human urine, after acute yellow atrophy of the liver and phosphorus poisoning.	Paroxybenzoic acid.
By fusing with KHO.		$C_6H_4{}^{OH}_{COOH}$.
In urine—		which passes into the urine as
In acute yellow atrophy of the liver and phosphorus poisoning.		Paroxybenzuric acid.
		$C_6H_4{}^{OH}_{CO . NH . CH_2 . COOH}$.

Phenylamidopropionic Acid.	Phenylpropionic Acid.	Phenylamidoacetic Acid.
$C_6H_5 . CH_2 . CH . NH_2 . COOH$.	$C_6H_5 . CH_2 . CH_2 . COOH$.	$C_6H_5 . CH . NH_2 . COOH$.
Decomposition of albumin in seedlings.	Decomposition product of albumin, oxidised in the organism to	Yields during putrefaction
	Benzoic acid.	Amygdalic acid.
	$C_6H_5 . COOH$.	$C_6H_5 . CH . OH . COOH$.
	which passes into the urine as	
	Hippuric acid.	
	$C_6H_5 . CO . NH . CH_2 . COOH$.	

AROMATIC SERIES.

Relation to the Decomposition Products of Albumin.

Oxyphenylacetic Acid.

$C_6H_4 \begin{matrix} OH \\ CH_2 . COOH \end{matrix}$.

Putrefactive product of tyrosin; normal urinary constituent. When given to an animal, it leaves the organism unchanged.

Parakresol.

$C_6H_4 \begin{matrix} OH \\ CH_3 \end{matrix}$.

Putrefactive product of tyrosin; occurs in urine as

$C_6H_4 \begin{matrix} CH_3 \\ OSO_2OH \end{matrix}$.

Phenol.

$C_6H_5 . OH$.

Putrefactive product of tyrosin; occurs in urine as

$C_6H_5 . OSO_2OH$.

In the organism, it is partly oxidised into

Pyrocatechin.

$C_6H_4 \begin{matrix} OH \\ OH \end{matrix}$

which occurs as a constituent of horses' urine, partly as an ether sulpho-compound, and partly free.

Phenylacetic Acid.

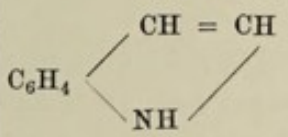
$C_6H_5 . CH_2 . COOH$.

Putrefactive product of phenylamidopropionic acid and of albumin, passes into the urine as

Phenaceturic acid.

$C_6H_5 . CH_2 . CO . NH . CH_2 . COOH$.

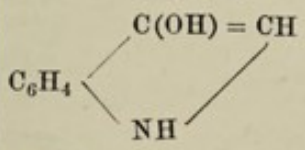
Indol.



Obtained from albumin by putrefaction, and heating with caustic potash.

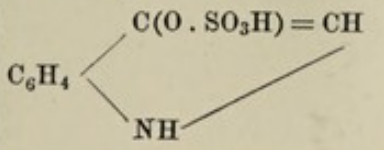
In the organism it is oxidised to

Indoxyl.

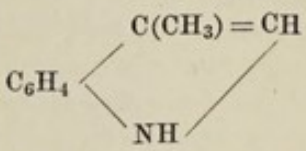


Pass into the urine as

Indoxylsulphuric acid.

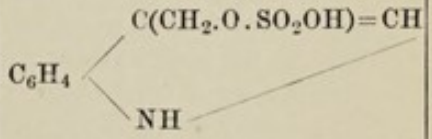


Skatol.



Putrefactive product of albumin, passes into the urine as

(Skatoxylsulphuric acid).



Röhmann.

Reagents and Some Solutions Required.

Acid—Acetic, glacial.
,, ,, 20 p.c.
,, ,, 2 p.c.
,, *Hydrochloric*, concentrated.
,, ,, 0·2 p.c. (add 6 c.c. HCl to 994 c.c. H_2O).
,, *Nitric* (i.) concentrated, (ii.) fuming—*i.e.*, with nitrous acid.
,, *Sulphuric*, concentrated, 20 p.c., $\frac{N}{1}$, 0·1 p.c.
Alcohol, methylated.
,, absolute.
Ammonium hydrate (S.G. 0·88).
Ammonium molybdate solution.—Dissolve 5 grms. in 20 c.c. strong ammonia. Heat, then pour it slowly into 80 c.c. diluted HNO_3, made by mixing 40 c.c. pure HNO_3 with 40 c.c. distilled water.
Ammonium oxalate, 10 p.c. solution.
,, *sulphate*, saturated solution.
,, ,, crystals.
,, *sulphide* solution.
Barfoed's reagent.—To 200 c.c. of a solution of neutral acetate of copper, containing 1 part of the salt to 15 water, add 5 c.c. of a 38 p.c. solution of acetic acid.
Barium chloride, saturated solution.
Baryta mixture.—(*a*) Make a saturated solution of barium hydrate in distilled water *in the cold.*
(*b*) Make a saturated solution of barium nitrate *in the cold.*
Mix 1 vol. *a* with 2 vols. *b* = "Baryta mixture."
Bromine, in tubes.
Calcium chloride, 2 p.c. solution.
Chloroform.
Cochineal, watery solution.
Congo red test papers.—Dissolve 1 decigrm. Congo-red in 100 c.c. distilled water. Dip strips of bibulous paper in it and dry them.
Copper sulphate, 1 p.c. solution.
Ether, methylated.
Esbach's reagent.—Dissolve 10 grms. picric acid and 20 grms. citric acid in 800 c.c. of boiling water. Add sufficient water to make up a litre.
Fehling's solution.—*Solution A.*—34·64 grms. of pure crystalline cupric sulphate are powdered and dissolved in 500 c.c. of distilled water. *Solution B.*—In another vessel dissolve 173 grms. of Rochelle salts (sodio-potassium tartrate) in 100 c.c. of pure caustic soda, S.G. 1·34, and add water to make 500 c.c. Keep the two solutions separate in stoppered bottles, and mix them as required. On mixing equal quantities of A and B, a clear deep blue fluid is obtained, the Rochelle salt holding the cupric hydrate in solution.
N.B.—Fehling's solution ought not to be kept too long; it is apt to decompose, and should therefore be kept away from the light, or protected with opaque paper pasted on the bottle. Some other substances in urine —*e.g.*, uric and glycuronic acids—reduce cupric oxide. *In all cases see that there is an excess of the test present.*
Ferric chloride.
Furfurol.
Guaiacum resin dissolved in alcohol.

Hayem's fluid for blood. Dissolve 1 grm. NaCl, 5 grms. Na_2SO_4, and 0·5 grm. $HgCl_2$ in 200 c.c. distilled water.

Hydrogen peroxide.

Iodine solution.—Dissolve 2 grms. of potassium iodide in 100 c.c. water, and 1 grm. iodine, and shake well.

Lacmoid.

Lead acetate basic.—Boil plumbic acetate with litharge for 10 minutes. The filtrate is basic lead acetate.

Lime-water.

Litmus, solution and papers (red and blue).

Magnesium sulphate, crystals, and saturated solution (for saturation 125 to 100 H_2O).

Mercuric chloride, saturated watery solution.

Methyl-orange, 1 p.c. watery solution.

Methyl-violet.

Millon's reagent.—Dissolve mercury in its own weight of strong HNO_3, S.G. 1·4, and add 2 volumes of water. Allow it to stand, and afterwards decant the clear fluid; or take 1 part of mercury, add 2 parts HNO_3, S.G. 1·4, and heat over a water-bath till complete solution occurs. Dilute with 2 volumes of water, and decant the clear fluid after 12 hours.

Pavy-Fehling solution.—Pure $CuSO_4$, 3·465 grms.; Rochelle salt, 17 grms.; pure KHO, 17 grms. Dissolve in distilled water, and make the solution up to 100 c.c., add 333 c.c. ammonia (S.G. 0·88), and then 396 c.c. distilled water. It is not necessary to dilute it when used for the volumetric estimation of sugar.

10 c.c. are equal to 1 c.c. Fehling—*i.e.*, 10 c.c. are reduced by 5 milligrms. dextrose. Use 50 c.c. for each volumetric estimation.

Phenol. See Uffelmann's Test, p. 84.

Phenyl-hydrazine and its hydrochloride.

Picric acid, saturated watery solution. Best to dissolve crystals in hot water, and filter when cold. Pure crystals are essential.

Phloroglucin-Vanillin test (yellowish colour).

Phloroglucin, ...	2 grms.
Vanillin, ...	1 grm.
Alcohol, ...	30 c.c.

Phosphomolybdic acid to precipitate proteids.—Dissolve 20 grms. of the acid (or phospho-tungstic) in 100 c.c. of water, and add 2 c.c. H_2SO_4.

Phosphoric acid, glacial, 0·5 p.c. solution.

Phospho-tungstic acid.

Potassio-mercuric iodide or Brücke's reagent.—Precipitate a saturated solution of potassium iodide with a similar solution of mercuric chloride; wash the precipitate, and dissolve it to saturation in a hot solution of potassium iodide.

Potassium, bichromate solution, 3 p.c.

,, *ferricyanide*, 10 p.c. solution.

,, *ferrocyanide*, ,, ,,

,, *hydrate solution* (20 grms. caustic potash in 100 c.c. H_2O), and 0·1 p.c.

,, *sulphate* for Kjeldahl's N estimation.

,, *sulpho-cyanide* solution.

Salicyl-sulphonic acid, watery solution.

Silver nitrate.—Dissolve 2 grms. $AgNO_3$ crystals in 20 c.c. distilled water.

Sodio-magnesium sulphate, crystals.

Sodium acetate, powder and solution.—Add 100 c.c. pure acetic acid (1·04 S.G.) to 70 c.c. distilled water. Dissolve 10 grms. pure sodium acetate in it, and add distilled water to make 100 c.c.

,, *carbonate*, saturated, and 2 p.c. solution.

,, *chloride*, crystals, saturated, and 10 p.c. solution.

,, *hydrate*, 40 p.c. solution and $\frac{N}{1}$ solution.

,, *hypobromite S.S. for urea.*—Dissolve 50 grms. sodium hydrate in a porcelain capsule in 100 c.c. distilled water. Place 25 c.c. distilled water in a tall thin glass jar, and add 12·5 c.c. bromine. Pour the soda into the diluted bromine solution, and cool the mixture. Use prepared solution sufficient for five estimations.

,, *nitro-prusside* solution (freshly prepared).

,, *phosphate* solution, 10 p.c.

Standard solutions.

Stokes' fluid.—Make a solution of ferrous sulphate; to it add ammonia after the previous addition of sufficient tartaric acid to prevent precipitation. Add about three parts by weight of tartaric acid to two of the iron salt. *Make it fresh when required.*

Sulphur, precipitated.

Syrup.—Dissolve 40 grms. cane sugar in 30 c.c. It is apt to ferment.

Talc, powdered (for N determination).

Tannin solution for proteids.—Dissolve 4 grms. tannic acid in 190 c.c. of 45 p.c. alcohol, and add 2 c.c. glacial acetic acid (*Arthus*).

Trichloracetic acid.

Uffelmann's test (for lactic acid). Clear violet solution.

Carbolic acid (1-20), ...	10 c.c.
Distilled water, ...	20 c.c.
Liq. ferri perchloridi, ...	1 or 2 drops.

Uranium acetate solution for testing.—Dissolve 2 grms. uranium acetate in about 80 c.c. ordinary pyroligneous acetic acid as may be required to dissolve it in the cold. Add distilled water to dilute the fluid to 100 c.c. and filter.

Uranium nitrate S.S. for H_3PO_4. 1 c.c. = 5 milligrms. phosphoric anhydride (P_2O_5).—Add 2·5 c.c. pure glacial acetic acid to 90 c.c. distilled water; dissolve it in 3·5 grms. uranium nitrate, and then add distilled water to make 100 c.c.

C.—APPENDIX ON INSTRUMENTS.

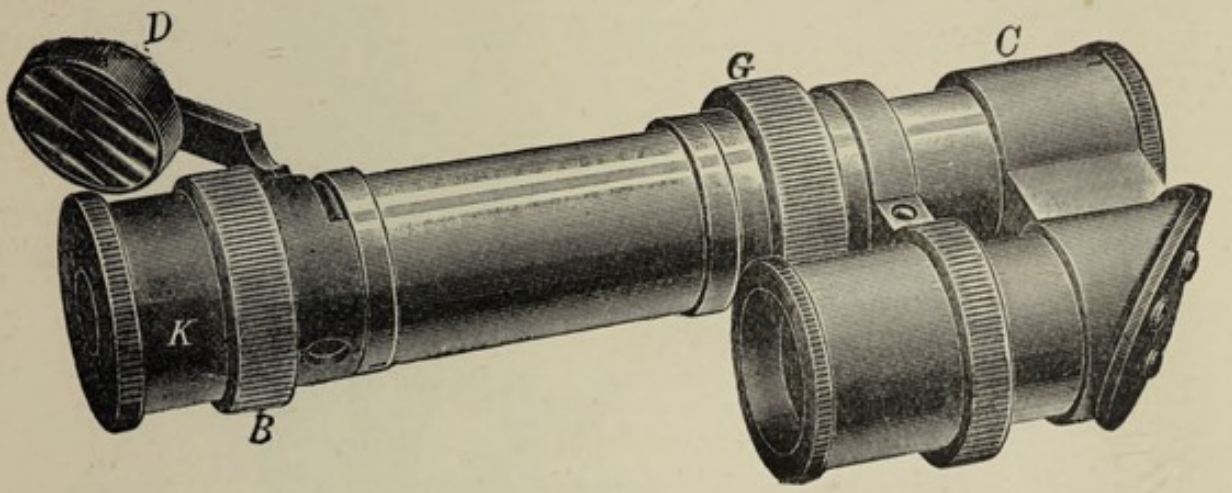


Fig. 455.—Spectroscope with comparison spectrum. Schmidt & Haensch, Berlin.

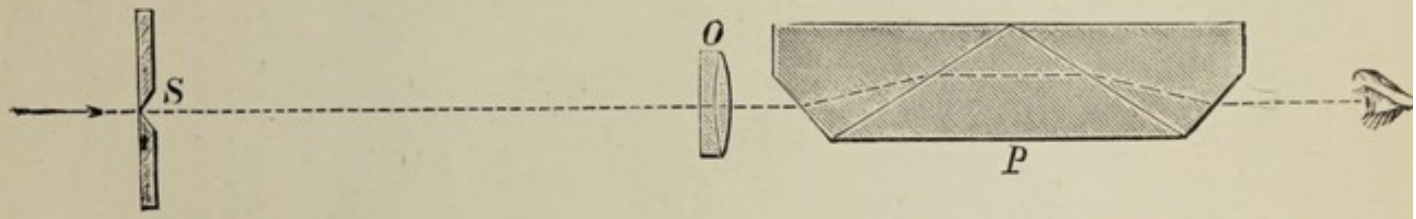


Fig. 456.—Arrangement of slit lens and prism in straight-vision spectroscope, as made by Schmidt & Haensch, Berlin.

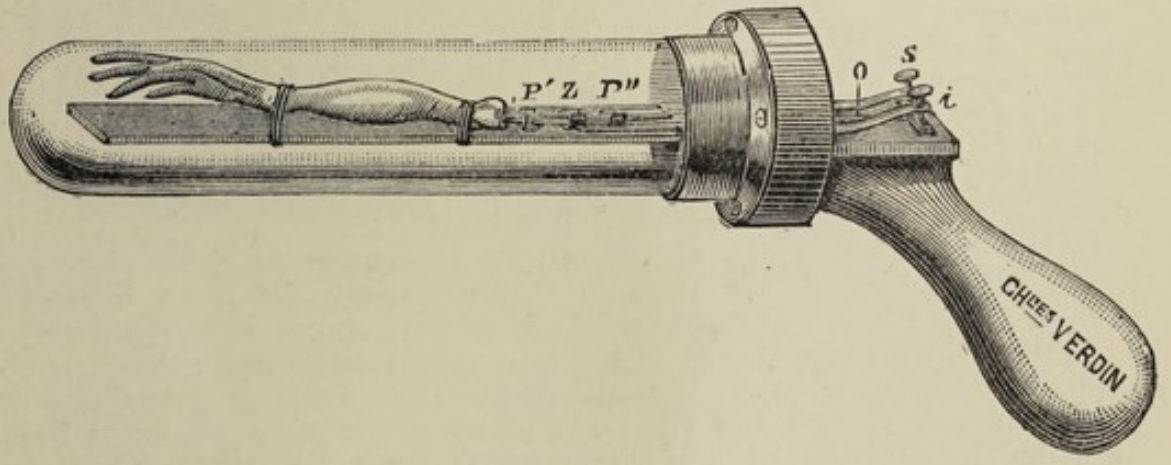


Fig. 457.—The classical "frog-pistol" of du Bois-Reymond. A N.M. preparation is made and enclosed in the glass tube. Connections are so made that by pressing the keys *S* and *i* the nerve is stimulated.

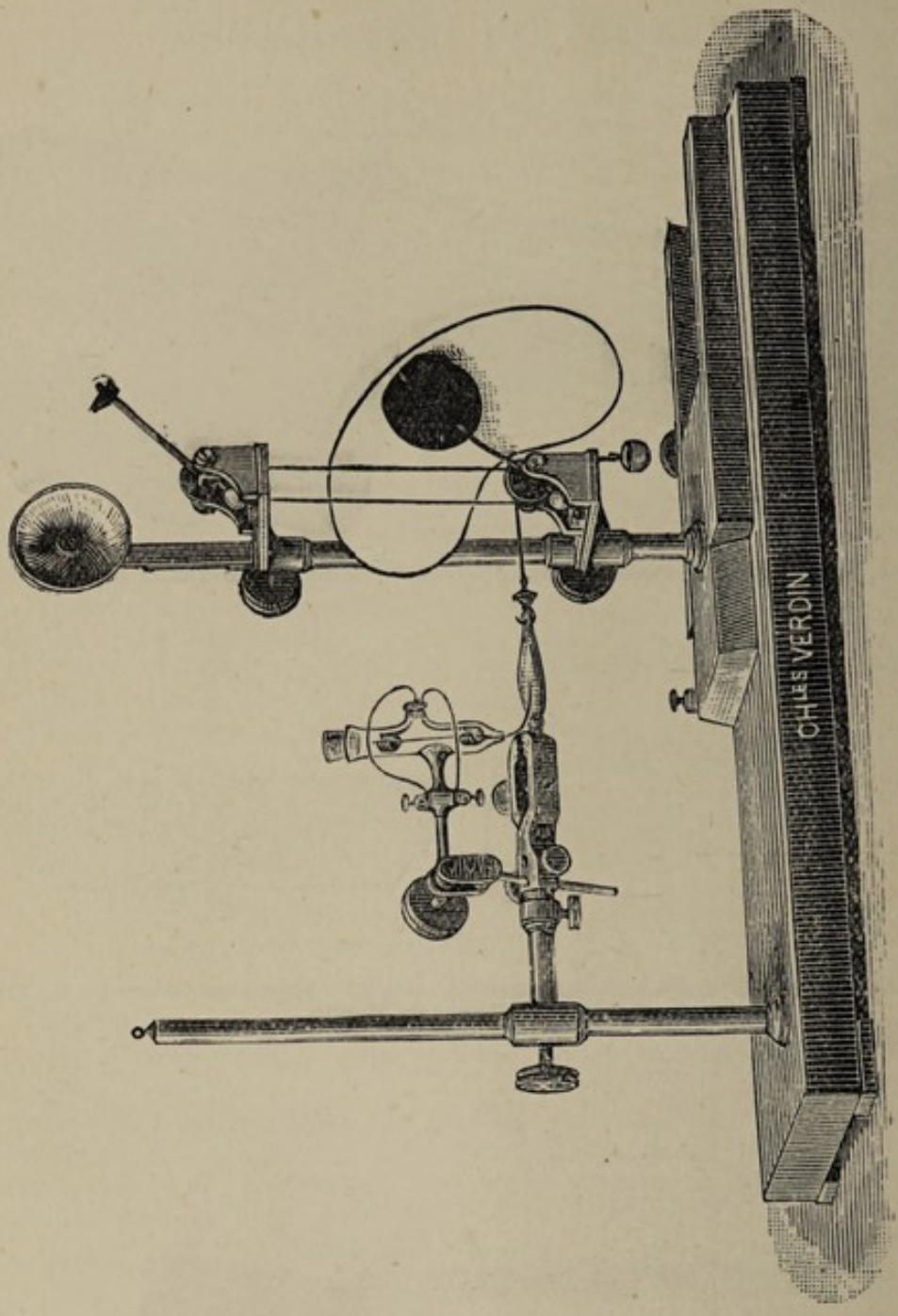


Fig. 458.—The "muscle telegraph" of du Bois-Reymond. The muscle, in contracting, raises a weight, pulls indirectly on a telegraph black disc and on a striker for a bell. The nerve of the muscle is enclosed and stimulated in a glass tube. For class demonstration, this is a most instructive experiment.

In the modification of the myograph shown in fig. 460, suggested by W. Charles, and now used in Owens College, the part *b* bearing the crank L is not continuous with the support *a*, but is by means of a cylindrical rod fitted fairly tight into a corresponding socket in the support *a*. Thus the whole crank and style recording lever can be gently turned so that the style can make a complete record without altering the position of the clamp which fixes the whole apparatus to the wooden support. The apparatus is made by C. F. Palmer (*Journ. of Phys.*, xxvii.).

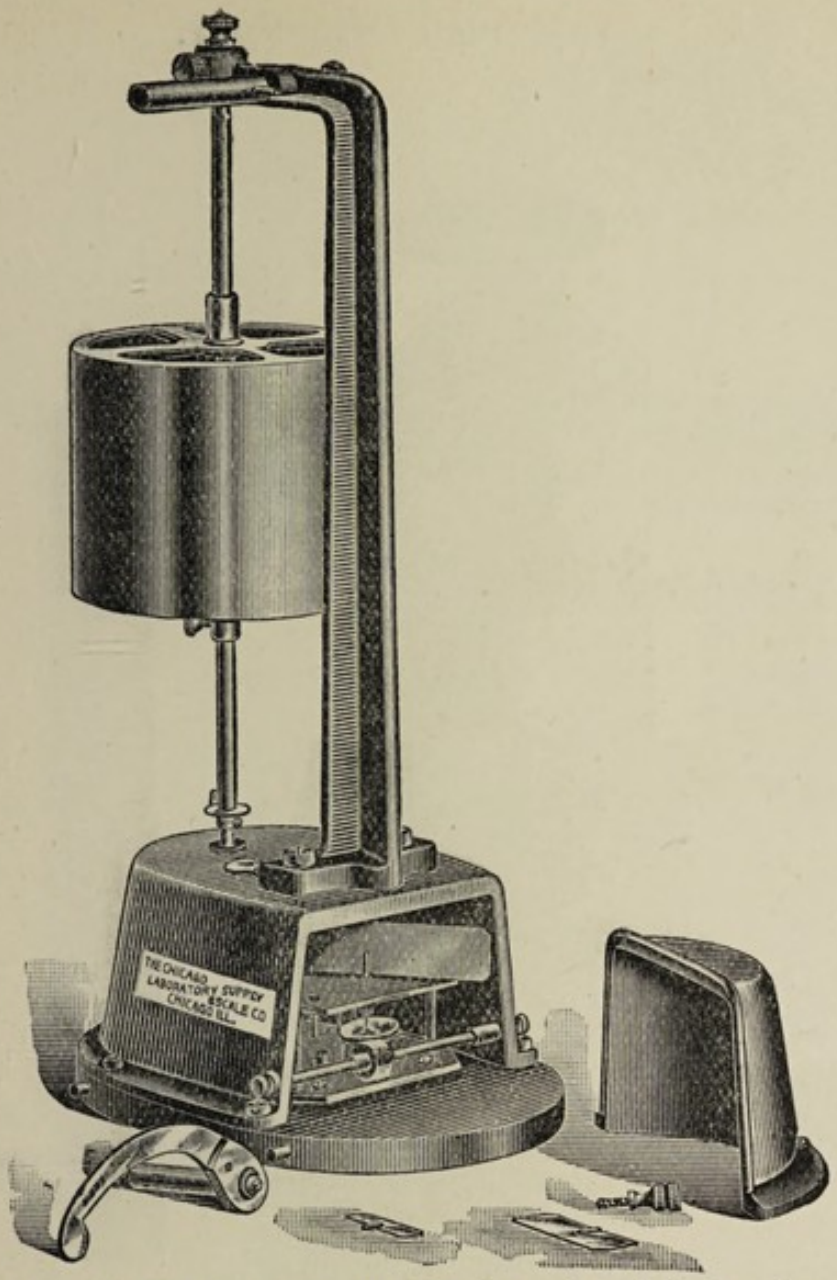


Fig. 459.—Form of recording apparatus largely used in U.S., America. Made by Chicago Supply and Scale Company. It is usually called a "kymographion."

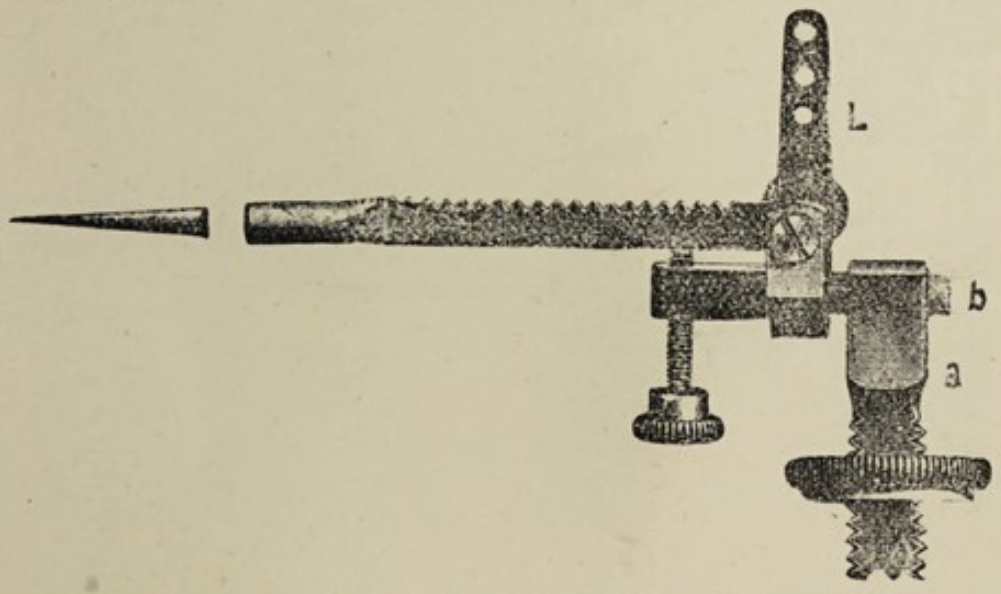


Fig. 460.—Modification of crank myograph, as used in Owens College.

Fig. 461.—Jaquet's graphic chronometer. It registers 0·2 second or 1 second. It is provided with two dials—one for indicating seconds, the other minutes. It is an excellent instrument, made by J. Jaquet, St. Imier, Switzerland.

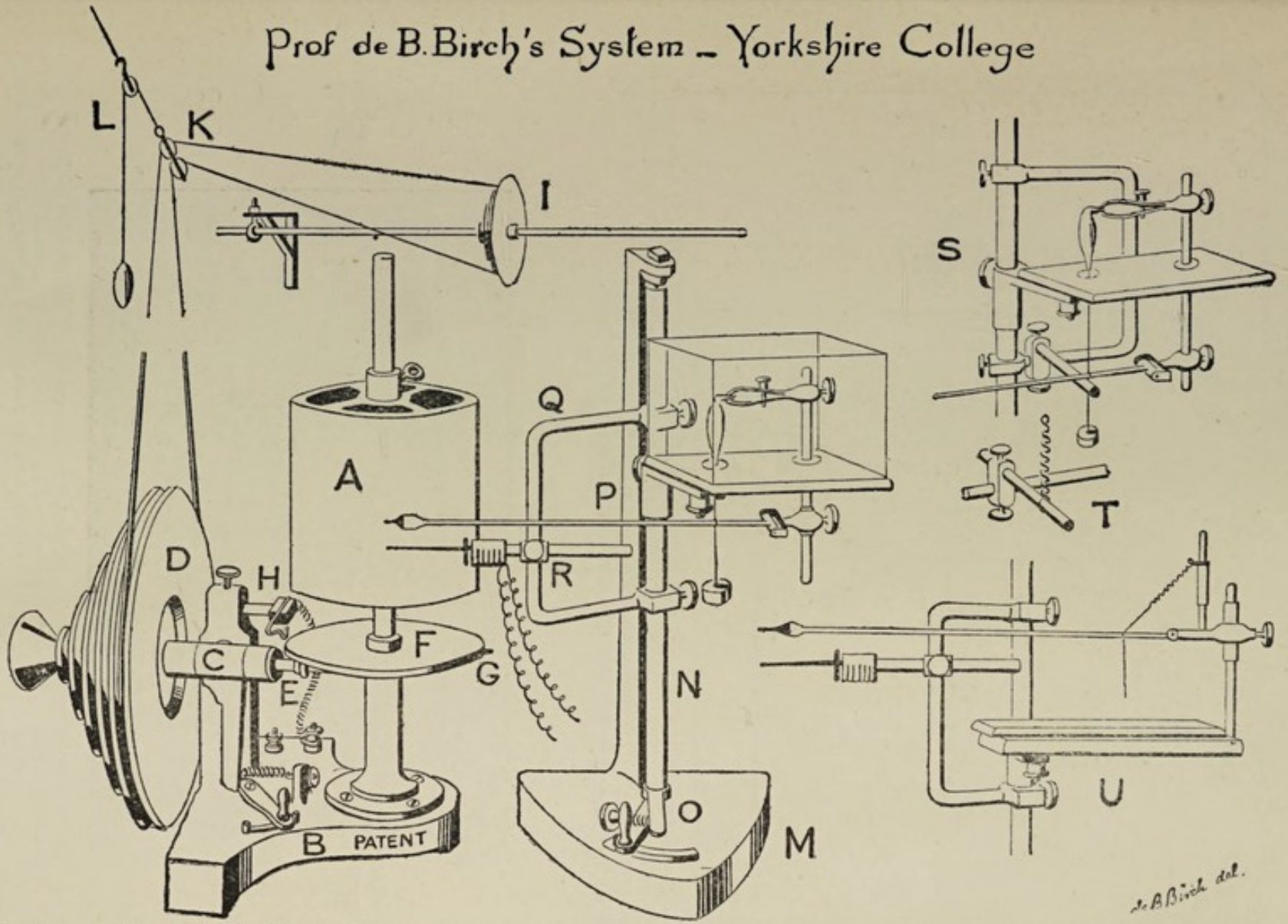


Fig. 462.—*A*, Drum; *B*, Starting and stopping lever which actuates *C*, The rocker; *D*, Driving cone; *E*, Driving pulley, which propels *F*, the disc on the drum axle; *G*, Pin for striking the contact, *H*; *I*, Cone on power shaft, cord connection to drum over guide pulleys, *K*; *L*, Tension pulley and counter-weight; *M*, Stand for muscle and nerve experiments; *N*, Pillar rotated by *O*, the lever with adjusting screw and stop; *P*, Bracket for supporting a muscle-chamber or frog heart plate; *Q*, Stirrup carrying a time-marker in a clamp, *R*; *S*, Arrangement for after-load; *T*, Spring for isometric muscle-curve; *U*, Arrangement with frog heart plate.

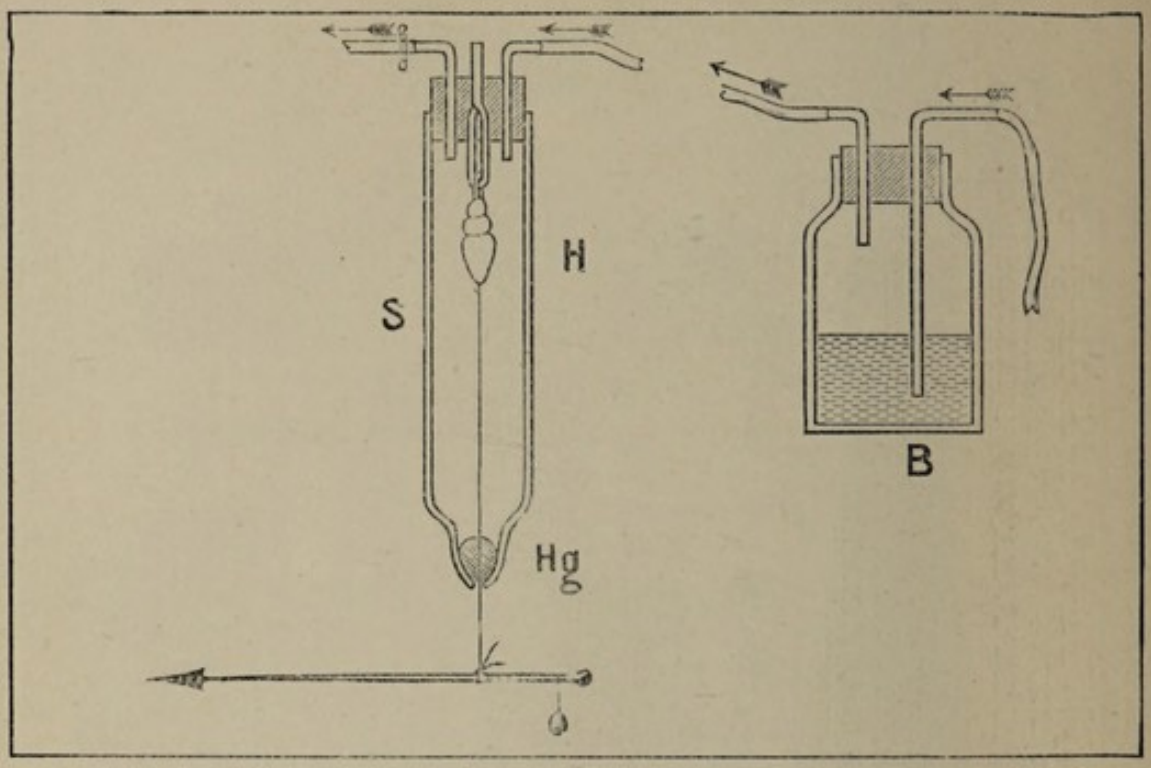


Fig. 463.—Sherrington's apparatus for applying gases or vapour to an excised frog's heart. S, Glass syringe-tube. H, Heart with thread to lever working through a drop of mercury (Hg). B, Vessel with water and a few drops of ether or chloroform. The vapour from B is blown through S by means of a spray producer.

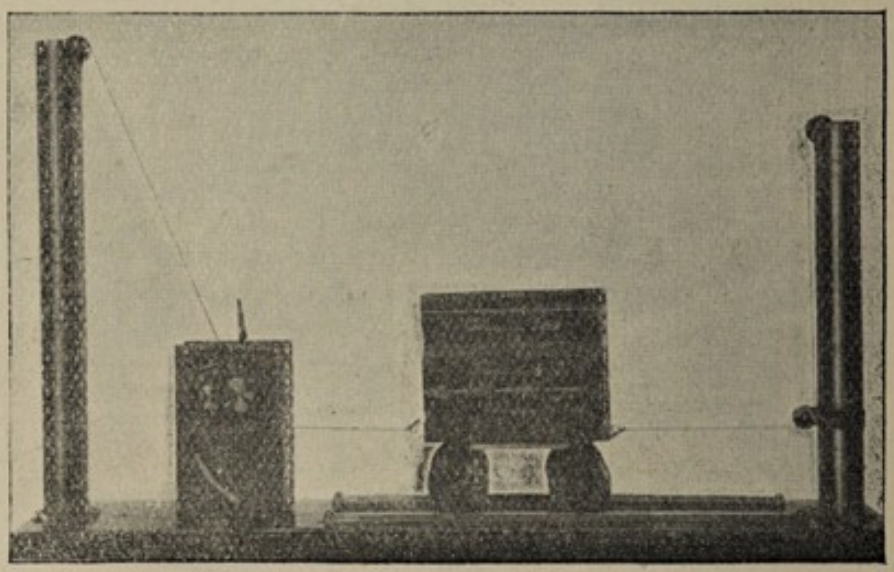


Fig. 464.—Lantern railway myograph. A bogie waggon carries a quarter ($3\frac{1}{4}$ inches by $4\frac{1}{4}$ inches) smoked plate, or Continental size, 9 by 12 cms. The motor has three speeds, approx. 1, 6, and 12 mm. per second; these can be varied by regulating the speed of clock (Waller's pattern, made by Hoyer).

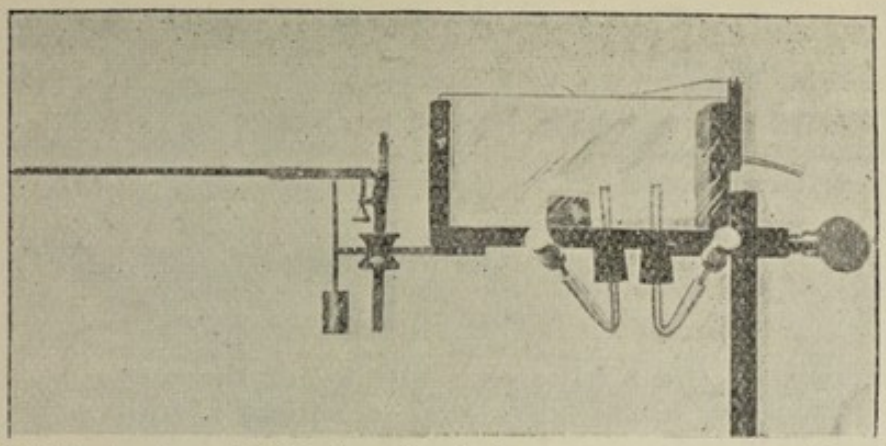


Fig. 465.—Moist chambers (Waller's pattern) for nerve or nerve-muscle. Made with removable glass sides and top. Fitted with one pair of non-polarisable electrodes, zincs, bell-crank writing lever (Isotonic or Isometric, as desired), gas inlet, &c., and made to fasten on a simple stand.

D.—APPENDIX.

Doses of Some Drugs Required to Produce their Physiological Effects.

Dog.—(1) Morphin sulph. subcutan., 0·01 grm. per kilo. half an hour before operation.

Then anæsthetic by chloroform-ether.

(2) Morph. sulph., 0·01 grm. per kilo.
Atropin sulph., 0·01 ,, ,, } subcutan.
(for work on circulation).

(3) Prof. Cushny prefers aceton-chloroform for dogs.

(4) *Curare*, 0·01 grm. per kilo.

(5) For work on the circulation Richet uses chloralose injected into the veins. It is rather insoluble, and so a large amount of fluid is required. It does not lower the blood pressure.

Rabbit.—(1) Chloral-hydrate, 1 to 2 grms. subcutan., or 0·6 to 0·7 grm. per kilo., by stomach-tube or rectum.

(2) Urethan, 2 grms. in 25 p.c. solution. Is without action on heart, and is very satisfactory (1 grm. per kilo.).

(3) Give by the mouth 25 c.c. of a saturated solution of chlorozone, and follow by inhalation of ether.

(4) The A.C.E.—*i.e.*, a mixture of alcohol, chloroform, and ether—is used by some for general anæsthesia.

Cat.—Levy-Dorn *Centrbl. für Physiologie*, 1895, p. 97.

Opossum.
Chloral-hydrate, 1 grm.
Morph. sulph., 0·03 to 0·04 grm.
(*Reid Hunt.*)

Solubilities in Water at 15° to 18° C.

Ammonium chloride,	36 per 100.
Sodium chloride,	36 ,,
Ammonium sulphate,	50 ,,
Magnesium sulphate,	125 ,,

Measures of Length

Metric System.

The standard is the metre; for multiples of the metre prefixes deca- hecto- and kilo- are used; for subdivisions thereof, milli- centi- and deci- are used just as in the case of the grm. in the table below.

1 millimetre = 0·001 metre = 0·03937 inch.
1 centimetre = 0·01 ,, = 0·3937 ,,
1 decimetre = 0·10 ,, = 3·93707 inches.
1 metre = 39·37079 ,,

English System.

1 inch = 25·4 millimetres.
1 foot = 12 inches = 304·8 ,,

Measures of Capacity.

Metric System.

A litre is the standard, and is equal to 1000 cubic centimetres (1000 c.c.); each cubic centimetre is the volume of 1 grm. of distilled water at 4° C.

1 cubic centimetre (1 c.c.) = 16·631 minims.
1 litre = 1000 c.c. = 1 pint 15 oz. 2 drs. 11 min. (35·2154 ozs.).

English System.

1 minim	=	0·059	cubic centimetre.
1 fluid drachm = 60 minims	=	3·549	cubic centimetres.
1 fluid ounce = 8 fluid drachms	=	28·398	,, ,,
1 pint = 20 fluid ounces	=	567·936	,, ,,
1 gallon = 8 pints	=	4·54837	litres.

Weights.

Metric System.

1 milligrm. =	0·001 grm.	=	0·015432 grain.
1 centigrm. =	0·01 ,,	=	0·154323 ,,
1 decigrm. =	0·1 ,,	=	1·543235 grains.
1 grm. =	1 ,,	=	15·43235 ,,
1 decagrm. =	10 grms.	=	154·33235 ,,
1 hectogrm. =	100 ,,	=	1543·235 ,,
1 kilogrm. =	1000 ,,	=	15432·35 ,,
		=	2 lb. 2 oz. 119·8 ,,

For practical purposes, the kilogrm. or kilo. is taken at 2·2 lbs.

English System.

1 grain = 0·0648 grm.
1 ounce = 437·5 grains = 28·3595 grms.
1 lb. = 16 oz. = 7000 ,, = 435·5925 ,,

Area of sphere = πr^2, or $3{\cdot}1416r^2$.
Surface ,, = $4\pi r^2$, or $12{\cdot}5664r^2$.
Volume ,, = $\frac{4\pi}{3} r^3$, or $4{\cdot}2r^3$.

THERMOMETRIC SCALES.

Fahrenheit scale, freezing point of water, 32°; boiling point, 212°.
Reáumur ,, ,, ,, ,, 0° ,, ,, 80°.
Centigrade ,, ,, ,, ,, 0° ,, ,, 100°.

To convert degrees F. into degrees C., subtract 32 and multiply by $\frac{5}{9}$ or $C = (F - 32)\frac{5}{9}$. To convert C.° into F.°, the formula is $F = \frac{9}{5} C + 32$.

SOME OF THE INSTRUMENT-MAKERS WHO SUPPLY PHYSIOLOGICAL APPARATUS.

Eugen Albrecht, Universitäts Mechanicus, Tübingen (Hürthle's hæmodynamic apparatus).
Backhouse, University College, London.
A. H. Baird, 33 Lothian Street, Edinburgh.
Baird & Tatlock, 14 Cross Street, Hatton Gardens, London, E.C.
Butler, Physiological Laboratory, Oxford.
Cambridge Scientific Instrument Co.
L. Castagna, Schwarzspanierstrasse 17, Wien IX./3 (Exner's app.).
Chicago Laboratory Supply and Scale Co., 31-45 Randalph Street, Chicago, Ill., U.S.
J. H. Harting-Bank, Utrecht (Zwaardemaker's instruments).
James J. Hicks, 8 Hatton Gardens, London.
J. C. Hoyer, St. Mary's Medical School, London (Waller's apparatus).
W. Hume, Lothian Street, Edinburgh.
Jaquet, St. Imier, Switzerland (Chronometric apparatus).
R. Jung, Landhausstrasse 12, Heidelberg.
D. B. Kagenaar, Utrecht.
A. Kershaw, Dorrington Street, Leeds (Birch's apparatus).
Fritz Köhler, Linnestrasse, Leipzig (Cryoscopic apparatus of Ostwald).
F. & M. Lautenschläger, Oranienburgerstrasse 54, Berlin, N.
J. F. Meyer, Seilergraben 7, Zurich.
Milton Bradley Co., Springfield, Mass., U.S., America (Optical).
C. F. Palmer, 6 Upper Tulse Hill, Brixton, London, S.W. (Brodie's apparatus).
W. Petzold, Schönenweg 6, Leipzig.
W. G. Pye & Co., Granta Works, Mill Lane, Cambridge.
Reynolds & Branson, Commercial Street, Leeds.
Max Rinck, Physiologisches Institut, Marburg (Kossel's apparatus).

W. J. Rohrbeck's Nachfolger, Kärtnerstrasse 59, I., Vienna (Simple psychological apparatus).
R. Rothe, 16 Liebig Strasse, Leipzig (Hering's instruments).
Runne, Heidelberg (Centrifuges).
Hilding Sandström, University, Lund, Sweden (App. of Blix and recording app.)
Schmidt & Haensch, Berlin (Optical).
Siedentopf, Würzburg.
Ch. Verdin, Rue Linné 7, Paris (Apparatus of Marey and the French School).
E. Zimmermann, 21 Emilienstrasse, Leipzig (Psychological apparatus of Wundt and others).

INDEX.

Aberration—Chromatic, 510.
,, spherical, 510.
Absorption-bands, 50.
,, from rectum, 490.
Accommodation, 512.
,, line of, 516.
Aceto-acetic acid, 178.
Aceton, 177.
Achroo-dextrin, 26, 27, 75.
Achromic point, 175.
Acid-albumin, 7, 79.
Acid-hæmatin, 55.
Acidulated brine, 167.
Acme sacchar-ureameter, 176.
Action-current of muscle, 316.
,, of nerve, 317.
Acuity of vision, 522.
Adamkiewicz, reaction of, 11.
Adaptation, 528.
Æsthesiometers, 566.
After-images, 528.
After-load, 255.
Air expired, 473.
,, analysis of, 478.
Albumin, 1, 40.
,, coagulation temperature of, 4.
,, derived, 16.
,, egg, 15.
,, estimation of, 168.
,, general reactions, 2.
,, in urine, 164.
,, native, 4.
,, nitrogen in, 11.
,, serum, 5, 40.
,, soluble, 5.
,, sulphur in, 11.
,, temp. of coagulation, 11.
,, tests for, 165.
,, vegetable, 113.
Albuminates, 6.
Albuminimeter, 168.
Albuminoids, 13.
Albuminuria, 165.
Albumoses, 8, 81, 86.
Albumosuria, 167.
Aldehyde, 19.
Algesometer, 576.
Alkali-albumin, 7.
Alkali-hæmatin, 55.
,, reduced, 56.
Alkaline phosphates, 133.
Almén's test, 169.
Alternating currents, 214.
Amalgamation of zinc, 198.
,, mixture, 199.
Amidulin, 26.
Ammonia as stimulus, 225.
Ammonium carbonate, 142.
,, urate, 155.
Ampère, 197.
Amyl nitrite, 428.
Amyloid substance, 13.
Amylopsin, 90.
Amyloses, 18.
Anæsthetics, 453.
Anaglyph, 556.
Analysis of a fluid, 35, 191.
,, of a solid, 193.
Animal starch, 27, 105.
Anti-peptone, 85.
Anode, 195.
Apex-beat, 414.
Apex-preparation, 396.
Apnœa, 468.
Apparent movements, 539.
Arginin, 11.
Aristotle's experiment, 568.
Aromatic compounds, 163.
Arterial pressure, 449.
Arteriometer, 436.
Artificial eye, 561, 563.
,, gastric juice, 477.
,, pancreatic juice, 90.

Astigmatism, 517, 565.
Associated movements of eye, 533.
Atropin on heart, 403.
Augmentor nerve, 385.
Auto-laryngoscopy, 484.
Automatic break excitation, 255.
Automatograph, 508.
Auxocardia, 420.

Barfoed's reagent, 20, 596.
Baryta mixture, 596.
Bayliss' writing-point, 376.
Beckmann's apparatus, 486.
Beef tea, 119.
Benham's top, 554.
Benzoic acid, 159.
Benzo-purpurin, 84.
Benzoyl chloride, 20.
Bergmann's experiment, 523.
Bernard's method for curare, 241.
,, ,, for glucose, 46.
Bernstein's method for heart, 363.
Bezold's experiment, 511.
Bichromate cell, 196.
Bidwell's illusion, 540.
Biedermann's modification, 327.
Bile, 98.
,, acids, 99, 170.
,, actions of, 102.
,, cholesterin in, 101.
,, crystallised, 99.
,, Gmelin's test, 100, 169.
,, in urine, 169.
,, mucin in, 98.
,, Pettenkofer's test, 99, 170.
,, pigments, 99, 169.
,, salts, 99.
,, solvent, 103.
Bile-acids in urine, 170.
Bile-fistula, 489.
Bile-pigments in urine, 169.
Bilin, 99.
Bilirubin, 100, 169.
Biliverdin, 100, 169.
Binocular contrast, 554.
,, vision, 532.
Bismuth test, 20.
Biuret reaction, 9, 142.
Black-band experiment, 526.
Black's experiment, 473.
Blind spot, 520.
Blix's myograph, 286.
Blood, 37.
Blood, action of saline solution, 38.
,, Buchanan's experiments, 43.
,, carbonic acid on, 59.
,, clot, 39, 45.
,, coagulation of, 39.
,, coagulation experiment, 43.
,, colour, 37.
,, corpuscles, 44.
,, ,, numeration of, 60.
,, defibrinated, 38.
,, diabetic, 47.
,, drugs on, 58.
,, frog's, 45.
,, gases, 474.
,, grape-sugar in, 46.
,, in urine, 168.
,, iron in, 47.
,, laky, 37.
,, mammalian, 44.
,, nitrites on, 58.
,, opacity, 37.
,, oxygen capacity, 477.
,, plasma, 41.
,, pressure, 449.
,, ,, tracings, 460.
,, reaction, 37.
,, red corpuscles of, 44.
,, serum of, 39.
,, sodium fluoride on, 57.
,, specific gravity of, 37.
,, spectroscopy of, 48.
,, stains, 56.
,, time of coagulation, 45.
,, transparent, 37.
,, vessels, 412.
Bone, 34.
Böttger's test, 20, 171.
Bowditch's rotating coil, 210.
Bread, 114.
Break extra-current, 215.
Break-shock, 213.
Brine-test, 167.
British gum, 27.
Bromelin, 91.
Brondgeest's tonus, 499.
Brücke's method for glycogen, 106.
Brush electrodes, 317.
Buchanan's experiments, 43.
Buffy coat, 39.
Burette, 136.

Calcium phosphate, 134.
,, presence of, 226.

Calcium salts on blood, 43.
,, ,, on milk, 115.
Calculi, urinary, 184.
Campimetry, 543.
Cane-sugar, 22.
,, estimation of, 23.
,, inversion of, 23.
Cannula, 414.
Capillaries, pressure in, 433, 443.
Capillary electrometer, 319.
Carbohydrates, 17.
,, classification of, 18.
,, general characters, 17.
,, precipitation by salts, 28.
,, rotatory power, 29.
Carbolo-chloride of iron test, 84.
Carboluria, 178.
Carbonic acid, 480.
Carbonic oxide, colour of, 59.
,, hæmoglobin, 52.
,, spectrum of, 52, 72.
Cardiac delay, 439.
,, impulse, 414.
Cardiographs, 421.
,, for frog, 372.
Carica papaya, 91.
Casein, 10, 115.
Caseinogen, 10, 110.
Cathode, 194.
,, as stimulus, 339.
Cellulose, 29.
Centrifuge, 47, 179.
Cerebrum, removal of, 505.
Charpentier's experiments, 526.
Chemical stimulation, 224.
Chemotaxis, 356.
Chloral, 605.
Cholesterin, 101, 103.
Cholic acid, 99.
Choluria, 169.
Chondrigen, 14.
Chondrin, 14.
Chordogram, 271.
Choroidal illumination, 554.
Christison's formula, 127.
Chromatic aberration, 330.
Chromogens in urine, 162.
Chromoproteids, 10.
Chronograms, 252.
Chronograph, 252, 262.
Ciliary motion, 354.
Circulation, microscopic examination of, 444.
,, scheme of, 437.
Circumpolarisation, 29.
Clerk-Maxwell's experiment, 523.
Clonus, 292.
Coagulated proteids, 9.
Coagulation of blood, 39.
,, action of neutral salts, 41.
,, ,, of cold, 45.
,, experiments, 43.
,, oxalates, 42.
,, temperature, 41.
Coagulative enzymes, 122.
Cocaine, 581.
Coil, induction, 208.
Cold—Effects on blood, 45.
,, ,, heart, 362.
,, ,, muscle, 278.
,, ,, nerve, 348.
Cold spots, 570.
Collagen, 13.
Colloid, 26, 76.
Colorimetric method, 65.
Colostrum, 109.
Colour-blindness, 546.
Colour-sensations, 541.
Coloured fringes, 510.
,, shadows, 553.
Combined sulphuric acid, 133.
Commercial peptones, 8.
Commutator, 204.
Compensation method, 321.
Compensatory movements, 585.
,, pause, 383.
Complementary colours, 541.
Condenser stimulation, 233.
Conduction in nerve, 338, 344.
Congo red, 83.
Constant current, 197, 229.
,, ,, action of, 331.
,, ,, ,, on heart 383.
,, ,, intensity, 229.
Contact key, 202.
,, sense of, 573.
Contraction—isometric, 266.
,, isotonic, 249, 266.
,, maximal, 249.
,, minimal, 250.
,, paradoxical, 326.
,, secondary, 324.
,, without metals, 323.

Contrast, 546.
,, binocular, 554.
,, simultaneous, 546, 550.
Corresponding points, 524.
Crank-myograph, 254, 601.
Crescent stimulation, 391.
Cresol, 163.
Croaking experiment, 506.
Cryoscopy, 47, 486.
Crystallin, 6.
Curare, 237, 241.
Curdling of milk, 112.
Current, demarcation, 314, 316.
,, of heart, 317.
,, of injury, 314.
Cystin, 184.

Daniell's cell, 194.
Darby's fluid meat, 9.
D'Arsonval's N.P. electrodes, 318.
Decalcified plasma, 42.
Defibrinated blood, 38.
Deglutition apnœa, 468.
Demarcation currents, 320, 323.
Deposits in urine—collecting, 179.
,, organised, 182.
,, unorganised, 183.
Depressor nerve, 457.
Derived albumins, 6.
Desprez's signal, 252.
Detector, 197.
Deutero-albumose, 79, 87.
Dextrin, 27.
,, preparation of, 29.
,, varieties of, 26.
Dextrose, 18, 170.
,, reducing power of, 76.
,, rotatory power of, 30.
,, urine, 174.
Diabetes, 174.
Diabetic blood, 47.
Dialyser, 86.
Diazo-reaction of Ehrlich, 179.
Diffusion, 510.
Digestion—Biliary, 98.
,, gastric, 77.
,, pancreatic, 89.
,, salivary, 73.
Dilemma, 503.
Dimethylamidoazobenzol, 83.
Diplopia monophthalmica, 517.
Direct stimulation, 234.
,, vision, 522.
Direction, judgment of, 539.
Disaccharides, 22.
Distance, judgment of, 538.
Distilled water, action of, 226.
Donné's test, 183.
Doremus urea apparatus, 149.
Double conduction in nerve, 345.
Dresslar's card, 569.
Drums, 266.
Drying of nerve, 226.
Du Bois electrodes, 212.
,, induction coil, 208.
,, key, 199.
,, rheochord, 208.
Dudgeon's sphygmograph, 424.
Dupré's urea apparatus, 148.
Duration of impressions, 526.
Dynamographs, 305.
Dynamometers, 235.

Ear, 583.
Earthy phosphates, 134.
Egg-albumin, 2, 5.
Eggs, 113.
Elasticity of artery, 439.
,, of lungs, 468.
,, of muscle, 284.
Elastin, 15.
Electrical keys—Brodie's, 217.
,, contact, 102.
,, Du Bois, 199.
,, mercury, 201.
,, Morse, 202.
,, Ostwald's, 203.
,, plug, 203.
,, spring, 202.
,, trigger, 203.
Electrical constant current, 197.
,, repeated shocks, 228.
,, single shocks, 212, 226.
,, stimulation, 226.
,, stimuli, 224.
,, units, 197.
Electrodes, 198.
,, d'Arsonval's, 318.
,, brush, 317.
,, Du Bois, 212.
,, for nerve, 317.
,, non-polarisable, 313.
,, polarisation of, 203.
,, shielded, 211.
Electrolysis, 199.
Electrometer, capillary, 319.

Electromotive force, 321.
Electro-motivity, electro-tonic variation of, 339.
,, of heart, 317.
,, of muscle, 314.
Electrotonic variation of electro-motivity, 339.
Electrotonic variation of excitability, 335.
Electrotonus, 335.
Ellis' air-piston recorder, 429.
Emulsification, 93.
Emulsion, 33, 93.
Endocardial nutrition, 410.
,, pressure, 394.
Engelmann's experiment, 332.
Entoptical vision, 525.
Enzymes, 85, 121.
Erdmann's float, 138.
Ergograph, 300.
Erythro-dextrin, 26, 27, 75.
Esbach's albuminimeter, 168.
Ethereal sulphates, 133, 163.
Ewald's coil, 210.
Examination of a fluid for proteids and carbohydrates, 35, 191.
Examination of a solid, 193.
Excitability, muscular, 232.
,, *v.* conductivity, 344.
,, in dying nerve, 232.
,, of nerve, 232, 347.
Exhaustion in nerve and muscle, 298.
Exner's models, 583.
Experimentum mirabile, 504.
Expired air, 478.
Extensibility of muscle, 284.
Extensors, excitability of, 348.
Extra-current, 215.

Far point, 573.
Faradic electricity, 208.
Faradisation, 214.
Fatigue of muscle, 296, 302.
,, of nerve, 298.
Fats, neutral, 32.
Fechner's experiments, 585.
Fehling's solution, 20, 172, 596.
Fermentation test, 22, 176.
Ferments—Amylopsin, 90.
,, coagulative, 122.
,, fibrin, 40.
,, inversive, 95.
,, milk curdling, 83, 95.
Ferments, organised, 121.
,, pancreatic, 89.
,, pepsin, 78.
,, pialyn, 94.
,, ptyalin, 74.
,, rennet, 83.
,, steapsin, 94.
,, trypsin, 94.
,, unorganised, 121.
,, vegetable, 91.
,, in urine, 163.
Ferratin, 104.
Fibrin, 10, 38.
Fibrin-ferment, 40.
Fibrinogen, 6, 42.
Fick's kymograph, 463.
,, manometer, 463.
,, ophthalmotonometer, 563.
,, tension recorder, 305.
Field of vision, 530.
Flexors, excitability of, 348.
Flicker, 566.
Flour, 113.
Formaldehyde, 19.
Fovea centralis, 522.
,, shadows on, 524.
Fractional heat-coagulation, 41.
Frog pistol, 599.
Furfurol, 21, 100.

Gad's emulsion experiment, 33.
Gall stones, 101.
Galton's whistle, 589.
Galvanic electricity, 194.
Galvani's experiments, 322.
Galvanometer, 311.
Galvanoscope, 197.
Galvanotaxis, 357.
Garrod's test, 155.
Gases in blood, 474.
Gaskell's clamp, 374.
,, heart-lever, 369.
Gas-pumps, 474.
Gas-sphygmoscope, 429.
Gastric action on milk, 82.
,, antiseptic, 82.
,, contents, examination of, 88.
,, digestion, 77, 79.
,, drugs on, 89.
,, juice, 77.
,, mechanical restraint, 88.
,, Mett's method, 88.
,, peptones, 79.

Gastric products of, 80, 85.
,, time of, 88.
Gelatin, 14.
Gentian violet, 13.
Geometrical illusions, 534.
Gerrard's urea apparatus, 150.
Girgensohn's method, 13.
Globin, 6.
Globulins, 5, 40.
Globulinuria, 167.
Glucoproteids, 10, 15.
Glucose, 18, 116.
,, in blood, 46.
,, in urine, 170.
,, rotatory power of, 30.
,, tests for, 19, 20.
,, to prepare, 26.
Glucoses, 18.
Gluten, 10, 113.
Glycerin, 29.
,, extracts, 78, 80.
Glyco-cholic acid, 99, 104.
Glycocol, 125.
Glycogen, 27, 105.
,, hydrolysis of, 108.
,, preparation of, 105.
,, tests for, 28, 107.
Glycosamin, 124.
Glycosuria, 170.
,, alimentary, 173.
Glycuronic acid, 176.
Gmelin's test, 100, 169.
Goltz's tapping experiment, 506.
Gotch on excised heart, 367.
Gotch's localised cold nerve, 348.
Gower's hæmocytometer, 60.
,, hæmoglobinometer, 64.
Gracilis, experiment on, 345.
Grape-sugar, 18.
,, in blood, 46.
,, in urine, 170.
Graphic method, 245.
Gravity on circulation, 449.
Grenet cell, 196.
Grove's cell, 196.
Grünhagen's experiment, 344.
Guaiacum test, 38, 123, 169.
Guanin, 125.
Gymnema sylvestris, 581.

Hæmatin, 58.
,, preparation of, 58.
Hæmatinometer, 57, 71.
Hæmatocrit, 47, 180.
Hæmatoporphyrin, 56, 161.
Hæmatoscope, 57.
Hæmaturia, 168.
Hæmin, 57.
Hæmochromogen, 56, 58.
Hæmocyanin, 10.
Hæmocytometer, 60.
,, Haldane's modification, 65.
Hæmodynamometers, 430.
Hæmoglobin, 38, 48, 50.
,, ash of, 47.
,, carbonic oxide, 52.
,, crystals of, 48.
,, estimation of, 59.
,, ferricyanide on, 52.
,, nitrites on, 54.
,, non-diffusibility, 38.
,, oxy-, 50.
,, ozone test, 48.
,, preparation of, 57.
,, reduced, 52, 57, 71.
,, scale, 69.
,, spectrum of, 50, 71.
,, sulph., 59.
Hæmoglobinometer, Gower's, 64.
,, Oliver's, 66.
Hæmoglobinuria, 169.
Hæmometer of Fleischl, 69.
Hamburger's method, 45.
Hand electrodes, 198.
Haploscope, 559.
Häser-Trapp's coefficient, 127.
Haycraft on S.G. of blood, 38.
,, uric acid, 156.
Hayem's solution, 64.
Hay's sulphur test, 100, 170.
Hearing, 584.
Heart—Acids on, 408.
,, action of drugs on, 401.
,, apex, 363, 396.
,, atropin on, 403.
,, augmentor fibres, 385.
,, caffein, 406.
,, casts of, 410.
,, clamp, 374.
,, cocain, 406.
,, cold on, 362, 382.
,, constant current on, 383.
,, curare on, 406.
,, currents, 326, 364.
,, digitalin on, 406.

Heart—distilled water, 401.
,, drugs on, 401.
,, effect of heat on, 382.
,, electro-motivity of, 317.
,, endocardial nutrition, 410.
,, ,, pressure, 394.
,, excised, 362, 366.
,, extract of meat on, 408.
,, frog's, 360.
,, ganglia of, 376.
,, graphic record of, 365.
,, heat on, 362, 382.
,, impulse, 414.
,, inhibition of, 386, 417.
,, innervation of, 385.
,, inorganic salts on, 402.
,, intracardiac ganglia, 386.
,, latent period, 380.
,, lever, 369.
,, mammal's, 408.
,, manometer, 394.
,, monopolar method, 334.
,, motor centres, 393.
,, movements of, 364.
,, muscarin, 403.
,, nervous system on, 385.
,, nicotin on, 404.
,, ox, 421.
,, perfusion, 396.
,, pilocarpin on, 406.
,, reflex inhibition, 271, 287.
,, refractory period, 383.
,, saline on, 402.
,, salts on, 402.
,, section of 364.
,, snip, 373.
,, sounds of, 414.
,, staircase of, 379.
,, Stannius' experiment, 377.
,, supra-renal extract on, 407.
,, suspension methods, 369.
,, swallowing on, 416.
,, sympathetic on, 392.
,, tonometer, 397.
,, tortoise's, 367.
,, vagus on, 388.
,, valves of, 408.
Heat on cilia, 355.
,, on heart, 362.
,, on muscle, 225.
,, on nerve, 347.
Heat-rigor of muscle, 270.
Heller's blood test, 169.
Heller's test, 166.
Helmholtz's modification, 215, 228.
Hemialbumose, 85.
Hemipeptone, 85.
Hempel's method, 478.
Hering's apparatus for contrast, 545, 551.
,, experiments on nerve, 327.
,, myograph, 330.
Hermann's scheme, 340.
Hetero-albumose, 79, 87.
Hippuric acid, 158.
Histidin, 11.
Histohæmatin, 10.
Holmgren's apparatus, 444.
,, worsteds, 546.
Hopkin's uric acid method, 155.
Hot spots, 571.
Hüfner's urea apparatus, 147, 151.
Huppert's test, 170.
Hydrocele fluid, 43.
Hydrochloric acid, dilute, 78.
Hydrogen peroxide, 38.
Hydrostatic test, 468.
Hyoglossus, 221, 259, 293.
Hypnosis, 505.
Hypobromite method, 147.
,, of sodium, 147.

Illuminated ox heart, 421.
Illusions connected with skin, 568, 572.
Image, formation of, in eye, 509.
Impressions, duration of, 526.
Indefatigability of nerve, 298.
Independent muscular excitability, 236.
Indican, 162, 163.
Indifferent fluids, 221.
Indigo-carmine test, 20, 173.
Indigo-forming substance, 162.
Indigogens, 162.
Indirect stimulation, 234.
,, vision, 339.
Indol, 93, 96, 162.
Induced electricity, 208.
Induction coil, 208.
,, Bowditch's form, 210.
,, Ewald's form, 210.
,, graduated form of, 210.
,, in nerves, 218.
,, new form of, 211.
,, shocks, effects of, 213.

Inflammation, 445.
Inhibition of rabbit's heart, 417.
Inhibitory heart arrest, 380.
Inhibitory nerve, 385.
Interference phenomenon, 311.
Interrupted current, 214, 228.
Intra-ocular pressure, 563.
Intra-pleural pressure, 470.
Intra-thoracic pressure, 470.
Invertase, 95.
Inverted image, 509.
Invertin, 95.
Invert-sugar, 29.
Iodine solution, 597.
,, in thyroid, 123.
Iodoform test, 178.
Iris movements, 518.
Iron in blood, 47.
,, inorganic, 104.
,, organic, 104.
,, test for, 104.
Irradiation, 494, 533.
Isometric contraction, 267.
Isotonic ,, 249.
,, solutions, 45, 69, 222, 486.

Jaffé's test, 160, 162.
Joints, sensations from, 579.
Judgment of direction, 539.
,, of distance, 538.
,, of size, 539.

Kathode, 194.
Kathodic stimulus, 339.
Keratin, 15.
Key—Brodie's, 217.
,, Du Bois-Reymond's, 199.
,, mercury, 201.
,, Morse, 202.
,, Ostwald's, 203.
,, plug, 203.
,, spring or contact, 202.
,, trigger, 203.
Kjeldahl's method, 143, 145.
Knee-jerk, 496.
Kœnig's flames, 483.
Kreatin, 118.
Kreatinin, 119, 160.
Kronecker's frog manometer, 394.
,, perfusion cannula, 396.
Kühne's artificial eye, 562.
,, curare experiment, 242.
Kühne's double conduction experiment, 345.
,, gastric juice, 78.
,, gracilis experiment, 345.
,, muscle-press, 327.
,, nerve current experiment, 327.
,, pancreas powder, 90.
,, sartorius experiment, 239, 345.
Külz's method for glycogen, 107.
Kymograph, 449, 601.
,, spring, 463.

Lact-albumin, 5, 110.
Lactic acid, 112.
Lactoscope, 115.
Lactose, 24, 28, 116.
Lambert's method, 542.
Lardacein, 13.
Laryngoscope, 481.
Latent period of heart, 380.
,, ,, muscle, 258.
,, ,, reflex, 493.
,, ,, vagus, 391.
Laurent's polarimeter, 30.
Lecithin, 124.
Leclanché cell, 195.
Legal's test, 178.
Leucin, 93, 96.
Leucocytes, counting, 62.
Levulose, 95.
Lieberkühn's jelly, 7.
Liebermann's reaction, 11, 102.
Liebig's extract of meat, 118.
,, process for urea, 150.
Lieben's test, 178.
Ligature, effect of, 410.
Liquor pancreaticus, 90.
,, pepticus, 78.
Listing's reduced eye, 519.
Lithates, 157.
Liver, extracts of, 93.
,, glycogen in, 105.
,, iron in, 104.
Load on muscle, 270.
,, ventricle, 379.
Localisation, 566, 568.
Locke's fluid, 396, 411.
Ludwig's kymograph, 449.
,, sphygmograph, 425.
Lungs, elasticity of, 468.
,, permeability of, 472.

Lustre, 558.
Lymph-hearts, 445.
,, sac, 446.
Lysatinin, 96.
Lysin, 11, 92, 96.

Magnesia mixture, 135.
Magnetic tuning-fork, 295.
Make shocks, 213.
Malt extract, 76.
Maltose, 23, 28, 75.
,, estimation of, 24.
,, reducing power, 24, 76.
,, salivary digestion, 75.
Manometer, frog, 394.
,, membrane, 464.
,, mercury, 449.
Manometric flames, 483.
Marey's cardiograph, 415.
,, myograph, 276.
,, sphygmograph, 423.
,, tambour, 264.
Maréchal's test, 169.
Mariotte's experiment, 520.
Maximal contraction, 227.
Maxwell's experiment, 523.
Measures of capacity, 606.
,, length, 606.
Meat, extract of, 118.
Mechanical stimulation, 224.
Meiocardia, 420.
Mercury key, 201.
Methæmoglobin, 53.
,, action of drugs, 58.
,, alkaline, 85.
,, crystals, 58.
,, neutral, 53.
Methylene blue, 47.
Methyl violet, 13, 83.
Metronome, 295.
Mett's method, 88.
Meyer on contrast, 549.
Microchemical tests, glycogen, 109.
,, ,, iron, 104.
,, ,, phosphorus, 104.
Micrococcus ureæ, 129.
Miles' experiment, 514.
Milk, 109.
,, casein, 115.
,, caseinogen, 110, 114.
,, coagulation of, 112.
,, curdling of, 112.
,, fats of, 111, 115.
Milk, gastric juice on, 82.
,, lactalbumin of, 110.
,, opacity of, 115.
,, pancreatic juice on, 95.
,, to peptonise, 82, 95.
,, rennet on, 83.
,, salts of, 111.
,, souring of, 112.
,, sugar of, 110.
Milk-curdling ferment, 94.
Milk-sugar, 24, 28, 110.
Millon's reagent, 2.
Mineral *v.* organic acids, 83.
Minimal contraction, 227.
,, stimulus, 379.
Mohr's test, 84.
Moist chambers, 247, 604.
Molisch's test, 20.
Monobutyrin, 94.
Monochord, 205.
Monopolar method, 334.
Monosaccharides, 18.
Moore's test, 22, 171.
Morse key, 202.
Mosso's ergograph, 300.
,, plethysmograph, 448.
,, sphygmomanometer, 434
Mucin, 10, 14.
Mucinoid, 105.
Mucoids, 10.
Mucus in urine, 163.
Mulberry calculus, 186.
Mulder's test, 20, 173.
Müller's experiment, 430.
,, valves, 473.
Münsterbeg phenomenon, 556.
Murexide test, 154.
Muscæ volitantes, 525.
Muscarin on heart, 403.
Muscle—Acidity of, 295.
,, action current of, 316.
,, ,, of heat, 278.
,, ,, of veratria, 282.
,, current of injury, 315.
,, curve of, 257.
,, demarcation current, 374.
,, direct excitability, 239.
,, ,, stimulation of, 234.
,, effect of load, 280.
,, ,, two shocks, 289.
,, elasticity, 284.
,, electrical stimulation, 226.
,, excitability, 236.

Muscle, extensibility of, 286.
,, extractives of, 118.
,, extracts of, 117.
,, fatigue, 296.
,, independent excitability of, 236.
,, indirect stimulation of, 234.
,, lever, 246.
,, load, 270, 280.
,, negative variation, 316.
,, on mercury, 230.
,, pigments of, 120.
,, plasma of, 119.
,, press, 327.
,, proteids, 119.
,, reaction of, 116.
,, recovery of, 296.
,, relative excitability, 232, 240.
,, rupturing strain, 235.
,, serum, 119.
,, simple contraction, 227, 257.
,, smooth, 351.
,, sound, 235.
,, specific gravity, 256.
,, stimulation of, 224.
,, successive shocks on, 289.
,, telegraph, 600.
,, temperature on, 278.
,, tension of, 267.
,, tetanus, 291.
,, thickening of, 306.
,, twitch, 227.
,, veratrin on, 282.
,, wave, 308.
,, work of, 259, 269.
Muscular contraction, 249.
,, load on, 280.
,, sense, 578
,, temperature on, 278.
,, veratrin on, 282.
Myelin-forms, 124.
Myographic experiments on man, 304.
Myographs, 246.
,, Blix's, 286.
,, crank, 254, 601.
,, Fredericq's, 277.
,, Marey's, 276.
,, pendulum, 271.
,, railway, 604.
,, spring, 274.
,, transmission, 276.
Myosin, 10, 119.
Myosinogen, 120.

Nail pulse, 428.
Native albumins, 4.
Neef's hammer, 209.
Negative after-images, 529.
,, variation, 316.
Nerve—Action current, 317.
,, cold on, 348.
,, demarcation current, 316.
,, double conduction, 345.
,, dying, 232.
,, ether, 350.
,, excitability of, 344.
,, fatigue of, 298.
,, indefatigability, 298.
,, localised cold on, 348.
,, relative excitability, 232, 240.
,, salt on, 225, 336, 347.
,, section of, 348.
,, unequal excitability, 346.
,, velocity of impulse, 341.
Nerve-muscle preparation, 219, 222.
Nervous tissue, chem. of, 123.
Neuramœbimeter, 503.
Neuro-keratin, 15.
New's apparatus, 333.
Nicotin on heart, 404.
Nitrites, 541.
,, in saliva, 74.
,, on muscle, 120.
Nitrogen, estimation of, 143, 146.
,, in albumin, 11.
Nitropropiol, 20.
Non-polarisable electrodes, 204.
Normal saline, 45, 221.
,, soda solution, 131.
,, urobilin, 161.
Nuclein, 124.
,, bases, 158.
Nucleo-albumin, 10, 592.
Nucleo-histon, 10.
Nucleo-proteids, 124.

Ohm, 198.
Oils, chemistry of, 32.
,, neutral, 94.
Olein, 32.
Olfactometer, 583.
Olfactory sensations, 582.
Oliver's hæmocytometer, 63.

Ophthalmometer, 515.
Ophthalmoscope, 560.
Ophthalmotonometer, 563.
Oppel's lines, 535.
Orcein, 21.
Organic acids as tests, 83.
Organised deposits in urine, 182.
Osazones, 21.
Osmotic pressure, 485.
Ossein, 34.
Ovomucoid, 113.
Ox-heart, 421.
Oxalate of lime, 139.
,, plasma, 42.
Oxidase, 122, 593.
Oxy-hæmoglobin, 50.
,, crystals of, 48.
,, reduction of, 52.
,, spectrum of, 50, 70.
Ozone test for Hb, 48.

Pain spots, 576.
Palmitin, 32.
Pancreatic action on fats, 93.
,, ,, on proteids, 91.
,, ,, on starch, 90.
,, digestion, 89.
,, extracts, 89.
,, juice, 89.
,, milk, 95.
,, peptones, 91.
,, powder, dry, 90.
Paradoxical contraction, 326.
Paraglobulin, 40.
Paramæcium, 356.
Pause, compensatory, 383.
Pavy's fluid, 175, 597.
Pea-meal, 114.
Pembrey, action of drugs, 408.
Pendulum myograph, 271, 343.
Pepsin, 77, 78, 163.
,, comparison of, 88.
Pepsinogen, 123.
Peptones, 8, 79.
,, commercial, 8.
,, diffusibility of, 9.
,, tests for, 9, 81.
,, Witte's, 8, 489.
Peptonised milk, 95.
Peptonuria, 167.
Perfusion, 395, 412.
Perimetry, 530.
Peripheral projection, 576.
Perrin's eye, 561.
Pettenkofer's test, 99, 170.
Pflüger's law of contraction, 328.
Phakoscope, 515.
Phenol in urine, 163.
,, tests for, 179.
Phenolphthalein, 73.
Phenyl-glucosazon, 21.
,, ,, tests for, 172.
,, hydrazin test, 21, 172.
,, maltosazon, 24.
Phillips, action of drugs, 408.
Phloro-glucin, 21.
,, vanillin, 76, 84.
Phonendoscope, 422.
Phosphates in urine, 133, 158.
,, mixed, 186.
Phosphenes, 523.
Phosphomolybdic acid, 3.
Phosphoric acid, 137.
,, volumetric process for, 137.
Phosphorus, test for, 104.
Phosphotungstic acid, 3.
Physiological anode, 333.
,, apparatus, list of makers of, 607.
,, rheoscope, 324.
Pialyn, 94.
Picric acid, 171.
Picro-carmine, spectrum of, 56.
Picro-saccharimeter, 175.
Pigments of bile, 100.
,, urine, 161.
Pilocarpin on heart, 406.
Pince myographique, 307.
Piotrowski's reaction, 2.
Piston-recorder, 429.
Pithing, 219.
Plasma decalcified, 42.
,, of blood, 41.
,, of muscle, 119.
,, oxalated, 42.
,, proteids of, 41.
,, salted, 41.
Plasmine, 42.
Plattner's bile, 99.
Plethysmograph, 446.
Plug key, 203.
Poggendorff's illusion, 537.
Pohl's commutator, 204.
Polarimeters, 29.

Polarisation of electrodes, 203, 335.
Polar stimulation, 331, 334, 353.
Polygraph, 419, 467.
,, clinical, 435.
Polysaccharides, 25.
Positive after-images, 528.
Potassio-mercuric iodide, 597.
Potassium bromide, 495.
,, chloride, 495.
,, sulphocyanate, 74.
Pratt, nutrition of heart, 410.
Preformed sulphuric acid, 132.
Pressure sense, 573, 575.
Protamines, 10.
Proteids, 1.
,, as emulsifiers, 94.
,, classification of, 4.
,, coagulated, 9.
,, coagulation by heat, 4.
,, combined, 10.
,, crystallisation of, 12.
,, effect of acids, &c., 3.
,, general reactions, 2.
,, halogen derivatives, 13.
,, non-diffusibility, 11.
,, precipitation by salts, 3.
,, removal of, 13.
,, rotatory power, 31.
,, smooth muscle, 354.
Protein-chromogen, 93.
Proteoses, 8, 81, 87.
,, primary, 19.
,, secondary, 79.
Proto-albumose, 78, 87.
Pseudoscope, 559.
Ptyalin, 73.
Pulse, 422.
,, delay, 439.
,, volume, 449.
,, wave, 440.
Pupil, 518.
,, albino, 518.
,, light on, 518.
,, reflex, 518.
Purkinje's figures, 525.
,, phenomenon, 544.
Purkinje-Sanson's images, 514.
Pus in urine, 183.
Putrefactive products of pancreatic digestion, 92.
Pyrocatechin, 179.
Pyuria, 183.

Quadriurates, 158.
Quantitative estimation of acidity, 130.
,, ,, of chlorides, 132.
,, ,, of phosphates, 137.
,, ,, of sugar, 174.
,, ,, of urea, 117.
,, ,, of uric acid, 155.

Rabbit-holder, 453.
Radial movement, 539.
Ragona Scina, 550.
Ranvier's emulsion experiment, 33.
Reaction, biuret, 9.
,, of Adamkiewicz, 11.
,, of Liebermann, 11, 102.
,, of Piotrowski, 2.
,, of Uffelmann, 84.
,, xanthoproteic, 2.
Reaction-time, 500.
Recording apparatus, 244, 454.
Reduced alkali-hæmatin, 56.
,, hæmoglobin, 52.
,, ,, spectrum of, 71.
Reflex action, 492.
,, tone, 506.
Rennet, 83.
,, vegetable, 94.
Repeated shocks, 228.
Resistance, paradoxes, 580.
,, sense of, 579.
Resorcin, 21.
Respiration, artificial, 472.
,, cannula, 472.
,, on pulse, 295.
,, voluntary, 468.
Respiratory movements, 466.
,, ,, of frog, 470.
Retinal rivalry, 557.
,, shadows, 524.
Reverser, 204.
Rheochords, 206, 208, 337.
Rheometer, 441.
Rheonome, 233.
Rheoscopic limb, 324.
Riegler's test, 22.
Rigor caloris, 117.
Ringer's fluid, 402.
Ritter's tetanus, 339.
Rollett on flexors, 348.

Rosenbach's test, 169.
Rosenthal's modification, 241.
Roy's tonometer, 397.

Saccharimeter, 174.
Saccharoses, 18.
Salicylates, 179.
Salicyl-sulphonic acid, 167.
Saliva, 73.
,, digestive action of, 74.
Salivary digestion, 74.
,, ,, drugs on, 77.
,, ,, effects on, 75.
,, extract, 73.
,, secretion, 487.
Salted meat, 120.
Salt tetanus, 225.
Saponification, 32.
Sartorius of frog, 224, 230, 345.
Schäfer's piston recorder, 397.
Scheiner's experiment, 512.
Schiff's test, 155.
Schweitzer's reagent, 29.
Sciatic plexus, 348.
Secondary contraction, 324, 325.
,, ,, Biedermann's modification, 327.
,, ,, Hering's, 328.
,, tetanus, 324.
Semi-circular canals, 585.
Semi-membranosus and gracilis, 221.
Serpent's urine, 158.
Serum of blood, 39.
,, proteids of, 40.
,, salts of, 44.
,, sugar of, 44.
,, to obtain, 39.
Serum-albumin, 5, 39, 40.
,, globulin, 6, 39, 40, 45.
,, proteids, 39, 41.
,, ,, coagulation of, 40.
,, ,, precipitation of, 41.
Shadows, coloured, 553.
,, on retina, 524.
Shielded electrodes, 211.
Shunt, 314.
Silhouette curve, 298.
Simple muscle-curve, 257.
Simultaneous contrast, 546, 551, 552.
Single contraction, 229, 248.
,, induction shocks, 212.
Size, 539.
Skatol, 96.
Skin, sensations from, 565.
Smell, 582.
Smooth muscle, 120.
Soap, 32.
Solubilities, table of, 606.
Soluble albumin, 5.
,, starch, 26, 75.
Spatial perception, 534.
Specific rotation, 29.
Spectroscope, 48.
Spherical aberration, 510.
Sphygmochronograph, 435.
Sphygmographs, 422.
Sphygmomanometer, 434.
Sphygmoscope, 429.
Spinal nerve, overlap, 499.
,, ,, roots, 498.
Spirometer, 473.
Spring key, 202.
,, kymograph, 463.
,, myograph, 274.
Staircase of heart, 297, 379, 384.
Stannius' experiment, 377.
Starch, 25.
,, action of malt, 76.
,, animal, 27, 105.
,, colloid, 28, 76.
,, conversion to sugar, 26.
,, potato-, 25.
,, soluble, 26.
,, stages to glucose, 26.
,, ,, maltose, 75.
,, under miscroscope, 25.
,, ,, polariscope, 25.
Steapsin, 94.
Stearin, 32.
Steell's apparatus, for urea, 149.
Stellar phosphate, 135.
Stenson's experiment, 497.
Stereognostic sense, 586.
Stereoscope, 557.
Stethographs, 466.
Stethometer, 468.
Stethoscope, 414.
Stimuli, 224.
Stokes' fluid, 52.
Strasburger's test, 170.

Strobic discs, 539.
Struggle of fields of vision, 557.
Strychnin, 494.
Submaxillary gland, 487.
Successive light induction, 552.
Successive shocks, 289.
Succus entericus, 95.
Sugar in urine, 170.
,, estimation of, 174.
,, fermentation method, 176.
,, tests for, 171.
Sulphates, 132.
,, ethereal, 132, 163.
Sulph-hæmoglobin, 59.
Sulphocyanides, 74.
Sulphur in albumin, 11.
,, test for bile, 100, 170.
Summation, 290.
,, of stimuli, 232.
Suprarenal extract, 408, 491.
Suspension method, 369, 371.
Swallowing on heart, 416.
Switch, 203.
Sympathetic of frog, 392.
,, nerve of neck, 484.
,, of rabbit, 455.
Syntonin, 7.

Tactile discrimination, 566.
Talbot's law, 526.
Tambour, 264.
Tapping experiment, 506.
Taste, 580.
Taurin, 99.
Taurocholic acid, 99.
Telephone experiment, 235.
Temperature, on muscle, 278.
,, sense of, 569.
,, spots, 569.
Tendon, to rupture, 235.
Tension of muscle, 267.
,, recorder, 305.
Test meal, 89.
,, types, 517.
Tetanomotor, 225.
Tetanus, 291, 294.
,, Ritter's, 339.
,, secondary, 324.
Thermal stimulation, 224.
Thermometric scales, 262.
Thermotaxis, 359.
Thoma-Zeiss hæmocytometer, 61.
Threshold for pain, 577.
,, for touch, 566.
Thrombase, 122.
Thymol, 91.
Thymus gland, 124.
Thyroid gland, 123.
Time-markers, 607.
Tissue-fibrinogen, 16.
Tœpfer's test, 83.
Toluylenediamin, 104.
Tonograms, 267.
Tonometer, 397.
Tonus, 499.
,, of auricles, 380.
Total N, estimation of, 143.
Touch, 566.
Trichloracetic acid, 107, 167.
Trigger key, 203.
Triple phosphate, 136.
Trommer's test, 19, 171.
Tropæolin, 83.
Tropostereoscope, 559.
Trypsin, 90, 93.
Tryptophan, 83.
Tryptic digestion, 90.
Tubes, rigid and elastic, 441.
Türck's method, 493.
Twitch, 229, 248, 255.
Tyrosin, 93, 96.

Uffelmann's reaction. 84.
Umikoff's reaction, 112.
Unipolar stimulation, 231, 348.
Unorganised deposits in urine, 183.
Urates, 157.
Urea, 140.
,, Kjeldahl's estimation of, 143.
,, nitrate, 141.
,, of serum, 44.
,, oxalate, 141.
,, preparation, 140.
,, quantity, 143.
,, reactions of, 142.
,, synthesis of, 152.
,, volumetric analysis, 147.
Ureameter, 148.
,, of Doremus, 149.
Urease, 122.
Uric acid, 153.
,, calculi, 185.

Uric acid, estimation of, 155.
,, Hopkins' method for, 155.
,, preparation, 153.
,, quantity, 154.
,, reactions, 154.
,, salts of, 157.
,, tests, 154.
Urinary calculi, 185.
,, deposits, 179, 182.
Urine, 125.
,, abnormal constituents, 164.
,, acidity, 128, 130.
,, albumin in, 164.
,, alkalinity, 128.
,, bile in, 169.
,, blood in, 168.
,, chlorides, 132.
,, colour, 125.
,, colouring-matters, 161.
,, diabetic, 171.
,, deposits in, 179, 182.
,, fermentation method, 176.
,, fermentations of, 129.
,, ferments in, 163.
,, general examination of, 188.
,, inorganic bodies, 131.
,, mucus in, 163.
,, odour, 127.
,, organic bodies, 140.
,, phenol in, 163, 179.
,, phosphates in, 133.
,, pigments of, 161.
,, pus in, 183.
,, quantity, 125.
,, reaction, 127.
,, reaction to reagents, 164.
,, solids in, 127.
,, specific gravity, 126.
,, sugar in, 170.
,, sulphates in, 132.
,, transparency, 129.
,, urates in, 156.
,, urea in, 140.
,, uric acid in, 153.
Urinometer, 126.
Urobilin, 161.
,, febrile, 162.
Urochrome, 161.
Uroerythrin, 161.

Vago-sympathetic, 385.
Vagus of frog, 390.
Vagus of frog, latent period of, 391.
,, of rabbit, 455.
,, on heart, 456, 462.
,, stimulation, 393.
Valsalva's experiment, 430.
Valves of heart, 408.
Vanillin, 84.
Varnish, 248.
Vascular tonus, 484.
Vaso-motor nerves, 484.
Vegetable albumin, 113.
,, enzymes, 91.
Veratrin, 282.
Vibrating reed, 275.
Vision, physiology of, 509.
Visual axes, 539.
,, judgments, 534.
Vital capacity, 473.
Vitellin, 10, 113.
Vogel's lactoscope, 115.
Volkmann's experiment, 521.
Volt, 198.
Volumetric process, 136.
,, for phosphoric acid, 137.
,, for sugar, 174.
,, for urea, 147.
Vomit, examination of, 88.
Vowel-sounds, 484.

Waller, knee jerk, 497.
Warm spots, 571.
Wave-lengths, 69.
Wave of muscle, 308.
Weber's circles, 574.
,, law, 532, 572.
,, test, 162.
Weights, 575, 579.
Wenz's method, 13.
Weyl's test, 160.
Wheaten bread, 114.
,, flour, 113.
Wheatstone's fluttering hearts, 511.
,, stereoscope, 558.
Wheel movements, 532.
Whistle, Galton's, 584.
White of egg, 1, 2.
Wild's apparatus, 310.
Wilke's reagent paper, 199.
Williams' heart apparatus, 400.

Witte's peptones, 8, 86.
,, action of, 490.
Wittich's method, 78.
Work done by muscle, 259, 269.
Writing point of Bayliss, 376.

Xanthin, 118, 158, 185.
,, bodies, 381.
Xanthoproteic reaction, 2.

Yellow spot, 523.
Yolk spheres, 113.

Zöllner's lines, 536.
Zymase, 121.
Zymogens, 89, 123.

THE END.

WELLCOME LIBRARY INSTITUTE

BELL AND BAIN, LIMITED, PRINTERS, GLASGOW.

A SMALLER CATALOGUE OF

MEDICAL WORKS

PUBLISHED BY

CHARLES GRIFFIN & COMPANY, LIMITED.

Being Sections 11 to 14 of Griffin's General Catalogue.

SECTIONS.

	PAGE
§ 11. Griffin's Standard Medical Series, . .	87
§ 12. Griffin's Reference Pocket-Books, . .	97
§ 13. Griffin's Medical Students' Text-Books, .	101
§ 14. Griffin's Practical Medical Handbooks:— Nursing, Ambulance, Sanitation, &c., .	107

MESSRS. CHARLES GRIFFIN & COMPANY'S PUBLICATIONS may be obtained through any Bookseller in the United Kingdom, or will be sent Post-free on receipt of a remittance to cover published price. To prevent delay, Orders should be accompanied by cheque or Postal Order crossed "SMITH, PAYNE & SMITHS."

***** General, Technical, and Full Illustrated Medical Catalogues Post-free on Application.**

LONDON:

EXETER STREET, STRAND.

1/1/02.

Eighteenth Annual Issue. Handsome cloth, 7s. 6d.

(To Subscribers, 6s.).

THE OFFICIAL YEAR-BOOK

OF THE

SCIENTIFIC AND LEARNED SOCIETIES OF GREAT BRITAIN AND IRELAND.

COMPILED FROM OFFICIAL SOURCES.

***Comprising** (together with other Official Information) **LISTS of the PAPERS** read during the Session 1900-1901 before all the **LEADING SOCIETIES** throughout the Kingdom engaged in the following Departments of Research:—*

§ 1. Science Generally: *i.e.*, Societies occupying themselves with several Branches of Science, or with Science and Literature jointly.
§ 2. Mathematics and Physics.
§ 3. Chemistry and Photography.
§ 4. Geology, Geography, and Mineralogy.
§ 5. Biology, including Microscopy and Anthropology.
§ 6. Economic Science and Statistics.
§ 7. Mechanical Science, Engineering, and Architecture.
§ 8. Naval and Military Science.
§ 9. Agriculture and Horticulture.
§ 10. Law.
§ 11. Literature.
§ 12. Psychology.
§ 13. Archæology.

§ 14. MEDICINE.

"FILLS A VERY REAL WANT."—*Engineering.*

"INDISPENSABLE to any one who may wish to keep himself abreast of the scientific work of the day."—*Edinburgh Medical Journal.*

"The YEAR-BOOK OF SOCIETIES is a Record which ought to be of the greatest use for the progress of Science."—*Lord Playfair, F.R.S., K.C.B., M.P., Past-President of the British Association.*

"It goes almost without saying that a Handbook of this subject will be in time one of the most generally useful works for the library or the desk."—*The Times.*

"British Societies are now well represented in the 'Year-Book of the Scientific and Learned Societies of Great Britain and Ireland.'"—(Art. "Societies" in New Edition of "Encyclopædia Britannica," vol. xxii.)

Copies of the FIRST ISSUE, giving an Account of the History, Organization, and Conditions of Membership of the various Societies, and forming the groundwork of the Series, may still be had, price 7/6. *Also Copies of the Issues following.*

The YEAR-BOOK OF SOCIETIES forms a complete INDEX TO THE SCIENTIFIC WORK of the sessional year in the various Departments. It is used as a HANDBOOK in all our great SCIENTIFIC CENTRES, MUSEUMS, and LIBRARIES throughout the Kingdom, and has become an INDISPENSABLE BOOK OF REFERENCE to every one engaged in Scientific Work.

READY IN OCTOBER EACH YEAR.

*** Owing to the alteration of the date of publication of this work, so as to make its appearance coincident with the commencement of the Sessional year, the present volume contains (as far as possible) lists of all papers read between January 1, 1900, and June 30, 1901 (eighteen months).

Charles Griffin & Co.'s Medical Series.

Standard Works of Reference for Practitioners and Students.

Issued in LIBRARY STYLE, large 8vo, Handsome Cloth, very fully Illustrated.

Larger Catalogue, with Specimen Illustrations, sent Post-free on application.

ANATOMY AND PHYSIOLOGY.

		PAGE
Human Anatomy,	PROF MACALISTER, M.D.,	88
Human Physiology,	PROFS. LANDOIS AND STIRLING,	88
Embryology,	PROF. HADDON,	88

DIAGNOSIS AND TREATMENT OF DISEASE.

Clinical Diagnosis,	DRS. V. JAKSCH AND CAGNEY,	89
Clinical Medicine,	JUDSON BURY, M.D.,	89
Fibroid Phthisis,	SIR AND. CLARK, M.D.,	90
Gout,	SIR DYCE DUCKWORTH, M.D.,	91
Rheumatism,	ARCH. GARROD, M.D.,	88
Pernicious Anæmia,	WM. HUNTER, M.D.,	90
Diseases of the Organs of Respiration,	SAMUEL WEST, M.D.,	89
,, Childhood,	WM. HUNTER, M.D.,	91
,, the Eye,	DRS. MEYER AND FERGUS,	90
,, the Heart,	A. E. SANSOM, M.D.,	91
,, the Skin,	PROF. M'CALL ANDERSON,	91
Atlas of Urinary Sediments,	PROFS. RIEDER AND DELÉPINE,	96

THE BRAIN, NERVOUS SYSTEM, LEGAL MEDICINE, &c.

The Brain and Spinal Cord,	VICTOR HORSLEY, F.R.C.S.,	92
Central Nervous Organs,	DRS. OBERSTEINER AND HILL,	92
Peripheral Neuritis,	DRS. ROSS AND BURY,	92
Mental Diseases,	BEVAN LEWIS, M.R.C.S.,	93
Asylum Management,	CHAS. MERCIER, M.D.,	93
Forensic Medicine and Toxicology,	PROF. DIXON MANN,	93
Poisons: Effects and Detection,	A. WYNTER BLYTH,	112
The Digestive Glands,	PROF. PAWLOW,	95

SURGERY.

Ruptures,	J. F. C. MACREADY, F.R.C.S.,	95
Surgery of the Kidneys,	KNOWSLEY THORNTON, F.R.C.S.,	94
,, Spinal Cord,	WM. THORBURN, F.R.C.S.,	94
Railway Injuries,	H. W. PAGE, F.R.C.S.,	94

The Journal of Anatomy and Physiology, ... 96

*** Other Volumes in active Preparation.

CHARLES GRIFFIN & CO.'S MEDICAL SERIES.

Standard Works of Reference for Practitioners and Students.

Issued in LIBRARY STYLE, large 8vo, Handsome Cloth, fully Illustrated.

HUMAN ANATOMY.

BY ALEXANDER MACALISTER, M.A., M.D., LL.D., D.Sc., F.R.S.,

Professor of Anatomy in the University of Cambridge, and Fellow of St. John's College.

"BY FAR THE MOST IMPORTANT WORK on this subject which has appeared in recent years."—*The Lancet.*

"Destined to be a main factor in the advancement of Scientific Anatomy. . . . The fine collection of Illustrations must be mentioned."—*Dublin Medical Journal.*

"This SPLENDID WORK."—*Saturday Review.*

HUMAN PHYSIOLOGY.

BY PROFESSOR LANDOIS OF GREIFSWALD

AND

WM. STIRLING, M.D., Sc.D.,

Brackenbury Professor of Physiology in Owens College and Victoria University, Manchester; Examiner in the Universities of Oxford, Edinburgh, and London.

FIFTH EDITION in preparation. With some of the Illustrations in Colours.

"The BOOK is the MOST COMPLETE *résumé* of all the facts in Physiology in the language. Admirably adapted for the PRACTITIONER. . . . With this Text-book at command, NO STUDENT COULD FAIL IN HIS EXAMINATION."—*The Lancet.*

"One of the MOST PRACTICAL WORKS on Physiology ever written. EXCELLENTLY CLEAR, ATTRACTIVE, and SUCCINCT."—*British Medical Journal.*

An Introduction to EMBRYOLOGY.

BY ALFRED C. HADDON, M.A., M.R.I.A.,

Professor of Zoology in the Royal College of Science, Dublin. 18s.

"AN EXCELLENT RÉSUMÉ OF RECENT RESEARCH, well adapted for self-study."—*The Lancet.*

RHEUMATISM AND RHEUMATOID ARTHRITIS.

BY ARCH. E. GARROD, M.A., M.D.OXON., F.R.C.P.,

Assistant-Physician to the Hosp. for Sick Children, Gt. Ormond St. 21s.

"We gladly welcome this Treatise. . . . The amount of information collected and the manner in which the facts are marshalled are deserving of ALL PRAISE."—*Lancet.*

GRIFFIN'S MEDICAL SERIES—*Continued.*

CLINICAL DIAGNOSIS.

The Chemical, Microscopical, and Bacteriological Evidence of Disease.

BY PROF. VON JAKSCH, OF PRAGUE,

AND

JAS. CAGNEY, M.A., M.D.

FOURTH ENGLISH EDITION. With many new Illustrations in Colours, and additions prepared specially by PROF. VON JAKSCH, who has also revised the work and brought it quite up to date. 24s.

"Prof. v. Jaksch's 'Clinical Diagnosis' stands almost alone in the width of its range, the THOROUGHNESS OF ITS EXPOSITION, and the clearness of its style. . . . A STANDARD WORK, AS TRUSTWORTHY as it is scientific."—*Lancet.*

CLINICAL MEDICINE.

A PRACTICAL HANDBOOK FOR PRACTITIONERS & STUDENTS.

BY JUDSON BURY, M.D., F.R.C.P.,

Physician to the Manchester Royal Infirmary.

SECOND EDITION, REVISED AND ENLARGED.

With Numerous Illustrations, Several in Colours. 21s.

With Additional Chapters on SKIN DISEASES, LARYNGOSCOPIC EXAMINATIONS, and on RÖNTGEN RAYS IN SURGERY.

"An accurate and up-to-date representation of Clinical Medicine, which must prove of the utmost use, and ONE THAT IS NOT SURPASSED IN THE ENGLISH LANGUAGE."—*British Medical Journal.*

"WE CAN CORDIALLY RECOMMEND THIS VOLUME to our readers, and congratulate the author on the success of his labours."—*The Lancet.*

"The Book is one of the best of its kind. . . . We see EVERY SIGN OF VITALITY ABOUT IT."—*Edinburgh Medical Journal.*

Important New Work. In Two Volumes. At Press.

In Large 8vo. Handsome Cloth. With numerous Diagrams and Illustrations.

DISEASES OF THE ORGANS OF RESPIRATION.

An Epitome of the Etiology, Pathology, Diagnosis, and Treatment of Diseases of the Lungs and Air Passages.

BY SAMUEL WEST, M.D., F.R.C.P.,

Senior Physician to the Royal Free Hospital; Consulting Physician to the New Hospital for Women; Assistant Physician and Demonstrator of Practical Medicine, St. Bartholomew's Hospital; Member of the Board of Faculty of Medicine in the University of Oxford, &c., &c.

GRIFFIN'S MEDICAL SERIES—*Continued.*

PERNICIOUS ANÆMIA.

ITS PATHOLOGY, INFECTIVE NATURE, SYMPTOMS, DIAGNOSIS AND TREATMENT.

Including investigations on

THE PHYSIOLOGY OF HÆMOLYSIS.

BY WILLIAM HUNTER, M.D., F.R.C.P., F.R.S.E.,

Assist.-Phys. London Fever Hospital; Arris and Gale Lect. R.C.S. Eng., &c., &c.

With Plates (4 coloured), Illustrations, and 2 Folding Diagrams. 24s. net.

CONTENTS.—Part I. Historical.—Part II. Morbid Anatomy.—Part III. Experimental.—Part IV. The Infective Nature of Pernicious Anæmia.—Part V. Etiology.—Part VI. Symptoms.—Part VII. Treatment.—Part VIII. The Physiology of Blood Destruction.—Part IX. Hæmolysis and Jaundice.—INDEX.

"We can speak in the highest terms as to Dr. Hunter's investigations on Hæmolysis, which are some of the most ELABORATE and INSTRUCTIVE yet carried out. . . . He has added greatly to what was previously known as to the nature of the disease."—*The Lancet.*

With Tables, and Eight Plates in Colours. Price One Guinea, Net.

FIBROID DISEASES OF THE LUNG, including FIBROID PHTHISIS.

BY

SIR ANDREW CLARK, BART., M.D., LL.D., F.R.S.,

Late President to the Royal College of Physicians, London; Consulting Physician to the London Hospital, and to the City of London Hospital for Diseases of the Chest,

AND

W. J. HADLEY, M.D., AND ARNOLD CHAPLIN, M.D.,

Assistant Physicians to the City of London Hospital for Diseases of the Chest.

"It was due to Sir ANDREW CLARK that a PERMANENT RECORD of his MOST IMPORTANT piece of PATHOLOGICAL AND CLINICAL WORK should be published. . . . A volume which will be HIGHLY VALUED BY EVERY CLINICAL PHYSICIAN."—*British Medical Journal.*

DISEASES OF THE EYE.

BY DR. ED. MEYER, OF PARIS.

From the Third French Edition,

BY A. FREELAND FERGUS, M.D.,

Surg., Eye Infirmary, Glasgow; Ophthalmic Surgeon, Glasgow Royal Infirmary. 25s.

With Coloured Plates.

"An EXCELLENT TRANSLATION of a Standard French Text-Book. . . . Essentially a PRACTICAL WORK. The publishers have done their part in the tasteful and substantial manner characteristic of their medical publications."—*Ophthalmic Review.*

GRIFFIN'S MEDICAL SERIES—*Continued.*

A Treatise on GOUT.

BY SIR DYCE DUCKWORTH, M.D., LL.D., F.R.C.P.,

Physician to, and Lecturer on Clinical Medicine at, St. Bartholomew's Hospital. 25s.

With a Coloured Plate.

"At once thoroughly practical and highly philosophical. The practitioner will find in it an ENORMOUS AMOUNT OF INFORMATION."—*Practitioner.*

THE DIAGNOSIS OF

DISEASES OF THE HEART.

BY A. ERNEST SANSOM, M.D., F.R.C.P.,

Physician to, and Lecturer on Clinical Medicine at, the London Hospital; Consulting Physician, North-Eastern Hospital for Children; Examiner in Medicine, Royal College of Physicians, Royal College of Surgeons, &c., &c. 28s.

"Dr. Sansom has opened to us a TREASURE-HOUSE OF KNOWLEDGE. . . . The originality of the work is shown on every page, an originality so complete as to mark it out from every other on the subject with which we are acquainted."—*Practitioner.*

"A book which does credit to British Scientific Medicine. We warmly commend it to all engaged in clinical work."—*The Lancet.*

DISEASES OF THE SKIN.

BY T. M'CALL ANDERSON, M.D.,

Professor of Systematic Medicine in the University of Glasgow; Examiner in Medicine and Pathology, H.M. British and Indian Army Medical Service.

SECOND EDITION. Revised and Enlarged. With four Coloured Plates. 25s.

"Beyond doubt, the MOST IMPORTANT WORK on Skin Diseases that has appeared in England for many years."—*British Medical Journal.*

THE DISEASES OF CHILDHOOD

(MEDICAL).

BY H. BRYAN DONKIN, M.A., M.D.OXON., F.R.C.P.;

Physician to the Westminster Hospital and to the East London Hospital for Children at Shadwell; Lecturer on Medicine and Clinical Medicine at Westminster Hospital Medical School; Examiner in Medicine to the Royal College of Physicians. 16s.

"In every sense of the word a FRESH and ORIGINAL work, recording the results of the author's OWN LARGE EXPERIENCE."—*The Lancet.*

GRIFFIN'S MEDICAL SERIES—*Continued.*

THE STRUCTURE AND FUNCTIONS OF
THE BRAIN AND SPINAL CORD.

BY VICTOR HORSLEY, F.R.C.S., F.R.S.,

Surgeon to University College Hospital, and to the National Hospital for Paralysed and Epileptic, &c., &c. 10s. 6d.

"We HEARTILY COMMEND the book to all readers and to ALL CLASSES OF STUDENTS ALIKE, as being almost the only lucid account extant, embodying the LATEST RESEARCHES and their conclusions."—*British Medical Journal.*

THE ANATOMY OF
THE CENTRAL NERVOUS ORGANS
IN HEALTH AND DISEASE.

BY PROFESSOR OBERSTEINER, OF VIENNA.

TRANSLATED BY ALEX HILL, M.A., M.D.,
Master of Downing College, Cambridge.

SECOND ENGLISH EDITION, Revised, Enlarged, and almost entirely Re-written. With additional Illustrations. Price 30s.

"Dr. Hill has enriched the work with many Notes of his own. . . . Dr. Obersteiner's work is admirable. . . . INVALUABLE AS A TEXT-BOOK."—*British Medical Journal.*

ON PERIPHERAL NEURITIS.

By JAS. ROSS, M.D., LL.D.,

Late Physician to the Manchester Royal Infirmary, and Joint Professor of Medicine at the Owens College; and

JUDSON BURY, M.D., F.R.C.P.,

Senior Assistant Physician to the Manchester Royal Infirmary.

With Illustrations, Large 8vo, Handsome Cloth, 21s.

"It will for many years remain the AUTHORITATIVE TEXT-BOOK on peripheral neuritis."—*British Medical Journal.*

"A monument of industry."—*Edinburgh Medical Journal.*

GRIFFIN'S MEDICAL SERIES—*Continued.*

MENTAL DISEASES:

With Special Reference to the Pathological Aspects of Insanity.

BY W. BEVAN LEWIS, L.R.C.P. (LOND.), M.R.C.S. (ENG.),

Medical Superintendent and Director of the West Riding Asylum, Wakefield; Examiner in Mental Diseases, Victoria University.

SECOND EDITION, Revised and in part Rewritten. With Additional Illustrations. 30s.

"Without doubt the BEST WORK in English of its kind."—*Journal of Mental Science.*

"This ADMIRABLE TEXT-BOOK places the study of Mental Diseases on a SOLID BASIS. . . . The plates are numerous and admirable. To the student the work is INDISPENSABLE."—*Practitioner.*

LUNATIC ASYLUMS: Their Organisation and Management.

By CHARLES MERCIER, M.B., F.R.C.S.,

Lecture on Neurology and Insanity, Westminster Hospital Medical School, &c., &c. 16s.

"WELL WORTHY of thoughtful study. . . . Contains an immense amount of useful and interesting information."—*Medical Press.*

"Will give a NEEDED IMPETUS to the study of Asylum Patients."—*Glasgow Med. Journal.*

FORENSIC MEDICINE AND TOXICOLOGY.

By J. DIXON MANN, M.D., F.R.C.P.,

Professor of Medical Jurisprudence and Toxicology, Owens College, Manchester; Examiner in Forensic Medicine, London University, and Victoria University, Manchester.

THIRD EDITION, Thoroughly Revised and Enlarged. 21s.

"By far the MOST RELIABLE, MOST SCIENTIFIC, and MOST MODERN book on Medical Jurisprudence with which we are acquainted."—*Dublin Medical Journal.*

POISONS: THEIR EFFECTS AND DETECTION.

BY A. WYNTER BLYTH, M.R.C.S., F.C.S.,

Barrister-at-Law, Public Analyst for Devonshire, and Med. Officer of Health for St. Marylebone.

THIRD EDITION, Revised and Enlarged. 21s. (See p. 112.)

GRIFFIN'S MEDICAL SERIES—*Continued.*

The SURGERY of the SPINAL CORD.

BY WILLIAM THORBURN, B.S., B.Sc., M.D., F.R.C.S.,

Honorary Surgeon to the Manchester Royal Infirmary; Assistant-Lecturer on Surgery, Owens College, Manchester. 12s. 6d.

"Really the FULLEST RECORD we have of Spinal Surgery, and marks an important advance."
—*British Medical Journal.*

RAILWAY INJURIES:

With Special Reference to those of the Back and Nervous System, in their Medico-Legal and Clinical Aspects.

BY HERBERT W. PAGE, M.A., M.C. (CANTAB), F.R.C.S. (ENG.),

Surgeon to St. Mary's Hospital; Lecturer on Surgery, St. Mary's Hospital Medical School; Consulting Surgeon L. & N.-W. Rwy. Co., &c., &c. 6s.

"A work INVALUABLE to those who have many railway cases under their care pending litigation. . . . A book which every lawyer as well as doctor should have on his shelves."
—*British Medical Journal.*

THE SURGERY OF THE KIDNEYS.

Being the Harveian Lectures for 1889.

By J. KNOWSLEY THORNTON, M.B., C.M.,

Cons. Surgeon to the Samaritan Free Hospital; to the Grosvenor Hospital for Women; to the New Hospital for Women and Children, &c., &c.

In Demy 8vo, with Illustrations, Cloth, 5s.

"The name and experience of the Author confer on the Lectures the stamp of authority."
—*British Medical Journal.*

GRIFFIN'S MEDICAL SERIES—*Continued.*

READY IMMEDIATELY. The Nobel Prize Researches in Physiology, 1901.

THE WORK OF THE DIGESTIVE GLANDS.

By PROFESSOR PAWLOW, OF ST. PETERSBURG.

TRANSLATED INTO ENGLISH BY

W. H. THOMPSON, M.D., M.Ch., F.R.C.S.,

Dunville Professor of Physiology, Queen's College, Belfast; Examiner in Physiology, R.C.S. Eng. and Royal Univ., Ireland.

The Publishers (through the Translator) have arranged with the distinguished Author for the publication of the English Edition of these important Lectures, embodying the results of Prof. Pawlow's Researches, which were recently awarded the Nobel Prize of £11,000. This edition will include the later volume, entitled "The Experiments," &c., together with the notes of the most recent researches of Prof. Pawlow, and will constitute the sole authorised edition for England and America of this important Work.

In Large 8vo, Handsome Cloth, with Illustrations and 24 Plates. 25s.

A TREATISE ON RUPTURES.

By JONATHAN F. C. H. MACREADY, F.R.C.S.,

Surgeon to the Great Northern Central Hospital; to the City of London Hospital for Diseases of the Chest, Victoria Park; to the City of London Truss Society, &c., &c.

"Certainly by far the most COMPLETE and AUTHORITATIVE WORK on the subject."—*Edin. Med. Journal.*

"Much THE MOST IMPORTANT work on Hernia which has appeared of late years. The Plates are of an order of ARTISTIC MERIT and SCIENTIFIC ACCURACY not often met with."—*Glasgow Medical Journal.*

MAMMALIAN DESCENT:

MAN'S PLACE IN NATURE.

Being the Hunterian Lectures for 1884. Adapted for General Readers.

By PROF. W. KITCHEN PARKER, F.R.S.,

Hunterian Professor, Royal College of Surgeons.

With Illustrations. In 8vo, Cloth. 10s. 6d.

GENERAL CONTENTS.—Introduction—Prototheria—The Marsupials or Pouched Animals—Edentata—Insectivora—Remaining Orders of Mammalia—Conclusion.

"The smallest details of science catch a LIVING GLOW from the ardour of the author's imagination, . . . we are led to compare it to some quickening spirit which makes all the dry bones of skulls and skeletons stand up around him as an exceeding great army."—Prof. Romanes in *Nature.*

HANDSOME CLOTH. PRICE 18s.

Beautifully Illustrated. With Thirty-six Coloured Plates, comprising 167 Figures.

ATLAS
OF
URINARY SEDIMENTS:
With Special Reference to their Clinical Significance.

EDITED AND ANNOTATED BY

SHERIDAN DELÉPINE, M.B., C.M. (EDIN.),
Professor of Pathology in Owens College and Victoria University, Manchester.

TRANSLATED BY

FREDERICK C. MOORE, M.Sc., M.B. (VICT.),

FROM THE GERMAN OF

DR. HERMANN RIEDER,
Of the University of Munich.

"The work gains much in value from the editorial notes of Prof. Delépine, which are, for the most part, EMINENTLY PRACTICAL."—*Edinburgh Medical Journal.*

"May be pronounced A SUCCESS IN EVERY WAY. . . . The plates are most BEAUTIFULLY EXECUTED AND REPRODUCED. . . . The drawings are excellent, and by FAR THE BEST WE HAVE SEEN. The additions to the text are considerable and valuable."—*The Lancet.*

Published Quarterly, Price 6s. Annual Subscription, 20s.; Post Free, 21s.
Subscriptions payable in advance.

THE JOURNAL OF ANATOMY AND PHYSIOLOGY:
NORMAL AND PATHOLOGICAL.
HUMAN AND COMPARATIVE.

CONDUCTED BY

SIR WILLIAM TURNER, M.B., LL.D., D.C.L., F.R.S.,
Prof. of Anatomy in the University of Edinburgh;

D. J. CUNNINGHAM, M.D., D.Sc., LL.D., D.C.L., F.R.S.,
Prof. of Anatomy and Chirurgery in the University of Dublin;

G. S. HUNTINGDON, A.M., M.D.
Professor of Anatomy in the Columbia University, New York;

ALEX. MACALISTER, M.A., M.D., LL.D., D.Sc., F.R.S.,
Prof. of Anatomy in the University of Cambridge;

AND J. G. M'KENDRICK, M.D., LL.D., F.R.S.,
Prof. of the Institutes of Medicine in the University of Glasgow.

*** MESSRS. GRIFFIN will be glad to receive at their Office Names of SUBSCRIBERS to the Journal.

---

§12. GRIFFINS' "POCKET" MEDICAL SERIES OF REFERENCE BOOKS.

Elegantly Bound in Leather, with Rounded Edges, for the Pocket.

		PAGE
A Surgical Handbook, . .	MM. CAIRD and CATHCART,	98
A Medical Handbook, . .	R. S. AITCHISON, M.D., .	98
A Handbook of Hygiene, .	Lt.-Colonel DAVIES, D.P.H.,	99
The Surgeon's Pocket-Book,	MM. PORTER and GODWIN, .	99
The Diseases of Children, .	MM. ELDER and FOWLER, .	100
Outlines of Bacteriology, .	THOINOT, MASSELIN, and Prof. SYMMERS, .	100

*** Other Volumes in Active Preparation.

*** The aim of the "POCKET" SERIES is to afford to the reader all that is **essential** in the **most handy** and **portable form.** Every aid to READY REFERENCE is afforded by Arrangement and Typography, so that the volumes can be carried about and consulted with ease by the Practitioner at any moment.

OPINION OF "THE LANCET" ON ONE OF THE "POCKET" SERIES.

"Such a work as this is really **necessary** for the busy practitioner. The field of medicine is so wide that even the best informed may at the moment miss the salient points in diagnosis . . . he needs to refresh and revise his knowledge, and to focus his mind on those things which are **essential.** . . . **Honestly Executed.** . . . No mere compilation, the scientific spirit and standard maintained throughout put it on a higher plane. . . . **Excellently** got up, handy and portable, and well adapted for **ready reference.**" —*The Lancet.*

Griffin's "Pocket-Book" Series.

THIRD EDITION, Revised and Enlarged. Pocket Size, Leather, 8s. 6d.

A MEDICAL HANDBOOK,

For the Use of Practitioners and Students.

By R. S. AITCHISON, M.D.Edin., F.R.C.P.

With Numerous Illustrations.

GENERAL CONTENTS.—Case-taking—Diseases of the Circulatory System—of the Respiratory System—of the Renal System—of the Digestive System—of the Nervous System—of the Hæmopoietic System—Fevers and Miasmatic Diseases—Constitutional Diseases—General Information and Tables Useful to the Practitioner—Rules for Prescribing—Prescriptions—Appendix.

"Such a work as this is really NECESSARY for the busy practitioner. The field of medicine is so wide that even the best informed may at the moment miss the salient points in diagnosis . . . he needs to refresh and revise his knowledge, and to focus his mind on those things which are ESSENTIAL. We can speak HIGHLY of Dr. Aitchison's Handbook. . . . HONESTLY EXECUTED. . . . No mere compilation, the scientific spirit and standard maintained throughout put it on a higher plane. . . . EXCELLENTLY got up, handy and portable, and well adapted for READY REFERENCE."—*The Lancet.*

"We STRONGLY RECOMMEND this little work as a reliable guide for medical practice. Elegantly got up."—*Liverpool Medico-Chirurgical Review.*

"As a means of ready reference, MOST COMPLETE. The busy practitioner will often turn to its pages."—*Journ. of the American Med. Association.*

ELEVENTH EDITION, *Thoroughly Revised. Pocket-Size, Leather, 8s. 6d. With very Numerous Illustrations.*

A SURGICAL HANDBOOK,

For Practitioners, Students, House-Surgeons, and Dressers.

BY

F. M. CAIRD, M.B., F.R.C.S. (Ed.), AND C. W. CATHCART, M.B., F.R.C.S. (Eng. & Ed.),

Assistant-Surgeons, Royal Infirmary, Edinburgh.

*** The NEW EDITION has been thoroughly Revised and partly Re-written. Much New Matter and many Illustrations of New Surgical Appliances have been introduced.

GENERAL CONTENTS.—Case-Taking—Treatment of Patients before and after Operation—Anæsthetics: General and Local—Antiseptics and Wound-Treatment—Arrest of Hæmorrhage—Shock and Wound Fever—Emergency Cases—Tracheotomy: Minor Surgical Operations—Bandaging—Fractures—Dislocations, Sprains, and Bruises—Extemporary Appliances and Civil Ambulance Work—Massage—Surgical Applications of Electricity—Joint-Fixation and Fixed Apparatus—The Syphon and its Uses—Trusses and Artificial Limbs—Plaster-Casting—Post-Mortem Examination—Sickroom Cookery, &c.

"THOROUGHLY PRACTICAL AND TRUSTWORTHY. Clear, accurate, succinct."—*The Lancet.*

"ADMIRABLY ARRANGED. The best practical little work we have seen. The matter is as good as the manner."—*Edinburgh Medical Journal.*

"THIS EXCELLENT LITTLE WORK. Clear, concise, and very readable. Gives attention to important details, often omitted, but ABSOLUTELY NECESSARY TO SUCCESS."—*Athenæum.*

"A dainty volume."—*Manchester Medical Chronicle.*

Griffin's "Pocket-Book" Series.

SECOND EDITION, Thoroughly Revised and partly Re-written. Pocket Size. Leather. With Illustrations. 8s. 6d. net.

A HAND-BOOK OF HYGIENE.

BY

LT.-COLONEL A. M. DAVIES, D.P.H.Camb.,

Late Assistant-Professor of Hygiene, Army Medical School.

General Contents.

Air and Ventilation—Water and Water Supply—Food and Dieting—Removal and Disposal of Sewage—Habitations—Personal Hygiene—Soils and Sites—Climate and Meteorology—Causation and Prevention of Disease—Disinfection.

"We are glad to welcome Surgeon-Major Davies' book . . . he has had ample opportunity to make himself A MASTER OF THE SCIENCE, and he has a right to speak. . . . WONDERFULLY WELL UP TO DATE, well and clearly written, pleasant to read."—*The Lancet.*

"This ADMIRABLE HANDBOOK . . . gives FULL information compressed into the smallest possible bulk."—*Edin. Med. Journal.*

"The elegant dress of the little volume before us is but the outer covering of A TRULY RICH KERNEL, and justly merits the praise it spontaneously calls forth. Attractive to the eye, Surgeon-Major DAVIES' volume is equally attractive to the mind. Students will find that its 590 pages comprise ALL information necessary. COMPACT, HANDY, COMPREHENSIVE, it certainly merits a high place among the text-books of the day."—*Sanitary Record.*

"Really an ADMIRABLE BOOK. . . . A MOST HANDY WORK OF REFERENCE full of information."—*The Hospital.*

"A singularly compact and elegant volume . . . contains an admirable *précis* of everything relating to Hygiene CLEARLY and LOGICALLY ARRANGED and easy of reference. Likely, we think, to be the favourite text-book."—*Public Health.*

FOURTH EDITION, *Revised and enlarged. Leather, with* 152 *Illustrations and Folding-plate.* 8s. 6d.

THE SURGEON'S POCKET-BOOK.

Specially adapted to the Public Medical Services

BY MAJOR J. H. PORTER.

Revised and in great part rewritten

BY BRIGADE-SURGEON C. H. Y. GODWIN,

Late Professor of Military Surgery in the Army Medical School.

"An INVALUABLE GUIDE to all engaged, or likely to be engaged, in Field Medical Service."—*Lancet.*

"A complete *vade mecum* to guide the military surgeon in the field."—*British Medical Journal.*

Griffin's "Pocket-Book" Series.

Pocket-size. Leather. With Illustrations, Many in Colours. 10*s.* 6*d.*

OUTLINES OF BACTERIOLOGY:

A Practical Handbook for Students.

ON THE BASIS OF THE

PRÉCIS DE MICROBIE

(OUVRAGE COURONNÉ PAR LA FACULTÉ DE MÉDECINE DE PARIS).

BY

DR. L. H. THOINOT, Professeur agrégé à la Faculté, Médecin des Hôpitaux.

AND

E. J. MASSELIN, Médecin Vétérinaire.

Translated and adapted for English Use, with Additions,

BY

WM. ST. CLAIR SYMMERS, M.B.(ABERD.),

Professor of Pathology and Bacteriology, Govt. Medical School, Cairo, late Assistant-Bacteriologist, British Institute of Preventive Medicine, London; Pathologist, Lancashire County Asylum, Prestwick; and Pathologist, General Hospital, Birmingham, &c., &c.

CONTENTS.—I. Glass Apparatus. II. Preparation and Sterilisation.—Culture Media. III. Autoclave and Incubator. IV. Experiments with Living Animals. V. VI. General Culture Methods. VII. Colouring and Staining. VIII. Microscopic Examination. Part II.—I. Diseases Common to Man and Lower Animals. II. Diseases Special to Higher Animals. III. Microbic Diseases peculiar to Man.

"Contains far more information than the original work."—*Literature.*

"The information conveyed is SINGULARLY FULL AND COMPLETE. The work compares VERY FAVOURABLY in this respect with manuals of much larger size. We recommend the book for its ACCURACY, CLEARNESS, NOVELTY, AND CONVENIENT SIZE."—*The Lancet.*

Pocket-Size. Leather. With Illustrations. 10*s.* 6*d.*

THE DISEASES OF CHILDREN:

A CLINICAL HANDBOOK.

BY

GEO. ELDER, M.D., F.R.C.P.(Ed.),

AND

J. S. FOWLER, M.B., F.R.C.P.(Ed.),

Clinical Tutors, Royal Infirmary, Edinburgh; Physicians for Out-patients, Leith Hospital.

"MUCH MORE is contained in it, than in many similar books professing more."—*Bristol Medical Journal.*

"May be cordially recommended."—*Edinburgh Medical Journal.*

§§ 13-14. STUDENTS' STANDARD TEXT-BOOKS.

Fully Illustrated.

		PAGE
Practical Physiology,	PROF. STIRLING, M.D.,	102
Practical Histology,	„ „	102
Physiologist's Note-book,	ALEX HILL, M.D.,	103
Biology (Vegetable),	PROF. AINSWORTH DAVIS,	104
Biology (Animal),	„ „	104
Botany: the Flowering Plant,	„ „	114
Zoological Pocket-book,	PROFS. SELENKA & DAVIS,	103
Inorganic Chemistry,	PROFS. DUPRÉ AND HAKE,	111
Quantitative Analysis,	PROF. HUMBOLDT SEXTON,	111
Qualitative Analysis,	„ „	111
Practical Pharmacy,	WM. ELBORNE, F.L.S., F.C.S.,	106
Physics,	PROFS. POYNTING AND THOMSON,	113
Midwifery,	ARCH. DONALD, M.D.,	105
Diseases of Women,	JOHN PHILLIPS, M.D.,	105
Wife and Mother,	A. WESTLAND, M.D.,	108
Infant Rearing,	J. B. HELLIER, M.D.,	108
Lunatic Asylums,	CHARLES MERCIER, M.B.,	106
Nursing, Medical and Surgical,	L. HUMPHRY, M.D.,	107
Foods and Dietaries for the Sick,	R. BURNET, M.D.,	107
Ambulance,	J. SCOTT RIDDELL, C.M.,	109
First Aid at Sea,	JOHNSON SMITH, M.D.,	109
Practical Sanitation,	GEO. REID, M.D., D.P.H.,	110
Hygienic Prevention of Consumption,	J. E. SQUIRE, M.D., D.P.H.,	110

WORKS

By WILLIAM STIRLING, M.D., Sc.D.,

Professor in the Victoria University, Brackenbury Professor of Physiology and Histology in the Owens College, Manchester; and Examiner in the Universities of Oxford, Edinburgh, and London; and for the Fellowship of the Royal College of Surgeons, England.

FOURTH EDITION, *at Press. Thoroughly Revised and largely Re-written. In Extra Crown 8vo, with 620 pp. and over 460 Illustrations.*

OUTLINES OF PRACTICAL PHYSIOLOGY:

Being a Manual for the Physiological Laboratory, including Chemical and Experimental Physiology, with Reference to Practical Medicine.

PART I.—CHEMICAL PHYSIOLOGY.

PART II.—EXPERIMENTAL PHYSIOLOGY.

"The BEST STUDENTS' book on the subject. . . . FULL of information and CAPITALLY illustrated."—*The Lancet.*

"The student is enabled to perform for himself most of the experiments usually shown in a systematic course of lectures on physiology, and the practice thus obtained must prove INVALUABLE. . . . May be confidently recommended as a guide to the student of physiology, and, we doubt not, will also find its way into the hands of many of our scientific and medical practitioners."—*Glasgow Medical Journal.*

"This valuable little manual. . . . The GENERAL CONCEPTION of the book is EXCELLENT; the arrangement of the exercises is all that can be desired; the descriptions of experiments are CLEAR, CONCISE, and to the point."—*British Medical Journal.*

SECOND EDITION. *In Extra Crown 8vo, with 368 Illustrations, Cloth* 12*s.* 6*d.*

OUTLINES OF PRACTICAL HISTOLOGY:

A Manual for Students.

*** Dr. Stirling's "Outlines of Practical Histology" is a compact Handbook for students, providing a COMPLETE LABORATORY COURSE, in which almost every exercise is accompanied by a drawing. Very many of the illustrations have been prepared expressly for the work.

"The volume proceeds from a MASTER in his craft. . . . We can confidently recommend this small but CONCISELY-WRITTEN and ADMIRABLY ILLUSTRATED work to students. They will find it to be a VERY USEFUL and RELIABLE GUIDE in the laboratory, or in their own room. All the principal METHODS of preparing tissues for section are given, with such precise directions that little or no difficulty can be felt in following them in their most minute details."—*Lancet.*

"The general plan of the work is ADMIRABLE. . . . It is very evident that the suggestions given are the outcome of a PROLONGED EXPERIENCE in teaching Practical Histology, combined with a REMARKABLE JUDGMENT in the selection of METHODS. . . . Merits the highest praise for the ILLUSTRATIONS, which are at once clear and faithful."—*British Medical Journal.*

In Large 8vo. With 36 Plates and Blank Pages for MS. Notes. Cloth, 12s. 6d.

THE PHYSIOLOGIST'S NOTE-BOOK:

A Summary of the Present State of Physiological Science for Students.

BY ALEX HILL, M.A., M.D.,

Master of Downing College and formerly Vice-Chancellor of the University of Cambridge.

GENERAL CONTENTS:—The Blood—Vascular System—Nerves—Muscle—Digestion—Skin—Kidneys—Respiration—The Senses—Voice and Speech—Central Nervous System—Reproduction—Chemistry of the Body.

CHIEF FEATURES OF DR. HILL'S NOTE-BOOK.

1. It helps the Student to CODIFY HIS KNOWLEDGE.
2. Gives a grasp of BOTH SIDES of an argument.
3. Is INDISPENSABLE for RAPID RECAPITULATION.

The Lancet says of it:—"The work which the Master of Downing College modestly compares to a Note-book is an ADMIRABLE COMPENDIUM of our present information. . . . Will be a REAL ACQUISITION to Students. . . . Gives all ESSENTIAL POINTS. . . . The TYPOGRAPHICAL ARRANGEMENT is a chief feature of the book. . . . Secures at a glance the EVIDENCE on both sides of a theory."

The Hospital says:—"The Physiologist's Note-book bears the hall-mark of the Cambridge School, and is the work of one of the most successful of her teachers. Will be INVALUABLE to Students."

The British Medical Journal commends in the volume—"Its admirable diagrams, its running Bibliography, its clear Tables, and its concise statement of the anatomical aspects of the subject."

*** For Dr. Hill's Translation of Prof. Obersteiner's *Central Nervous Organs*, see p. 92.

A ZOOLOGICAL POCKET-BOOK; Or,

Synopsis of Animal Classification. Comprising Definitions of the Phyla, Classes, and Orders, with Explanatory Remarks and Tables. By Dr. EMIL SELENKA, Professor in the University of Erlangen. Authorised English translation from the Third German Edition. By Prof. AINSWORTH DAVIS. In Small Post 8vo, Interleaved for the use of Students. Limp Covers, 4s.

"Dr. Selenka's Manual will be found useful by all Students of Zoology. It is a COMPREHENSIVE and SUCCESSFUL attempt to present us with a scheme of the natural arrangement of the animal world."—*Edin. Med. Journal.*

"Will prove very serviceable to those who are attending Biology Lectures. . . . The translation is accurate and clear."—*Lancet.*

LONDON: CHARLES GRIFFIN & CO., LIMITED, EXETER STREET, STRAND.

An Elementary Text-Book

OF

BIOLOGY.

By J. R. AINSWORTH DAVIS, M.A., F.Z.S.,
PROFESSOR OF BIOLOGY, UNIVERSITY COLLEGE, ABERYSTWYTH;
EXAMINER IN ZOOLOGY, UNIVERSITY OF ABERDEEN.

SECOND EDITION. In Two Volumes. Sold Separately.

I. VEGETABLE MORPHOLOGY AND PHYSIOLOGY.

General Contents.—UNICELLULAR PLANTS (Yeast-plant, Germs and Microbes, White Mould, Green Mould, &c., &c.)—SIMPLE MULTICELLULAR PLANTS (Wrack, Stoneworts, &c.)—THE MOSS—PTERIDOPHYTES (Bracken and Male Shield Ferns, &c.)—PLANT CELLS AND TISSUES—GYMNOSPERMS (the Fir)—ANGIOSPERMS (Buttercup, Snowdrop)—VEGETATIVE ORGANS OF SPERMAPHYTES—REPRODUCTIVE ORGANS ON ANGIOSPERMS—PHYSIOLOGY OF HIGHER PLANTS—DEVELOPMENT OF ANGIOSPERMS—COMPARATIVE VEGETABLE MORPHOLOGY AND PHYSIOLOGY—CLASSIFICATION OF PLANTS.

With Complete Index-Glossary and 128 *Illustrations.* 8s. 6d.

II. ANIMAL MORPHOLOGY AND PHYSIOLOGY.

General Contents.—PROTOZOA: Amœba (Proteus Animalcule), Vorticella (Bell Animalcule), Gregarina—CŒLENTERATA: Hydra (Fresh-Water Polype), &c.—PLATYHELMIA (Flat-worms): Liver-fluke, Tapeworm—NEMATHELMIA (Thread-worms)—ANNELIDA (Segmented Worms): Earthworm, Leech—ARTHROPODA: Crayfish—MOLLUSCA: Fresh-Water Mussels, Snail—VERTEBRATA ACRANIA: Amphioxus (Lancelet)—PISCES (Fishes): Dogfish—AMPHIBIA: Frog—AVES (Birds): Pigeon and Fowl—MAMMALIA: Rabbit—COMPARATIVE ANIMAL MORPHOLOGY AND PHYSIOLOGY—MAN—CLASSIFICATION and DISTRIBUTION OF ANIMALS.

With Complete Index-Glossary and 108 *Illustrations.* 10s. 6d.

NOTE—The SECOND EDITION has been thoroughly Revised and Enlarged, and includes all the leading selected TYPES in the various Organic Groups.

"Certainly THE BEST 'BIOLOGY' with which we are acquainted. It owes its pre-eminence to the fact that it is an EXCELLENT attempt to present Biology to the Student as a CORRELATED AND COMPLETE SCIENCE."—*British Medical Journal.*

"CLEAR and COMPREHENSIVE."—*Saturday Review.*

"Literally PACKED with information."—*Glasgow Medical Journal.*

DAVIS (Prof): THE FLOWERING PLANT.
(See p. 114.)

DAVIS AND SELENKA: ZOOLOGICAL POCKET-BOOK. (See p. 103.)

THIRD EDITION, Revised. With Numerous Illustrations. 5s.

AN INTRODUCTION TO THE STUDY OF

MIDWIFERY.

For the Use of Young Practitioners, Students, and Midwives.

BY

ARCHIBALD DONALD, M.A., M.D., C.M.EDIN.,

Obstetric Physician to the Manchester Royal Infirmary; Honorary Surgeon to St. Mary's Hospital for Women, Manchester.

HIGHLY CREDITABLE to the Author. . . . Should prove of GREAT VALUE to Midwifery Students and Junior Practitioners."—*British Gynæcological Journal.*

"As an Introduction to the study of Midwifery NO BETTER BOOK could be placed in the hands of the Student."—*Sheffield Med. Journal.*

THIRD EDITION, Thoroughly Revised. With Illustrations. 7s. 6d.

OUTLINES OF THE

DISEASES OF WOMEN.

A CONCISE HANDBOOK FOR STUDENTS.

By JOHN PHILLIPS, M.A., M.D., F.R.C.P.,

Senior Physician, British Lying-in Hospital; Obstetric Physician, and Lecturer on Practical Obstetrics at King's College Hospital; Examiner in Midwifery to the University of Cambridge; Examiner in Midwifery and Diseases of Women to the Royal College of Physicians.

*** Dr. Phillips' work is ESSENTIALLY PRACTICAL in its nature, and will be found invaluable to the student and young practitioner.

"Contains a GREAT DEAL OF INFORMATION in a VERY CONDENSED form. . . . The value of the work is increased by the number of sketch diagrams, some of which are HIGHLY INGENIOUS."—*Edin. Med. Journal.*

"Dr. PHILLIPS' MANUAL is written in a SUCCINCT style. He rightly lays stress on Anatomy. The passages on CASE-TAKING are EXCELLENT. Dr. Phillips is very trustworthy throughout in his views on THERAPEUTICS. He supplies an excellent series of SIMPLE but VALUABLE PRESCRIPTIONS, an INDISPENSABLE REQUIREMENT for students."—*Brit. Med. Journal.*

"This EXCELLENT TEXT-BOOK . . . gives just what the student requires. . . . The prescriptions cannot but be helpful."—*Medical Press.*

In Extra Crown 8vo, with Litho-plates and Numerous Illustrations. Cloth, 8s. 6d.

ELEMENTS OF
PRACTICAL PHARMACY AND DISPENSING.

By W. ELBORNE, B.A.Cantab., F.L.S., F.C.S.,
Demonstrator of Materia Medica and Teacher of Pharmacy at University College, London; Pharmacist to University College Hospital.

GENERAL CONTENTS.

Part I.—Chemical Pharmacy and Laboratory Course.
Part II.—Galenical Pharmacy and Laboratory Course.
Part III.—A Practical Course of Dispensing, with Fac-Similes of Autograph Prescriptions.

"A work which we can very highly recommend to the perusal of all Students of Medicine. . . . Admirably adapted to their requirements."—*Edinburgh Medical Journal.*

"The system . . . which Mr. Elborne here sketches is thoroughly sound."—*Chemist and Druggist.*

*** Formerly published under the title of "Pharmacy and Materia Medica."

Large 8vo, Handsome Cloth. 16*s.*

LUNATIC ASYLUMS:
THEIR ORGANISATION AND MANAGEMENT.

By CHARLES MERCIER, M.B.,
Lecturer on Neurology and Insanity, Westminster Hospital Medical School; Late Senior Assistant-Medical Officer at Leavesden Asylum, and at the City of London Asylum.

ABSTRACT OF CONTENTS.

Part I.—Housing.—General Principles.—Supervision.—Cost.—Wards and Ward Offices.—Day Rooms.—The Dining and Recreation Halls, Chapel, &c.—Communication.—Administrative Portion.—Accommodation for the Staff.—Airing Courts.

Part II.—Food and Clothing.—Beverages.—Dietaries.—Testing.—Storing and Keeping.—Serving.—Extra Diets.—Clothing.

Part III.—Occupation and Amusement.—Occupation.—Amusements.—Airing Courts.—Grounds.—Open-Air Amusements.—The Recreation Hall.—Dancing.—Theatricals.—Concerts.

Part IV.—Detention and Care.—Detention.—Care.—Suicidal Tendency.—Supervision.—Violence.—Restraint.—Dispersion.—Change of Surroundings.—Pretended Violence.—Accidents.—Falls.—Epileptic Fits.—Epileptics at Night.—Provisions for Safety of Patients.—Fire-Extinguishing Apparatus.—Cleanliness.—Bathing.—Dirty Habits.

Part V.—The Staff.—Responsibility.—Awards.—Punishment.—Supervision.—Inspection.—Surprise Visits.—Reports.—The Chaplain.—The Superintendent.—Assistant Medical Officer.—Matron.—Warders.—Nurses.—&c., &c.

"Will give a much-needed impetus to the study of Asylum Patients."—*Glasgow Medical Journal.*

TWENTY-SECOND EDITION. *Entirely Revised. With Numerous Illustrations*, 3s. 6d.

A MANUAL OF NURSING:

Medical and Surgical.

BY LAURENCE HUMPHRY, M.A., M.D., M.R.C.S.,

Assistant-Physician to, late Lecturer to Probationers at, Addenbrooke's Hospital, Cambridge; Teacher of Pathology and Examiner in Medicine, University of Cambridge.

GENERAL CONTENTS.—The General Management of the Sick Room in Private Houses—General Plan of the Human Body—Diseases of the Nervous System—Respiratory System—Heart and Blood-Vessels—Digestive System—Skin and Kidneys—Fevers—Diseases of Children—Wounds and Fractures—Management of Child-Bed—Sick-Room Cookery, &c., &c.

"In the fullest sense Dr. Humphry's book is a DISTINCT ADVANCE on all previous Manuals. . . . Its value is greatly enhanced by copious Woodcuts and diagrams of the bones and internal organs, by many Illustrations of the art of BANDAGING, by Temperature charts indicative of the course of some of the most characteristic diseases, and by a goodly array of SICK-ROOM APPLIANCES with which EVERY NURSE should endeavour to become acquainted."—*British Medical Journal.*

"We should advise ALL NURSES to possess a copy of the work. We can confidently recommend it as an EXCELLENT GUIDE and companion."—*Hospital.*

THIRD EDITION. *Handsome Cloth*, 4s.

FOODS AND DIETARIES:

A Manual of Clinical Dietetics.

BY R. W. BURNET, M.D., F.R.C.P.,

Physician in Ordinary to H.R.H. the Duke of Cornwall and York; Senior Physician to the Great Northern Central Hospital, &c.

GENERAL CONTENTS.—DIET in Diseases of the Stomach, Intestinal Tract, Liver, Lungs, Heart, Kidneys, &c.; in Diabetes, Scurvy, Anæmia, Scrofula, Gout, Rheumatism, Obesity, Alcoholism, Influenza, Nervous Disorders, Diathetic Diseases, Diseases of Children, with a Section on Predigested Foods, and Appendix on Invalid Cookery.

"The directions given are UNIFORMLY JUDICIOUS. . . . May be confidently taken as a RELIABLE GUIDE in the art of feeding the sick."—*Brit. Med. Journal.*

"Dr. Burnet's book will be of great use. . . . Deals with BROAD and ACCEPTED VIEWS. . . . Gout, Scurvy, Rickets, and Alcoholism are instances in point, and they are all TREATED WITH ADMIRABLE SENSE and JUDGMENT by Dr. Burnet. The careful study of such books as this will very much help the Practitioner in the Treatment of cases, and powerfully aid the action of remedies."—*Lancet.*

"Dr. Burnet gives a Time-table of Diet, with Bill of Fare for each meal, quantities, and beverages . . . will help the nurse when at her wits' end for a change of diet, to meet the urgency of the moment or tempt the appetite of the patient."—*Glasgow Herald.*

LONDON: CHARLES GRIFFIN & CO., LIMITED, EXETER STREET, STRAND.

"Should be in the hands of EVERY MOTHER AND NURSE."—*The Nurse* (Boston).

THIRD EDITION, REVISED. *Handsome Cloth. Price 5s.*

THE WIFE AND MOTHER:

A Medical Guide to the Care of Health and the Management of Children.

BY ALBERT WESTLAND, M.A., M.D., C.M.

GENERAL CONTENTS:—PART I.—Early Married Life. PART II.—Early Motherhood. PART III.—The Child, in Health and Sickness. PART IV.—Later Married Life.

"WELL-ARRANGED, and CLEARLY WRITTEN."—*Lancet.*

"THE BEST BOOK of its kind. . . . May be read from cover to cover with advantage."—*Medical Press.*

"A REALLY EXCELLENT BOOK."—*Aberdeen Journal.*

"EXCELLENT AND JUDICIOUS."—*Western Daily Press.*

"The best book I can recommend is 'THE WIFE AND MOTHER,' by Dr. ALBERT WESTLAND, published by Messrs. Charles Griffin & Co. It is a MOST VALUABLE work, written with discretion and refinement."—*Hearth and Home.*

"Will be WELCOMED by every young wife . . . abounds with valuable advice."—*Glasgow Herald.*

In Large Crown 8vo. Handsome Cloth. 3s.

INFANCY AND INFANT-REARING:

A Guide to the Care of Children in Early Life.

BY JOHN BENJ. HELLIER, M.D.,

Surg. to the Hosp. for Women and Children, Leeds; Lect. on Diseases of Women and Children, Yorkshire College, Leeds; Examiner in the Victoria University.

With Illustrations.

GENERAL CONTENTS.—Normal Growth and Development in the first Two Years of Life: The New-born Child—Signs of Maturity—The Bony System—Nervous System—Development of Special Senses—Teething. **Difficulties of Infant-Rearing:** Statistics of Mortality. **Causes of Mortality:** Improper Feeding—Sour and Diseased Milk. **Special Diseases of Infancy:** Gastro-enteric Catarrh—Rickets—Scurvy Rickets—Thrush—Rheumatism—Causes which hinder Thriving. **Infant-Feeding: Natural Feeding**—Wet Nurses and their Diet—Influence of Alcohol and Drugs. **Artificial Feeding:** Humanising Cow's Milk—Peptonising—Sterilising—Precautions—Feeding-Bottles—Milk of other Animals—Diet in Scurvy Rickets, &c. **General Hygiene of Infants:** Cleanliness—Warmth—Ventilation—Sleep—Out-door Exercise—Nursery Arrangements—Shortening. **Special Care of New-born Children—Care of Premature Infant:** Tarnier's Couveuse and Mode of using it. **Hygiene of Dentition—Special Conditions observed in Infancy.**

"THOROUGHLY PRACTICAL. . . . A MINE of information."—*Public Health.*

GRIFFIN'S "FIRST AID" PUBLICATIONS.

On Land.

FOURTH EDITION. *Large Crown 8vo. Handsome Cloth.* 4s.

A MANUAL OF AMBULANCE.

BY J. SCOTT RIDDELL, C.M., M.B., M.A.,

Senior Asst.-Surgeon, Aberdeen Royal Infirmary; Lecturer and Examiner to the Aberdeen Ambulance Association; Examiner to the St. Andrew's Ambulance Association, Glasgow, and the St. John Ambulance Association, London.

With Numerous Illustrations and Full Page Plates.

General Contents.—Outlines of Human Anatomy and Physiology—The Triangular Bandage and its Uses—The Roller Bandage and its Uses—Fractures—Dislocations and Sprains—Hæmorrhage—Wounds—Insensibility and Fits—Asphyxia and Drowning—Suffocation—Poisoning—Burns, Frost-bite, and Sunstroke—Removal of Foreign Bodies from (*a*) The Eye; (*b*) The Ear; (*c*) The Nose; (*d*) The Throat; (*e*) The Tissues—Ambulance Transport and Stretcher Drill—The After-treatment of Ambulance Patients—Organisation and Management of Ambulance Classes—Appendix: Examination Papers on First Aid.

"A CAPITAL BOOK. . . . The directions are SHORT and CLEAR, and testify to the hand of an able surgeon."—*Edin. Med. Journal.*

"This little volume seems to us about as good as it could possibly be. . . . Contains practically every piece of information necessary to render First aid. . . . Should find its place in EVERY HOUSEHOLD LIBRARY."—*Daily Chronicle.*

"So ADMIRABLE is this work, that it is difficult to imagine how it could be better."—*olliery Guardian.*

At Sea.

SECOND EDITION, *Revised. Crown 8vo, Extra. Handsome Cloth.* 6s.

A MEDICAL AND SURGICAL HELP
FOR SHIPMASTERS AND OFFICERS IN THE MERCHANT NAVY.

INCLUDING

FIRST AID TO THE INJURED.

BY WM. JOHNSON SMITH, F.R.C.S.,

Principal Medical Officer, Seamen's Hospital, Greenwich.

With 2 Coloured Plates, Numerous Illustrations, and latest Regulations respecting Medical Stores on Board Ship.

*** The attention of all interested in our Merchant Navy is requested to this exceedingly useful and valuable work. It is needless to say that it is the outcome of many year's PRACTICAL EXPERIENCE amongst Seamen.

"SOUND, JUDICIOUS, REALLY HELPFUL."—*The Lancet.*

"It would be difficult to find a Medical and Surgical Guide more clear and comprehensive than Mr. JOHNSON SMITH, whose experience at the GREENWICH HOSPITAL eminently qualifies him for the task. . . . A MOST ATTRACTIVE WORK. . . . We have read it from cover to cover. . . . It gives clearly written advice to Masters and Officers in all medical and surgical matters likely to come before them when remote from the land and without a doctor. . . . We RECOMMEND the work to EVERY Shipmaster and Officer."—*Liverpool Journal of Commerce.*

In Crown 8vo. With Frontispiece. Handsome Cloth. 6s.

THE HYGIENIC PREVENTION OF

CONSUMPTION.

BY

J. EDWARD SQUIRE, M.D., D.P.H. Camb.,

Physician to the North London Hospital for Consumption and Diseases of the Chest; Fellow of the Royal Med.-Chirurg. Society, and of the British Institute of Public Health, &c., &c.

"If the terrible prevalence of Consumption is to be lessened, it can only be by the intelligent co-operation of the laity with the Medical Profession. A conviction that the disease is PREVENTIBLE is of first importance, and some knowledge of its nature, and of the mode in which it spreads, is necessary to direct the preventive measures required."—*Extr. from Author's Preface.*

GENERAL CONTENTS:—THE NATURE OF CONSUMPTION—PREVENTIVE MEASURES: In Infancy, Childhood, School Life, Adult Life; Exercise, Clothing, Diet; the Household, Choice of Occupation, Residence—STATE HYGIENE—MANAGEMENT OF EARLY CONSUMPTION:—Question of Curability, Climatic Conditions, Travelling, &c.

"We can safely say that Dr. SQUIRE'S work WILL REPAY STUDY even by the most cultivated physician. . . . Although the book is not a large one, it is FULL OF INSTRUCTIVE MATTER, and is written in a judicious spirit, besides being VERY READABLE."—*The Lancet.*

EIGHTH EDITION, Revised. Price 6s.

PRACTICAL SANITATION:

A HAND-BOOK FOR SANITARY INSPECTORS AND OTHERS INTERESTED IN SANITATION.

BY GEORGE REID, M.D., D.P.H.,

Fellow, Mem. Council, and Examiner, Sanitary Institute of Great Britain, and Medical Officer to the Staffordshire County Council.

WITH AN APPENDIX ON SANITARY LAW.

BY HERBERT MANLEY, M.A., M.B., D.P.H.,

Medical Officer of Health for the County Borough of West Bromwich.

GENERAL CONTENTS.—Introduction.—Water Supply: Drinking Water, Pollution of Water.—Ventilation and Warming.—Principles of Sewage Removal.—Details of Drainage; Refuse Removal and Disposal—Sanitary and Insanitary Work and Appliances.—Details of Plumbers' Work.—House Construction.—Infection and Disinfection.—Food, Inspection of; Characteristics of Good Meat; Meat, Milk, Fish, &c., unfit for Human Food.—Appendix: Sanitary Law; Model Bye-Laws, &c.

"Dr. Reid's very useful Manual . . . ABOUNDS IN PRACTICAL DETAIL.'—*British Medical Journal.*

"A VERY USEFUL HANDBOOK, with a very useful Appendix. We recommend it not only to SANITARY INSPECTORS, but to HOUSEHOLDERS and ALL interested in Sanitary matters."—*Sanitary Record.*

A SHORT MANUAL OF INORGANIC CHEMISTRY.

BY

A. DUPRÉ, Ph.D., F.R.S.,

AND

WILSON HAKE, Ph.D., F.I.C., F.C.S.,
Of the Westminster Hospital Medical School.

THIRD EDITION, Revised, Enlarged, and brought up-to-date. Price 9s. net.

"A well-written, clear and accurate Elementary Manual of Inorganic Chemistry. . . We agree heartily with the system adopted by Drs. Dupré and Hake. WILL MAKE EXPERIMENTAL WORK TREBLY INTERESTING BECAUSE INTELLIGIBLE."—*Saturday Review.*

"There is no question that, given the PERFECT GROUNDING of the Student in his Science, the remainder comes afterwards to him in a manner much more simple and easily acquired. The work IS AN EXAMPLE OF THE ADVANTAGES OF THE SYSTEMATIC TREATMENT of a Science over the fragmentary style so generally followed. BY A LONG WAY THE BEST of the small Manuals for Students."—*Analyst.*

LABORATORY HANDBOOKS BY A. HUMBOLDT SEXTON,

Professor of Metallurgy in the Glasgow and West of Scotland Technical College.

OUTLINES OF QUANTITATIVE ANALYSIS.

FOR THE USE OF STUDENTS.

With Illustrations. FOURTH EDITION. Crown 8vo, Cloth, 3s.

"A COMPACT LABORATORY GUIDE for beginners was wanted, and the want has been WELL SUPPLIED. . . . A good and useful book."—*Lancet.*

OUTLINES OF QUALITATIVE ANALYSIS.

FOR THE USE OF STUDENTS.

With Illustrations. THIRD EDITION. Crown 8vo, Cloth, 3s. 6d.

"The work of a thoroughly practical chemist."—*British Medical Journal.*
"Compiled with great care, and will supply a want."—*Journal of Education.*

ELEMENTARY METALLURGY:

Including the Author's Practical Laboratory Course. With many Illustrations. [See p. 66 *General Catalogue.*

SECOND EDITION, Revised. Crown 8vo. Cloth, 6s.

"Just the kind of work for students commencing the study of metallurgy."—*Practical Engineer.*

WORKS BY A. WYNTER BLYTH, M.R.C.S., F.C.S.,

Barrister at Law, Public Analyst for the County of Devon, and Medical Officer of Health for St. Marylebone.

FOODS:

THEIR COMPOSITION AND ANALYSIS.

In Demy 8vo, with Elaborate Tables, Diagrams, and Plates. Handsome Cloth. FOURTH EDITION. Price 21s.

GENERAL CONTENTS.

History of Adulteration—Legislation, Past and Present—Apparatus useful to the Food-Analyst—"Ash"—Sugar—Confectionery—Honey—Treacle—Jams and Preserved Fruits—Starches—Wheaten-Flour—Bread—Oats—Barley—Rye—Rice—Maize—Millet—Potato—Peas—Chinese Peas—Lentils—Beans—MILK—Cream—Butter—Oleo-Margarine—Butterine—Cheese—Lard—Tea—Coffee—Cocoa and Chocolate—Alcohol—Brandy—Rum—Whisky—Gin—Arrack—Liqueurs—Absinthe—Principles of Fermentation—Yeast—Beer—Wine—Vinegar—Lemon and Lime Juice—Mustard—Pepper—Sweet and Bitter Almond—Annatto—Olive Oil—WATER—Standard Solutions and Reagents. *Appendix:* Text of English and American Adulteration Acts.

PRESS NOTICES OF THE FOURTH EDITION.

"Simply INDISPENSABLE in the Analyst's laboratory."—*The Lancet.*

"THE STANDARD WORK on the subject. . . . Every chapter and every page gives abundant proof of the strict revision to which the work has been subjected. . . . The section on MILK is, we believe, the most exhaustive study of the subject extant. . . . An INDISPENSABLE MANUAL for Analysts and Medical Officers of Health."—*Public Health.*

"A new edition of Mr. Wynter Blyth's Standard work, ENRICHED WITH ALL THE RECENT DISCOVERIES AND IMPROVEMENTS, will be accepted as a boon."—*Chemical News.*

POISONS:

THEIR EFFECTS AND DETECTION.

THIRD EDITION. In Large 8vo, Cloth, with Tables and Illustrations. Price 21s.

GENERAL CONTENTS.

I.—Historical Introduction. II.—Classification—Statistics—Connection between Toxic Action and Chemical Composition—Life Tests—General Method of Procedure—The Spectroscope—Examination of Blood and Blood Stains. III.—Poisonous Gases. IV.—Acids and Alkalies. V.—More or less Volatile Poisonous Substances. VI.—Alkaloids and Poisonous Vegetable Principles. VII.—Poisons derived from Living or Dead Animal Substances. VIII.—The Oxalic Acid Group. IX.—Inorganic Poisons. *Appendix:* Treatment, by Antidotes or otherwise, of Cases of Poisoning.

"Undoubtedly THE MOST COMPLETE WORK on Toxicology in our language."—*The Analyst (on the Third Edition).*

"AS A PRACTICAL GUIDE, we know NO BETTER work."—*The Lancet (on the Third Edition).*

*** In the THIRD EDITION, Enlarged and partly Re-written, NEW ANALYTICAL METHODS have been introduced, and the CADAVERIC ALKALOIDS, or PTOMAINES, bodies playing so great a part in Food-poisoning and in the Manifestations of Disease, have received special attention.

GRIFFIN'S INTRODUCTORY SCIENCE SERIES.

FULLY ILLUSTRATED.

BOTANY.

OPEN-AIR STUDIES IN BOTANY. By R. LLOYD PRAEGER, B.A., M.R.I.A., 7/6

THE FLOWERING PLANT. By Prof. AINSWORTH DAVIS. THIRD EDITION, Revised, ... 3/6

HOW PLANTS LIVE AND WORK. By ELEANOR HUGHES-GIBB, . . . 2/6

THE MAKING OF A DAISY. By ELEANOR HUGHES-GIBB, . . . 2/6

CHEMISTRY.

A SHORT MANUAL OF INORGANIC CHEMISTRY. By Prof. DUPRÉ, F.R.S., and Dr. WILSON HAKE. THIRD EDITION, ... 9/ net.

THE THRESHOLD OF SCIENCE. By C. R. ALDER WRIGHT, D.Sc., F.R.S., SECOND EDITION, ... 6/

CHEMICAL RECREATIONS. By J. J. GRIFFIN, F.C.S. TENTH EDITION, Complete, ... 12/6
(Or in 2 parts, sold separately.)

GEOLOGY.

OPEN-AIR STUDIES IN GEOLOGY. By GRENVILLE COLE, F.G.S., M.R.I.A., 8/6

ENGINEERING DRAWING.

I. PRACTICAL GEOMETRY, THIRD EDITION, ... 3/

II. MACHINE DESIGN, ... 4/6
THIRD EDITION. By Principal S. H. WELLS, A.M.Inst.C.E.

MAGNETISM & ELECTRICITY.

By Prof. JAMIESON, late of the Glasgow and West of Scotland Technical College. FIFTH EDITION, ... 3/6

MECHANICS.

By Prof. JAMIESON. FOURTH EDITION, . . 3/6

THE STEAM ENGINE.

By Prof. JAMIESON. EIGHTH EDITION, . 3/6

METALLURGY.

By Prof. HUMBOLDT SEXTON, Glasgow and West of Scotland Technical College. SECOND EDITION, Revised, . . . 6/

PHYSICS.

A TEXT-BOOK OF PHYSICS: By J. H. POYNTING, Sc.D., F.R.S., and J. J. THOMSON, M.A., F.R.S.
Vol. I.—PROPERTIES OF MATTER.
Vol. II.—SOUND (SECOND EDITION), . 8/6

THIRD EDITION, Revised and Enlarged. Large Crown 8vo, with numerous Illustrations. 3s. 6d.

THE FLOWERING PLANT,

WITH A SUPPLEMENTARY CHAPTER ON FERNS AND MOSSES,

As Illustrating the First Principles of Botany.

BY J. R. AINSWORTH DAVIS, M.A., F.Z.S.,

Prof. of Biology, University College, Aberystwyth; Examiner in Zoology, University of Aberdeen.

"It would be hard to find a Text-book which would better guide the student to an accurate knowledge of modern discoveries in Botany. . . . The SCIENTIFIC ACCURACY of statement, and the concise exposition of FIRST PRINCIPLES make it valuable for educational purposes. In the chapter on the Physiology of Flowers, an *admirable résumé*, drawn from Darwin, Hermann Müller, Kerner, and Lubbock, of what is known of the Fertilization of Flowers, is given."—*Journal of Botany.*

POPULAR WORKS ON BOTANY BY MRS. HUGHES-GIBB.

With Illustrations. Crown 8vo. Cloth. 2s. 6d.

HOW PLANTS LIVE AND WORK:

A Simple Introduction to Real Life in the Plant-world, Based on Lessons originally given to Country Children.

BY ELEANOR HUGHES-GIBB.

*** The attention of all interested in the Scientific Training of the Young is requested to this DELIGHTFULLY FRESH and CHARMING LITTLE BOOK. It ought to be in the hands of every Mother and Teacher throughout the land.

"The child's attention is first secured, and then, in language SIMPLE, YET SCIENTIFICALLY ACCURATE, the first lessons in plant-life are set before it."—*Natural Science.*

"In every way well calculated to make the study of Botany ATTRACTIVE to the young."—*Scotsman*

With Illustrations. Crown 8vo. Gilt, 2s. 6d.

THE MAKING OF A DAISY;

"WHEAT OUT OF LILIES;"

And other Studies from the Plant World.

A Popular Introduction to Botany.

BY ELEANOR HUGHES-GIBB,

Author of *How Plants Live and Work.*

"A BRIGHT little introduction to the study of Flowers."—*Journal of Botany.*

"The book will afford real assistance to those who can derive pleasure from the study of Nature in the open. . . . The literary style is commendable."—*Knowledge.*

"Boys COULD NOT HAVE A MORE ALLURING INTRODUCTION to scientific pursuits than these charming-looking volumes."—Letter to the Publishers from the Headmaster of one of our great Public Schools.

OPEN-AIR STUDIES IN BOTANY:

SKETCHES OF BRITISH WILD FLOWERS IN THEIR HOMES.

BY

R. LLOYD PRAEGER, B.A., M.R.I.A.

Illustrated by Drawings from Nature by S. Rosamond Praeger, and Photographs by R. Welch.

Handsome Cloth, 7s. 6d. Gilt, for Presentation, 8s. 6d.

GENERAL CONTENTS.—A Daisy-Starred Pasture—Under the Hawthorns—By the River—Along the Shingle—A Fragrant Hedgerow—A Connemara Bog—Where the Samphire grows—A Flowery Meadow—Among the Corn (a Study in Weeds)—In the Home of the Alpines—A City Rubbish-Heap—Glossary.

"A FRESH AND STIMULATING book . . . should take a high place . . . The Illustrations are drawn with much skill."—*The Times.*

"BEAUTIFULLY ILLUSTRATED. . . . One of the MOST ACCURATE as well as INTERESTING books of the kind we have seen."—*Athenæum.*

"Redolent with the scent of woodland and meadow."—*The Standard.*

"A Series of STIMULATING and DELIGHTFUL Chapters on Field-Botany."—*The Scotsman.*

"A work as FRESH in many ways as the flowers themselves of which it treats. The RICH STORE of information which the book contains . . ."—*The Garden.*

OPEN-AIR STUDIES IN GEOLOGY:

An Introduction to Geology Out-of-doors.

BY

GRENVILLE A. J. COLE, F.G.S., M.R.I.A.,

Professor of Geology in the Royal College of Science for Ireland, and Examiner in the University of London.

With 12 Full-Page Illustrations from Photographs. Cloth. 3s. 6d.

GENERAL CONTENTS.—The Materials of the Earth—A Mountain Hollow—Down the Valley—Along the Shore—Across the Plains—Dead Volcanoes—A Granite Highland—The Annals of the Earth—The Surrey Hills—The Folds of the Mountains.

"The FASCINATING 'OPEN-AIR STUDIES' of PROF. COLE give the subject a GLOW OF ANIMATION . . . cannot fail to arouse keen interest in geology."—*Geological Magazine.*

"EMINENTLY READABLE . . . every small detail in a scene touched with a sympathetic kindly pen that reminds one of the lingering brush of a Constable."—*Nature.*

"The work of Prof. Cole combines ELEGANCE of STYLE with SCIENTIFIC THOROUGHNESS."—*Petermann's Mittheilungen.*

"The book is worthy of its title: from cover to cover it is STRONG with bracing freshness of the mountain and the field, while its ACCURACY and THOROUGHNESS show that it is the work of an earnest and conscientious student. . . . Full of picturesque touches which are most welcome."—*Natural Science.*

"A CHARMING BOOK, beautifully illustrated."—*Athenæum.*

A BOOK NO FAMILY SHOULD BE WITHOUT.

THIRTY-SIXTH EDITION. *Royal 8vo, Handsome Cloth,* 10*s.* 6*d.*

A DICTIONARY OF
Domestic Medicine and Household Surgery,

BY

SPENCER THOMSON, M.D., L.R.C.S. (Edin.),

AND

J. CHARLES STEELE, M.D., Late of Guy's Hospital.

THOROUGHLY REVISED AND BROUGHT DOWN TO THE PRESENT STATE OF MEDICAL SCIENCE

BY

ALBERT WESTLAND, M.A., M.D.,
AUTHOR OF "THE WIFE AND MOTHER";

AND

GEO. REID, M.D., D.P.H.,
MEDICAL OFFICER TO THE STAFFORDSHIRE COUNTY COUNCIL.

With a Section on the Maintenance of Health and the Management of Disease in Warm Climates by JAS. CANTLIE, M.A., M.B., F.R.C.S., Lecturer on Applied Anatomy, Charing Cross Hospital; Surgeon, West-end Hospital for Nervous Diseases; formerly Surgeon, Charing Cross Hospital; Dean of the College of Medicine, Hong Kong, &c., &c.;

AND AN

Appendix on the Management of the Sick-room, and many Hints for the Diet and Comfort of Invalids.

In its new Form, DR. SPENCER THOMSON'S "DICTIONARY OF DOMESTIC MEDICINE" fully sustains its reputation as the "Representative Book of the Medical Knowledge and Practice of the Day" applied to Domestic Requirements.

The most recent IMPROVEMENTS in the TREATMENT OF THE SICK—in APPLIANCES for the RELIEF OF PAIN—and in all matters connected with SANITATION, HYGIENE, and the MAINTENANCE of the GENERAL HEALTH—will be found in the Present Issue in clear and full detail; the experience of the Editors in the Spheres of Private Practice, of Hospital Treatment, of Sanitary Supervision, and of Life in the Tropics respectively, combining to render the Dictionary perhaps the most thoroughtly practical work of the kind in the English Language. Many new Engravings have been included—improved Diagrams of different parts of the Human Body, and Illustrations of the newest Medical, Surgical, and Sanitary Apparatus.

*** *All Directions given in such a form as to be readily and safely followed.*

FROM THE AUTHOR'S PREFATORY ADDRESS.

"Without entering upon that difficult ground which correct professional knowledge and educated judgment can alone permit to be safely trodden, there is a wide and extensive field for exertion, and for usefulness, open to the unprofessional, in the kindly offices of a *true* DOMESTIC MEDICINE, the timely help and solace of a simple HOUSEHOLD SURGERY, or, better still, in the watchful care more generally known as 'SANITARY PRECAUTION,' which tends rather to preserve health than to cure disease. 'The touch of a gentle hand' will not be less gentle because guided by knowledge, nor will the *safe* domestic remedies be less anxiously or carefully administered. Life may be saved, suffering may always be alleviated. Even to the resident in the midst of civilisation, the 'KNOWLEDGE IS POWER,' to do good; to the settler and emigrant it is INVALUABLE."

"Dr. Thomson has fully succeeded in conveying to the public a vast amount of useful professional knowledge."—*Dublin Journal of Medical Science.*

"The amount of useful knowledge conveyed in this Work is surprising."—*Medical Times and Gazette.*

"WORTH ITS WEIGHT IN GOLD TO FAMILIES AND THE CLERGY."—*Oxford Herald.*

LONDON: CHARLES GRIFFIN & CO., LIMITED, EXETER STREET, STRAND.

ΕΟ/5
ΣΚ/5