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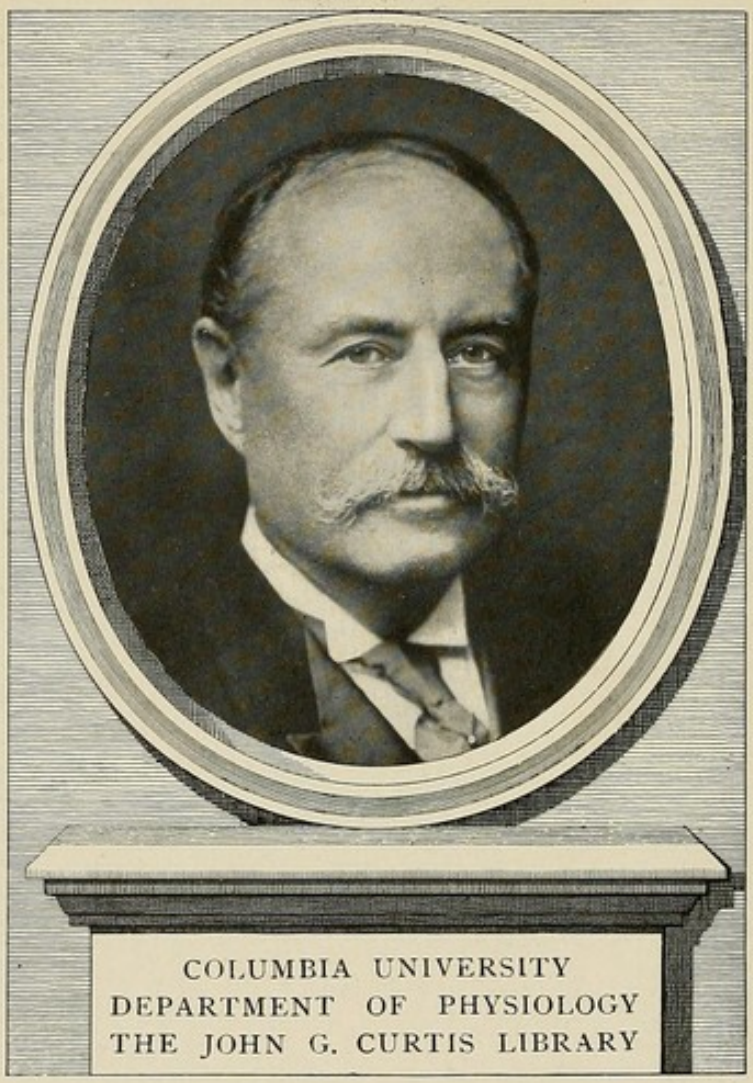

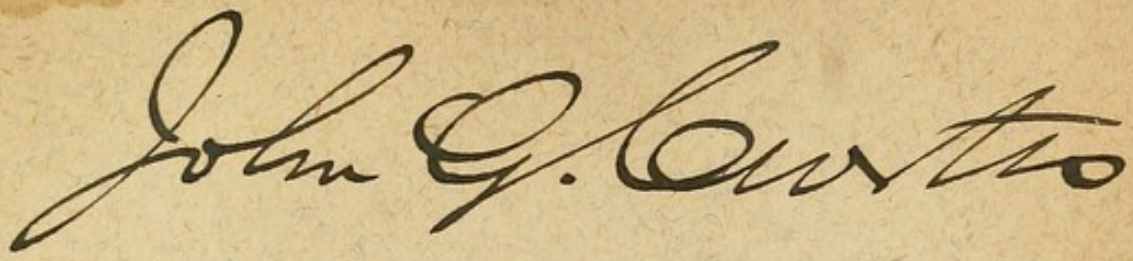

EXERCISES

IN

PRACTICAL PHYSIOLOGY

BY

AUGUSTUS D. WALLER, M.D., F.R.S.

PART I.

ELEMENTARY PHYSIOLOGICAL CHEMISTRY

BY

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LONGMANS, GREEN, AND Co.

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ELEMENTARY PHYSIOLOGICAL CHEMISTRY.

GENERAL DIRECTIONS.

Ascertain the reaction of every fluid that you test, by dipping strips of litmus paper in it. Acid fluids make neutral, or blue litmus paper red. Alkaline fluids make neutral, or red litmus paper blue.

To neutralise a fluid,—add a ·1 per cent. solution of caustic soda, or a ·1 per cent. solution of hydrochloric acid, until it does not change the colour of neutral litmus paper.

The specific gravity of a fluid is measured by immersing a hydrometer in it, and noting to what degree the instrument sinks.

Unless otherwise directed, you are to use fresh material for each reaction.

EXAMINATION OF THE MORE IMPORTANT PROXIMATE PRINCIPLES.

PROTEIDS.

General Reactions.

Directions.	*Result.*
1 Burn a little dry proteid with soda-lime in a spoon	It becomes charred, and smells of ammonia.
The charring proves the presence of carbon, the smell shows that nitrogen is present.	
2 *Xanthoproteic reaction.* Add nitric acid to a proteid solution and boil	A yellow colouration or precipitate appears.
then cool, and add ammonia	The colour deepens to orange.
3 *Biuret reaction.*[1] Add one drop of copper sulphate solution, and one volume[2] of strong caustic soda solution	A violet or pink colour results.
4 Add $\frac{1}{4}$ volume of Millon's re-agent ..	A precipitate is formed.
then boil the solution	The precipitate becomes reddish.
5 Add $\frac{1}{4}$ volume of potassium ferrocyanide solution, and excess (one volume or more) of acetic acid	A precipitate appears.
Peptones and some albumoses do not give this reaction.	
6 Saturate[3] with sulphate of ammonium ..	A precipitate forms.
Peptones are not precipitated.	

[1] Properly speaking, the term "biuret reaction" is applicable only to the pink colour obtained with digested proteids, and with biuret itself.

[2] "One volume" means an equal bulk.

[3] You will not really *saturate* the fluid. To do so would require prolonged agitation. It will be enough for you to shake the solution for five minutes with half its bulk of crystals. You may see the gradual formation of precipitate by pouring fluid on to a layer of crystals and leaving it undisturbed in a test tube. As the salt dissolves a turbid ring forms at the junction of fluid and crystals. This applies also to the globulin test by magnesium sulphate.

Special Reactions.

	Directions.	*Result.*
	Native albumins (*solutions of egg albumin, and of serum albumin free from globulins*).	
7	Faintly acidify and boil	The albumin is coagulated.
8	Saturate with sulphate of magnesium ..	The albumin is *not* precipitated.
9	Acidify, add one volume of ether, and shake	Egg albumin is usually coagulated. Serum albumin is *not* usually coagulated.
	Globulins (*solution of serum globulin, or of muscle globulin*).	
10	Faintly acidify, and boil	The globulin is coagulated.
11	Saturate with sulphate of magnesium ..	The globulin is precipitated.
12	Let a few drops fall into distilled water ..	A cloud, *i.e.*, a faint precipitate of globulin appears.
	Derived albumins (*prepared by treating native albumins, or globulins with dilute acid or alkali*).	
13	Take reaction	It is acid, or alkaline, *not* neutral.
14	Boil	Derived albumins are not coagulated.
15	Cautiously neutralise, shaking after each addition of neutralising re-agent,	Derived albumins are precipitated.
	then add slight excess of the neutralising re-agent	The precipitate re-dissolves.
	Albumoses—	
16	Faintly acidify, and boil	Albumoses are not coagulated.
17	Apply biuret test	A pink colour results.
18	Saturate with sulphate of magnesium ..	Some albumoses (primary albumoses) are precipitated.
19	Cautiously add nitric acid, or salicyl-sulphonic acid, and shake	A precipitate forms.
	then heat the test tube	The precipitate dissolves.
	and then cool the test tube under the tap	The precipitate re-appears.
	Peptones—	
20	Faintly acidify, and boil	Peptones are not coagulated.
21	Apply the biuret test	A pink colour results.
22	Add nitric acid	Peptones are not precipitated.

COMPOUND PROTEIDS.

	Directions.	*Result.*
	Mucin (*a compound of proteid and sugar*).	
23	Boil	Mucin is not coagulated.
24	Apply xanthoproteic test	Yellow colour results.
25	Add acetic acid	Mucin is precipitated, and does not re-dissolve in excess of the acid.
	Nucleo-albumins (*compounds of proteid with nuclein*).	
26	Apply the xanthoproteic test	An orange colour results.
27	Add acetic acid	Nucleo-albumins are precipitated.
28	Saturate with magnesium sulphate ..	Nucleo-albumins are precipitated.

Nucleo-albumins react very much like globulins, and like mucin.

	Oxyhæmoglobin (*a compound of proteid with hæmatin, and oxygen.*	
29	Boil	The proteid is coagulated, and the hæmatin (brownish pigment) liberated.
30	Dilute and examine with a spectroscope ..	Two dark absorption bands are seen between the yellow and green of the spectrum.
31	Add sulphide of ammonia to remove the oxygen, and then examine as in 30	The two bands vanish, and a single, broader, fainter band appears in their place. The oxyhæmoglobin is reduced to hæmoglobin.
32	Add tincture of guaiacum, and ozonic ether	A blue colour is developed.

ALBUMINOID.

	Gelatin—	
33	Warm the jelly	It becomes fluid.
	then cool the fluid under the tap	It gelatinises.
34	Apply the biuret test	A violet colour results.
35	Add nitric acid	Gelatin is not precipitated.
	then complete the xanthoproteic reaction	A yellow colour results.
36	Apply reaction No. 5	Gelatin is not precipitated.

If you do not dilute sufficiently, you may see a single very distinct broad absorption band. You must not confuse this with the single faint band of reduced hæmoglobin.

SUMMARY OF THE CHIEF REACTIONS OF THE PROTEIDS.

Coagulate on boiling	Egg albumin	Coagulated by ether, not precipitated by sulphate of magnesium.
	Serum albumin....	Not coagulated by ether, not precipitated by sulphate of magnesium.
	Globulins	Precipitated by saturation with sulphate of magnesium.
Do *not* coagulate on boiling	Derived albumins..	*Violet* biuret reaction. Precipitated on neutralisation.
	Albumoses	*Pink* biuret reaction. Precipitated by nitric acid and by salicyl-sulphonic acid, the precipitate disappearing with heat, and re-appearing on cooling the solution. Precipitated on saturation with sulphate of ammonium.[1]
	Peptones.....	Pink biuret re-action in filtrate after saturation with sulphate of ammonium.

FAT.

Directions.	*Result.*
37 Shake up some rancid oil (*i.e.*, liquid fat *plus* fatty acid) with water	The fat is neither dissolved nor emulsified.
then add some alkali and shake again	The fat is not dissolved, but is emulsified.
38 Shake up one volume of oil with four or five volumes of ether	The fat dissolves.
then pour a little of the ethereal solution on a piece of paper	The ether evaporates leaving a permanent greasy stain.
39 Take one or two drops of emulsified oil, and boil with alkali	The fat dissolves, yielding glycerine, and forming soap.

[1] Subject to the qualifications adverted to in Part II.

CARBO-HYDRATES.

	Directions.	*Result.*
	Starch—	
40	Acidify and add iodine solution	A blue colour results.
	then heat the blue fluid	The colour disappears.
	and then cool the colourless solution ..	The colour comes back.
	Glycogen—	
41	Acidify and add iodine solution	Reddish brown colour.
	then heat the brown fluid	The colour vanishes.
	and then cool the colourless solution ..	The colour *often* reappears.
42	Add two or three volumes of alcohol ..	Glycogen is precipitated.
	Dextrin –	
43	Acidify and add iodine solution	Reddish brown colour.
	then heat the brown fluid	The colour vanishes.
	and then cool the colourless solution ..	The colour *may* reappear.
	Dextrose—	
44	*Trommer's test.*	
	To some caustic soda in a test tube, add one or two drops of copper sulphate ..	A bluish precipitate (cupric hydrate) forms.
	then add a little solution of dextrose ..	The precipitate dissolves, giving a blue solution.
	and then boil	The cupric hydrate is reduced giving a red precipitate of cuprous oxide.
45	*Moore's test.*	
	Add one volume of caustic soda solution and boil	A yellow, or brownish colour (caramel) results, according to the amount of sugar present.

Moore's test is only to be used as a rough test for dextrose in the presence of proteids.

	Lactose—	
46	Apply Trommer's test	The cupric hydrate is reduced as by dextrose.
	Saccharose—	
47	Apply Trommer's test	The cupric hydrate is *not* reduced, and so no red precipitate is formed.
48	Boil with a few drops of dilute hydrochloric acid, to convert the saccharose into reducing sugar, and apply Trommer's test.	A red precipitate results, as with dextrose.

EXAMINATION OF THE MORE IMPORTANT ARTICLES OF DIET.

BREAD.

Directions.	*Result.*	*Inference.*
49 Pour a drop of iodine solution on bread	A blue colour is developed	Starch is present.
50 Apply the xanthoproteic reaction to another piece	An orange colour results	Proteid is present.

POTATO.

51 Boil a bit of potato in water, cool, and add some iodine solution	A blue colour appears	Starch is present.

You could not detect the starch without boiling because the granules are enclosed in a coating of cellulose.

52 Apply the xanthoproteic test	Only a faint orange colour appears	Very little proteid is present.

BUTTER.

53 Shake up a little butter with some ether	It dissolves, leaving little or no residue	It is composed almost entirely of fat.

COMMERCIAL "PEPTONE."

54 To a little solution of commercial peptone, apply the biuret test	Pink colour results	The only proteids that can be present are peptones and albumoses.
55 Saturate some of the solution with ammonic sulphate.	A copious precipitate falls	Much albumose is present.
56 Remove the precipitated albumose by filtration and apply the biuret test to the filtrate.[1]	A faint pink colour may develop	If so a little peptone is present.

[1] Great excess (4 or 5 volumes or more) of caustic soda is necessary.

BEEF TEA (HOT) MADE BY BOILING MEAT AND BONES.

Directions.	*Result.*	*Inference.*
57 Cool some under the tap	It gelatinises ..	Gelatin is present.
58 Apply the xantho-proteic test	A faint yellow colour results, *not* usually *orange*	Little, if any, proteid is present.
59 Shake a little with ether and pour the ether on some paper.	A slight greasy stain may remain	If so a little fat is present.
60 Add a little sodium nitro-prusside and caustic soda	A red colour appears	
then boil	The colour fades	
and while boiling add acetic acid	A blue or green colour results	Creatinin is present.

BEEF TEA MADE WITH EXTRACT OF MEAT[1] ('LIEBIG').

61 Apply the xanthoproteic test	A faint yellow colour appears	Little, if any, proteid is present.
62 Shake a little with ether, and pour the ether on a filter paper	No greasy stain results	Fat is absent.
63 Add some iodine solution	A faint brown colour may appear	If so, a little glycogen or dextrin[2] is present.
64 To another portion add sodic nitro-prusside and caustic soda	A red colour develops	
then boil	The colour fades	
and while boiling add acetic acid	A blue or green colour appears	Creatinin is present.

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[1] This fluid has been decolourised by shaking it with animal charcoal and filtering.

[2] The body is present in such small quantity that you cannot tell whether it is glycogen or dextrin. You would expect to find the former rather than the latter.

EXAMINATION OF THE MORE IMPORTANT TISSUES AND FLUIDS OF THE BODY.

SALINE EXTRACT OF LIVER, *i.e.*, OF EPITHELIAL CELLS.

Directions.	*Result.*	*Inference.*
65 Apply the xanthoproteic test	Deep orange colour	Proteid is present.
66 Faintly acidify, and boil	Coagulation occurs	This proteid is either native albumin, or globulin, or both.
67 Saturate with sulphate of magnesium	Precipitation[1] occurs	Some of the proteid may be globulin.
68 Filter the mixture obtained in test 68 and boil	Little or no coagulation occurs	Little or no albumin is present.

BONE ASH.

69 Dissolve bone ash in dilute nitric acid, warming, to accelerate solution	Effervescence occurs, the gas being colourless	Some carbonate is present.
70 To the solution add ammonic molybdate, and boil	A copious crystalline yellow precipitate falls	Much phosphate is present.

SALINE EXTRACT OF NERVOUS TISSUE.

71 To a saline extract of brain, apply the xanthoproteic test	Orange colour results	Proteid is present.
72 Faintly acidify another portion, and boil	The proteid is coagulated	The proteid is native albumin or globulin or both.

The solids of a saline extract of brain are derived chiefly from the grey matter.

ETHEREAL EXTRACT OF NERVOUS TISSUE.

73 Pour a little of an ethereal extract of brain on a filter paper	A permanent greasy stain results	Fat is present.[2]
74 To another portion add *cautiously* some strong sulphuric acid	A cherry red colour results at the junction of the two fluids	Cholesterin is present.

The solids of an ethereal extract of brain are derived chiefly from the white matter.

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[1] The precipitation is not entirely due to globulin, but in part to nucleo-albumin.

[2] Remember that this is not wholly ordinary fat, but largely a nitrogenous, phosphorised fat,—lecithin.

BLOOD (DEFIBRINATED).

	Directions.	*Result.*	*Inference.*
	Observe the specific gravity of defibrinated blood	It is between 1050 and 1060.	
75	Dip a piece of neutral glazed litmus paper in the blood, and wash away the blood-stain.	The paper is turned blue	Blood is alkaline.
76	Dilute freely, add a little freshly made tincture of guaiacum, and ozonic ether	A blue colour is formed.	Hæmoglobin[1] is present.
77	Dilute freely[2] and examine with the spectroscope	Two dark absorption bands are seen between the yellow and green of the spectrum	Oxy-hæmoglobin was present and has been deoxygenated.
78	Add sulphide of ammonium (a reducing agent) and warm gently	The two bands vanish and a single, broader, fainter band appears in their place	
79	Pour the fluid backwards and forwards in two test tubes	The two bands temporarily reappear	The hæmoglobin has been temporarily re-oxygenated.
80	Dilute and examine with the spectroscope, blood saturated with coal gas	Two bands like those seen in No. 77 appear	Carboxy-hæmoglobin is present.
81	Add sulphide of ammonium, and warm gently	The spectrum is not altered	
82	Dry a little blood on a slide, add glacial acetic acid, cover, heat until bubbles appear, and examine with microscope	Nut-brown crystals of hæmin are seen	Hæmoglobin is present.

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[1] This is not a distinctive test for hæmoglobin.

[2] If you do not dilute sufficiently, you may see a single very distinct broad absorption band. You must not confuse this with the single faint band of reduced hæmoglobin.

BLOOD ASH.

Directions.	*Result.*	*Inference.*
83 Place blood-ash in a test tube, dissolve it in hydro-chloric acid, and add ferrocyanide of potassium	A blue colour forms	Iron is present in blood.

BLOOD SERUM (DILUTED).

84 Apply the xanthoproteic test to some of the fluid	Orange colour results	Proteid is present.
85 Boil another portion ..	Coagulation takes place	The proteid is native albumin, or globulin, or both.
86 Saturate with sulphate of magnesium	A precipitate falls	Globulin is present ("serum globulin").
87 Remove the precipitated globulin by filtration, acidify the filtrate and boil	Coagulation occurs	Native albumin is present ("serum albumin").

SALINE EXTRACT OF MUSCLE.

88 Apply the xanthoproteic test to one portion	An orange colour results	Proteid is present.
89 Boil another portion..	Coagulation occurs	The proteid is native albumin, globulin, or both.
90 Saturate with sulphate of magnesium	A precipitate falls	Globulin is present (chiefly myosin).
91 Remove the precipitated globulin by filtration, acidify the filtrate and boil	Coagulation occurs	A native albumin is present (muscle-albumin).

MILK.

Directions.	*Result.*	*Inference.*
92 Observe specific gravity	It is between 1025 and 1035	
93 Dip neutral litmus paper in the milk	It should become blue	Normal milk is alkaline.
London Milk is frequently acid.		
94 Dilute a little milk 3 or 4 times, add a few drops of *dilute* acetic acid, and warm Filter off the precipitate and put it aside.	A precipitate[1] falls.	
95 To some of the *filtrate* obtained in No. 94 apply Trommer's reaction	A red precipitate is formed	A reducing sugar is present (lactose).
96 To another portion of the filtrate obtained in No. 94 apply the xanthoproteic reaction	Orange colour results	Proteid is present (milk albumin).
97 Take the precipitate obtained in No. 94 and wash it with ether. Pour a little of the ethereal solution on a piece of paper	Permanent greasy stain	Fat is present.
98 Dissolve the residue left from No. 97, in dilute alkali and apply the xanthoproteic test	Orange colour results	Proteid is present (caseinogen).
99 To some warmed milk add a little rennet and set aside for 5 or 10 minutes	The milk clots[1] and forms "curd and whey"	

[1] The precipitate obtained by acid and the clot obtained by rennet are different bodies. The acid precipitate is "caseinogen" and is freely soluble in dilute alkali; the rennet clot is "casein" and is much less soluble in dilute alkali. Cheese is made with rennet and cannot be made with acid. Caseinogen is a nucleo-albumin.

URINE.

	Directions.	*Result.*	*Inference.*
	Observe the specific gravity	It is between 1015 and 1025.	
100	Dip a neutral litmus paper into some normal urine	It is turned red..	Normal urine is acid.
101	Do the same with some stale urine	It is turned blue..	Stale urine is alkaline.
102	Warm the paper used in No. 101	The blue fades ..	The alkalinity of stale urine is not due to fixed alkali, but to a volatile alkali (ammonium carbonate).
103	Add a few drops of silver nitrate solution and excess of nitric acid	A precipitate forms	Chlorides are present.
104	Add a few drops of barium chloride solution and excess of hydrochloric acid	A precipitate forms	Sulphates are present.
105	Add nitric acid and boil, then add ammonium molybdate solution and boil again	A yellow precipitate forms	Phosphates are present.
106	Add solution of sodium hypobromite	Bubbles of gas (nitrogen) are evolved	A nitrogenous body is present.
107	Remove the sulphates and phosphates of urine by adding $\frac{1}{2}$ volume of baryta mixture, and filtering. To a portion of the filtrate add some mercuric nitrate solution	A precipitate forms (a compound of urea and mercury)	Urea is present.
108	To another portion of the filtrate obtained in No. 107, add some sodium chloride and then mercuric nitrate as before	No precipitate forms until excess of mercuric nitrate has been added	The presence of sodium chloride hinders the precipitation of urea.

	Directions.	*Result.*	*Inference.*
109	Add a little sodium nitro-prusside and caustic soda	A red colour develops.	
	then boil	The colour fades.	
	and while boiling add acetic acid	Colour changes to blue	Creatinin is present.
110	*Murexide test.*— Take some deposit obtained from a highly coloured, acid urine, and evaporate to dryness[1] with a few drops of *yellow* nitric acid	As it dries it becomes reddish.	
	Then add a little dilute ammonia	The colour changes to violet	Uric acid is present.
111	*Schiff's test.*—Let a drop of an alkaline solution of uric acid fall on a filter paper, and then let a drop of silver nitrate solution fall on the paper near the other, so that they may touch	A dark line forms at the junction of the drops	Uric acid is a reducing agent.
112	Apply Trommer's test to the alkaline solution of uric acid, *not* to urine.	A red precipitate may be formed	Uric acid is a reducing agent. (This is a possible fallacy in testin urine for sugar.)

[1] Great care must be taken not to overheat the residue.

BILE.

	Directions.	*Result.*	*Inference.*
	Observe specific gravity	It is between 1020 and 1030	
113	Dip a neutral litmus paper in bile	It becomes blue..	Bile is alkaline.
114	Add acetic acid ..	A precipitate falls	Mucin[1] is present.
115	*Pettenkofer's test.*—Shake up a little bile with a grain[2] of cane sugar, and then add some strong sulphuric acid or a solution of furfural[2] may be substituted for the sugar	The froth and fluid below it are coloured purple	Bile-salts are present.
116	*Gmelin's test.*—Smear bile evenly over a white tile and let a drop of yellow nitric acid fall into the film	Concentric rings of colour appear round the drop	Bile pigments are present.
117	To a solution of white gall stones in chloroform add some strong sulphuric acid	Cherry red colour appears	Cholesterin is present.

[1] This is not wholly mucin but in part nucleo-albumin.

[2] The purple colour tends to become black if too much sugar or furfural are used.

SALIVA.

Directions.	*Result.*	*Inference.*
118 Take two test tubes A and B, and label them with your name. In A put starch solution and saliva, and in B starch solution and boiled saliva. Place both in the water bath for ten minutes. Meanwhile test saliva as follows: –		
119 Add excess of acetic acid	A precipitate falls	Mucin is present.
120 Apply the xanthoproteic test	An orange colour appears	Proteid is present.
121 Add silver nitrate and nitric acid	A precipitate falls	Chlorides[1] are present.
122 Add ferric chloride ..	A red colour appears	A thiocyanate is present.[2]
123 Divide the contents of test tube A into two parts, acidify one part and add iodine solution	A somewhat purple colour appears	Starch and dextrin are present (reddish brown, plus blue = purple).
	Or a reddish brown colour develops	In this case erythrodextrin and no starch is present.
	Or the solution remains colourless	In this case neither erythrodextrin nor starch is present.
124 To the other part apply Trommer's test	A red precipitate falls	Sugar is present (chiefly maltose).
125 Test some of the contents of tube B as in No. 123	A blue colour appears	Starch is unaltered by *boiled* saliva
126 To the remainder apply Trommer's test	No red precipitate[3] falls	

[1] Silver nitrate precipitates both mucin and albumin, and so these bodies ought, properly speaking, to be removed before testing for chlorides, but the precipitate here is too copious to be entirely due to mucin and albumin.

[2] Assuming the absence of acetate and meconate.

[3] A small precipitate *may* fall owing to presence of sugar in your saliva.

GASTRIC JUICE.

Vessels are placed on the tables labelled X and Y and containing products of gastric digestion.

	Directions.	*Result.*	*Inference.*
127	Take two test tubes C and D and place a pellet of fibrin in each. To C add dilute gastric glycerin plus an equal volume of ·3 per cent. hydrochloric acid, and to D add only ·15 per cent. hydrochloric acid. Put them in the water bath and leave for 30 minutes Meanwhile examine the fluids X and Y. In X albumin has been digested with gastric juice, for a short time, in Y for a longer time.		
128	Neutralise a little of the fluid X	A precipitate falls on neutralisation	Acid albumin is present.
129	Apply the biuret test to a little of the fluid Y	A pink colour results	The only proteids present are albumose and peptone.
130	Neutralise, and saturate another portion with ammonic sulphate	A precipitate falls	Albumose is present.
131	Remove the precipitated albumose by filtration and apply biuret test to the filtrate[1]	A pink colour results	Peptone is present.
132	Examine the tubes C and D. The fibrin in both is swollen.		
133	Filter their contents and apply biuret test to both filtrates	A pink colour results in C; a violet, in D	The fibrin in C is digested, that in D is *not* digested.

[1] In applying the biuret test to a filtrate after saturating with ammonic sulphate it is necessary to add *great* excess (three or four volumes or more) of caustic soda. This dilutes the peptone considerably and so the pink will be much less distinct than in No. 129. For this reason you would probably fail to find peptone in the fluid X.

PANCREATIC JUICE.

Vessels labelled Z are placed on the tables containing products of the pancreatic digestion of proteids.

	Directions.	*Result.*	*Inference.*
134	In a test tube E place a pellet of fibrin with a little dilute pancreatic glycerin, and an equal bulk of a 2 per cent. solution of sodic carbonate. Place in the water bath for 30 minutes.		
135	In another test tube F place some starch mucilage with pancreatic glycerin and sodic carbonate as above. Put it in the water bath for 10 minutes.		
136	In other test tubes G and H place some oil and add some blue litmus. To G add a small fragment of pancreas and place both in the water bath for 30 minutes. Whilst these " digests " are progressing, examine the fluid placed on the table ; notice its smell.		
137	Neutralise some of it, and saturate it with sulphate of ammonium	Very little precipitate falls, if any	Digestion is practically complete.
138	Remove this precipitate, if necessary, by filtration and apply biuret test	A pink colour results	Peptone is present.

	Directions.	*Result.*	*Inference.*
139	Dip the end of a wooden match into hydrochloric acid, and then into the fluid	The match may be stained red	If so indol is present.
140	Boil a little of the fluid with an equal volume of Millon's re-agent	A port wine colour may appear	If so, tyrosin is present
141	Examine the test tube E. The fibrin in it is eroded, not swollen as it was in No. 132		
142	Filter the contents of E and apply the biuret test to the filtrate	A pink colour results	The fibrin has been digested.
143	To the contents of F apply Moore's test	A yellow or brown colour results	The starch has been digested.
144	Compare the contents of G and H	In G the litmus has become red	Acid has been liberated by the pancreas.

ACTION OF INTESTINAL JUICE.

145	The vessel K contains saccharose. Apply Trommer's test to some of its contents	No red precipitate falls	The sugar present is not a reducing sugar.
146	The vessel L contained saccharose. But it has been acted on by succus entericus. To some of it apply Trommer's re-action	A red precipitate falls	The saccharose has been converted into a reducing sugar.

GENERAL HINTS FOR THE QUALITATIVE EXAMINATION OF SIMPLE FLUIDS CONTAINING SUBSTANCES OF PHYSIOLOGICAL INTEREST.

Take the reaction of the fluid. If it is coloured, examine it with the spectroscope, before and after the addition of ammonic[1] sulphide.

Then decolourise with animal charcoal, and test the fluid for proteids. If proteids are present try and find out which proteids they are.

Test also for the albuminoids and ferments.

Whether proteids are present or not, test the original fluid for—

Starch, Dextrin, Glycogen } acidifying if necessary.

Bile pigments

Uric acid { using Schiff's test if the solution is alkaline, and the murexide in any case.

Digestive ferments (proteolytic alone, and with amylolytic ferments)

Sulphates, phosphates, chlorides.

If proteids are absent test also for

Urea

Sugars

Bile salts

But if proteids are present take 25-50 cc. of the fluid, evaporate almost to dryness, extract residue with alcohol, filter, evaporate alcohol from filtrate, dissolve this residue in water, and then test for urea, sugars, and bile salts.

GENERAL HINTS FOR THE QUALITATIVE EXAMINATION OF SIMPLE SOLIDS CONTAINING SUBSTANCES OF PHYSIOLOGICAL INTEREST.

Examine microscopically, looking especially for—

Blood corpuscles,

Starch granules,

Crystals.

Make extracts with—

1 Distilled water
2 Normal saline, and a 10 per cent. solution of sodic chloride
3 Alcohol

} and examine them as directed above.

4 Ether, and look for fat.

5 Dilute acid, and look for phosphates.

If any insoluble residue remain it may be

Coagulated proteid,

Elastic tissue.

---

[1] Ammonic sulphide must not be added to an acid solution. If necessary therefore neutralise before reducing.

ESTIMATION OF SODIUM CHLORIDE IN URINE.

Mohr's Method—

You are given a burette containing a standard solution of silver nitrate, and a small flask. Place 10 cc. of urine in the flask with 100 cc. (half a flask), of distilled water, and 10 or 12 drops of potassium chromate solution. Let the silver solution run gradually into the urine, shaking the latter frequently until a *permanent* orange tinge appears in the precipitate.

Before calculating the amount of sodium chloride present, you must deduct 1 cc. from the amount of the standard solution used, because some of the silver is precipitated by the phosphates of the urine.[1] Then calculate as follows:—

Suppose that you required 10 cc. from the burette.

1 cc. of the standard solution precipitates ·01 gram of sodic chloride.

∴ 9 cc. ,, ,, ,, ·09 ,, ,, ,,

∴ ·09 gram of sodic chloride are present in 10 cc. of urine.

∴ ·9 ,, ,, ,, ,, 100 cc. ,, ,,

ESTIMATION OF THE PHOSPHORIC ACID IN URINE.

You are given a burette containing a standard solution of uranium acetate, a white basin, a white tile, and a vessel of potassium ferrocyanide solution.

Place a few drops of the ferrocyanide solution on different points of the tile. Place 50 cc. of urine in the basin, under the burette, and boil the urine; when it boils run in the standard solution gradually, stopping frequently, and testing some of the urine by dipping a glass rod in it, and then touching one of the ferrocyanide drops on the tile. When the ferrocyanide is tinged brown by the drop on the rod, the precipitation of the phosphoric acid is complete, and an excess of the uranium salt has been added. Suppose that you use 15 cc. from the burette, calculate as follows:—

1 cc. of standard solution = ·005 gram of P_2O_5.

∴ 15 cc. ,, = ·005 × 15 gram of P_2O_5.

∴ ·005 × 15 grams of P_2O_5 are present in 50 cc. of urine.

Multiply this amount by 2 and you get the percentage of P_2O_5 in the urine. Remember that P_2O_5 does not exist *free* in the urine, but in combination with bases.

---

[1] In qualitative testing for the presence of chlorides, you prevent the formation of silver phosphate by adding nitric acid to the urine. You cannot add nitric acid here, because it would prevent the appearance of the orange colour, and you would not know when the precipitation was complete.

ESTIMATION OF DEXTROSE IN URINE.

Fehling's Method—

You are given a bottle of Fehling's solution, a white evaporating dish, and a burette containing a diabetic urine diluted ten times. Place the dish on a tripod under the burette, add 30 cc. of distilled water to it, and apply heat with a Bunsen's burner. Note the level at which the solution in the burette stands, and when the Fehling boils run in the dilute urine, a few cc. at a time boiling after each addition, until the blue colour has nearly gone. Then work more cautiously, adding a few drops at a time until the Fehling is quite colourless. When all the blue is destroyed, note how much dilute urine you have used, and you will have a rough idea of the quantity required to decolourise 10 cc. of Fehling's solution. Now wash out the white dish and repeat the process adding, when the Fehling first boils, nearly enough dilute urine to decolourise it, and working gradually until you obtain exact decolourisation. To be accurate you ought to make a third determination, but for rough work two are enough.[1] Then find the average amount of solution used in your two or three determinations, and calculate the amount of sugar present in the urine as follows. Suppose that you used 12 cc. from the burette.

10 cc. of Fehling are decolourised by ·05 gram dextrose

∴ 12 cc. of *diluted* urine contain .. ·05 ,, ,,

∴ 1 cc. ,, ,, ,, .. $\frac{\cdot 05}{12}$,, ,,

∴ 100 cc. ,, ,, ,, .. $\frac{\cdot 05}{12} \times 100$ grams dextrose

∴ 100 cc. *undiluted* ,, ,, .. $\frac{\cdot 05}{12} \times 100 \times 10$ grams dextrose

In estimating sugar observe the following cautions—

1.—Do not allow the Fehling to cool before looking to see whether it is colourless, because as it cools, it gets blue again.

2.—Do not trust a determination in which you require less than 10 cc. from the burette. If less than 10 cc. of the fluid decolourise your Fehling, the urine must be still further diluted, and of course this extra dilution must be allowed for in the calculation.

3.—Do not try to be *too accurate* in the determination of the end.

---

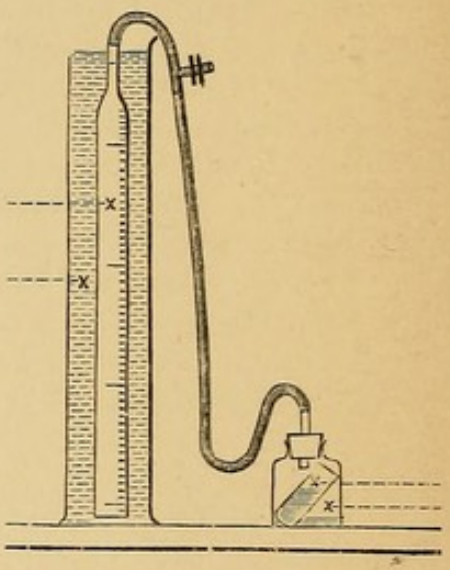
[1] If you have to do with a fluid, in which the percentage of sugar is *entirely* unknown to you, you may advantageously commence by estimating roughly and rapidly, applying the test to 1 cc. of Fehling diluted three times in a test-tube. If this is decolourised by less than 1 cc. of urine the amount of sugar is above 5 per 1,000, and the urine must be diluted. If, for example, you find with urine diluted ten times, that 2 cc. *do not*, and that 4 cc. *do* completely reduce 1 cc. of Fehling, you know that the sugar percentage in the diluted urine is between 2·5 and 1·25 per 1,000, and can then proceed to a more accurate determination within these limits.

ESTIMATION OF UREA IN URINE.

Hypobromite Method—

You are given a gas burette, standing in a tall jar of water, and connected by an india-rubber tube with a bottle, which has a small test tube in it, graduated to hold 5 cc.

Take out the graduated tube, and place 25 cc. of hypobromite of soda in the bottle. Pour urine into the graduated tube, up to the 5 cc. mark, and lower the tube carefully into the bottle containing the hypobromite solution. Then cork the bottle. This will force the level of the water inside the gas burette, below that outside it. Open the brass clip and the water in the burette will rise to the proper level. Then close the clip and note the level of the water in the burette. Now tilt the bottle gently so as to mix the urine and hypobromite solution, and the urea will be decomposed into nitrogen, water, and carbonic acid gas. The carbonic acid will be absorbed by the free alkali in the hypobromite, and the nitrogen will pass over into the burette, and depress the water inside it. Allow the bottle to cool and then read the amount of nitrogen evolved. This is done by lifting the burette until the water inside it is at the same level as the water outside it, and then noting the level of the water. Then, knowing the level at which the water stood before the experiment, you can ascertain the quantity of nitrogen evolved, and calculate the amount of urea present, as follows. Suppose that you obtain 30 cc. of nitrogen,



35·5 cc. of nitrogen is yielded by . . . . . ·1 gram of urea

$\therefore$ 1 cc. of nitrogen ,, ... $\frac{\cdot 1}{35\cdot 5}$,,

$\therefore$ 30 cc. of nitrogen ,, ... $\frac{\cdot 1}{35\cdot 5} \times 30$,,

$\therefore$ $\frac{\cdot 1}{35\cdot 5} \times 30$ gram of urea is present in 5 cc. of urine.

$\therefore$ $\frac{\cdot 1}{35\cdot 5} \times 30 \times 20$ grams of urea are present in 100 cc. of the urine, *i.e.*, the percentage of urea in the urine is 1·69.

John G. Curtis.

EXERCISES

IN

PRACTICAL PHYSIOLOGY

BY

AUGUSTUS D. WALLER, M.D., F.R.S.

PART II.

Exercises and Demonstrations in Chemical and Physical Physiology

BY

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AND

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PART II.

EXERCISES AND DEMONSTRATIONS IN CHEMICAL AND PHYSICAL PHYSIOLOGY.

CHAPTER I.

Blood.

I.—**The Hydrocele Experiment** (Dem.).—A hydrocele fluid (man) or a pericardial fluid (horse) is mixed with serum and left over night. A gelatinous clot is formed.

Some shreds of fibrin thrown into the hydrocele fluid will have the same effect.

N.B.—The experiment fails with certain specimens of hydrocele fluid. To give a positive result the fluid must contain fibrinogen, which is not always the case.

II.—**Prevention of Coagulation in Vivo** (Dem.).—A solution of albumoses is injected, *per jugulam*, into the vascular system of a dog. (For a 10 kg. dog the solution should contain between 3 and 5 gr. of albumose.) A few minutes later the dog is bled. A sample of blood taken before the albumose injection clotted quickly and firmly; a sample of blood taken after injection does not clot at all.

N.B.—The animal is deeply anæsthetised throughout the experiment, and killed during anæsthesia. This practice is observed in all cases where operations must be made in vivo.

III.—**Production of Coagulation in Vivo** (Dem.).—An *extract of thymus* is prepared as follows:—

The "thymus" of a calf, fresh from the butchers, is minced and extracted for twenty-four hours in three to four litres of a 2 per 1,000 solution of sodium carbonate, to which 15 to 20 c.c. of chloroform are added, then strained through fine calico. The extract contains about 1 per cent. of nucleo-albumin, and may be kept indefinitely if the chloroform is not allowed to evaporate off entirely. (Wright.)

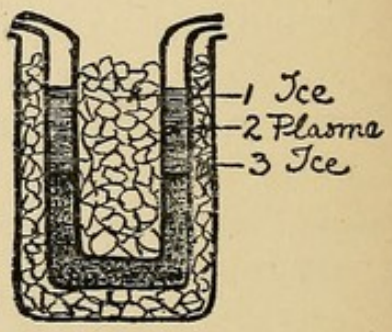
Or the gland is finely chopped and ground up in a mortar with an equal bulk of sodic chloride. The resulting sticky mass is then thrown into a large vessel of water and left for some hours. The nucleo-albumin will float, whilst the salt will sink. The nucleo-albumin is dissolved in a 1 per cent. solution of sodic carbonate. (Halliburton.)

The jugular vein of a rabbit is exposed and a cannula inserted in its cardiac end; from a burette 30 to 50 c.c. of thymus extract are injected. Observe the dyspnœa, mydriasis, and proptosis. Open up the abdomen immediately after death and examine the portal system; the blood within it is found coagulated.

IV.—**Separation of Blood-Plasma** (Dem.), "cold" plasma, "peptone" plasma, "salt" plasma, "decalcified" plasma.

To prepare blood-plasma, the coagulation of the blood is to be retarded or prevented, and the corpuscles allowed to settle down in the fluid, or made to separate by centrifugal force.

(A) "*Cold*" *Plasma* is best obtained from horses' blood. Blood from the freshly killed animal is *quickly* transferred to a nest of three metal vessels, the first and third of which are packed with ice; the blood is quickly poured into the not too roomy space between the first and second, so as to be thoroughly cooled before it has had time to coagulate. The vessels are at once put aside and left perfectly undisturbed for twenty-four hours. At the end of this time the corpuscles will have subsided, leaving an upper stratum of plasma. This plasma decanted off into a beaker at ordinary



temperature coagulates; it may be kept fluid until required in a stoppered bottle surrounded by ice.

(B) "*Salt*" *Plasma* is prepared from any small animal—dog, cat, rabbit. A cannula is inserted (during anæsthesia by chloroform) into the cardiac end of the carotid artery, and the blood is made to run into a 100 c.c. flask containing 30 c.c. of a saturated solution of magnesium sulphate. The contents are at once well mixed and then left undisturbed in a cool place for twenty-four hours. The upper layer of "salt" plasma is then pipetted or syphoned off.

(C) "*Peptone*" *plasma* is the laboratory name for what should more properly be termed "*albumose*" plasma. A dog is treated as in Dem. II., and plasma is separated by the centrifuge.

(D) "*Decalcified*" *plasma*. An animal is anæsthetised, bled from the carotid artery, and the blood received into one-tenth of its volume of a 2 per cent. solution of potassic oxalate. A second portion of the blood is drawn off, *not* treated with the oxalate, and a sample of each placed in a test tube in a water bath. The non-oxalated blood clots rapidly and firmly, whilst that treated with the oxalate remains fluid. Its coagulability can be restored by the addition of enough calcic chloride to replace the lime salts precipitated by the oxalate of potassium.

Plasma may be obtained from the remainder of the oxalated blood by removing the corpuscles with the centrifuge, and its coagulability restored by replacing the calcium salts that were rendered insoluble by the oxalate.

V.—**Centrifuge.**—Take some of the non-coagulable blood, fill the four glass tubes with it, place them in the metal buckets, and keep the disc revolving at a high speed for at least five minutes; then let the disc run down and come to rest without interference. A red cake of corpuscles is formed at the bottom of the tubes; clear plasma occupies the top of the tubes; an intermediate milky pink stratum contains a large number of leucocytes.

N.B.—The disc must be in good equilibrium, i.e., *all four test tubes must be in use and equally filled.*

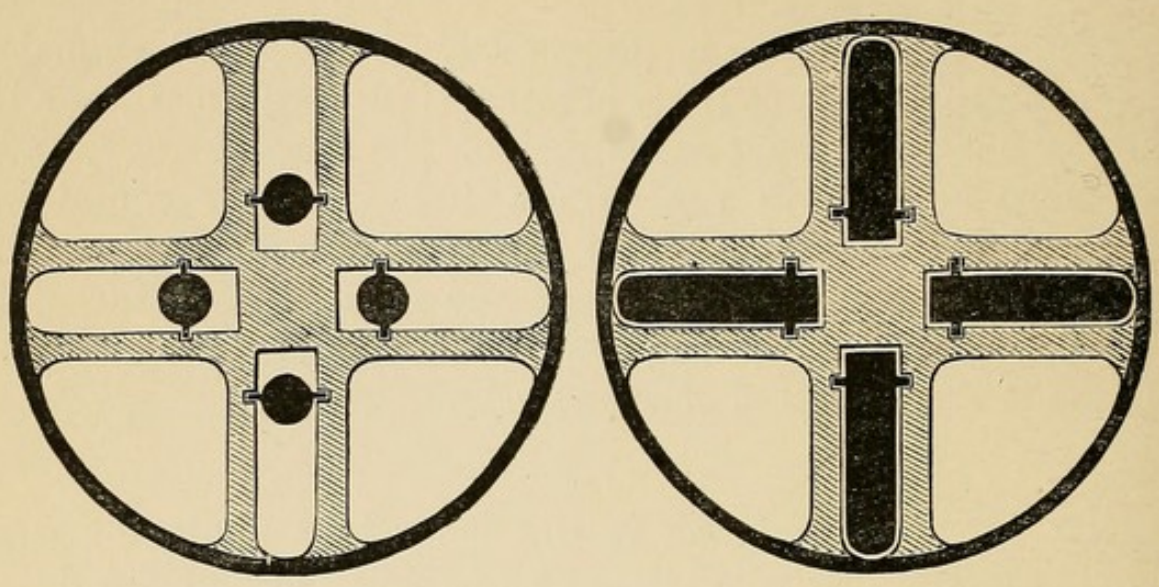


FIG. 1.—PLAN OF CENTRIFUGE AS USED FOR THE SEPARATION OF PLASMA.

Centrifugal Apparatus. Disc fitted with four long buckets pivoted so that they hang vertically when the disc is at rest, and assume a horizontal position as the disc revolves. Large test-tubes containing the blood-mixture fit into the buckets. By centrifugal force, the corpuscles collect towards the circumference, while the disc is revolving, *i.e.*, at the bottom of the test tubes; the plasma is left nearer the centre, *i.e.*, at the top of the test-tubes.

Stationary. The buckets (represented in transverse section) hang vertically from the disc.

Revolving. The buckets (represented in longitudinal section) swing out to a horizontal plane.

The centrifuge is of clinical use for the rapid separation of sediments and for the determination of the proportion of blood corpuscles in a sample of blood. For this last purpose, blood mixed with a solution of potassium bichromate is introduced into a measured capillary pipette, and then centrifuged for three minutes; the corpuscles collect at the peripheral end of the pipette, and the length of the corpuscular column thus formed is the index to the proportion of corpuscles present in the blood investigated. This instrument has received the name of "*hæmatocrite*" (Gärtner).

VI.—**Extraction of the Blood-gases** (Dem.). The determination of the volumes of carbon dioxide and oxygen that can be extracted (*e.g.*, from arterial and venous blood) with anything approaching accuracy is a very difficult matter. To this end

it would be necessary to simultaneously fill two blood-bulbs directly from the artery and vein of a living animal, without exposure of the blood to the air, and to extract the gases at once in both samples before any great alteration in the proportion of the two gases has had time to take place, owing to respiratory changes within the blood itself.

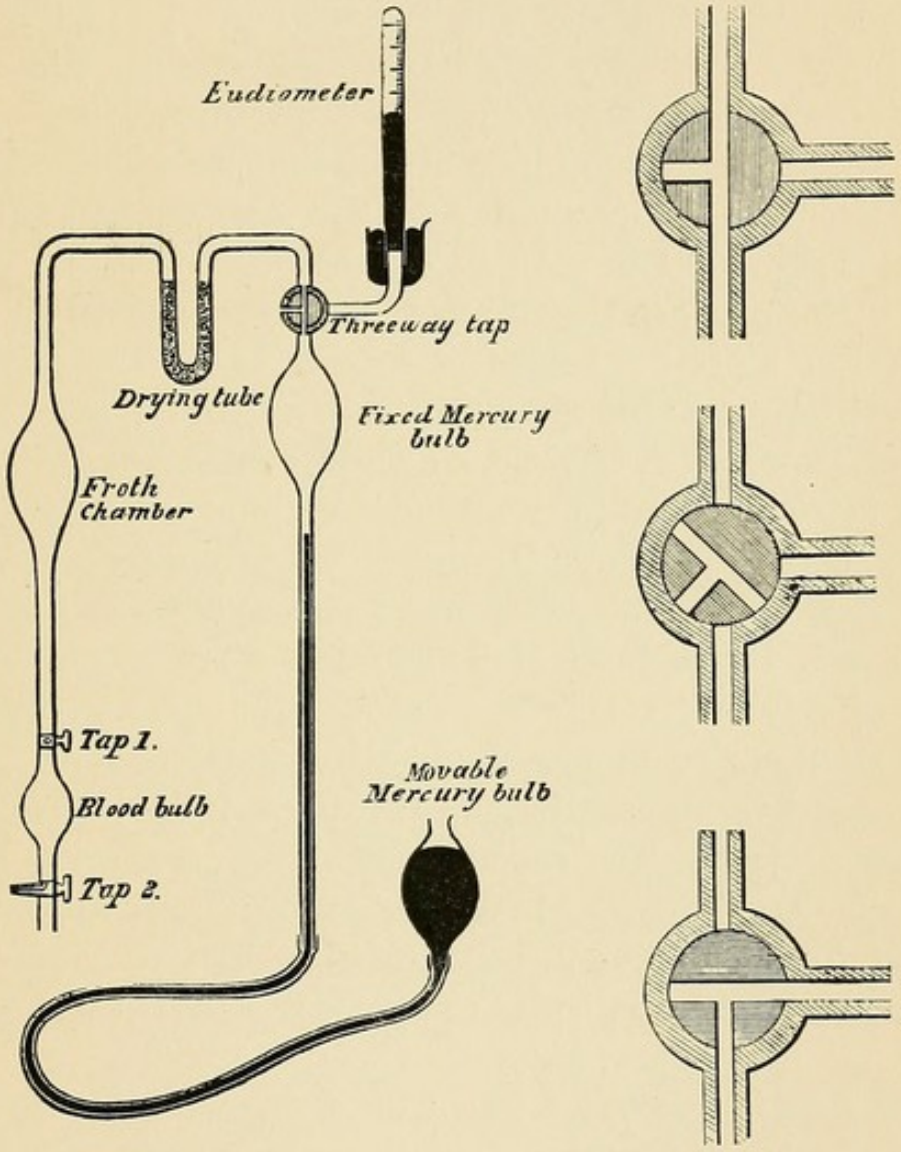


FIG. 2.—PLAN OF MERCURY PUMP FOR THE EXTRACTION OF THE GASES OF THE BLOOD.

All that is aimed at in the following experiment is a demonstration of the method of extraction and analysis, using for the purpose "Alvergniat's small blood pump" and defibrinated blood. The steps of the demonstration are as follows:—

Vacuum is established within the apparatus down to tap 1 by repeatedly (*a*) lowering the movable mercury bulb while the three-way tap is in position I., thus drawing air from the

apparatus into the fixed mercury bulb; (*b*) raising the movable mercury bulb while the three-way tap is in position III., thus driving out air from the fixed mercury bulb through the delivery tube. Vacuum having been established, the blood bulb (capacity about 20 c.c.) previously filled with blood is now put into communication with the froth chamber, &c., by opening tap 1. The gases begin to bubble out; their disengagement being promoted by immersing the bulb in warm water. The eudiometer previously filled with mercury is adjusted over the delivery tube. By lowering and raising the movable mercury bulb, as before, they are drawn off and expelled through the delivery tube into the eudiometer.

The nature and quantities of the gases collected in the eudiometer are determined as follows:—

The eudiometer is transferred to a mercury vessel, and by bringing the mercury inside the tube on a level with that outside, the volume of gas within the tube is read at atmospheric pressure. A few c.c. of strong caustic potash solution are then introduced into the eudiometer, and at the end of a few minutes, when all the CO_2 present has combined with the potash, a second reading is taken; the difference between the first and second readings gives the volume of CO_2. Finally, a few c.c. of pyrogallol solution are introduced, and at the end of half an hour or more, when all the O_2 present has combined with the pyrogallol, a third reading is taken; the difference between the second and third readings gives the volume of O_2. The residual gas is nitrogen, in small part extracted from the blood, but probably in larger part due to the introduction of air through imperfect joints of the apparatus itself.

Great care is necessary to make the taps and other joints of the apparatus air-tight. The three-way tap especially must be greased and tested before the apparatus is used; any india-rubber connection must be carefully secured and tested. Still, in spite of all care, it is often not possible to absolutely avoid leakage, which will be manifested as a too great excess of residual gas (nitrogen) after the third reading.

VII. — **The Spectroscope.** A direct vision spectroscope consists essentially of (1) a slit which controls the amount of ight that enters the instrument; (2) an achromatic lens which

parallelises the light transmitted by the slit; and (3) a train of prisms which "disperse" the constituents of the white light.

The slit is placed vertically, and its edges are parallel to one another. In the better instruments the width of the slit can be altered by turning a milled collar, but in the smaller models the width of the slit is constant. In the better spectroscopes again, the lower half of the slit is covered by a small right-angled prism so arranged as to reflect light situated at the side of the instrument through the slit, and so to enable comparison of the absorption spectra of two solutions.

The lens is movable towards and from the slit, its purpose has already been indicated.

The prisms are five in number, three of them are made of crown glass, the remaining two of flint glass. This combination makes it possible to obtain "dispersion" without "deviation," and enables you to see a spectrum by looking "directly" through the instrument at the solution under examination. The instrument is most conveniently used on a stand provided with two lights, and two niches to hold flat bottles, which contain the coloured solutions, between the spectroscope and the light (1 oz. white medicine bottles with parallel sides about 1 c.m. apart answer very well as spectroscope bottles).

(A) *Oxyhæmoglobin.*—Take defibrinated blood, dilute it ten times, and keep it as a stock solution. To identify the position of the D line, light a match previously moistened with Na_2CO_3 solution, and look at the spectrum through the flame.

Fill three or four bottles with blood diluted 10, 50, 100 times respectively, and examine—or fill a wedge-shaped vessel with blood diluted 25 times, and look through a thinner or a thicker layer. Observe that the two bands become darker and broader, and coalesce, and that the violet end of the spectrum becomes obscured with increasing strength of blood solution (in the bottles), or with increasing thickness of solution (in the wedge-vessel).

(B) *Reduced Hæmoglobin.*—Add a few drops of ammonium sulphide to an oxyhæmoglobin bottle in which the two bands are clear, and warm gently. Observe that in a few minutes the double band gives way to a single far less distinct absorption band. Notice that the colour of the solution in the bottle has changed as reduction has been effected.

(C) *Carboxyhæmoglobin.*—Pass coal-gas through blood. Notice the change of colour. Compare its spectrum with that of oxyhæmoglobin. They are almost identical, the double band of carboxyhæmoglobin being slightly nearer the violet end than that of oxyhæmoglobin.

Add ammonium sulphide and observe that the bands of carboxyhæmoglobin remain unaltered.

(D) *Methæmoglobin.*—Add a few drops of potassium ferricyanide solution, to blood ten times diluted. Notice the change of colour. Cautiously dilute further and compare the spectrum with that of oxyhæmoglobin, and notice a dark band on the red side of D. Compare with the next spectrum.

(E) *Acid Hæmatin.*—Take equal parts of acetic acid and ether in a test-tube and gradually drop into it diluted blood (1 in 10). The layer of ether takes acid hæmatin into solution; the characteristic band of its spectrum is between C and D, in the same position as that of methæmoglobin. The two bodies are distinguished by the effect of a reducing agent (ammonium sulphide), which causes the methæmoglobin spectrum to give place to that of reduced hæmoglobin, which on shaking gives way in turn to that of oxyhæmoglobin. Notice the accompanying changes of colour.

(F) *Alkaline Hæmatin.*—Mix ammonia with 3 vols. absolute alcohol in a test-tube. Drop diluted blood (1 in 10) into this solution. The characteristic band of the spectrum is on the red side of D.

(G) *Reduced Alkaline Hæmatin (Hæmochromogen).*—Add ammonium sulphide to the above solution of alkaline hæmatin. Observe that the single band in the red disappears, and that two distinct bands appear to the violet side of D.

(H) *Hæmatoporphyrin.*—Add blood drop by drop to strong sulphuric acid. Observe two dark bands in its spectrum nearer the red end than those of oxyhæmoglobin. Notice the colour.

To distinguish and identify with certainty the eight spectra enumerated above, they should be carefully compared two by two, and where necessary tested by a reducing agent (ammonium sulphide).

A spectrum with *two* characteristic bands might be produced by *oxyhæmoglobin*, *carboxyhæmoglobin*, *hæmochromogen*, or *hæmatoporphyrin*.

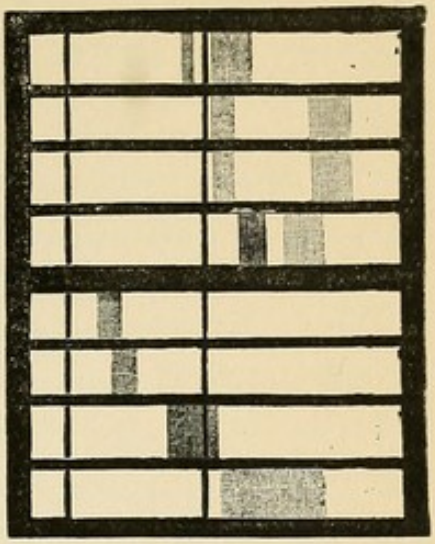


Fig. 3.

Oxyhæmoglobin and *carboxyhæmoglobin* would be distinguished from each other by adding a reducing agent. *Hæmochromogen* bands would be to the violet side, and *hæmatoporphyrin* bands to the red side of oxyhæmoglobin bands.

A spectrum with *one* distinct band line, might be due to *acid hæmatin*, *alkaline hæmatin*, *reduced hæmoglobin*, or *methæmoglobin*. A reducing agent would wipe out this band in the case of methæmoglobin or alkaline hæmatin, giving reduced hæmoglobin or reduced hæmatin; reduced hæmoglobin could be temporarily re-oxygenated by shaking the fluid.

VIII. — **The Blood Spectrum in vivo.** — Look obliquely through the spectroscope at any portion of flushed skin — finger, cheek, finger-nail—so that a strong light (direct sunlight if possible), may be reflected from the skin through the instrument to your eye.

Under these circumstances, when quite favourable, the two bands of oxyhæmoglobin will be visible. In the case of a finger, if the circulation is arrested by a ligature they may be observed to fade away owing to the reduction of oxyhæmoglobin by the living tissues.

Examine the blood (*a*) of a mouse poisoned by coal-gas, (*b*) of a mouse poisoned by nitrite of amyl vapour. The mice are decapitated with a pair of scissors, and the blood which escapes is smeared on a white plate, when it will be obvious that the blood of (*a*) is bright red in colour, while that of (*b*) is chocolate brown.

Now dilute a little of each kind of blood for spectroscopic examination, and verify that the blood of (*a*) exhibits the spectrum of carboxyhæmoglobin, and that of (*b*) the spectrum of methæmoglobin.

Finally, test these two statements :—

(1) A two-banded spectrum *per se* is not proof of the presence of blood ;

(2) Blood derivatives may fail to give any obvious absorption bands until after a reducing agent has been used ;

by examining—

(1) A solution of picro-carmine.

(2) A solution made from commercial dried blood.

The carmine solution gives a double-banded spectrum, which, however, on comparison with the oxyhæmoglobin spectrum, occupies a different position and is found to be unaltered by ammonium sulphide. The solution of dried blood may give no obvious bands, but (if so) on addition of ammonium sulphide the double band of *reduced hæmatin* becomes apparent.

Not all samples of dried blood can be counted upon for this reaction, although all will probably answer at least as regards the most prominent band of reduced hæmatin ; some samples give an oxyhæmoglobin spectrum.

IX.—**Hæmoglobinometers.**—Calculate the hæmoglobin value of your blood by means of Gower's or Oliver's hæmoglobinometer.

(A) *Gower's Hæmoglobinometer* consists of two glass tubes of equal bore, of which one contains a jelly tinted so as to represent blood diluted 100 times, the other being graduated. The apparatus is also accompanied by a blood pipette (capacity 20 cub. mm.) and a dropping bottle. Prick your finger at the root of the nail and fill the blood pipette, eject the blood into the graduated tube and add distilled water until the tint seems to you to be equal to that of the standard.

$$\text{Then : } \frac{\text{Height at which column of diluted blood stands}}{100} = \frac{\text{Quantity of Hb. present}}{\text{Normal quantity}}$$

(B) *Oliver's Hæmoglobinometer* consists of :

(1) Blood-measuring tube, capacity $= 5$ mm^3.

(2) Pipette with rubber nozzle.

(3) Blood-cell and cover glass.

(4) Set of standard tinted glasses, graduated in tens from 10 to 120 (100 being the normal).

(5) Set of "riders" graduated in units from 1 to 10.

(6) Camera tube.

Prick the finger, apply the blood-measuring tube (cleaned and dried by drawing a piece of darning cotton through it) to the drop, and let it fill by capillary attraction. Wipe the ends of the tube, and then wash out the blood into the blood-cell by means of the pipette and water. Fill the cell exactly level with the brim, mix thoroughly and cover. Place it under the camera tube by the side of the tinted scale, and match the two discs to the nearest ten, and then, by means of the "riders" placed upon the scale, to the nearest unit.

N.B.—Separate scales are to be used according as the observations are taken by natural or by artificial light. The green cover of the camera tube is for the purpose of resting the eye and rendering it more sensitive to difference of redness.

X.—**Hæmacytometers or Blood-Corpuscle Counters.**—Use either Gowers' or Thoma's counter; both these instruments are arranged to give you under a microscope a minute cubic space of diluted blood in which the corpuscles can easily be counted.

(A) In **Gowers' counter**, the blood is to be diluted 200 times, *i.e.*, fresh blood drawn into the capillary pipette up to the mark indicating 5 cub. mm. is mixed with 995 cub. mm. of sodium sulphate solution (S. G. 1025), or of normal saline solution. A drop of this diluted blood is placed in the microscopic cell, and the cover glass is put in place. The cell is 0·2 mm. deep, and its floor is divided into squares, the sides of which are 0·1 mm.; the cubic space over each square is thus 0·2 × 0·1 × 0·1 mm.^{3} or 0·002 mm.^{3}.

(B) **Thoma's counter** consists of:

(*a*) A measured capillary tube and small bulb containing a glass bead, for the proper mixture of a minute volume of blood with 100 times its volume of diluent fluid (3 per cent. NaCl.).

(*b*) A graduated counting chamber 0·1 mm. deep, with its floor marked in squares of 0·05 × 0·05 mm. Thus the cubic space overlying each square is 0·1 × 0·05 × 0·05 mm.^{3} or 0·00025 mm.^{3}; that overlying a group of 16 squares (as marked out by distinct lines) is 0·004 mm.^{3}.

Prick your finger, dilute the blood, fill the microscopic cell and count the corpuscles in one or several squares, from which strike an average and calculate what the number of corpuscles is per 1 mm.^{3} of undiluted blood.

In *Gowers' counter* the dilution is by 200 and the cubic space per square is $\frac{1}{5\,0\,0}$ mm.^{3}; so that the number per square is to be multiplied by 100,000 to give the final value per 1 mm.^{3}; With normal blood you may expect to find 40 to 50 corpuscles per square.

In *Thoma's counter* the dilution is by 100 and the cubic space per square is $\frac{1}{4\,0\,0\,0}$ mm.^{3}, per 16 squares $\frac{1}{2\,5\,0}$ mm.^{3}; so that the number per square is to be multiplied by 400,000, or the number per 16 squares by 25,000 to give the final value per 1 mm.^{3}. With normal blood you may expect to find about 10 corpuscles per square.

To count the leucocytes the blood is diluted only 10 times (in a separate pipette and bulb provided for this purpose with Thoma's instrument). A ·5 per cent. solution of acetic acid is used for this purpose, in order to clear away the red corpuscles, leaving the leucocytes alone visible. With normal blood diluted 10 times you may expect to find 4 or 5 leucocytes per group of 16 squares, on an average of several such groups, but in leucocythæmic samples you will find much higher numbers.

(C) **Oliver's Hæmacytometer.**—The percentage of blood-corpuscles is more conveniently estimated by means of Oliver's tube. This is a flattened tube to be held edgewise between the eye and a candle flame, so as to bring into view a horizontal row of flame images caused by vertical flutings of the glass when the tube contains a transparent or semi-transparent fluid.

Fill the tube with water and observe the clear row of images. Then empty and dry the tube thoroughly.

From a fresh finger prick let the blood measuring tube (capacity 10 mm.^{3}) fill with blood, and wash it into the flat tube with Hayem's fluid[1]. Continue to add fluid until a horizontal bright line (due to the row of images mentioned above) is just apparent (the blood-mixture in the tube must be made uniform by inversion after each addition of fluid; the outside of the tube must be wiped dry). Now read off the percentage on the scale, which is an empirical one of which the normal point is 100.

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[1] *Hayem's Fluid.*—Mercuric chloride, 1; sodic chloride, 2; sodic sulphate, 10; water, 400.

XI. — **Determination of the Specific Gravity of Human Blood.** You will require a series of solutions of specific gravities 1050,1051, 1052, 1053, 1054, 1055, 1056, 1057, 1058, 1059, 1060,[1] several freshly drawn out dry capillary pipettes with the points bent at right angles, a clean sharp surgical needle, a series of small cylindrical glass vessels.

Fill the series of glass vessels with appropriate solutions. Prick the skin of the finger freely at the root of the nail, avoiding any ligature or compression. Quickly draw blood into a pipette from the exuded drop, and as quickly place the pipette in the 1050 and in the 1060 solutions with its bent end horizontal and expel some blood. If the S.G. of the blood under examination is between normal limits, the red stream issuing from the pipette will fall in the 1050 fluid and rise in the 1060 fluid. This being so, proceed to a more exact determination by finding in which fluids the stream just tends to rise or to fall.

If at the first trial the blood should rise in 1050 fluid or sink in 1060, you would require to use a more extended scale of fluids (up to say 1070 and down to 1030).

[1] A convenient stock solution is composed of Barff's boroglyceride, glycerin and magnesium sulphate in distilled water with a little mercuric perchloride. From such a stock solution with S.G. 1040 a suitable series between 1030 and 1070 is made by addition of distilled water or of glycerin to lower or raise the S.G.

CHAPTER II.

Circulation.

I.—The Stethoscope. Heart sounds.
II.—The Cardiograph. The Chronograph.
III.—The Sphygmograph. Amyl Nitrite. Inhibition of Inhibition.
IV.—The Sphygmomanometer.
V.—The Plethysmograph.
VI.—Blood-pressure. The Manometer.
VII.—Action of the Cervical Sympathetic (rabbit).
VIII.—Action of the Vagus (rabbit).
IX.—Action of the Depressor (rabbit).
X.—Relation between Blood-pressure and Blood-flow. The Dromometer. The Stromuhr.
XI.—Stannius Experiments on frog's heart.
XII.—Action of the Vagus on frog's heart.
XIII.—Period of latent Stimulation (frog's heart).
XIV.—Wave of Construction (frog).
XV.—Staircase (frog's heart).
XVI.—Refractory Period (frog's heart).
XVII.—Effect of Faradisation (frog's heart).
XVIII.—Goltz's Experiment—reflex arrest of heart (frog).
XIX.—Perfusion experiments. Action of Drugs (frog's heart). Frog's web or Mesentery under microscope.

I.—**The Stethoscope** (Man).—Listen to the heart sounds at the "apex-beat," and at the second right, and third left costal cartilages.

Learn to distinguish the first and second sounds of the heart by their rhythm, and notice which of the two sounds is best heard at the two places named above.

Feel the radial pulse while you are listening to the heart's sounds, and notice that it occurs between the two, *i.e.*, after the first and before the second sound.

II.—**The Cardiograph** (Man).—A cardiograph consists of the following parts: (1) an exploring tambour, which is applied to the spot where the impulse is best felt; this tambour is

joined by a tube with (2) a recording tambour which carries a lever. The lever marks its movements on a cylinder covered with smooth paper and blackened by a smoky flame; the point of the lever rubs lightly against this blackened surface and makes a white line, or "tracing." The rate at which the cylinder rotates is determined by means of a time-marker or "chronograph." Convenient speeds for ordinary use are about 10 mm., 50 mm., and 250 mm. per second.

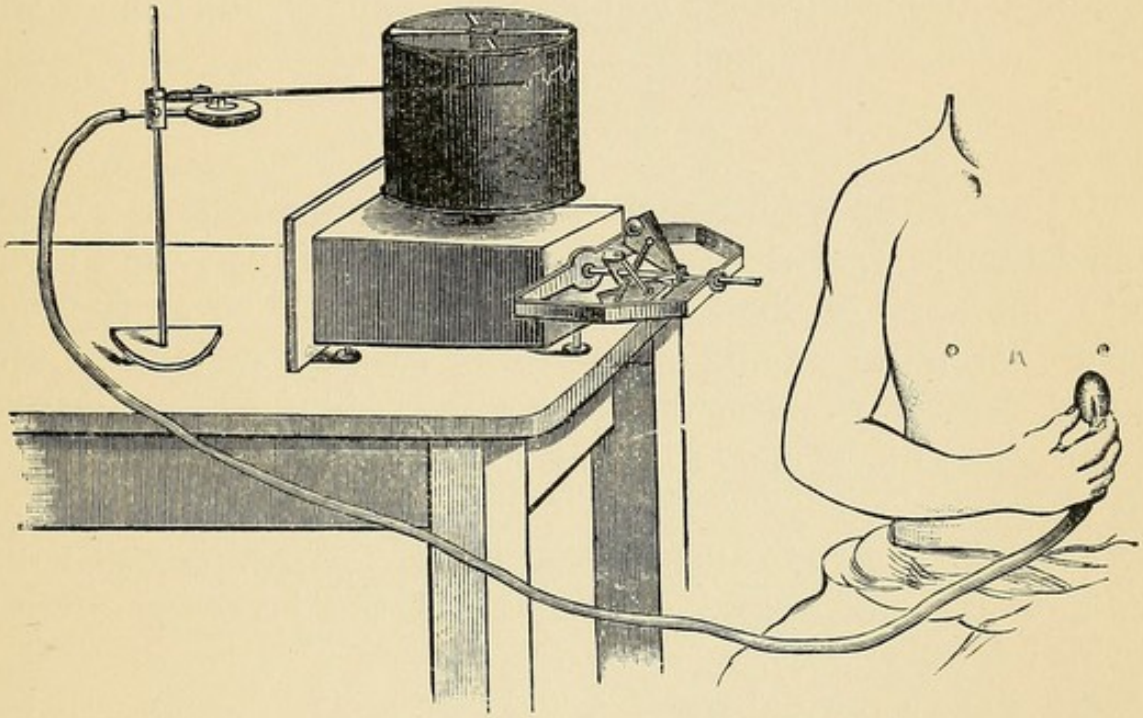

FIG. 4.

Chronographs.—First realise the principle of a time-marker as follows:—Arrange the smoked cylinder to rotate slowly. Connect a piece of india-rubber tubing closed at one end with a recording tambour and arrange the lever of the tambour against it; pinch or strike the tubing connected with it in the rhythm of a clock or watch; find out what that rhythm is (it will probably be seconds or half seconds), and measure on the cylinder the length between ten or twenty marks thus made. From this datum ascertain what length of recording surface is the equivalent of one second.

A simple chronograph is in use in the laboratory for slow rate of cylinder on the plan just adopted. The pendulum of a metronome strikes against a fixed air drum, which is joined by a tube to a recording tambour. Before you use it adjust the sliding weight on the pendulum until it strikes sixty times in the minute. To time the cylinder at higher speed use a

tuning fork vibrating 100 per second. Stick a light pen on one prong with modelling wax (a pointed scrap of paper will do), adjust the stand and fork close to the cylinder and tangential to it. Strike the fork while the cylinder is at full speed, and lightly bring the pen on the fork against it for a second or two.

It is most correct to time the surface while the record you want to measure is being taken, but you would have to use an electrical chronograph, and the means above adopted will be sufficiently accurate if you take the timing and record with the cylinder at full speed.

III.—**The Sphygmograph** (Man).—The instrument put into your hands is Ludwig's model; the lever being at right angles to the long axis of the forearm can be adjusted so as to record the radial pulse-tracing against the smoked cylinder. Prolonged records of observation are thus obtained, by means of which you will be able to study the effects of drugs, &c., upon the pulse.

Arrange the instrument over the radial artery and adjust the pressure until good excursions of the lever are obtained with each pulse beat.

(1) Take a normal tracing.

(2) While a normal tracing is in progress, sip water through a straw in order to make several acts of deglutition.

(3) While a normal tracing is in progress, inhale nitrite of amyl.

(4) Record the effects of Müller's experiment (strong inspiration with closed mouth and nose) and of Valsalva's experiment (strong expiration with closed mouth and nose) ("Introduction to Human Physiology," pp. 106, 86, and 147).

IV.—**Sphygmomanometer** (Man).—A mercurial manometer connected by thick-walled elastic tubing with an elastic finger-stall; T piece and side tube guarded by a spring clip.

To take observations of pulse-tension, first inflate the stall through the side tube and close the clip; notice the height at which the mercury now stands. Adjust the inflated finger-stall over the radial artery of the patient, and while feeling the pulse beyond the point of pressure, press the finger-stall upon

the vessel with the other hand. Read off the pressures at which the pulse is well felt and not felt. The mean between these values, *minus* the original pressure made by inflation, is an approximate value of the arterial pressure or pulse-tension in

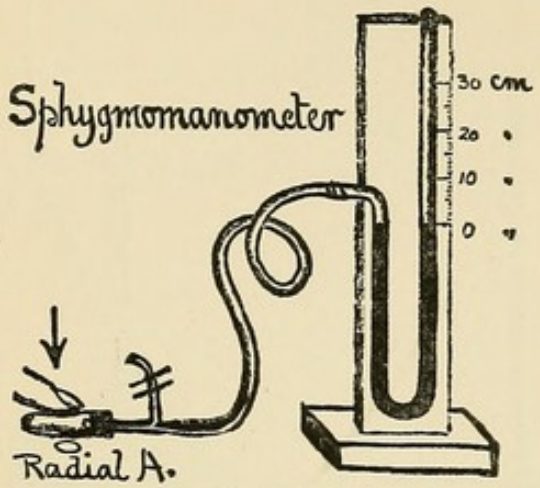

FIG. 5.

centimetres of mercury. Do not attempt too fine a determination, and remember that it may be necessary to compress the ulnar artery as well as the radial to obliterate the distal pulse in the latter vessel.

V.—**The Plethysmograph.**—The instrument supplied to you is intended for the forearm, and you may use it (*a*) filled with air or (*b*) filled with water, effecting perfect closure by inflating a double india-rubber armlet fitting between the tube and the arm.

In the first case you will connect the plethysmograph by a tube with an ordinary Marey tympanum, or preferably with a piston-recorder, and take a record of the variations of volume coinciding with the arterial pulse.

In the second case you will connect it with a large vessel of water carrying a float, that rises and falls as the arm swells and shrinks; the float raises and lets down a lever that marks against a slowly revolving smoked cylinder. In this way you may take a prolonged observation of the slow variations of volume of the forearm.

The position of the several parts of the apparatus is sufficiently indicated by the figure; movements of the limb must as far as possible, be avoided; and with the apparatus filled

with water, the two parts should be at the same level to avoid pressure of fluid on the limb.

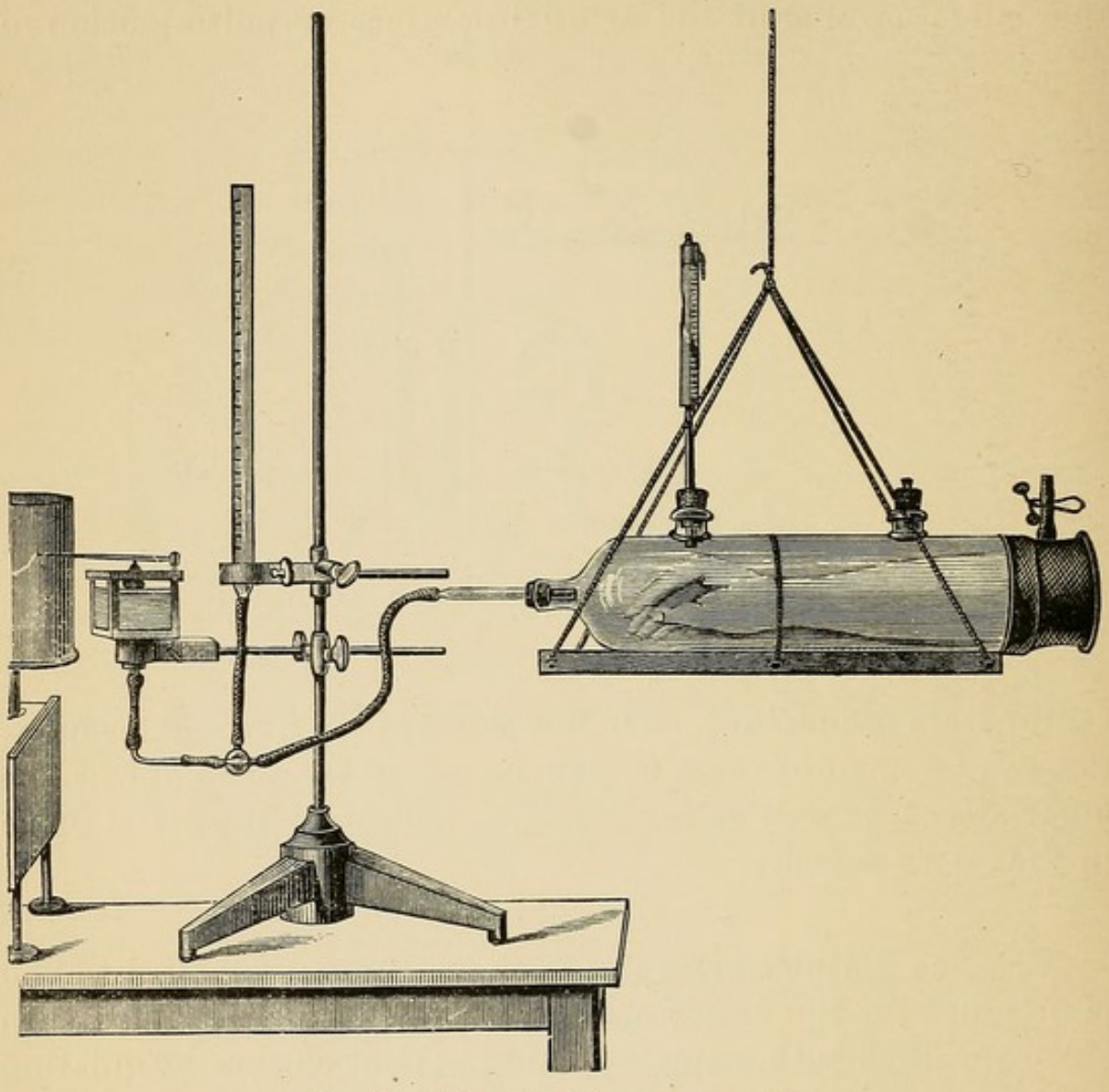

FIG. 6.—MOSSO'S PLETHYSMOGRAPH.

Test the effects upon the volume of the limb—

(1) Of Müller's experiment (strong effort of inspiration with closed air passages).

(2) Of Valsalva's experiment (strong effort of expiration with closed air passages.)

(3) Muscular contractions of the forearm.

To take this last observation in a regular and known manner, an ordinary clinical dynamometer (with the index arranged so that it works to and fro) is pressed at regular intervals to a regular amount—say at two-second intervals for a period of two minutes to a pressure of 22 lbs. (10 kg.). The limb shrinks during the contraction and then undergoes a dilatation from which it slowly subsides.

VI. — **Measurement of Blood-Pressure. The Manometer** (Dem.)—In preparing this demonstration it will be convenient to clear the vago-sympathetic and pass a loose ligature round, in order to subsequently show the effect upon the blood-pressure (*a*) of section, (*b*) of excitation of the vagus nerves.

Blood-pressure in animals (dog, cat or rabbit), is measured, and its variations recorded by means of a manometer or pressure-gauge, provided with a pen arranged to write on a moving surface. Such an apparatus is known as a kymograph.

Hering's model is represented diagrammatically below.

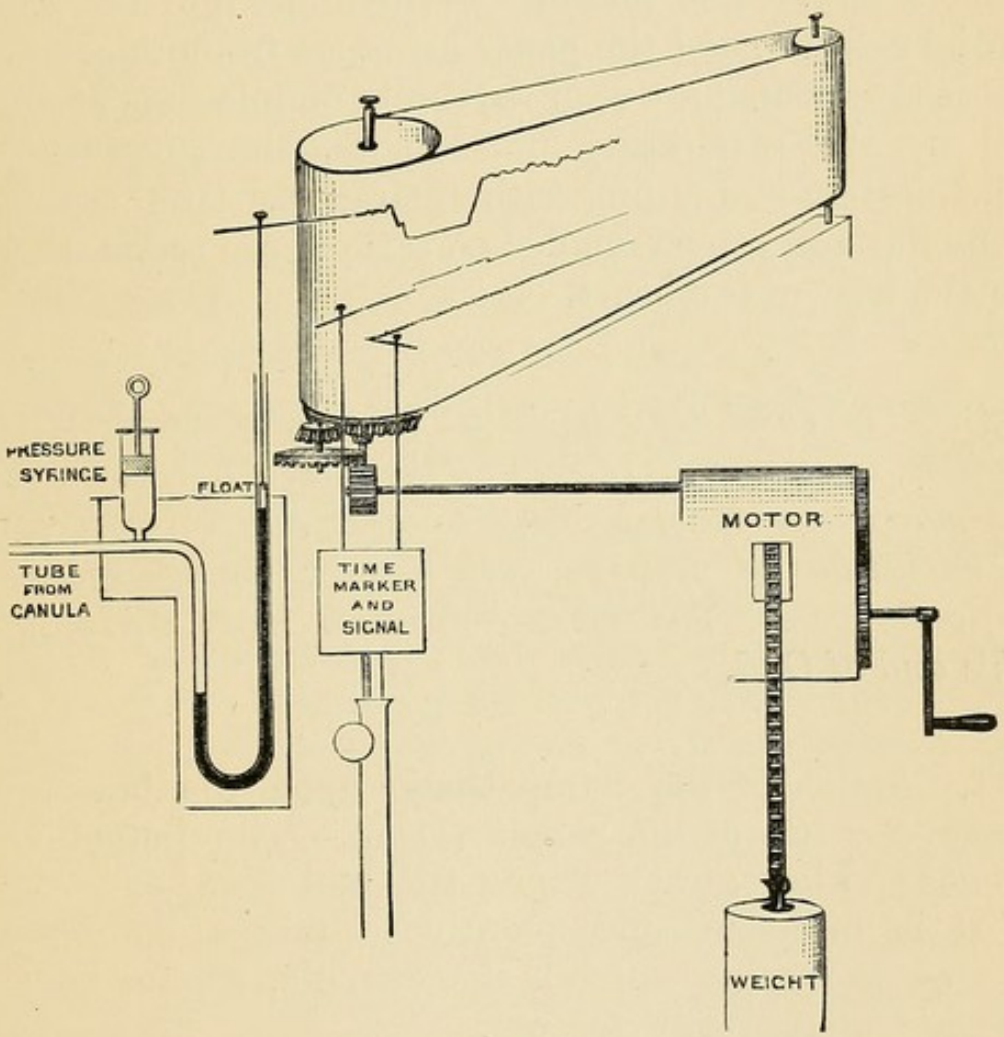

FIG. 7.

In it the manometer is a mercurial manometer, on the mercury of which, in the distal limb, floats a vertical rod, to which is attached a writing style, so arranged as to press against the smoked paper covering the rotating cylinder. The motive power is provided by a weight which in falling causes the large cylinder to rotate, and the smoked paper to move. The method of using it is as follows:—The paper is smoked,

the weight raised, the time recorder wound up, and the mercury raised by means of a syringe containing a semi-saturated solution of sodium sulphate to an extent rather less than that to which the blood-pressure would raise it. A flexible, but not distensible, pipe is then fastened to the proximal limb of the manometer and filled by the syringe with the sodium sulphate. The animal, anæsthetised by morphine and ether, is arranged conveniently, and the carotid artery exposed and clamped near its origin. An oblique cut is now made into the vessel and a glass cannula ligatured in it. The cannula is joined to the pipe leading to the manometer, and then the clamp is removed from the artery. The pressure of the blood now bears on the mercurial column, and the paper being set in motion, a tracing is produced in which are seen rhythmic undulations of pressure caused by the respiratory movements, the pressure rising with inspiration and falling with expiration. On these respiratory undulations other smaller elevations are seen; these are due to the beat of the heart.

N.B.—The chief draw-back to the use of a simple mercurial manometer, is the great inertia of the mercury. Some form of spring manometer must be used to give any correct record of rapid variations of pressure, such as occur in the systolic and diastolic periods. The most convenient models are those of Hürthle and of Gad.

VII.—**Action of the Sympathetic upon the blood-vessels and upon the iris of the rabbit** (Dem).—The induction coil, electrodes and keys, are first prepared and tested.

If it is intended subsequently to take a blood-pressure tracing on the same animal, the cannula, tubing and manometer are made ready for use.

A large rabbit is etherised under a bell-jar and fixed on its back on the rabbit-holder.

Examine the eyes and ears of the animal before exposure and section of any nerves.

A free incision is made in the middle line of the neck and the muscles separated with forceps to expose the carotid artery and accompanying nerves. The latter are freed for an inch to an inch and a half with forceps and scissors. Two ligatures are applied, a quarter of an inch apart to the middle of the ex-

posed portion of nerve, of, say, the *left* side, and the nerve divided between the two ligatures, which are left of such length that either end can be conveniently raised and laid across electrodes.

Effects of Section.—The animal is turned so that light falls equally upon both eyes. Compare the two pupils; that of the *left* side is contracted.

Examine the vessels of the two ears, holding them up between you and the light; that of the *left* side is the more congested, its vessels are dilated.

Take the temperature of the two ears, wrapping each ear similarly round the bulb of an ordinary thermometer; the temperature of the *left* is higher than that of the right ear, and the difference may be so great that you can appreciate it by holding the ears in the hand.

Effects of Excitation.—The cephalic end of the vago-sympathetic is raised from its bed and placed across the electrodes; and the exciting current sent into the nerve by raising the short circuiting key in the secondary circuit.

Examine the eyes; the left pupil is widely dilated.

Examine the ears; the left ear is pale and anæmic, its vessels are constricted.

The animal is killed at once by laying open the chest, or first utilised for the following demonstration.

VIII.—**Action of the vagus upon the heart and upon the blood-pressure. The depressor nerve (rabbit or cat)** (Dem.).—The animal used for the preceding demonstration may be further utilised as follows:—

(*A*) If it is killed at once by opening the thorax, the movements of the still beating heart may be watched, and before they have come to a stop the action of the vagus should be tested by placing the thoracic end of the vago-sympathetic across the electrodes and passing an interrupted current. The heart's movements are arrested, and on careful examination it will be seen that the arrest is in diastole. The time during which this experiment is possible may be prolonged by using artificial respiration. To this end the trachæa is laid bare, a cannula introduced into it through a scissor cut, and tied in by a strong ligature. Air is then rhythmically injected and withdrawn from the lungs by an ordinary bellows connected with the cannula by an india-rubber tube provided with a lateral opening of suitable size.

If time allows, and if the animal is vigorous, a blood-pressure tracing may be taken, postponing the above until the animal has to be killed.

(*B*) The carotid artery is exposed, (VI.) and a glass cannula introduced, and connected to the manometer.

After you have seen that the index of the manometer responds to the movements of the heart and of the chest, the thoracic end of the vagus or vago-sympathetic is laid across the electrodes, and stimulated. Observe a sudden fall of pressure; this is due to an arrest of the heart's action by the direct action of the vagus.[1]

(*C*) The cephalic end of the vagus (or vago-sympathetic) is laid across the electrodes and excited. Observe a fall of blood-pressure; this fall is due to an arrest of the heart's action by reflex action through the intact vagus, or to a reflex dilatation of blood vessels—splanchnic and other—by the excitation of "depressor" fibres. To distinguish between these two causes it would be necessary to repeat the excitation of the cephalic end of the vagus after section of the opposite vagus. A fall of blood-pressure would then be a true depressor effect.

IX.—**The Depressor.**—In the rabbit (also in the cat or dog) a particular branch of the vagus or of its branch (the superior laryngeal) can be identified and isolated. Excitation of its cephalic end causes a gradual fall of blood-pressure (after section of both vagi, *i.e.*, without reflex cardiac inhibition) which is due to reflex dilatation of the splanchnic and other vessels. The anatomy of the "depressor" nerve varies; the experiment has been successfully performed when, after section of both vagi, excitation of the central end of one vagus, or of one of its branches, has produced a fall of blood-pressure.

X.—**Experiments illustrating the relation between blood-pressure and blood-flow.**—You have learned that the velocity of the blood-flow increases with increasing heart's force and with increased freedom of the peripheral outlet.

Under the heading "Further Physical Considerations"

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[1] As here described the left vagus has been taken; it is not uncommon, however, for the right vagus to be the more effectual in cardiac inhibition.

("Introduction to Human Physiology,") you have learned further that the total heart's force (*a*) overcomes resistance; (*b*) produces onward flow.

The *lateral* or *resistance pressure* (*h*) is directly measured by a manometer, the *velocity pressure h'* is calculated from the velocity observed.

The velocity is directly measured (from dromometer indications) or calculated (from stromuhr indications). It is equal to

$$\frac{\text{Volume of outflow}}{\text{Time} \times \text{sectional area}}.$$

The sectional area of a tube is equa to the radius squared multiplied by π (viz., 3·14) or the relation of the circumference to the diameter of a circle. Sectional area $= \pi r^2$.

Exp. 1.—Attach a stiff india-rubber tube of known internal diameter (say 5 mm.) to a water tap, and time by watch how many seconds are required to fill a litre flask. You find the time to be, say ten seconds. From these data find the velocity of current through the tube.

Diameter = 5 mm. ∴Radius = 2·5 mm. ∴Sectional area $\pi r^2 = 3{\cdot}14 \times 2{\cdot}5 \times 2{\cdot}5$ sq. mm. = 16·625 sq. mm. = 0·19625 sq. cm.

Volume of outflow = 1 litre = 1000 c.c. Time = 10 secs.

$$\therefore \text{Velocity (in cm. per sec.)} = \frac{1000}{10 \times 0{\cdot}19625} = \text{about } 510$$

This is a high velocity as compared with that of the blood-stream; you have in this experiment hardly any resistance, and a higher pressure than that in the arteries.

Exp. 2.—Repeat Exp. 1 with a stromuhr on the course of the tube and a resistance at the outlet. An ordinary gas tap or a bit of glass tubing drawn out in the blow-pipe flame to give a constricted outlet, may serve the purpose of a resistance.

Assuming that this outlet is out of your reach (as it would be in the case of a blood-vessel), use the stromuhr to measure the volume of water passing through the tube in a given time, and calculate the velocity from your observed data.

Exp. 3.—Verify (approximately) that through a large tube volume of outflow and velocity of flow vary as square root of pressure.

A large Mariotte bottle is suspended from the ceiling by

a cord passing over a pulley. By india-rubber tubing and a spring clip the water from the bottle is let into a litre measure at various pressures.

Take an observation either by counting how many seconds it takes to fill the litre measure, or how many cubic centimeters are discharged into it in a given number of seconds. In either way determine volume of outflow per second at pressures of, say, 1, 4, and 9 feet.

You should find these volumes to be approximately in the proportion, 1, 2, 3, *i.e.*, that they vary as square root of pressures, $\sqrt{1}$, $\sqrt{4}$, $\sqrt{9}$.

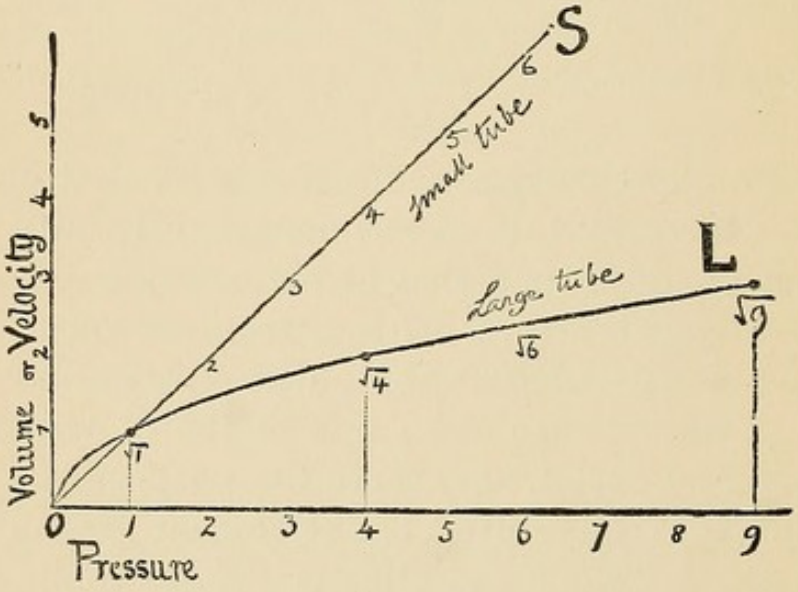
You know that velocity varies as volume, and having found that volume varies as $\sqrt{}$pressure, you also know that velocity varies as $\sqrt{}$pressure.

Exp. 4.—Verify (approximately) that in a small tube volume and velocity vary as pressure.

Apparatus as before *plus* a fine glass nozzle fixed to the end of the tube. Measure the outflow at different pressures and calculate as before.

You should find the volumes of outflow to be approximately proportional to the pressures given.

Velocity varies as volume, volume varies as pressure, therefore velocity varies as pressure.



Plot out the results of Exps. 3 and 4 on millimeter paper (or on any square scale paper), marking pressures along the abscissa and volumes or velocities along the ordinates. You will not obtain results coinciding with the ideal line O S, O L. but only results of Exp. 3 approximating to O L, and of Exp. 4 approximating to O S.

Remember that in the animal body blood-flow is ultimately governed by *small tubes*, and that therefore in the body greater pressure through means a proportionate increase of blood-flow through any vascular district in which the vessels are not altered, and a much greater increase in any district where the vessels are dilated.

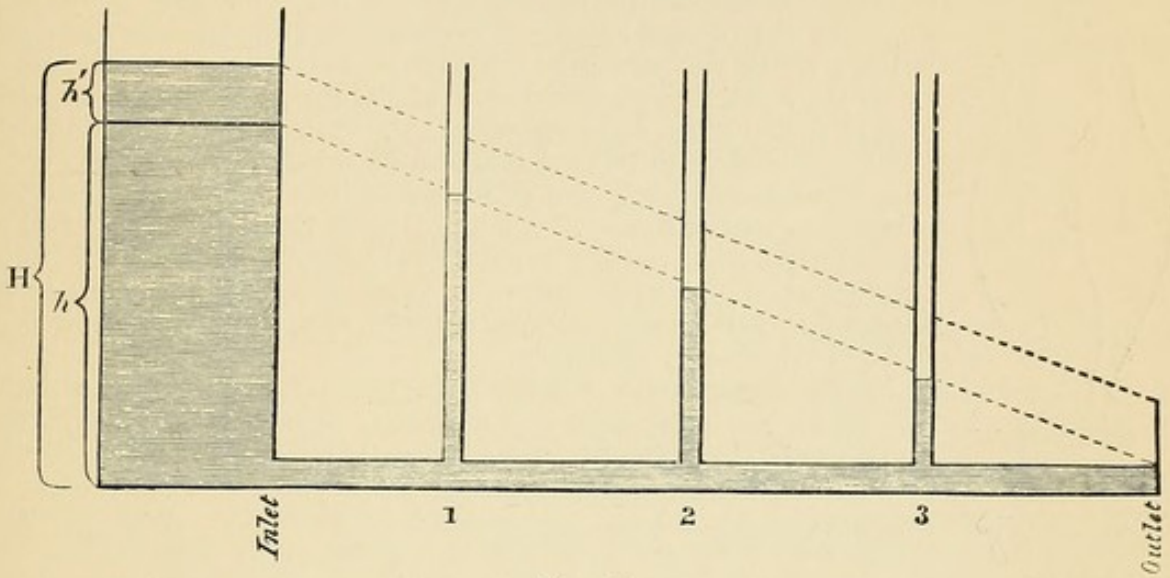


FIG. 8.

Demonstration.—Reservoir and vertical tubes to illustrate the following points. :—

(1) Decomposition of total pressure H into resistance pressure h (= lateral pressure) and velocity pressure h'.

(2) Fall of lateral pressure along a horizontal tube as shown in vertical tubes.

(3) Greater and smaller steepness of fall with greater and smaller velocity caused by smaller or greater resistance at outlet.

(4) Greater and smaller lateral pressure with greater and smaller resistance at outlet ("Introduction to Human Physiology," p. 67).

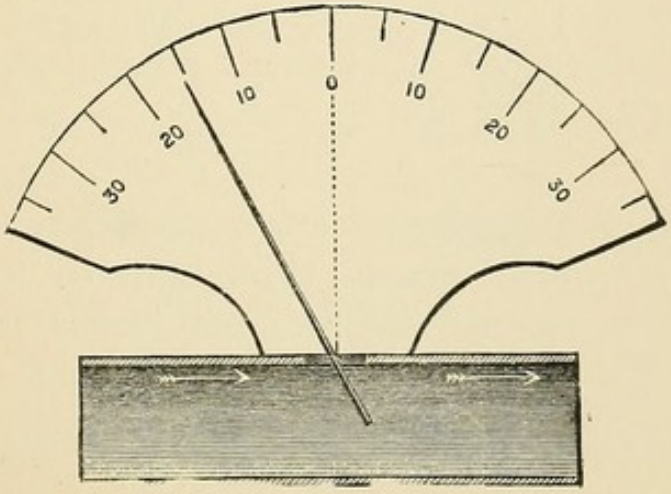
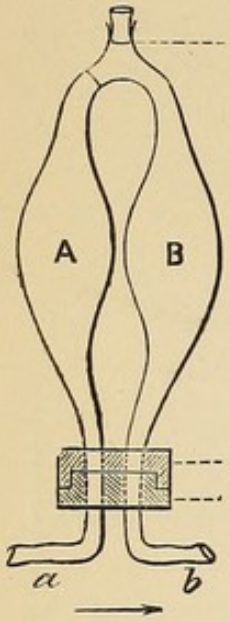


FIG. 9—CHAUVEAU'S HÆMODROMOMETER.

Demonstration.—Large model of Chauveau's hæmodromometer on a large tube fixed to a water tap. Variations of velocity with variations of peripheral resistance.

LUDWIG'S STROMUHR.



Two bulbs, A and B, fixed to the upper metal disc (1); two cannulæ, *a*, *b*, fixed to the lower metal disc (2); the upper disc movable round the lower, so that the connection between the bulbs A B and the cannulæ *a b* can be reversed, or interrupted. Cannula *a* fixed in central end of carotid, cannula *b* in peripheral end: bulb A filled with oil, bulb B with defibrinated blood. Blood from the central end of artery enters A, drives oil over to B, from which the defibrinated blood is driven into the peripheral end of artery. A being full of fresh blood, B is full of oil: the position of the bulbs is suddenly reversed by a half-revolution of the upper disc. B (full of oil) is now connected with *a*; A (full of blood) is connected with *b*. The manœuvre is repeated several times.

Given the capacity of a bulb, the number of times it has been filled and emptied, and the sectional area of the artery, the *velocity* of the blood-current is calculated—*e.g.* a stromuhr placed on a carotid artery of a small dog showed a flow of 90 c.c. per minute, *i.e.* 1·5 c.c. per second. The sectional area of the vessel was 5 square mm., *i.e.* $\frac{1}{20}$ square cm. The rapidity of the current is, then 1·5 divide by $\frac{1}{20}$, *i.e.* 30 cm. per second.

Demonstration (Ludwig's stromuhr).—A stromuhr ("Introduction to Human Physiology," p. 70), is connected to a water tap by a tube of about 2 mm. internal diameter; a similar tube leads off from the stromuhr and terminates by a tap or clamp, by means of which the flow of water can be regulated. The capacity of each bulb of the stromuhr is 10 c.c.

Having arranged the instrument with the inlet bulb full of oil and the outlet bulb full of water, start the stream by turning the tap. As soon as the oil has all been driven over from one bulb into the other, quickly reverse the bulbs. Count the number of reversals per minute.

From this datum calculate the velocity of water-flow through the tube:—*e.g.*, if 6 reversals per minute were needed, the quantity per minute was 60 c.c., and the velocity per second was about 32 c.m.[1]

XI.—**Stannius' Experiment** (Frog).—Expose the heart by removing the sternum. Pass a thick ligature under the aortic

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[1] Velocity per sec. $= \frac{\text{quantity of outflow}}{\text{time in secs.} \times \text{sectional area}}$. Sectional area $= \pi r^2$.

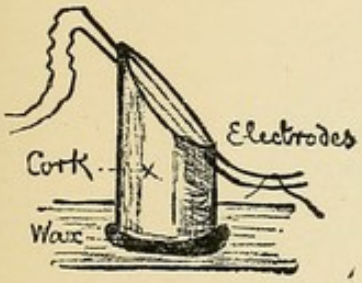
Velocity in centimetres $= \frac{60}{60 \times \cdot 0314} = 31{\cdot}8$ c.m.

bifurcation and above the superior venæ cavæ. Turn up the heart over the ligature which now lies under the sinus. Divide the frænum. Notice a white crescentic line, which is the guide to the junction of sinus and auricle. Tighten the ligature just over this line — arrest of auricle and ventricle in diastole, lasting for more than ten minutes if successful.

The ventricle of a successfully stanniused heart will recommence beating if it is divided from the auricle, by a second ligature, or by a cut.

On removal of the upper third of the ventricle the remaining portion (*i.e.*, "ventricle-apex preparation") will remain quiescent.

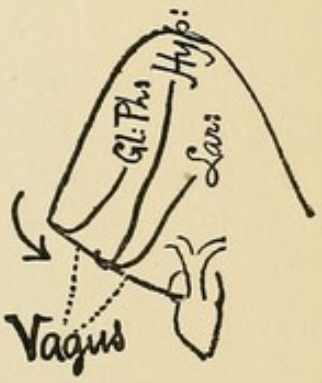
XII. — **Action of Frog's Vagus on Heart.**—Use a frog from which you have made nerve-muscle preparations for dissecting out and identifying vagi.



Arrange a cork and fine wires to serve as electrodes. Cut the cork obliquely, fix the wires in two knife cuts, fasten the cork to the frog-board by a lump of modelling wax, so that the electrodes can be conveniently adjusted to receive the nerve. Get ready the coil and adjust it to give a current just perceptible on the tongue.

Do not at first attempt to record the heart's contractions. Later, if desired you can obtain a record in the usual way, in which case see that all accessories (smoked cylinder, lever, pen, &c.) are ready before you expose the vagus. Expose the heart. Cut through the pericardium, slip a fine ligature under the heart and tie the apex, attach the ligature to a lever arranged to write on a smoked surface.

Dissection from the back and side.— Remove the skin of the back. Cut through and remove the scapula and attached muscles, including the sterno-mastoid; cut through the brachial nerves; then fix the frog on its side. The following are exposed: the petrohyoid muscle (the lower border of which is the guide to the vagus), the hypoglossal, glosso-pharyngeal, laryngeal and vagus nerves; of these the hypoglossal is the most prominent, it is easily recognised



as a large white cord running to the tongue; the vagus is an insignificant greyish filament, accompanied by a vein and pigmented connective tissue; it runs along the lower border of the petrohyoid muscles to the posterior or venous end of the heart.

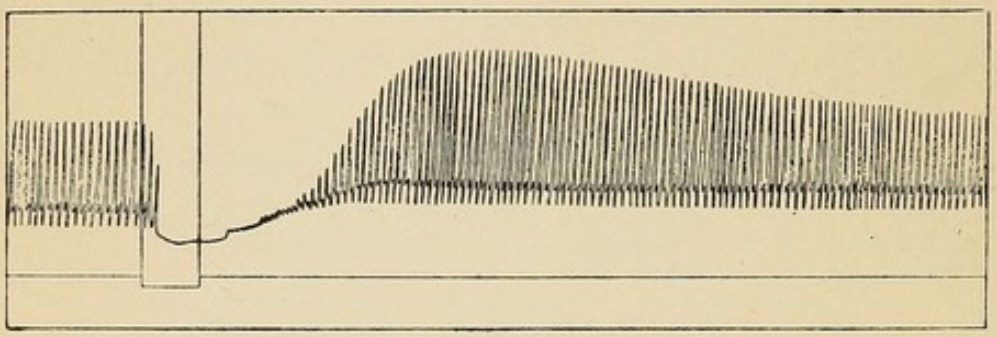


FIG. 10.—BRIEF STIMULATION OF VAGUS.

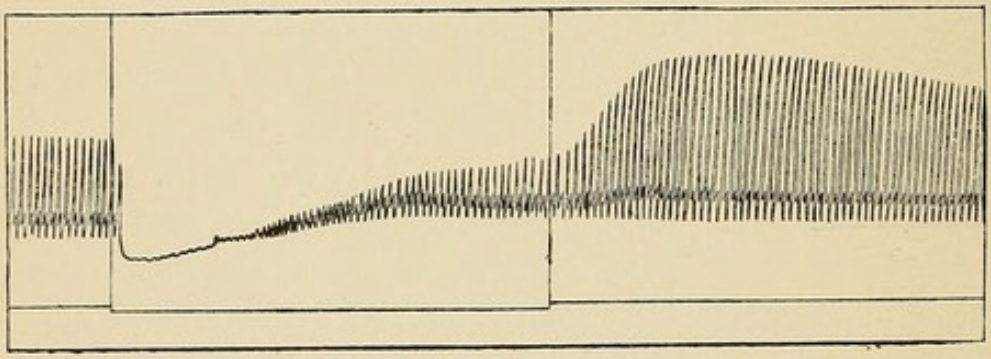


FIG. 11.—PROLONGED STIMULATION OF VAGUS.

The isolation of the nerve will be much facilitated by distending the œsophagus and by removing the projection of the lower jaw. Isolate the nerve, tie a fine thread round it as near the cranium as possible, and cut it above the ligature. As in small frogs the nerve is too delicate to isolate completely, it can be left attached to the petrohyoid muscle, in which case, cut away part of the tympanic and squamosal bones of the skull to form a handle to hold the nerve and muscle, which can then be carefully dissected out. Lay the nerve across the electrodes, and when you have satisfied yourself that the heart is beating steadily, excite the nerve by opening the key in the secondary circuit.

Observe the arrest of the heart during vagus excitation if not too prolonged.

XIII.—**Period of latent stimulation. Frog's heart.**—A. —*With electrical stimulation.*—Prepare a slip of wood and

fine wires to serve as electrodes. Fix the wires round the slip about 1 mm. distant from each other. Fix the slip in a clamp and adjust a light lever above it to be placed on the heart.

Prepare the cylinder, coil and battery, arrange a " trigger " key in the primary circuit, adjust the lever to the cylinder, and see that all is in working order.

Stannius a heart, remove it, place it on the slip of wood so that it rests upon the electrodes, and adjust the lever upon it.

If the Stannius arrest is complete, take the record of a single beat excited by an induction shock, and mark the latent period as directed in Part III.

If the Stannius arrest has failed, cut off the lower two-thirds of the ventricle and proceed as before, but with a ventricle-apex preparation instead of with a Stanniused heart.

B.—*With Mechanical Stimulation.*—Same arrangements as above with the exception of the exciting apparatus, which are not required.

FIG. 12.—LATENCY OF MECHANICALLY EXCITED BEAT (FROG'S VENTRICLE). TIME TRACING SHOWS SECONDS.

Gently touch the quiescent heart with the point of a needle while the lever upon it is in contact with the revolving cylinder. The effect of the touch will be recognisable on the record, a notch or wavelet preceding the rise of the lever caused by the contraction.

This is the simplest way of showing the latent period ; the record can be taken at any part of the cylinder ; the stimulus may be effectively applied even on the spontaneously but not too rapidly beating heart during a diastolic pause, and the difference in length of the latent period according as the touch is applied (*a*) to the auricle, or (*b*) to the ventricle, is easily tested.

The actual time values of the latent period are ascertained by a chronograph.

XIV. — **The Wave of Contraction—normal and reversed. Frog's Heart.**—Remove the heart and lay it on a slip of wood fixed in a clamp. Arrange two levers to rest upon the heart and adjust their points to write against a quick cylinder vertically above one another.[1]

Take a record of a spontaneously beating heart with :—

(*a*) One lever on the auricle, the other on the ventricle.

(*b*) Both levers on the ventricle.

Notice (*a*) that the auricular begins to rise sooner than the ventricular lever ; (*b*) that the lever near the base of the ventricle begins to rise before the lever near the apex.

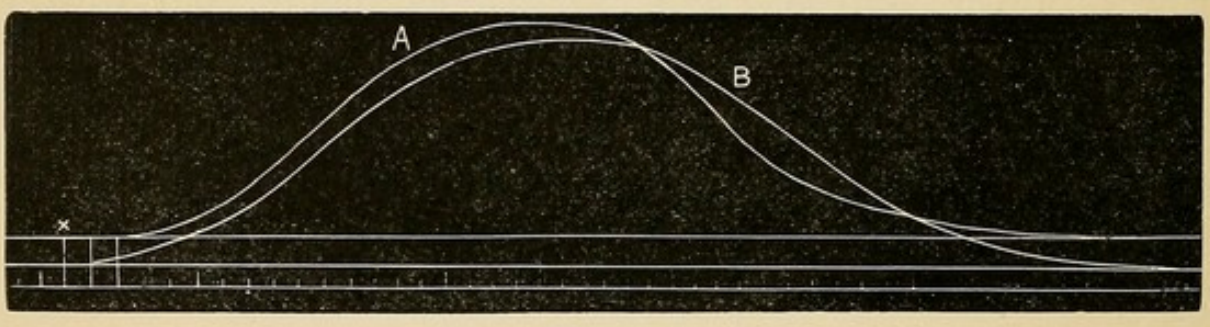


Fig. 13.

(*c*) With the levers respectively upon the auricle and the ventricle, and recording steadily choose a favourable moment, *i.e.*, the middle of a pause between two beats

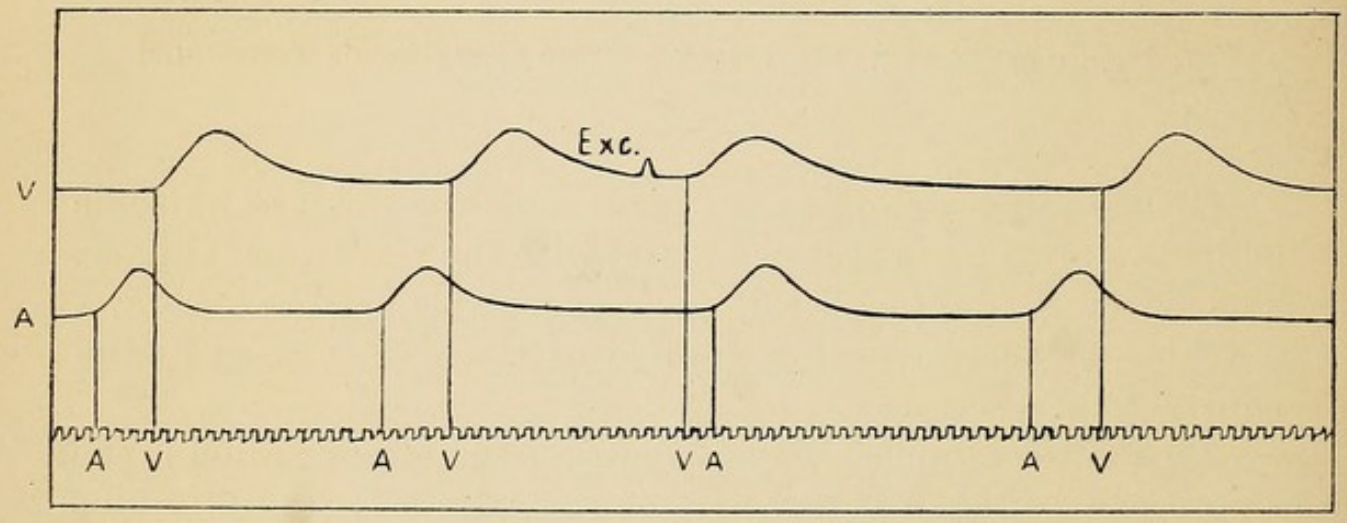


Fig. 14.

[1] The lever points need not be in the same vertical plane if "corresponding points" are carefully indicated. This is done by gently tapping the lever stand or table with the levers in position against the stationary cylinder.

for the application of a mechanical stimulus to the ventricle, *i.e.*, touch its apex with a needle point. Notice that in the beat thus provoked the ventricular has begun to rise sooner than the auricular lever. (*d*) Repeat this observation with both levers on the ventricle; notice that in a beat provoked by mechanical excitation near the apex, the nearer lever has begun to rise before the more distant lever. If the ventricle is excited near the base the two levers rise as in the normal beat. Calculate the rate of propagation of the contraction wave in the ventricle, *e.g.*, the levers being 5 mm. apart, the difference between the two initial points of rise being 2·5 mm. and the rate of revolution being 50 mm. per second the rate of propagation of the contraction wave is 100 mm. per second.

XV.—**The "Staircase Effect"—Frog's Heart.**—Use the same slip of wood, wires and lever as in the preceding exercise, and either a Stanniused or a ventricle apex preparation.

Arrange the lever to write against a slowly moving surface —about 20 mm. per minute.

Place a spring key in the primary circuit instead of the knock-over key.

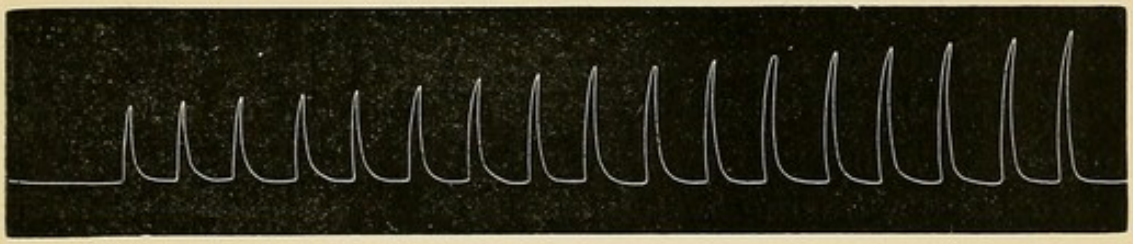


Fig. 15.

Record the effect of a series of single induction shocks applied at intervals of, say, four seconds by means of the spring key.

XVI.—**The Refractory Period. Frog's Heart.**—Same apparatus as is in preceding exercise, but with a more rapidly moving cylinder (about 5 mm. per second). Use a Stanniused heart, or an excised heart that is spontaneously beating.

With the Stanniused heart excite by induction shocks applied at longer or shorter intervals. Find a strength of shock such that the heart responds "infallibly" when stimulated every two or three seconds. Then apply two successive

shocks with a shorter and shorter interval between them, and notice that with a very short interval the second shock of each pair fails to produce any effect.

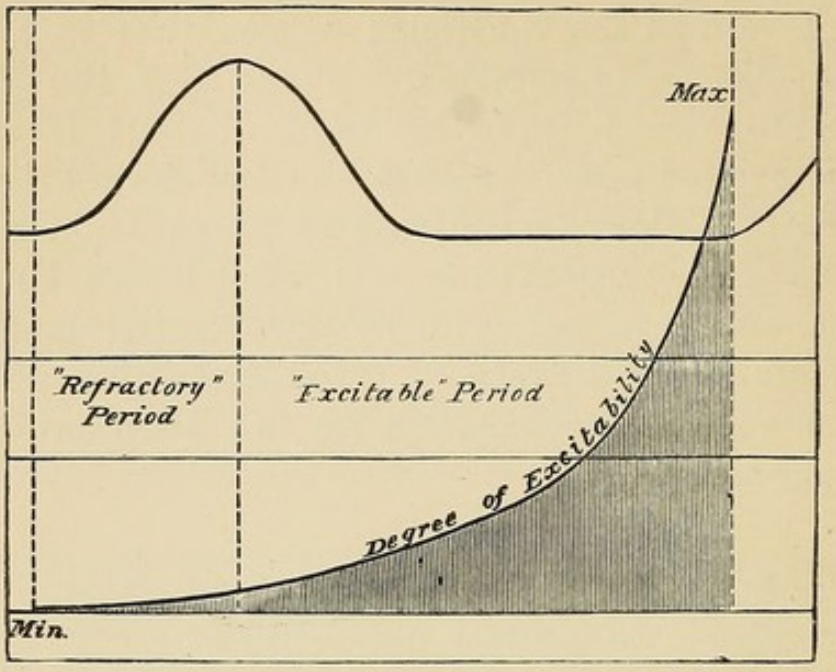


FIG. 16.

To illustrate the varying excitability of a frog's heart at different periods of systole and of diastole. The excitability is lowest during the first half of systole, greatest during the second half of diastole.

With the spontaneously beating heart watch the record and apply a single induction shock (*a*) during the diastole (*b*) during the systole, and notice that with an appropriate strength of shock a response is obtained during diastole, not during systole. Choosing your time carefully and with a somewhat stronger stimulus, you may further observe that the excitability during systole is less at the beginning than at the end of systole.

N.B.—It is convenient (but not essential) to make the stimulating key record its movement below the cardiogram; this may easily be done by fixing a lever to it and bringing the lever point vertically below that of the heart lever; the time-relation of your stimuli to the heart's phases is thus exactly recorded.

XVII.—**Effect of Faradisation. Frog's Heart.**—Same apparatus as in previous exercises. Preferably a slowly revolving cylinder.

Faradisation of a Stanniused heart gives rise to beats, not to any tonic contraction. Faradisation of a spontaneously

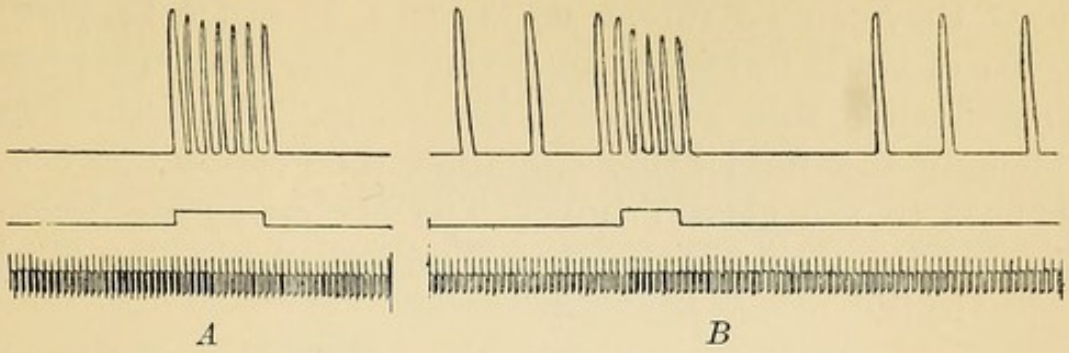


FIG. 17.—FARADISATION *A* OF QUIESCENT, *B* OF SLOWLY BEATING VENTRICLE. TIME TRACING SHOWS SECONDS.

beating heart causes either an arrest or an accelerated frequency of beat. Arrest occurs when the electrodes are in contact with the auricle, acceleration when they are in contact with the ventricle.

XVIII.—**Reflex Inhibition of the Frog's Heart** (Goltz).—Decerebrate a frog without injuring the spinal bulb and cord. Fix the frog on its back and expose the heart and the intestines. Arrange a loop of intestine over a small block of wood fixed with sealing wax to the frog-board.

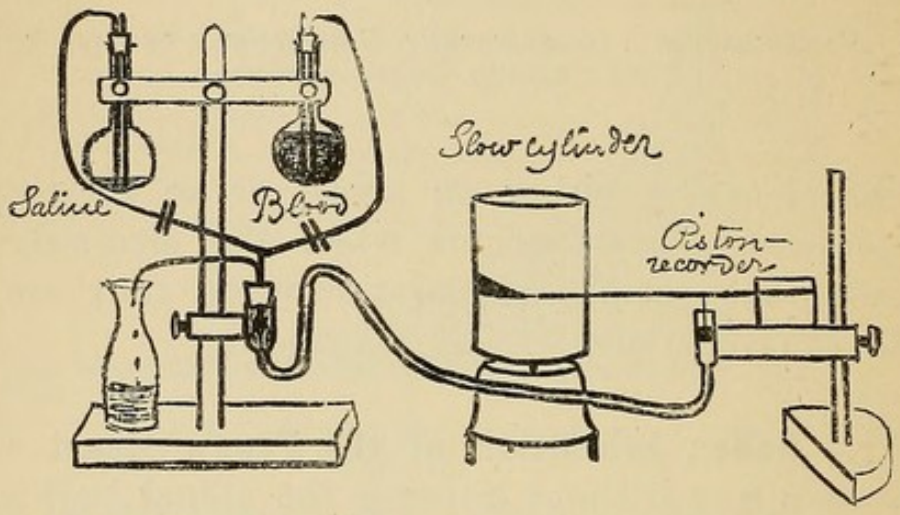
While the heart is observed to be beating regularly, tap the intestine sharply with the handle of a scalpel and notice that the heart is temporarily arrested in diastole. Then pith the frog completely and repeat the experiment; the heart is now unaffected by tapping the intestine.

N.B.—If desired, you may record the effect by connecting the heart with a light lever.

Before pithing you may test the effect of other strong centripetal stimuli, *e.g.*, section of the sciatic and "crimping" of its central end.

XIX.—**Heart Perfusion Experiments** (frog).—Cut through the sternum and expose the heart. Remove the pericardium. Turn up the heart and cut through the frænum. Insert a blunt-pointed instrument (*e.g.*, aneurism needle) through the thin wall of the sinus venosus and into the ventricle, after which it will be found easy to introduce the small nozzle of the heart cannula. Tie a ligature round the cannula as near as possible to the base of the ventricle and free the heart from the body by cutting through the aortæ and veins.

Apparatus.—Two flasks fitted with syphons fixed on a stand so that they can be raised or lowered in order to vary the pressure of liquid flowing from them. Each syphon is provided with a flexible tube on which is a clamp. Start the syphon by sucking the fluid along the tube, then clamp it. Fix the two tubes to the glass T tube. The three-way heart cannula is divided by a septum so that fluid can flow in by one tube



Frog's heart—Perfusion apparatus.

and out by the other. Avoid forcing air through the heart by filling the tubes with fluid before starting the experiment. The cannula is fixed through an india rubber cork into a wide tube containing salt solution connected with a U-shaped narrow tube which forms a manometer, and connected by india-rubber tubing to a piston recorder carrying a light glass pen by which the variations in the fluid caused by the heart beat can be indicated on a smoked surface.

The heart having been set up as above described, and a circulation of normal saline having been established through it by the two-way cannula, the order of procedure is as follows :

(1) The heart is "washed out" by the normal saline. In this process the spontaneous beats gradually become smaller and cease altogether ; when it is complete the heart should give no response to a strong break induction shock.

(2) Upon the now "washed out" heart the nutrient or non-nutrient action of a fluid can now be ascertained. A solution of dried blood or of blood-serum or dilute milk is found to restore first the electrical excitability, then the spontaneous

beats which with the progress of renutrition gradually become larger.

A solution of egg-albumin is non-nutrient. Dilute acid solutions (*e.g.*, one part H_2SO^4 to 20,000 parts fluid) quickly cause arrest in diastole, *i.e.*, have an anti-tonic action. Dilute alkaline solutions one part KHO to 20,000 parts fluid) cause arrest in systole Ringers fluid[1] has a restorative action upon a partially-washed out heart, especially if the saline used has a slightly acid reactlon.

Action of Drugs on the Heart.—The next four experiments may be made by perfusion. (Exp. XIX), or by the direct application of fluids to the outer surface of a frog's heart, simply exposed, or excised. In the latter case a record can be taken by arranging a light lever upon the heart, or by suspending a light lever from the heart; in either case the lever point is raised with systole; the record should be taken on a slowly travelling surface, either a cylinder or a glass plate. All the four observations may with care be made upon a single heart.

Muscarin and Atropin.—Apply a drop of *muscarin* solution. Observe decline of the beats and arrest in diastole.

Fig. 18.

Wash the heart with normal saline by means of a camel hair brush.

Apply a drop of *atropin* solution. Observe recovery of the beats.

Acid and alkali.—Apply a drop of an *acid* solution (1 per cent. nitric acid). Observe decline of the beats and arrest in diastole.

[1] $Ca_3(PO_4)_2$ sat. sol. in normal saline ... ... 100 c.c.
KCl 1 per cent. sol. ... ... ·5 to 1 c.c.
$NaHCO_3$ 1 per cent. sol. ... ... 1 c.c.

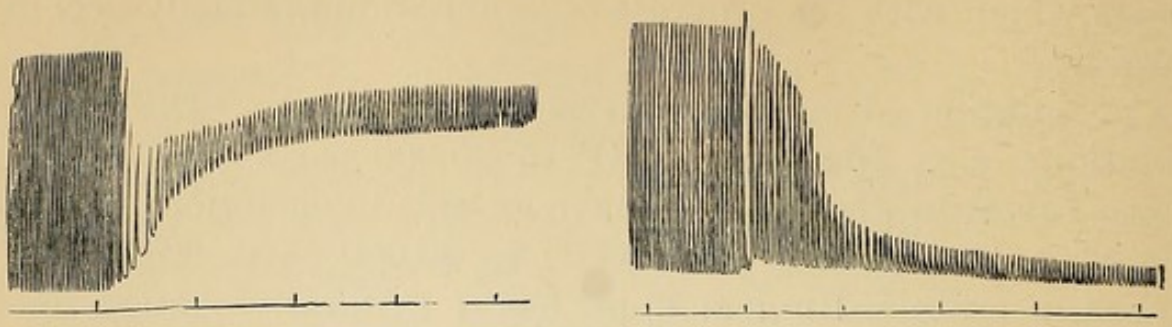


FIG. 19.

Quickly wash the heart with normal saline, and apply a drop of an alkaline solution (1 per cent. caustic potash). Observe recovery of the beats, followed by arrest in systole.

Action of Gases and Vapours on the Heart (frog).—(CO_2, Et_2O. $CHCl_3$, N_2O, NH_3, &c.). Put up a frog's heart in a simple

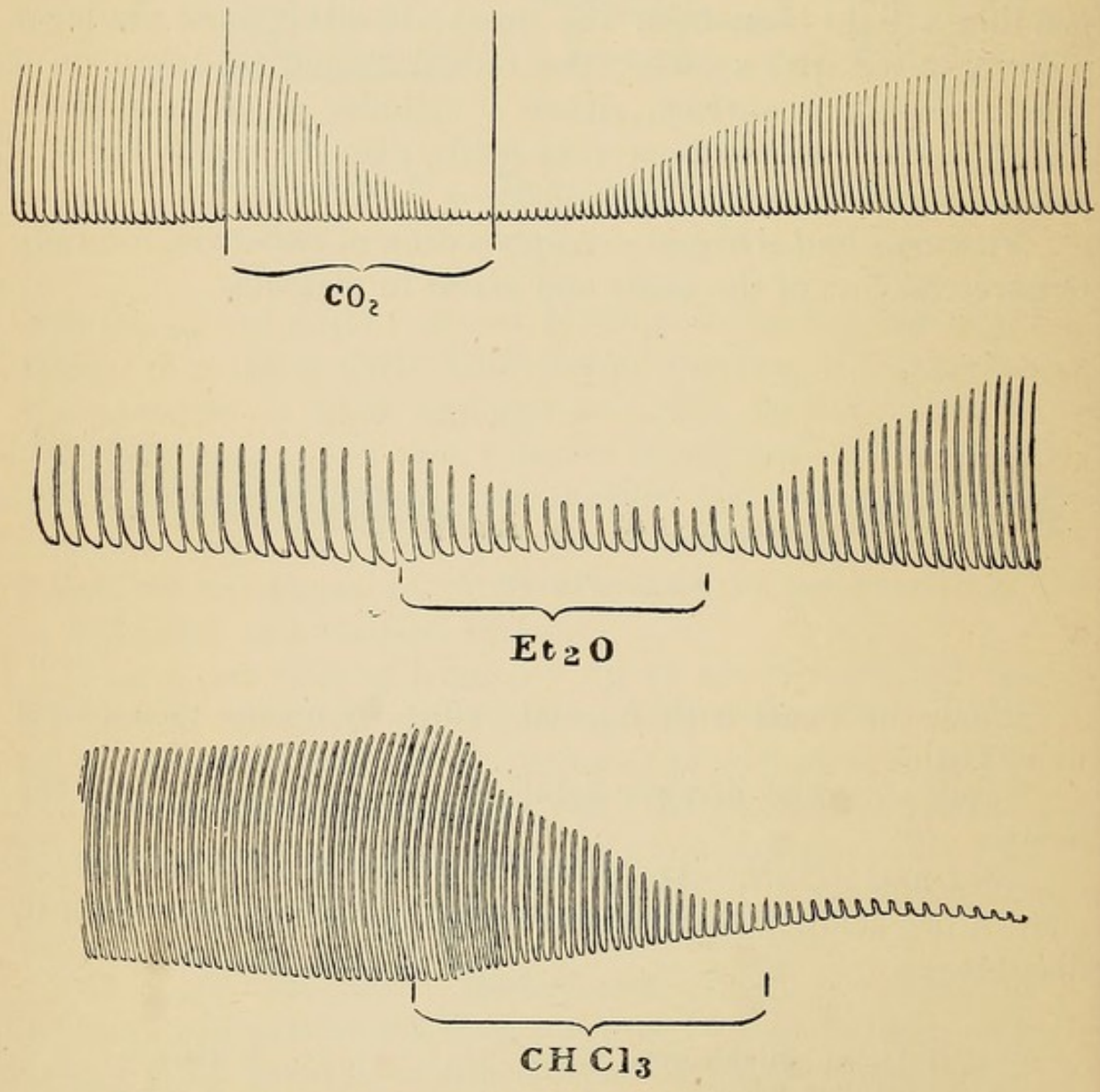


FIG. 20.—EFFECT OF GASES AND VAPOURS ON HEART (FROG).

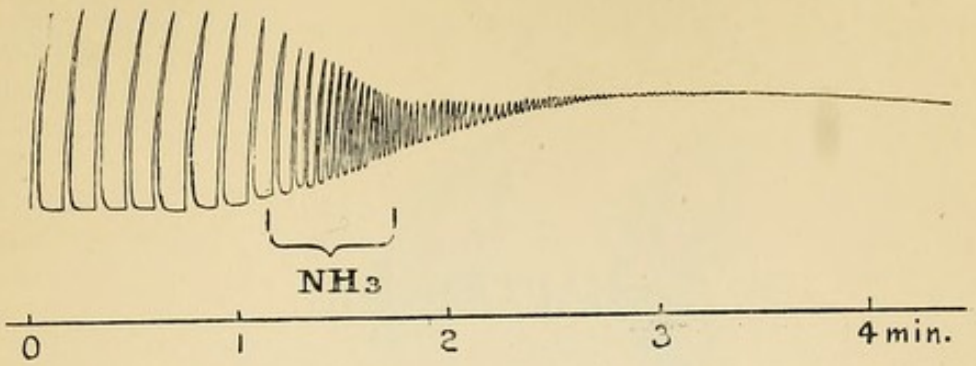


FIG. 21.—EFFECT OF GASES AND VAPOURS ON HEART (FROG).

moist chamber as in figure. Use a slow moving surface, preferably a railway myograph with smoked glass plate travelling at about 25 mm. per minute.

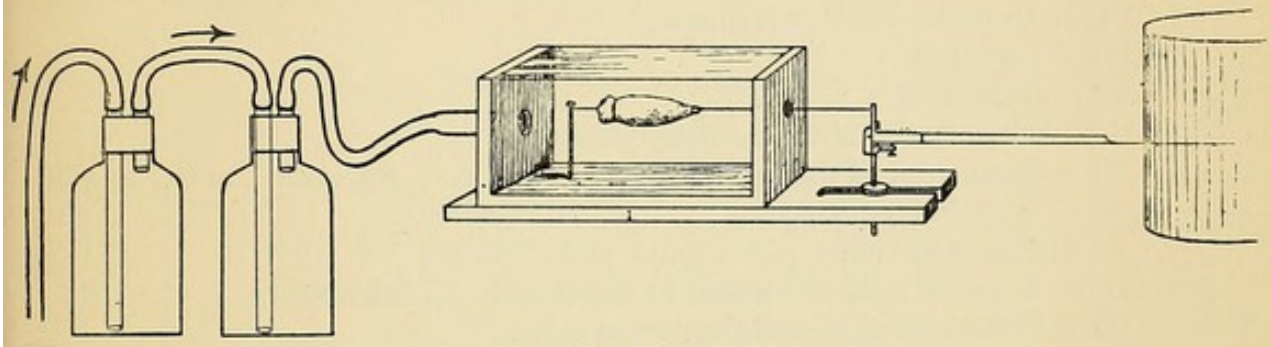


FIG. 22.

Make records demonstrative of the effects of the reagents named above, and of any other gases you may think fit to test.

In each case drive the gas into the heart chamber through a wash bottle as figured.

CHAPTER III.

The Carbohydrates and Salivary Digestion.

I.—Starch: the polariscope.
II.—Dextrins.
III.—Glycogen: its preparation.
IV.—Maltose: preparation of osazone.
V.—Saccharose.
VI.—Lactose.
VII.—Dextrose: the polarimeter.
VIII.—Lævulose.
IX.—Galactose.
X.—Estimation of the carbohydrates.
XI.—Dissection of the submaxillary gland, &c. Excitation of the lingual. chorda tympani, and sympathetic nerves. Effect of atropine, pilocarpine, and nicotine.
XII.—Effect of acid and alkali in the mouth, on salivation.
XIII.—Collection and examination of saliva.
XIV.—Diastasimetry.

I.—**Starch.**—Cut open a potato, scrape it, and diffuse the scrapings in water. Examine a drop of this with a

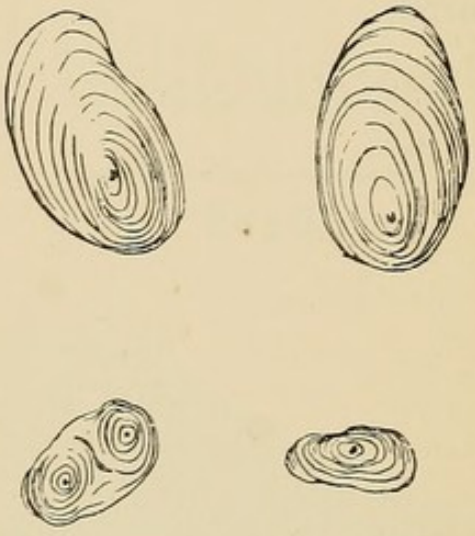


Fig. 23.—Starch Granules from Potato.

microscope, and observe the concentrically marked granules. These granules are composed of granulose (starch proper) enclosed in a capsule of cellulose. Examine another drop

with a polariscope, and note the appearance of the granules when the "Nicols" are "crossed."

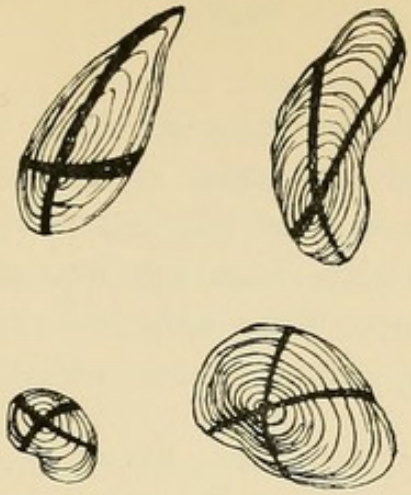


FIG. 24.—STARCH GRANULES (POTATO) SEEN WITH CROSSED NICOLS.

You are given a 1 per cent. solution of starch. Test it at different dilutions with iodine, and observe the effect on the resulting colour, of warming and subsequent cooling. Show that alkali interferes with the iodine reaction, and that starch is precipitated by two volumes of strong alcohol.

The Polarising Microscope (*Polariscope*). (Dem.).—The apparatus serves to distinguish isotropous (singly refracting) from anisotropous (doubly refracting) material. It is an ordinary microscope fitted with two Nicols' prisms known respectively as "polariser" and "analyser." The polariser is fixed below the stage of the microscope in the path of the light reflected by the mirror, and plane-polarises the light passing through it. The ocular of the microscope is removed and the collar belonging to the analyser placed on the microscope, but not yet screwed to it. The ocular is then re-inserted and the analyser placed on it, so that the index of the analyser is opposite 90° on the collar scale. The collar is now rotated until the field of the microscope is at its darkest,[1] and then screwed on to the tube. In this position the "Nicols" are "crossed." The analyser is next rotated through 90°, when the illumination becomes maximal, in which case the "Nicols" are "parallel." On examining, *e.g.*, starch granules, crystals, or muscle with the microscope and parallel "Nicols," the struc-

[1] If no light passed into the tube, other than through the polariser, the field would become quite dark. Some, however, is reflected from the stage, &c., so the field is always somewhat illuminated.

tures appear as though seen in ordinary light, but on rotating the analyser through 90° (*i.e.*, on "crossing" the "Nicols") isotropous material becomes dark, whilst anisotropous substances remain illuminated.

II.—**Dextrins.**—You are given a 1 per cent. solution of erythrodextrin. If pure, the solution will be colourless and odourless. Perform the same chemical reactions as you did with starch and show that dextrin is less readily precipitated by alcohol. Show also, that basic acetate of lead[1] does not precipitate dextrin. The achroodextrins, which are not coloured by iodine, are also precipitated by alcohol.

III.—**Glycogen.**—You are given a 1 per cent. solution. Observe its opalescence. Examine it as you did the dextrins, showing that it is readily precipitated by alcohol, and also by basic acetate of lead.

Preparation of Glycogen (*Rabbit, Dog*). (Dem.)—The fasting animal is fed (a rabbit on carrots—a dog on a pudding made of arrowroot, sugar, and milk), and killed by bleeding, six hours after the meal. The liver is plunged into boiling water faintly acidulated with acetic acid, until opaque throughout, and then minced, triturated with sand, and returned to the water. After boiling for five minutes, the coagulated proteid is strained and filtered off and the filtrate tested for proteids. If any are present they are removed by adding hydrochloric acid and Brücke's reagent (potassio-mercuric iodide) and filtering. The glycogen solution is concentrated until a "skin" forms on it, when the glycogen is precipitated by excess of alcohol, washed with alcohol, alcohol-ether, ether, and finally dried and powdered.

More glycogen may be obtained from the coagulated proteid by dissolving it in a hot 2 per cent. solution of caustic potash, removing the bulk of the resulting alkali albumin by neutralisation and filtration, and the remainder by hydrochloric acid and Brücke's reagent as above. This second lot is precipitated, washed and dried, like the first, but is not quite so pure.

IV.—**Maltose.**—(*The Product of Salivary and Pancreatic Digestion of Starch.*)—You are given a ·5 per cent. solution.

---

[1] The solution of basic lead acetate is seldom clear, and is readily clouded by atmospheric CO_2. Do not mistake such a turbidity for a precipitate of dextrin.

Show that it dissolves cupric hydrate, and that on boiling it reduces the cupric hydrate to cuprous oxide (Trommer's test). Show also that maltose will *not* reduce an acid cupric solution (Barfoed's reagent). On hydrolysis maltose yields two molecules of dextrose.

Prepare phenyl-maltosazone as follows: Take 10 cc. maltose solution, add to it one "knife-pointful" (about ·2 gm.) of phenyl-hydrazine hydrochloride and double as much acetate of soda, and place in a test tube in boiling water for half an hour. Phenyl-hydrazine acetate and sodium chloride are formed, and the maltose reacting with the first of these forms ultimately phenyl-maltosazone, which on cooling, if not sooner, is deposited in more or less stellate groups of yellowish "knife-blade" crystals. Should the deposit be amorphous, add alcohol to dissolve the precipitate, boil the alcohol off, and let cool gradually. The crystals of phenyl-maltosazone melt at 206° C.

V.—**Saccharose** (*Cane Sugar*).—You are given a ·5 per cent. solution. Show that it dissolves cupric hydrate, and that it will not reduce it. On hydrolysis cane sugar yields one molecule of dextrose and one of lævulose.

VI.—**Lactose** (*Milk Sugar*).—You are given a ·5 per cent. solution. Show that it dissolves cupric hydrate, and (like maltose) reduces it on boiling. On hydrolysis lactose yields one molecule of dextrose and one of galactose.

Prepare phenyl-lactosazone as you did the maltosazone, and compare the hedgehog-like masses of acicular crystals with the larger crystals of the maltosazone. Phenyl-lactosazone melts at 200° C.

VII.—**Dextrose.** (*Formed in the small intestine by hydrolysis of maltose, saccharose, and lactose.*)—You are given a ·5 per cen. solution. Examine it as you did maltose, showing that (unlike maltose) it reduces Barfoed's reagent. Prepare its osazone and observe the star and feather-like clusters of acicular crystals. They are smaller than the maltosazone crystals and larger than those obtained from lactose. They melt at 205° C. Examine a stronger (15 per cent.) solution in a polarimeter. The specific rotatory power of dextrose is greatest immediately after solution is completed. On standing, and still more on warming, it falls rapidly. At 20° C. it averages 52·6°.

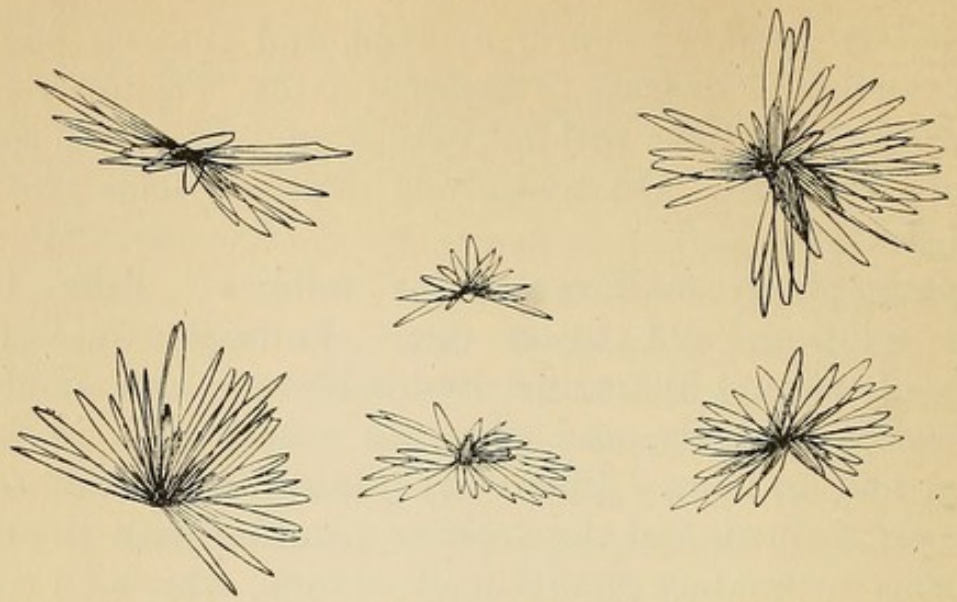


FIG. 25.—CRYSTALS OF PHENYL-MALTOSAZONE (Halliburton).

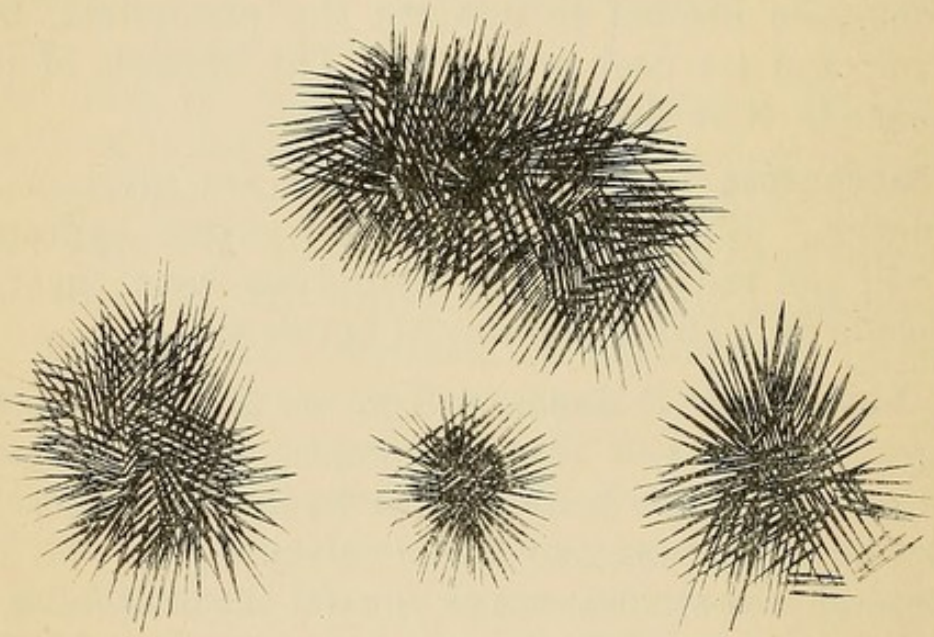


FIG. 26.—CRYSTALS OF PHENYL-LACTOSAZONE (Halliburton).

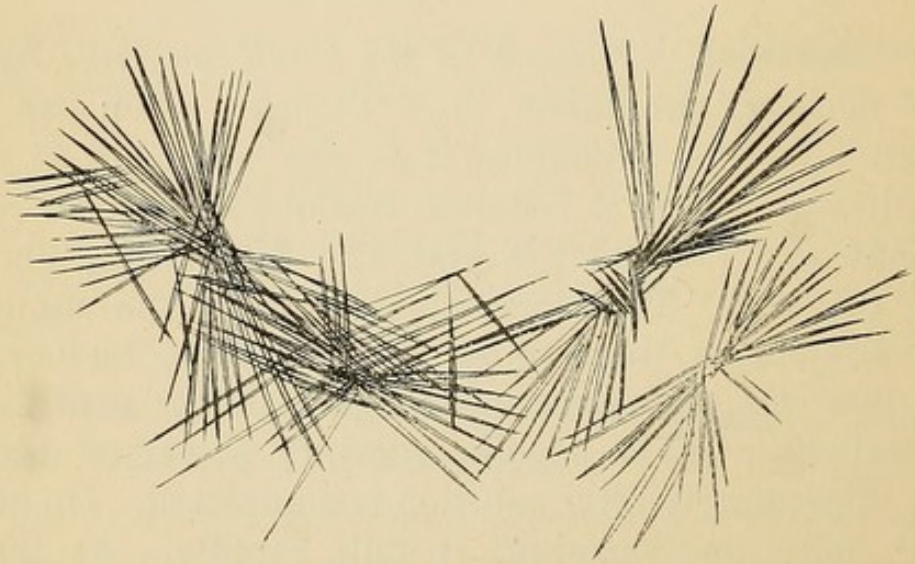


FIG. 27.—CRYSTALS OF PHENYL-GLUCOSAZONE (Halliburton).

The Polarimeter. (Dem.)—When plane polarised light passes through, *e.g.*, a solution of sugar or proteid, the plane of vibration is rotated to an extent dependent on—

(1) The wave length of the light.

(2) The rotatory power of the optically-active substance.

(3) The concentration of the solution.

(4) The thickness of the layer of optically-active solution through which the light passes.

Polarimeters are instruments for detecting and measuring the amount of this rotation, and the term "specific" is applied to the rotatory power of a solution containing one gram of the active substance per cc., when used in a layer one decimetre thick.

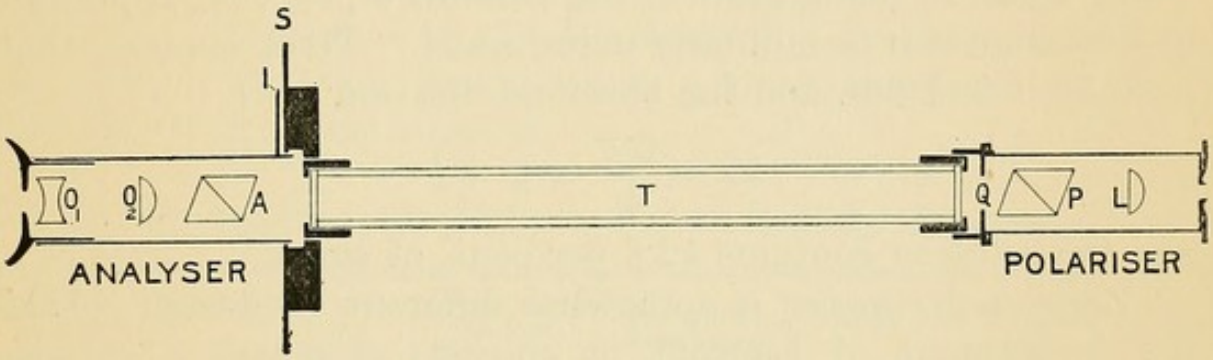


FIG. 28.—POLARIMETER.

O_1, O_2, lenses of eyepiece; A, analysing Nicol; I, index; S, scale; T, tube to contain the fluid under examination; Q, position of quartz; P, polariser; L, parallelising lens.

This "specific rotatory power" is denoted by the symbol a_D or a_J, according as it is measured by sodium light or by ordinary yellow (*jaune*) gaslight.

Laurent's polarimeter is used with a sodium lamp, viz., a Bunsen burner with fused sodium chloride suspended in the flame.

The instrument consists essentially of a polariser, a quartz plate, a tube to hold the solution under examination, and an analyser which can be rotated and which carries an index movable along a circular scale graduated in degrees. When the Nicols are parallel, and the tube contains no active substance, the field, seen through the analyser, is uniformly illuminated. The quartz plate covers half the field only, and is so cut that insertion of an optically-active substance between it and the analyser (with Nicols parallel) darkens one half of the field. Rotation of the analyser restores uniform illumination, and the direction and amount of this observed rotation,

indicates the direction and measure of the rotatory effect of the active substance.

Before using the instrument its zero must be determined by observing the position of the analyser index, when, in absence of active substance, its field is uniformly lighted.

The specific rotatory power (a_D) of a substance may be deduced from the equation—

$$a_D = \pm \frac{a}{p\,l}$$

in which a = the rotation observed with the polarimeter, p = weight of active substance per 1 cc. solution, and l. = length of the tube in decimetres. The signs + and — signify respectively rotation to right and left.

Or a_D and l being known, the amount of, *e.g.*, sugar present in a solution can be similarly determined. Thus, suppose that $a_D = 52$, l = 1 dm, and the observed rotation = + 6·5,

$$52 = \frac{6{\cdot}5}{p} \text{ or } p = \tfrac{1}{8}$$

and the solution contains 12·5 per cent. of sugar.

Zeiss' polarimeter is somewhat different in design. Like the instrument of Laurent, it consists of polariser, quartz, tube, and analyser, but differs from it in the effect of the quartz, and in being used with ordinary gaslight. The quartz here consists of a dextro- and a lævo-rotatory half, each covering half the field. These, in the absence of optically-active substance, give the field viewed through the analyser a uniform rose-violet tint. If a tube of active substance be inserted in the instrument the two halves of the field differ in colour. Rotation of the analyser restores the uniform tint, and calculation is made as with Laurent's instrument. Roughly speaking—

$$\frac{a_D}{a_j} = \frac{10}{11}$$

Value of a_D in some substances of physiological importance (Hammarsten).

Substance.	a_D.
Dextrose	+ 52·6
Saccharose	+ 66·5
Maltose	+ 137
Serum Albumin	− 62 − 65
Serum Globulin	− 47·5

VIII.—**Lævulose.** (*Formed together with Dextrose in the small intestine, from Saccharose.*)—Lævulose is readily prepared as follows : a 10 per cent. solution of inulin,[1] is boiled for five minutes with sulphuric acid (·1 cc. H_2SO_4 in 100 cc. of inulin solution). A pinch of barium carbonate is added to neutralise the acid, and the fluid filtered. Examine some of the solution in a polarimeter, and observe its lævo-rotatory effect. The specific rotatory power of lævulose is not exactly known. It is, however, known to be greater than that of dextrose. Dilute some of the solution ten times and examine it as you did dextrose. It forms the same osazone as does dextrose.

IX.—**Galactose.** (*Formed together with Dextrose in the small intestine, from Lactose.*)—You are given a ·5 per cent. solution. Examine it as you did dextrose. Its osazone is very like that of dextrose but melts at a lower temperature, viz., 193° C. It is dextro-rotatory.

Classification of the Sugars and their Chief Reactions.

		Boiled with Fehling's solution.	Boiled with Barfoed's reagent.	Examined in Polarimeter.	Boiled with Phenyl-Hydrazine Acetate.
Disaccharides.	Saccharose	No Change	No Change	Dextro-rotation	No compound formed.
	Maltose ..	Reduction	No Change	Dextro-rotation	Phenyl-maltosazone formed. Stellate clumps of "knife-blade" crystals melting at 206° C.
	Lactose ..	Reduction	No Change	Dextro-rotation	Phenyl-lactosazone formed. Stellate clumps of minute acicular crystals melting at 200° C.
Monosaccharides.	Dextrose..	Reduction	Reduction	Dextro-rotation	Phenyl-glucosazone formed. Stellate and feather-like clumps of acicular crystals, larger than those of the lactose, but smaller than those of the maltose compound. Melt at 205° C.
	Lævulose	Reduction	Reduction	Lævo-rotation	
	Galactose	Reduction	Reduction	Dextro-rotation	Phenyl-galactosazone formed. Crystals very like those of the glucosazone but melt at 193° C.

[1] A polysaccharide obtained from dahlia bulbs.

X.—**Estimation of Carbohydrates.**—The reducing sugars are most conveniently estimated by Fehling's method, Part I., p. 24. Although at first attempts, the end point is somewhat obscure, it is found that those who have worked with solutions of known strength, can by working under sufficiently identical conditions as to dilution, duration of boiling, nature of vessel, &c., obtain concordant results.

The remaining carbohydrates may be similarly estimated after hydration. In the case of cane sugar this is best done by boiling with dilute sulphuric acid for ten to fifteen minutes. The product is then neutralised and estimated in the usual way. Its reducing power which is somewhat higher than that of pure dextrose, is given in the table below.

In the case of starch, dextrin, and glycogen, hydration is most conveniently effected by ptyalin at 40° C. the digestion being left in progress for fully fifteen minutes after the "achromic point"[1] is reached. The resulting sugar is maltose. In all cases and especially in those of the above polysaccharides, the solution must be sufficiently diluted before hydration. The subjoined table shows the reducing power of various sugars when 10-20 cc. of their solution are employed to effect reduction of 10 cc. Fehling's fluid.

10 *cc. of Fehling's fluid are completely reduced by*—

·050 grams of dextrose, lævulose, or galactose.
·047 ,, ,, hydrolysed saccharose.
·068 ,, ,, lactose.
·081 ,, ,, maltose.
·075 ,, ,, starch, dextrin, or glycogen hydrolysed to maltose.

XI.—**Preparation of the Submaxillary Gland and its Secretory Nerves.** (Dem.)—A dog or large cat is anæsthetised and fixed supine, with extended head. Tracheotomy is performed and anæsthesia maintained *via* the tracheal cannula. This done, the skin is incised just below the inferior maxilla, near the junction of the anterior and middle thirds of the bone, and cut (on a director) backwards as far as the vertebral column. The platysma and deep fascia are next divided, exposing the jugular vein and the digastric and masseter muscles. The veins (excepting the glandular ones) are tied and cut as they appear, and the digastric is cleaned and separated from the facial artery which lies between it and the masseter.

[1] *Vide* p. 49.

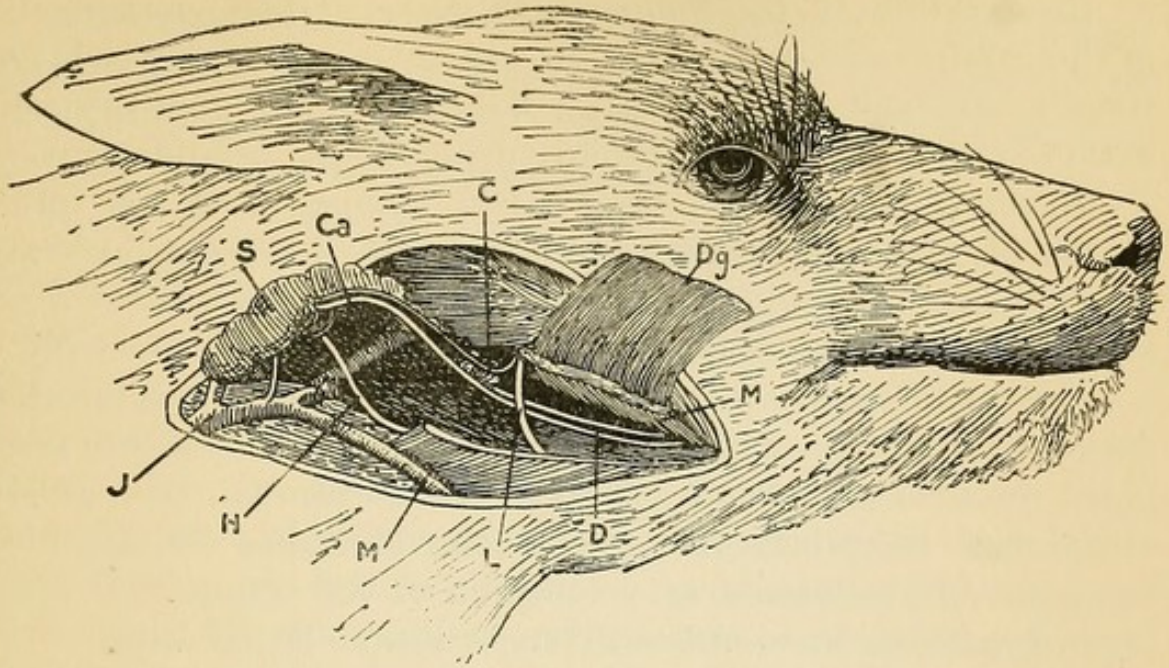


FIG. 29.—THE DUCT AND NERVES OF THE SUBMAXILLARY GLAND IN THE DOG (Brodie, after Bernard).

The digastric is now to be cut near the jaw, and as far as possible removed, thus exposing the mylohyoid muscle anteriorly, and the submaxillary gland, duct, artery and nerves posteriorly. The sublingual gland is closely attached to the submaxillary and its duct (the smaller of the two) runs parallel and ventral to the submaxillary duct. The mylohyoid muscle is carefully removed, and the chordo-lingual nerve seen running dorso-ventrally from the anterior border of the masseter. From this nerve the chorda tympani may be traced at first dorso-ventrally, and then antero-posteriorly. The submaxillary duct is cut as far from the gland as possible, and its end inserted in a test tube, or laid on a piece of white paper.

In the cat the sympathetic is isolated in the neck, immediately below the superior cervical ganglion, but in the dog its glandular branches must be found. These are to be sought near the gland, running along the carotid artery.

Stimulation of the Central End of the Lingual Nerve.—The lingual nerve is ligatured near its entrance to the mouth, cut and stimulated. Unless the animal is too deeply anæsthetised, salivation is accelerated.

Stimulation of the Peripheral End of the Chorda Tympani.—The chorda is ligatured as near the lingual as possible, and cut. The submaxillary gland is cleaned and the chorda excited. On excitation, salivation is increased and the gland flushes.

Stimulation of the Sympathetic Nerve.—The cephalic end of the cervical sympathetic in the cat, or its glandular twigs in the dog are excited. The gland becomes pallid and the salivation is much less copious than during chorda stimulation.

Effect of Atropine on the Chorda Tympani.—A few milligrams of atropine sulphate (4 for a cat, 12 for a dog) are injected into a vein. On excitation of the chorda, little or no salivation is produced, but vaso-dilatation occurs as normally.

Effect of Pilocarpine.—A milligram of pilocarpine is injected into the submaxillary gland. If atropine has not already been given profuse salivation occurs. If the animal be already atropinised the pilocarpine may so far neutralise the atropine as to enable salivation on excitation of the chorda. It may, however, fail to do so unless given in almost lethal dose.

Effect of Nicotine.—A 1 per cent. solution of nicotine in normal saline solution is painted—

(*a*) On the "submaxillary ganglion," which lies between the chorda and lingual nerves. This does not alter the effect of chorda stimulation on the submaxillary gland.

(*b*) On the hilus of the submaxillary gland. Excitation of the chorda now produces no salivation, whilst excitation applied directly to the gland hilus produces salivation as before application of the alkaloid.

XII.—**Effect of Acid and Alkali on Salivary Secretion.**—Wash out the mouth with a ·5 per cent. solution of sodium carbonate. Unpleasant dryness of mouth and fauces indicates interference of the drug with normal salivation. Alkalies are anti-sialogogues.

Wash the mouth with a ·5 per cent. solution of acetic acid. Profuse salivation occurs. Acids are sialogogues. The effect of atropine and pilocarpine on salivation is described above.

XIII.—**Collection of Saliva.**—Wash the mouth with water, and inhale the vapour of acetic acid, preferably through the mouth. Previous administration of $\frac{1}{20}$ grain of pilocarpine nitrate will materially increase the flow of saliva.

Examination of Saliva.—Take reaction. Test for mucin with acetic acid, filter off the precipitated mucin and test the filtrate for proteid, and show the proteid to be coagulable on heating. Remove the coagulated proteid by filtration, and demonstrate the presence of chloride, phosphate, and sulphate in the filtrate. Test saliva for thio-cyanate.

Show that saliva contains an amylolytic ferment whose activity is destroyed by heat, and hindered or destroyed by acidification, v. Part 1, p. 17. Prepare and identify the osazone of the sugar formed by saliva from starch.

XIV.—**Diastasimetry** (Roberts).—You are given a ·1 per cent. "solution" of pure starch. Take 100 cc. of this, warm to 40° C., and place drops of iodine solution on a white tile. Stir 1 cc. saliva into the warm starch, and at intervals of thirty seconds bring a drop of the digest to one of the drops of iodine. The (at first) blue reaction gives place in turn to violet, brown, and yellow, which are succeeded by an "achromic point," when no further colour is produced. The interval that elapses between addition of the saliva to the starch and the appearance of the achromic point is a rough measure of the diastatic activity of the saliva. Compare in this way the diastatic activities of—

(1) Normal saliva.
(2) Saliva diluted with nine volumes of water.
(3) Neutralised saliva.

CHAPTER IV.

The Proteids and Gastric Digestion.

I.—Elementary analysis.
II.—Colour reactions.
III.—Precipitability.
IV.—Diffusibility.
V.—Native proteids—Heat coagulation.
VI.—Mechanical coagulation.
VII.—Crystallisation of egg albumin.
VIII.—Derived albumins.
IX.—The albumoses: their separation.
X.—The peptones: their separation.
XI.—Preparation of artificial gastric juice.
XII.—Estimation of the acidity of gastric juice.
XIII.—Identification of the gastric acid.
XIV.—Process of gastric digestion.
XV.—Comparison of the activity of different gastric juices.

I.—Elementary Analysis of Proteid. (*Use dried white of egg.*)

Detection of Carbon.—Burn some of the proteid in a spoon. Charring indicates presence of carbon.

Detection of Nitrogen.—Powder the proteid with about four parts of soda-lime, and heat in a dry test-tube. Evolution of ammonia, detected by a moistened litmus paper, shows nitrogen.

Detection of Sulphur.—Boil some of the powder with strong caustic soda solution, and add acetate of lead. A black precipitate (PbS) shows sulphur.

Detection of Phosphorus (in nucleo-proteids).—Take dried yolk of egg, boil with strong nitric acid in an evaporating dish for fifteen to thirty minutes. Then dilute, filter, and test for phosphates (Part I., Expt. 117).

The carbon and hydrogen in proteid are estimated by combustion, whereby carbon dioxide and water are produced. The former is absorbed in a weighed soda-lime tube or potash bulb; the latter in a weighed calcium chloride tube, the increase of weight in these tubes resulting from an experiment being the measure of the carbon dioxide and water produced, and so of the carbon and hydrogen

present. Fat and carbohydrate may be similarly treated. Nitrogen in proteid and other physiological nitrogenous bodies is most conveniently estimated by the oxidation method of Kjeldahl, which will be described in the chapter on Urine.

II.—**Colour Reactions of Proteids.** (*Use undiluted white of egg.*)—Compare the xantho-proteic, biuret, and Millon's reactions (Part I., Expts. 2, 3, and 4) with the subjoined.

Adamkiewic's Reaction.—Dissolve egg-white in glacial acetic acid [1] with aid of heat, and then cautiously add sulphuric acid. A bluish or purple colour develops, sometimes associated with fluorescence.

Pettenkofer's Reaction (*cf.* bile).—Shake up egg-white with a crystal (or a drop of a 10 per cent. solution) of cane sugar. Then add sulphuric acid, holding the test-tube under the water-tap to keep it cool. A purple-violet colour develops, which, to the unaided aye, is identical with that yielded by bile salts (*cf.* p. 64).

III.—**Precipitation of Proteid.** (*Use solutions of albumin and of commercial peptone.*)—Add acetic acid and ten volumes of alcohol. All proteid is precipitated on standing.

Add one volume of a 10 per cent. solution of tri-chloracetic acid. All proteids, except peptones, are precipitated.

Add solution of mercuric choloride. All undigested proteids and some albumoses are precipitated.

IV.—**Diffusibility of Proteids.** — Prepare two U-shaped dialysers of sausage paper, and suspend each in a vessel of distilled water. Place solution of albumin in one, and of commercial peptone in the other. Set aside for a week, and then test the water for proteid by the biuret and tannin tests (Part I., p. 3).

V.—**Heat Coagulation.**—Take a test-tube containing a thermometer and enough proteid solution to cover the bulb. Place in a double glycerin bath (two concentrically-fitted beakers containing glycerin) heated by a small gas flame, keeping the test-tube moving in the inner beaker and the thermometer moving in the test-tube.

The temperature at which opalescence first appears is the "coagulation temperature" of the proteid under the conditions of the experiment.

For a given proteid this temperature is lower when the solution is strong and when it is *slightly* acid, and higher when

[1] The reaction is due to the presence of glyoxylic acid in the glacial acetic acid.

the solution is weaker or less acid. Excessive acidity or slight alkalinity abolishes coagulation. The presence of neutral salts generally raises the coagulation temperature. The onset of heat coagulation is gradual, so the fluid must be kept at the coagulation temperature for some time (minutes, or occasionally hours) before the whole of the proteid is coagulated. When a proteid undergoes heat-coagulation its solution becomes less acid.

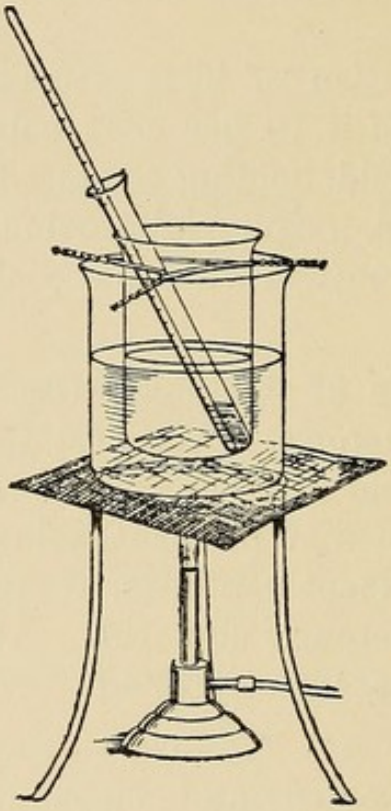


Fig. 30.

Observe the coagulation temperature of serum albumin dissolved in a saturated solution of magnesium sulphate, and observe the effects of—

(1) Dilution, (2) addition of a little HCl., ·1 per cent., (3) NaOH ·1 per cent.—on the coagulation temperature.

VI.—**Mechanical Coagulation of Proteids.**—Take the white from a new laid egg, and pour from test-tube to test-tube. Fibrin-like threads appear in the course of a few minutes. The appearance of this mechanical coagulum may be hastened by vigorously shaking the egg-white. Examine some of the coagulum under the low power of a microscope.

VII.—**Crystallisation of Egg Albumin** (Hofmeister, Hopkins). (Dem.)—100 cc. of egg-white from fresh eggs are

well whisked whilst an equal volume of a saturated solution of ammonic sulphate is gradually added to it. The mixture is set aside for a night.

Next day it is filtered, and more (5 to 15 cc.) ammonic sulphate solution added, until permanent turbidity ensues, which is then removed by adding distilled water, almost drop by drop. The mixture is then placed in a stoppered bottle and 10 per cent. acetic acid added until a definite precipitate appears, when the stopper is inserted and the bottle set aside.

Examine the resulting deposit with a microscope, noting the rosette and sheaf-like clusters of acicular crystals.

VIII.—**Acid- and Alkali-Albuminate.**—Take two test-tubes, and place solution of native albumin or globulin in each. Add an equal volume of ·4 per cent. hydrochloric acid to one, and a like quantity of ·1 per cent. caustic soda to the other. Place both in a water bath at 40° C. for half an hour. Then ascertain that the proteid is not coagulable by heat, that it is precipitated on neutralisation, that this neutralisation-precipitate is soluble in excess of the neutralising reagent, and that it (the ppt.) is rendered insoluble by boiling. Show also that saturation with magnesium sulphate precipitates both the above derived albumins.

Lieberkühn's Jelly (alkali-albumin).—Stir powdered caustic soda into undiluted egg-white. It forms a stiff jelly. Dissolve the jelly in water and test as above.

Acid Jelly (acid-albumin).—Dissolve egg-white in glacial acetic acid with aid of heat. It forms a jelly on cooling. Dissolve and test as above.

IX.—**Albumoses.** — Take a 10 per cent. solution of commercial "peptone." Saturate it with sodium chloride. Some of the proto- and all of the hetero-albumose are precipitated.[1] Filter, and save both precipitate and filtrate. Labe the filtrate "A."

Separation of Proto- and Hetero-albumose (*Neumeister*). (Dem.)—Some of the above precipitate is washed with a saturated solution of sodium chloride, and dissolved in a

[1] The best *test* between primary and secondary albumoses is afforded by adding a few drops of $CuSO_4$ solution. Primary albumoses are precipitated, secondary albumoses are not.

minimum of water. The resulting solution is placed in a running-water dialyser and left for a week. Most of the salt and some of the proto-albumose dialyse away. The hetero-albumose is completely precipitated, and is then removed by filtration. Proto-albumose may be precipitated from this filtrate by saturating it with sulphate of ammonium.

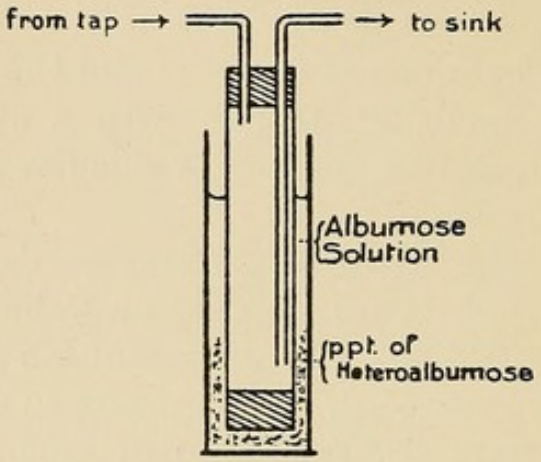


FIG. 31.—DIALYSER.

A tube of parchment paper, corked at both ends, through which a stream of water is maintained, and which is suspended in the albumose solution. The salts diffuse (dialyse) from the solution, through the parchment, into the water stream.

Separation of Deutero-Albumose (*Neumeister*).—To the filtrate "A" add acetic acid until no further precipitate falls. The precipitate consists of the residue of the proto-albumose, and some, but not all, of the deutero-albumose. Filter, and saturate the filtrate with sulphate of ammonium—much of the deutero-albumose is thrown down, and may be separated by filtration.

X.—*Separation of Peptones* (*Kühne*). (Dem).—Two hundred grams of commercial peptone are digested in 10 litres of strong artificial gastric juice for at least a week.[1] This solution is then neutralised, boiled, and (whilst boiling) saturated with ammonium sulphate. On cooling it is filtered, alkalised with ammonia and ammonium carbonate, boiled, and again saturated with ammonium sulphate. The precipitate is removed by skimming and filtration, and the filtrate boiled until all smell of ammonia has disappeared. It is then made distinctly acid with acetic acid, and boiled with more ammonic sulphate, when a third crop of albumoses is separated. The filtrate from this crop is said to contain pure peptone, which may be separated by removing the ammonium salt by crystallisation, followed by boiling with barium carbonate and ammonia; and precipitating the peptone itself with alcohol, after first concentrating its solution to workable proportions.

[1] Six or seven weeks may be allowed with advantage.

THE CHIEF REACTIONS OF ALBUMOSES AND PEPTONES.

	Proto-albumose.	Hetero-albumose.	Deutero-albumose.	Peptones.
$CuSO_4$ in solution ..	Ppt.	Ppt.	—	—
Biuret test	Pink	Pink	Pink	Pink
Dialysis	—	Ppt.	—	—
Saturation with NaCl or $MgSO_4$	Ppt.	Ppt.	—	—
Nitric acid	Ppt.[1]	Ppt.	Ppt. only in presence of enough NaCl	—
Saturation[2] with Am_2SO_4	Ppt.	Ppt.	Ppt.	—

XI.—**Preparation of Artificial Gastric Juice.** (Kühne's method).—A pig's or dog's stomach is opened, gently sponged, and the cardiac region scraped with a not too sharp instrument. The scrapings are triturated with powdered glass, shaken with about 100 cc. of water and filtered. An equal bulk of ·4 per cent. HCl is added. If a larger quantity is required and the presence of digestive products not objected to, the mucosa may be stripped off, minced, placed in two litres of ·4 per cent. hydrochloric acid, and kept at 40° C. until dissolved. Fifty cubic centimetres of the resulting solution added to 500 cc. of ·2 per cent. hydrochloric acid make an active juice.

Gastric juice may also be prepared by mixing 50 cc. of gastric glycerin (Part I., appendix) with a litre of ·2 per cent. hydrochloric acid.

XII.—**Estimation of the Acidity of Gastic Juice** (Leo).—Use artificial juice. Take 20 cc., add 10 to 15 cc. of strong solution of calcium chloride and titrate with $\frac{N}{10}$ solution of caustic soda using litmus as indicator, either in the form of solution or paper. You will thus ascertain the *total* acidity, and may express it in terms of hydrochloric acid thus: suppose that you used 12 cc. of $\frac{N}{10}$ alkali in neutralising. Then your 20 cc.

[1] See Part I., Expt. 20.

[2] See p. 54.

of gastric juice contains as much hydrochloric acid as do 12 cc. of a $\frac{N}{10}$ solution, *i.e.* (12 × ·00365) gram = ·0438 gram, and 100 cc. of gastric juice contains five times as much, viz., ·219 gram.

Some of the acidity may be due to acid phosphate.

To determine this take 30 cc. of the gastric juice, shake it with freshly precipitated calcium carbonate, thus neutralising the *free* acid, and filter. Then take the filtrate, pass air through it to wash out the liberated CO_2, measure 20 cc. from it, add calcium chloride as above and titrate again with the same indicator. You thus ascertain how much $\frac{N}{10}$ alkali is neutralised by the acid phosphate, and by subtracting this from the amount used in the first titration, you calculate how much went to neutralise the free acid. Artificial gastric juice contains but little phosphate, so the total acidity, and the acidity due to free acid, are practically the same thing.

XIII.—**Identification of the Gastric Acid.**—Test gastric juice with a solution of sodic thiosulphate. A precipitate (sulphur) indicates presence of *free* acid.

Günzburg's Test.—Take a few drops of Günzburg's reagent (phloroglucin and vanillin dissolved in alcohol) and dry at a temperature not exceeding 100° C., with an equal quantity of gastric juice. A carmine stain, sometimes crystalline, indicates presence of *mineral* acid. This test is hindered by proteids (digested or otherwise), so that whilst its positive results are trustworthy, absence of colouration does not necessarily mean absence of mineral acid.

To ascertain that the free mineral acid is hydrochloric, you would have to estimate the total bases present in the gastric juice, and then the amount of chlorine, when you would find more chlorine than could combine with the bases.

Absence of Lactic Acid from Gastric Juice.—Take some Uffelmann's reagent (dilute solution of phenol tinged blue, by addition of ferric chloride), and add some dilute lactic acid to it, a yellow colour results. Show that hydrochloric acid does not hinder this reaction, and then repeat it with gastric juice instead of lactic acid. Gastric juice merely bleaches the fluid, no trace of yellow being observable.

The tropœolin reaction of Gastric Juice.—Take two or three drops of a saturated alcoholic solution of tropœolin O O and evaporate to dryness in a white dish at 40° C. Add a drop or two of gastric juice and keep warm. A reddish violet colour appears. A ·2 per cent. solution of hydrochloric acid behaves in the same way as gastric juice. Diluted lactic acid, however, gives no such colour.

XIV.—**Process of Gastric Digestion.**—Take some fibrin, or minced and washed muscle, and place some in three test tubes with artificial gastric juice. Test after 15, 45, and 90 minutes' stay in a water bath at 40° C., as follows: Filter, test for neutralisation precipitate (acid albumin) which filter off. Test this filtrate (*a*) with the biuret test, (*b*) with nitric or salicyl-sulphonic acid as in Part I., Expt. 20.

With the small bulk of fluid at your command, it would be useless to test for peptones. *Vide* p. 54.

The effect of gastric rennin on milk is described in another chapter.

XV.—**Comparison of the Proteolytic Activity of Different Gastric Juices.** (Grützner.)—Fresh fibrin is washed in running water, and the cleanest of the colourless pieces selected and cut small with scissors. The selected pieces are stained in a not too ammoniacal solution of carmine for twenty-four hours, washed, dipped in a large quantity of ·2 per cent. hydrochloric acid, and washed again. Equal volumes of the samples of gastric juice are placed in test tubes of equal diameter, with as nearly as possible equal bulks of carminated fibrin in each, and all kept at 40° C. In a given time the most active juice will be the most deeply tinged by the carmine liberated by solution of the fibrin.

Compare in this way the digestive activity of (1) gastric juice; (2) boiled gastric juice; (3) hydrochloric acid of the same strength as in the gastric juice.

CHAPTER V.

Fat and Pancreatic Digestion.

I.—Extraction from adipose tissue: Soxhlet's apparatus.
II.—Emulsification.
III.—Identification of the glycerylradicle.
IV.—Saponification.
V.—Preparation of artificial pancreatic juice.
VI.—Pancreatic digestion of starch.
VII.—Pancreatic digestion of proteid. Leucin, tyrosin, indol.
VIII.—Pancreatic digestion of fat.
IX.—Absorption of fat.
X.—Identification of fat in villus.

I.—**Extraction of Fat from Adipose Tissue.** (Dem.)—Adipose tissue is minced, and placed on a pad of glass wool, in a Soxhlet's apparatus. The apparatus is then fixed below

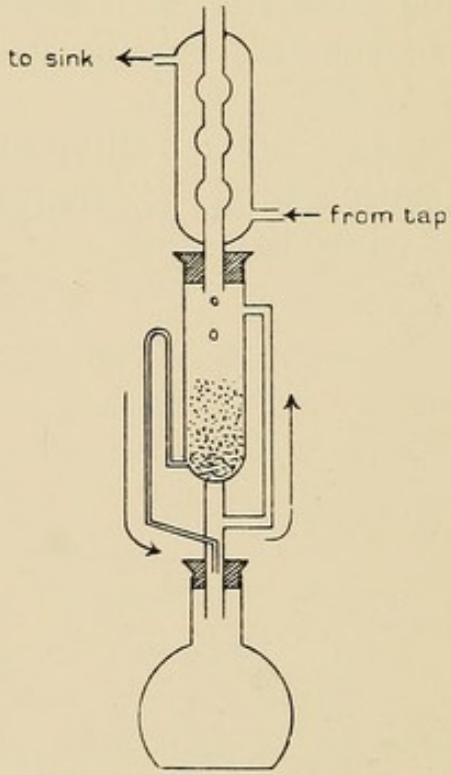


Fig. 32.—Soxhlet's Apparatus, fitted with Flask below, and Reflux Condenser above.

in the cork of a flask containing about 100 cc. of ether, and above to an upright Liebig's condenser. The whole is carefully fixed on a water bath heated to 70° or 80° C. The ether

vapour rising through the apparatus is condensed and so drips on the adipose tissue, where it accumulates until it reaches the top of the small siphon tube, when it is siphoned off into the flask below.

Repetition of the process extracts ultimately all the fat, which is freed from ether by carefully heating on a water bath in an open basin.

Compare the consistency of olive oil (chiefly olein) with that of fat extracted (*a*) from mutton suet (largely stearin) and (*b*) from human adipose tissue. Compare also the rate of solution of each in ether. Take some methyl orange, ascertain the respective effects of acid and alkali upon it, and then test the three fats with it. They are all acid.

II.—**Preparation of Neutral Fat. Emulsification.**—Olive oil has been shaken with caustic soda, and the resulting rough emulsion dissolved in (not acid) ether. The ethereal solution has been washed by shaking with water, separated from the water by a pipette, and the ether driven off by gentle heat.

Shake some of this neutral fat with alkali, emulsification is but imperfect and transitory. Acidify some of the neutral fat with oleic acid and shake with alkali; emulsification is much more perfect and permanent. The addition of proteid solution before shaking would have still further improved the emulsion. Examine the emulsion with a microscope.

III.—**Acrolein Test for Glyceryl of Neutral Fat.**—Take some glycerin in a test-tube, add a crystal of sodium hydrogen sulphate ($NaHSO_4$), and heat. Acrolein is evolved: smell it cautiously.

Heat neutral fat with $NaHSO_4$ as above. Acrolein is evolved, showing presence of glyceryl in the neutral fat.

IV.—**Saponification of Fat.**—Boil a few drops of fat in a mixture of 10 per cent. solution of caustic potash and an equal volume of alcohol. The fat dissolves, forming soap, the glyceryl being liberated in the form of glycerin. Saturate the soap solution with sodium chloride: the soap is precipitated. Collect some of the soap, dissolve in distilled water, add sulphuric acid, and warm. The fatty acid is liberated, and is seen as an oily fluid on the surface of the water.

V.—**Preparation of Artificial Pancreatic Juice.**—(The glycerin method.)—The pancreas, removed from an animal

killed five or six hours after a meal, is freed from adherent fat and connective tissue, and left at the temperature of the laboratory for twenty-four hours. It is then minced and placed in glycerin. Pig's pancreas yields most amylopsin, whilst that of the ox is more suitable when trypsin is required. Glycerin must not be relied on to extract or preserve steapsin. Fifty cubic centimetres of pancreatic glycerin dissolved in a litre of water or 1 per cent. sodium carbonate makes a fairly strong juice.

VI.—**Pancreatic digestion of Starch.**—Artificial pancreatic juice is made by mixing 50 cc. of pancreatic glycerin with a litre of water, or by the following method of Roberts: Pig's pancreas, cleansed and minced as above, is placed in five times its bulk of 20 per cent. alcohol, where it remains, occasionally shaken, for a week. To each litre of this mixture 1 cc. of acetic acid (B.P.) is then added and the whole filtered. The filtrate diluted with five volumes of water is strongly amylolytic. Show this, with starch mucilage, as you showed the similar property of saliva.

VII.—**Pancreatic digestion of Proteid.**—Pancreatic juice is prepared for this purpose by mixing 50 cc. of pancreatic glycerin with a litre of 1 per cent. sodium carbonate, or by the following method of Hammarsten: The pancreas of an ox or dog is cleansed, minced, lightly washed with water to remove blood, and then extracted for twenty-four hours with a weak solution of ammonia[1] and filtered. Acetic acid is cautiously added, and the resulting precipitate collected and dissolved in 1 per cent. sodium carbonate.

Take some fibrin, or minced and washed muscle, and place some in three test-tubes with artificial pancreatic juice. Test as you did in XIV. In this case you will find peptones.

Examine prepared pancreatic digests as follows: Fluid 1 is a proteid digest which has been three to six days in progress, and in which putrefaction has been prevented by thymol. Set aside 20 cc. to concentrate on a boiling-water bath, and (when almost dry) extract it with a minimum of alcohol, filter, and allow the filtrate to crystallise. Spherical clumps of leucin crystals will be deposited, with perhaps a few "sheaves" of tyrosin. The bulk of the tyrosin, however, is not dissolved by

[1] Prepared by diluting the concentrated commercial solution (S.G. ·88) one thousand times.

the alcohol. Take the residue left by the alcohol, extract it with a little faintly ammoniated alcohol, filter, and let crystallise. Sheaf-like clumps of tyrosin crystals are deposited.

Whilst the separation of leucin and tyrosin is in progress, test the fluid 1 for products of proteid digestion, by the biuret test before and after saturation with sulphate of ammonium.

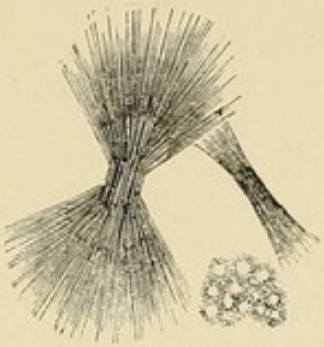


FIG. 33.—CRYSTALS OF LEUCIN (IN SPHERICAL CLUMPS), AND TYROSIN (IN SHEAVES)

Fluid 2 is prepared like fluid 1, save that putrefaction has not been hindered.[1]

Smell it and test for indol and skatol (methyl-indol) as follows :—

Add some dilute solution of sodium nitrite ($NaNO_2$) and nitric acid. Indol gives a red solution or crystalline precipitate of nitroso-indol nitrate, skatol giving merely a milky turbidity which is usually masked by the indol reaction.

To another portion add enough sodium nitro-prusside to tinge the fluid yellow, and then caustic soda. Indol gives a blue-violet solution, whilst skatol yields a yellow which may be masked by the indol.

To another portion add strong sulphuric acid and warm. Skatol produces a purple-red colouration.

VIII.—**Pancreatic digestion of Fat.**—For this, owing to the instability of steapsin, it is better to work with fresh pancreatic tissue than with an artificial juice.

Bernard's saponification experiment.—Shake up a few cc. of water, with about an equal bulk of minced pancreas from a just-killed dog or cat, add a piece of butter about equal in bulk to the pancreas used, and keep at 40° C. The smell of

[1] It is advantageous to start putrefaction by adding a little putrid flesh to the digest.

butyric acid, which soon appears, indicates saponification of the butyrin.

Take two test-tubes, A and B. In A place equal bulks of (1) neutralised oil (p. 22), (2) minced pancreas, and (3) neutral litmus solution, and in B pancreas and neutral litmus only. Place both in a warm bath at 40° C. and examine at intervals of fifteen minutes. If B changes colour, the pancreatic tissue has become acid and the experiment is worthless,[1] but if B remains neutral and A becomes acid you have observed saponification of the neutral oil.

IX.—**Absorption of Fat (Bernard).**—A rabbit is killed two to five hours after a meal of maize flour wetted with milk. The abdomen is opened, the junction of the pancreatic duct with the duodenum exposed, and the mesenteric lacteals observed above and below it. Above, they are almost invisible; below, they are conspicuous owing to the opaque milky chyle within them.

X.—**Identification of Fat in Villi.**—Pieces of the fat-absorbing portion of the intestine examined in IX. are "fixed" for twenty-four hours in Hermann's fluid, or other solution of osmic acid, and then washed (twenty-four hours) in running water. Villi are then scraped off, teased in glycerin, and examined with a high power objective. Another piece may be microtomed by the gum method, and the resulting sections stained with safranin and mounted in glycerin or lævulose syrup.

[1] With acidification of the pancreas, the steapsin is destroyed.

CHAPTER VI.

Milk.

I.—Take reaction—it should be amphoteric. Observe the specific gravity. Skimming raises the specific gravity, dilution lowers it.

II.—**Coagulation of Milk.**—Place 5 cc. of milk in three test tubes. "A," "B," and "C." To "A" add rennet, to "B" boiled rennet, and to "C" potassic oxalate and rennet. Place them in a water bath at 40° C. for fifteen minutes, and proceed with the next experiment.

III.—**Separation and Coagulation of Caseinogen.**—Take 25 cc. milk, add 75 cc. water, and saturate with sodium chloride. Filter, and save both filtrate and precipitate.

The precipitate contains the caseinogen. Dissolve it in a minimum of lime water, add a few drops of calcium chloride solution, as much dilute phosphoric acid, and some rennet. Label this "D," and place in the warm bath.

Take the filtrate, show that it contains a proteid, coagulable by heat, filter off the coagulated proteid, and test the filtrate from it for sugar, salts, and urea.

Now examine "A," "B," "C" and "D." In "A" the milk has clotted, in "B" and "C" it has not, in "D" the caseinogen is clotted. In "C" coagulation was prevented by removal of the soluble calcium salts of the milk. Divide "C" into two parts, "C_1" and "C_2". Add calcium chloride to "C_1" and (if necessary) warm again. "C_1" coagulates. Boil "C_2," and then cool, and add calcium chloride. Coagulation occurs as in "C_1," showing that although the rennet had not actually completed coagulation in "C," it had yet done something towards it.

IV.—**Fermentation of Milk.**—Examine fermented milk. Take reaction. Note the curdling. Warm to facilitate

separation of the curd, and filter. Test the filtrate for lactic acid (p. 19).

V.—**Pancreatic Coagulation of Milk.**—To 5 cc. milk a few drops of pancreatic extract, best prepared by the alcohol method, and keep at 40° C. Coagulation ensues, as with rennet.

VI.—**Identification of Butyrin in Butter.** (Dem.)—Dissolve solid caustic potash in a minimum of water, add four volumes of alcohol and a little butter. Heat carefully, and when a drop or two of the mixture causes no turbidity in distilled water, gradually add sulphuric acid. Butyric acid is evolved. Smell it. Butyric acid is also liberated from butter by steapsin, *vide* p. 61.

Bile.

VII.—**Separation of Bile Salts.**—Bile mixed with animal charcoal has been desiccated and powdered. Extract some of the powder with not too much hot alcohol, and filter. The filtrate contains the bile salts together with cholesterin, lecithin, &c. Take a small quantity of the filtrate, and add excess of anhydrous ether—the bile salts are precipitated, the cholesterin, &c., remaining in solution. Filter or decant off the ether, dry the precipitate, and dissolve it in water, and test by Pettenkofer's test (Part I., p. 16). Examine the spectrum of the coloured fluid, diluting with alcohol if necessary. Note the two bands covering "E" and "F" respectively. These serve to distinguish the bile-salt reaction from the similar colouration yielded by proteid solutions (p. 51). Sometimes a third band near "D" is visible.

VIII.—**Separation of Glycocholic Acid.** (Dem.)—The mixed bile salts, prepared as in VII., are dissolved in water, dilute sulphuric acid is added until the fluid becomes permanently turbid, and the mixture set aside in a cool place. Glycocholic acid crystallises out. Or the procedure may be varied as follows:—

Hüfner's Method. (Dem.)—Fifty cubic centimetres of ox bile are freed from proteid by addition of a few drops of hydrochloric acid and filtration. The filtrate is placed in a stoppered cylinder with 2 to 3 cc. of strong hydrochloric acid and 15 cc.

ether, a crystal or two of glycocholic acid are added, and the vessel placed in iced water. Within a few hours crystals of glycocholic acid form. These are filtered from the mother liquor, and washed with iced water.

IX.—**Separation of Taurocholic Acid.** (Dem.)—The mixed bile salts from ox, or better from dog's bile, are dissolved in water, and neutral acetate of lead is added in excess. This precipitates the glycocholic acid as glycocholate of lead, which is filtered off. The filtrate is treated with more lead acetate, and ammonia—taurocholate of lead falls, is separated by filtration, washed, and dissolved in alcohol. Sulphuretted hydrogen is passed through the alcoholic solution to remove the lead, which is filtered off, and the filtrate is concentrated on a water bath. The taurocholic acid is then thrown down with ether.

X.—**Separation of Bile Pigments.** (Dem.)—*Preparation of Bilirubin.* Red gall stones are finely powdered, washed with ether[1] to remove cholesterin, and then with boiling water. The residue is thrown on a filter paper and washed with dilute hydrochloric acid, which liberates the bilirubin from the calcium compound in which it exists, without, however, dissolving it. The acid is washed away with distilled water, and the filter paper dried, when the bilirubin is extracted with chloroform or alkali.

Preparation of Biliverdin.—Bilirubin prepared as above is dissolved in 10 per cent. sodium hydrate, when it quickly becomes green, especially when poured into a wide flat vessel. It is precipitated by hydrochloric acid, washed on a filter paper with distilled water, and finally dissolved in alcohol.

XI.—**Products of Oxidation of Bile Pigments.** *Preparation of bilicyanin.*—Take a chloroform solution of bilirubin, or an alkaline solution of bilirubin, and add drop by drop strong nitric acid. When a blue colour commences to appear excess of alcohol is to be added, when the oxidation becomes slower, and the true blue is gradually produced.

Preparation of Choletelin.—Add excess of nitric acid to a solution of either bile pigment. Bilicyanin appears transitorily quickly giving place to a yellowish red solution of choletelin.

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[1] Best done in a Soxhlet's apparatus.

XII.—**Product of Reduction of Bilirubin.** (Dem.)—Bilirubin is suspended in water, and sodium amalgum added. The resulting sodium hydrate dissolves the pigment, and the nascent hydrogen gradually reduces it to hydrobilirubin. After two or three days' reduction, during which more of the amalgam is periodically added, the mixture is gently warmed until no further change in colour is observable. Hydrochloric acid is then added, and the precipitated hydrobilirubin filtered off and dissolved in alcohol. Examine its absorption spectrum, noting the distinct band between "B" and "F."

XIII.—**Reactions of Bile Pigments.** *Perform Gmelin's Test* (Part I., p. 16).

Ehrlich's Test for Bilirubin. To a solution of bilirubin in chloroform is added an equal volume of an aqueous solution of diazobenzene[1] sulphonic acid, and enough alcohol to enable the fluids to mix; a red colour gradually develops, which on gradual addition of hydrochloric acid passes through violet to blue.

Preparation of tri-bromobilirubin (Dem.)—A dilute solution of bromine in chloroform is added drop by drop to a solution of bilirubin also in chloroform. On shaking the mixture it becomes at first green, and on adding more bromine changes to a pure blue. The green colour is due to the mixture of unaltered bilirubin with blue tri-bromobilirubin. Excess of bromine destroys the blue colour.

Cholesterin.—The solution obtained by washing gall stones with ether has been evaporated to dryness.

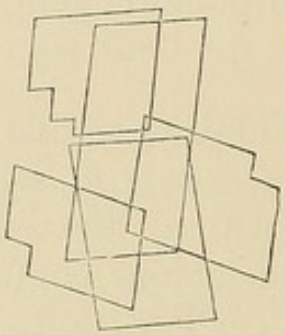

FIG. 34.—CRYSTALS OF CHOLESTERIN.

Take a small piece of the dry residue, place it on a slide with a little alcohol, warm cautiously, cool, and examine with a microscope. Note the notched rhombs of cholesterin.

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[1] Or of the following reagent:—Sulphanilic acid 1 gramme, hydrochloric acid 15 cc., sodium nitrite ·1 gramme, water to 1 litre.

Another portion of the dry residue has been dissolved in chloroform. Test it with sulphuric acid as in Part I., p. 16.

The residue under examination is not pure cholesterin, but contains lecithin, &c.

Muscle.

Take the reaction of living frog's muscle—it is amphoteric. Plunge the muscle into hot water, and when *rigor caloris* is established test the reaction again. Take the reaction of minced butcher's meat.

Make an aqueous extract of minced butcher's meat, filter and test it for albumin, albumose, sugar, creatinin, lactic acid, and salts. Note that the bulk of the muscle is insoluble in water. Dissolve the residue in a 10 per cent. solution of sodium chloride, strain through muslin, and test it for globulin, proving the magnesium sulphate precipitate to be soluble in saline solutions, and to be coagulable by heat. Filter a small quantity of your saline extract of muscle and ascertain at what temperature heat-coagulation commences. (p. 5). This will be below 50° C., and will be due to paramyosinogen (musculin).

XIV.—**Preparation of Muscle Plasma.** (Dem.)—Living muscle either from frog or mammal is cut into small pieces, wrapped in gutta-percha tissue, and frozen in a mixture of ice and salt. When frozen it is removed, minced, and squeezed in a press. A few drops of muscle plasma exude; note their alkaline reaction. On standing an acid reaction is developed. On falling into distilled water each drop gives a precipitate.

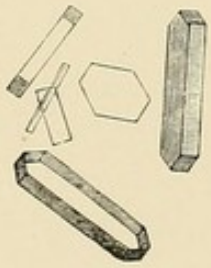

Fig. 35.—Creatin (Funke).

Separation of Creatin.—Take solution of Liebig's extract of meat, precipitate the phosphates with baryta mixture, filter, pass carbon dioxide through the filtrate to remove the barium salts, and filter again. Concentrate on a water bath, and set aside until the next meeting of the class. Crystals of creatin will be found in it.

XV.—**The Pigments of Muscle.**—A rabbit has been bled to death and its vessels washed out with normal saline solution. Compare its "red" and "pale" muscles. The chief pigment is hæmoglobin. Identify it with a spectroscope on a piece of diaphragm pressed between two glass plates. Examine in the same way a piece of pectoral or cardiac muscle from a pigeon. Look for the spectrum of myohæmatin.

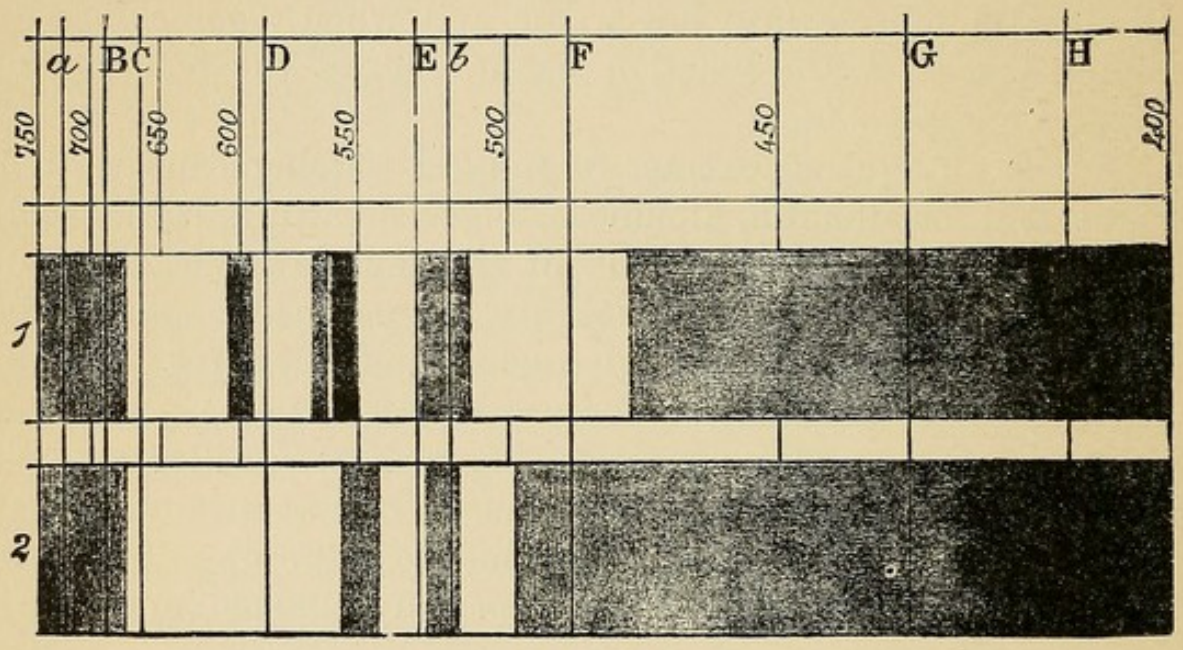

FIG. 36.

(1) Absorption spectrum of myohæmatin.
(2) Absorption spectrum of "modified myohæmatin." (Halliburton).

Examine the spectrum of "modified myohæmatin" in the red fluid which exudes from a muscle soaked in ether. It is very like that of hæmochromogen. The individuality of myohæmatin is a matter of doubt.

CHAPTER VII.

URINE.

I.—Take the specific gravity. Multiply its last two figures by 2·33—the product indicates roughly the number of grammes of solids present per litre.

Take reaction. Prove absence of free acid by means of sodic thiosulphate, *cf.* gastric juice.

II.—**The Inorganic Salts of Urine.**—Test for chlorides. Estimate the chlorides, expressed as NaCl, by Volhard's method as follows :—

Volhard's Method of estimating NaCl in Urine.—In a 100 cc. flask place about 70 cc. of distilled water, 2 cc. of nitric acid, and then, from pipette or burette 10 cc. urine and 20 cc. $\frac{N}{10}$ solution of silver nitrate. All the urinary chloride is precipitated as AgCl, and some of the silver nitrate left unchanged. The amount of unaltered $AgNO_3$ estimated as follows, and subtracted from the 20 cc. $\frac{N}{10}$ solution, originally taken, gives the amount of $\frac{N}{10}$ $AgNO_3$ decomposed by the urine.

To the contents of the flask add 5 cc. of saturated solution of iron alum, and run in from a burette $\frac{N}{10}$ ammonic thiocyanate until after complete admixture and subsidence of the precipitate, the fluid is tinged permanently red by ferric thiocyanate.

If, *e.g.*, you used 5 cc. of the thiocyanate solution, then 5 cc. of $\frac{N}{10}$ $AgNO_3$ were left unchanged, and 20 minus 5 cc. (*i.e.*, 15 cc.) were decomposed by the urinary chloride. Hence the 10 cc. urine contain the equivalent of 15 cc. $\frac{N}{10}$ NaCl = (15 × ·00585) grammes NaCl = ·08775 grammes, and 100 cc. contain ·8775 grammes NaCl if we assume the whole of the urinary chloride to be that of sodium.

Test the urine for phosphates. Take 10 cc. urine, add

ammonia, and boil—earthy phosphates are precipitated. Estimate the total phosphates in urine (v. Part I., p. 23).

Test the urine for sulphates, filter off the precipitated barium sulphate, and boil. Turbidity indicates liberation of sulphuric acid from the "conjugated" sulphates.

Estimate the total sulphates in urine as follows:—

Take 100 cc. urine in a beaker, add 5 cc. of hydrochloric acid, and boil to decompose the "conjugated" sulphates. Then run in from a burette the standard solution of barium chloride, stirring to ensure admixture. The heating is to be discontinued at intervals and, when the precipitate has subsided, a drop of the mixture brought on a glass rod to a drop of a strong solution of sodic sulphate previously placed on a black tile. Faint turbidity indicates that all the sulphate is precipitated. The standard solution employed is of such strength that 1 cc. exactly precipitates ·01 gramme SO_3. You may expect to use 15 to 20 cc. from the burette. The comparative indistinctness of the end point makes volumetric estimation of sulphates inexact. The gravimetric method ("Human Physiology," p. 238) is more accurate.

III.—**Separation of Urea from Urine.**—Take 25 cc. of concentrated urine, in a 100 cc. flask, add excess of nitric acid, cooling the flask under the tap—crystals of urea nitrate are

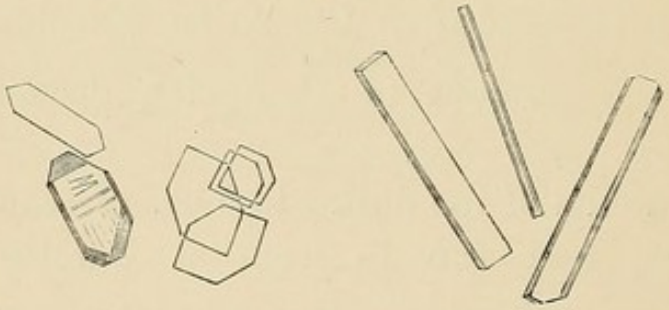

FIG. 37.—CRYSTALS OF NITRATE AND OXALATE OF UREA.

deposited. Examine some with microscope. Filter through a "hardened" filter, scrape the crystals into a mortar, and grind them into a paste with barium carbonate and a minimum of alcohol. Dry this over boiling water, extract with alcohol, filter, and drive off the alcohol from the filtrate by heat as above, when fairly pure urea will be left.

Whilst this is in progress estimate the urea in 5 cc. urine by the hypobromite method (Part I., p. 25). Repeat the estimation after adding glucose to the urine. You will then obtain a greater quantity of nitrogen.

Estimation of Urinary Nitrogen. (Kjeldahl.) *Principle of the method.*—The nitrogenous bodies are oxidised to sulphate of ammonia or allied substances. The ammonia is distilled from these, and collected in a known quantity of decinormal acid. The loss of acidity due to the ammonia is ascertained by titration, and the nitrogen calculated from it.

Oxidation.—Five cubic centimetres of urine are placed with 20 cc. pure sulphuric acid and a globule of mercury in a flask, and gently heated until the initial violence of the reaction has subsided. The fluid is now further heated to boiling for fifteen minutes, when about 10 grammes of pure potassic sulphate are added. The boiling is resumed and maintained until the fluid is almost colourless. It is then cooled, transferred to a larger flask, and diluted to about ten times its volume.

Distillation.—There are then quickly added: (1) 50 cc. of saturated solution of caustic soda to alkalise the fluid. (2) 25 cc. of saturated solution of potassic sulphide to decompose mercurammonium compounds. (3) Granulated zinc to prevent bumping, and the flask is rapidly connected to a condenser, which delivers into[1] a vessel containing 50 cc. of $\frac{N}{10}$ sulphuric

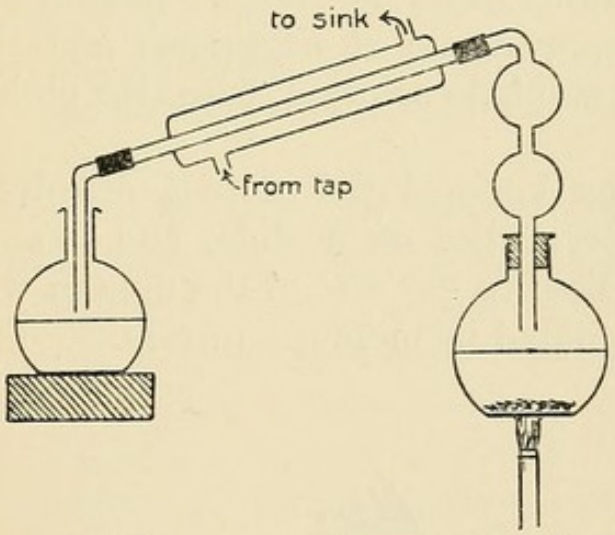

FIG. 38.—ESTIMATION OF NITROGEN BY KJELDAHL'S METHOD. DISTILLATION OF THE AMMONIA INTO $\frac{N}{10}$ SULPHURIC ACID.

acid. Heat is applied to the large flask, and distillation is permitted for half an hour, in course of which all the nitrogen distils as ammonia in aqueous solution into the decinormal acid.

[1] The delivery tube should not dip into the decinormal acid. The condenser may be dispensed with, if the delivery tube of the flask be long enough, but omission of the condenser is risky.

Titration.—When distillation is over, the acid is titrated with $\frac{N}{10}$ alkali, with methyl orange as indicator.

You are given a flask which contained 50 cc. $\frac{N}{10}$ sulphuric acid, and which has received the products of oxidation and distillation of 5 cc. of urine. Estimate its acidity as above. Suppose that 20 cc. of $\frac{N}{10}$ alkali suffice to neutralise it. Then the products of distillation had neutralised the other 30 cc., *i.e.*, these products contained the equivalent of 30 cc. of $\frac{N}{10}$ ammonia.

$$= (\cdot0017 \times 30) \text{ grammes} = \cdot051 \text{ grammes } NH_3.$$

$$= \frac{\cdot051 \times 14}{17} \text{ grammes nitrogen} = \cdot042 \text{ grammes.}$$

Preparation of Biuret from Urea.—Take the urea which you have extracted from urine, place a knifepointful in a dry test tube, and heat cautiously. The crystals melt, and the fluid gradually becomes opalescent, and finally dries to an opaque white mass. Meanwhile carbonate of ammonia is produced, and condenses as a whitish film on the cooler part of the tube. The opaque residue contains biuret. Dissolve this in solution of caustic soda, warming if necessary, and then add a few drops of copper sulphate solution, when the pink biuret reaction ensues.

Take another knifepointful of urea, dissolve in a minimum of water, let crystallise on a slide, and examine under the microscope. Dissolve the rest of the urea in water, and show that it is precipitated by mercuric nitrate.

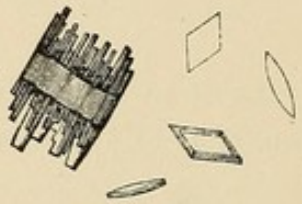

Fig. 39.—Crystals of Uric Acid.

IV.—**Separation of Uric Acid from Urine.**—Take 20 cc. urine in an evaporating dish, add 5 cc. hydrochloric acid, and set aside until next lesson. Then scrape the clumps of crystals on to a slide, and examine with a microscope.

V.—**Reactions of Uric Acid and Urates.**—Take commercial uric acid, dissolve in a minimum of 1 per cent. solution of sodic carbonate, concentrate, and let crystallise on a slide. Examine the resulting crystals of sodium biurate.

Dissolve a knifepointful of uric acid in 10 per cent. caustic soda solution, and test by Trommer's test (Part I., p. 7).

To another portion of the above add phosphomolybdic acid. A blue precipitate falls.

Take a knifepointful of serpent's excrement, mix with tap water, on a slide, and examine microscopically. Crystals of uric acid will form, liberated from the "quadri-urate" of the excrement.

The Murexide Test.—Apply the murexide test (1) to the uric acid obtained in IV., and (2) on concentrated urine. It will be less easy than with the large amount of uric used by you in Part I. The resulting purple is due to purpurate of ammonia. Substitution of soda for the ammonia gives a bluer purpurate of soda.

Estimation of Uric Acid. (Hopkins.) (Dem.)—One hundred cubic centimetres of urine are saturated[1] with ammonic chloride, and let stand at least two hours. The precipitated ammonic urate is filtered off, and washed from the filter into a basin with hot distilled water, after which 1 cc. of hydrochloric acid is added, and the vessel set aside for crystallisation of the thus liberated uric acid.

Next day the fluid is measured, and filtered through a small, dried, and weighed filter paper. The crystals are washed with distilled water, and the filter and its contents dried and weighed, when an increase in weight of 40 to 80 milligrammes may be found. Some of the uric acid remained in solution in the mother liquor. This is allowed for by adding 1 milligramme of uric acid for every 15 cc. of mother liquor, measured as above. The uric acid may be estimated[2] volumetrically with potassic permanganate, but the gravimetric method is probably the more accurate.

VI.—**Creatinin.**—Test urine for creatinin (Part I., p. 9). Test solution of acetone in the same way, and also a distillate from a diabetic urine. In urine it is not possible to dis-

[1] Thirty-five grammes will suffice. Saturation will not be quite complete, and so excess of undissolved salt is avoided.

[2] Hopkins' *Journal of Pathology and Bacteriology*, Vol. I.

tinguish between creatinin and acetone, but in a distillate only the latter of the two can be present.

Preparation of Creatinin—Zinc Chloride.—Urine has been treated with milk of lime and calcium chloride, filtered and concentrated. Some of the concentrated residue is given you, extract it with alcohol, filter, add a few drops of a concentrated and neutral alcoholic solution of zinc chloride to the filtrate and set aside until next lesson. Then scrape some of the crystalline deposit on to a slide, and examine the spherical clumps of radially arranged crystals with a microscope.

FIG. 40.—CRYSTALS OF CREATININ—ZINC CHLORIDE.

Preparation of Creatinin—Mercuric Chloride. (Johnson.)—To 100 cc. of urine add 5 cc. of a saturated solution of sodium acetate, and 20 to 25 cc. of saturated aqueous solution of mercuric chloride. Filter, and set aside until next lesson. Examine the deposit with the microscope. By collecting, washing, and weighing the whole of the deposit the amount of creatinin present is estimated. It is assumed that 20 per cent. of the weight of the compound is due to creatinin itself.

VII.—**Urinary Pigments and Chromogens.** *Separation of Urochrome.* (Garrod.)—Saturate 20 cc. urine with ammonic sulphate. Filter, and shake the filtrate with alcohol. On standing, the alcohol will separate from the remainder of the fluid, carrying some of the urochrome with it. Repeated shaking is said to cause extraction of nearly all the urochrome.

Preparation and Separation of Urobilin. (Méhu.)—To 50 cc. urine add a few drops of sulphuric acid, and saturate with ammonic sulphate. Filter, shake the precipitate with 20 cc. alcohol containing two drops of sulphuric acid, and filter again. The acid converts the chromogen (urobilinogen) to urobilin. The salt precipitates the pigment, which the acid alcohol dissolves. Examine the acid alcohol with a spectroscope and note the band of urobilin.

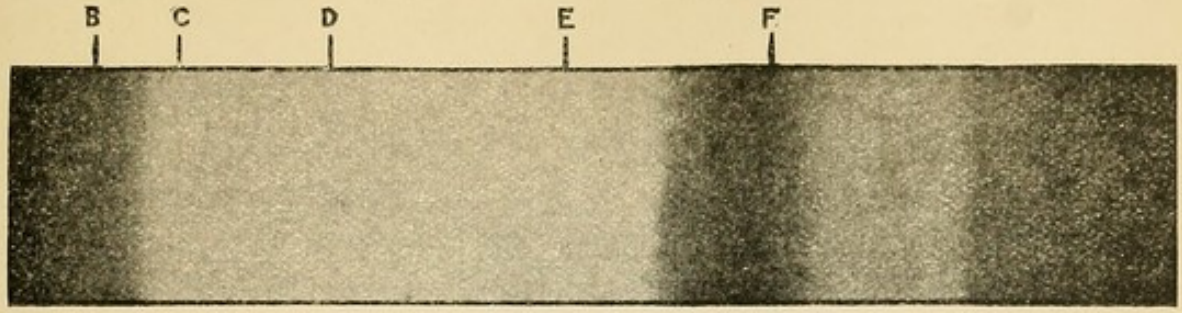

FIG. 41.—ABSORPTION SPECTRUM OF UROBILIN.

Separation of Hæmatoporphyrin. (Salkowski.)—Take 50 cc. urine, add 20 cc. baryta mixture, and filter. Wash the precipitate, on the filter, with distilled water, then scrape it into a dish, add 15 cc. of alcohol acidified with sulphuric acid, let stand for half an hour, filter, and examine the filtrate with spectroscope. Administration of sulphonal increases the urinary hæmatoporphyrin.

Preparation and Separation of Indigo-blue.—Take 20 cc. urine[1], add an equal volume of strong hydrochloric acid to decompose the chromogen ("indican" = potassium indoxyl sulphate), and 5 to 10 cc. of chloroform. Then add ·5 per cent. solution of potassic permanganate (a few drops at a time), shaking after each addition. The indoxyl of the chromogen is oxidised to indigo blue (occasionally to indigo red) by the permanganate, and is dissolved by the chloroform, to which it imparts its colour. Excess of the oxidising fluid destroys the colour.

[1] Preferably horses' urine. Any alkali present must be neutralised before decomposing the chromogen.

CHAPTER VIII.

Respiration.

I.—Volumetric estimation of carbon dioxide and oxygen in air.
II.—Collection and examination of expired air.
III.—Calculation of respiratory quotient.
IV.—The spirometer.

I.—Volumetric Estimation of Carbon Dioxide and Oxygen in Air.—*Principle.*—One hundred cubic centimetres of air are collected, the carbon dioxide is removed by caustic potash, and the residue measured. This gives the volume of carbon dioxide that was present. Oxygen is then absorbed from this residue by phosphorus, and its volume similarly determined.

Practice.—The apparatus consists of a gas burette for collecting and measuring the air and the residues left after each absorption, and of two gas pipettes, containing caustic potash and phosphorus respectively, in which the absorptions take place.

The gas burette consists of a vertical tube joined by rubber tubing to a movable filling bulb, and is graduated to hold 100 cc. of air. It is connected above to its inlet, and to the two gas pipettes, the connections being provided with taps, 1, 2 and 3. It is filled with distilled water containing 5 per cent. of sulphuric acid which has been saturated with carbon dioxide and with oxygen. Air is collected by opening tap 1, driving out any gas that may be in the burette by raising the filling bulb until the water reaches the zero mark, connecting the inlet with the gas to be examined, lowering the filling bulb until rather more than 100 cc. of gas has been drawn in, and then raising the filling bulb again until excess over 100 cc. has been expelled.

The gas pipettes consist of two bulbs, one cylindrical, the other spherical. The carbon dioxide pipette is charged as follows :—Pieces of glass tube about 5 cm. long and ½ cm. in diameter are placed in the cylindrical bulb until it is about one quarter full, when it is corked with a well-fitting rubber stopper, and charged with a 50 per cent. solution of caustic potash.

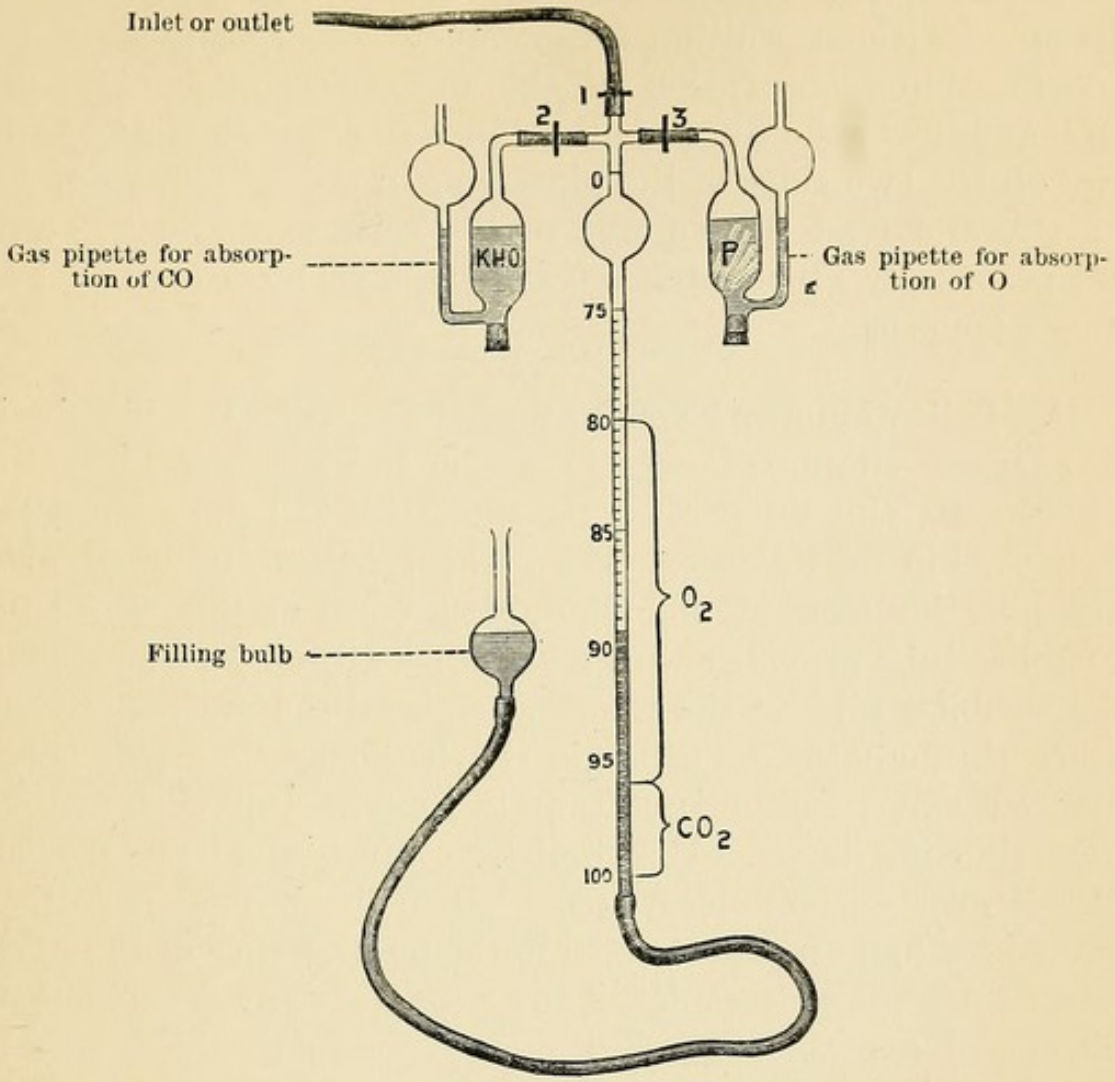

FIG. 42.

The oxygen pipette is similarly prepared, substituting clean sticks of phosphorus for the glass tube and water for the caustic potash solution.

Examination of atmospheric air.—The amount of carbon dioxide in the atmosphere is so small (·04 volumes per cent.) that it cannot be measured by the apparatus, and may be calculated as zero.

Collect 100 cc. of atmospheric air as above, close tap 1, and pass it into the oxygen pipette as follows:—Open tap 3 and raise the filling bulb until the water reaches the zero mark, then close the tap. Absorption of oxygen commences immediately, and at 20° C. is complete in 3 to 5 minutes. At 15° C. 15 to 20 minutes are necessary, and at 10° C. 30 to 40 minutes must be allowed. In a dark room, disappearance of the glow which accompanies oxidation of the phosphorus may be taken as indicating that absorption of oxygen is complete. The oxides of phosphorus are so freely soluble in water that they

exercise no appreciable tension, and the residue may be measured without waiting for complete disappearance of the fumes. When absorption of the oxygen is complete, open tap 3 and lower the filling bulb until water stands at the same level on the two sides of the pipette or in the two limbs of the burette, and read the volume of the residue. You will find that practically 21 cc. of gas (oxygen) have been absorbed by the phosphorus.

II.—Collection and Examination of Expired Air.—To obtain an average sample of expired air it is necessary to collect between 10 and 20 litres. Do this with a balloon of gold-beaters' skin as follows:—Tie a short length of wide glass tubing in the neck of the balloon, and connect this to a piece of rubber tube provided with a clamp. Holding the free end of the rubber tube in the mouth, inspire through the nose and expire through the mouth into the balloon. The soft palate acts as a valve, and ensures that the expired air is not mixed with atmospheric air. It is better to empty the balloon again after about a dozen expirations. When the balloon is full, let it stand for half an hour to cool to the temperature of the room. Then connect it to the inlet of the apparatus and draw in about 100 cc. of expired air. Reject this sample, which will be diluted with the atmospheric air in the dead space of the apparatus, then draw in an accurately-measured 100 cc. of the expired air, and examine it as above. You will find between 3 and 4 cc. of CO_2 and 16 or 17 cc. of oxygen. Note the residue of nitrogen left in the burette; it tells you from how much atmospheric air the 100 cc. of expired air was derived, since nitrogen is neither absorbed nor excreted by the lungs. Suppose that you find:—

CO_2	4 cc.
O_2	16 cc.
N_2	80 cc.
	100

the 100 cc. of expired air was derived from $(100 \times \frac{80}{79})$[1] cc. = 101·3 cc. of atmospheric air, and therefore contained $\frac{21}{100} \times 101{\cdot}3 = 21{\cdot}26$ cc. of oxygen.[1]

[1] Assuming atmospheric air to consist of 79 volumes N_2 and 21 volumes O_2 per cent.

III.—Calculation of Respiratory Quotient.—Calculate your respiratory quotient $\frac{\text{Vol. } CO_2 \text{ expired}}{\text{Vol. } O_2 \text{ absorbed}}$ as follows. The volume of CO_2 in the 100 cc. of expired air was 4 cc.; that of the O_2 absorbed was (21·26 — 16) cc. = 5·26 cc. Your respiratory quotient with the supposed sample of expired air was therefore $\frac{4}{5 \cdot 26} = \cdot 76$.

IV.—The Spirometer.—The spirometer consists of a movable metal air chamber, hanging in a vessel of water, over a tube through which air is introduced or withdrawn. The air chamber, suspended by a cord passing over a pulley, is counterpoised by a weight, so that it moves freely up or down when air enters or leaves it, and is provided with an index moving against a fixed scale, showing the volume of air in the chamber. The air-tube is provided with a tap and mouthpiece.

Vital capacity.—Determine your vital capacity as follows: Open the tap, and gently and slowly push the air chamber to its lowest point. Take as deep an inspiration as possible, close the nares, and then expire as fully as you can through the air tube into the apparatus. Close the tap and note the volume of air expired. You will find between three and four litres.

Tidal air.—Determine your tidal air in the same way, by measuring the volume of an ordinary expiration, after an ordinary inspiration. Do this three or four times. You will find it to average rather less than half a litre.

Supplemental air.—Measure the volume of a maximal expiration, after an ordinary inspiration. Subtract the volume of your tidal air from this, and the difference is your supplemental air. It averages a litre and a half.

Complemental air.—Calculate your complemental air by subtracting the volume of a maximal expiration found as above from your vital capacity. It amounts to nearly two litres.

Carbon dioxide dyspnœa.—Empty the spirometer and half fill it with oxygen. Breathe into and out of it, with closed nostrils, until dyspnœa compels you to cease. Draw off 100 cc. of its contents into the gas apparatus, and estimate the oxygen and carbon dioxide. You will find more than 20 cc. of oxygen, and about 8 cc. of carbon dioxide. Your dyspnœa was therefore not due to insufficiency of the former gas, but to excess of the latter.

EXERCISES IN

PRACTICAL PHYSIOLOGY

BY

AUGUSTUS D. WALLER, M.D., F.R.S.

LECTURER ON PHYSIOLOGY TO ST. MARY'S HOSPITAL MEDICAL SCHOOL

PART III.

PHYSIOLOGY OF THE NERVOUS SYSTEM

ELECTRO-PHYSIOLOGY

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PREFATORY NOTE.

The following pages form Part III. of a series of Exercises and Demonstrations to accompany "An Introduction to Human Physiology," *and are primarily intended to facilitate the class-work of this Laboratory.*

The Directions given in them are addressed to "advanced" students who have properly expended one year in the study of Physiology.

In some cases it will be found that an exercise may be carried out by each student working independently; in others, that the student will require much assistance from a skilled demonstrator; in others still, that the student will at most take some part in a carefully-prepared demonstration.

A. D. Waller.

PART III.

EXERCISES AND DEMONSTRATIONS IN THE PHYSIOLOGY OF THE NERVOUS SYSTEM.

ELECTRO-PHYSIOLOGY.

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1 Galvanic Cells; Daniell, Leclanché.
2 Du Bois-Reymond Key; Tumbler Key.
3 Pohl's Commutator; Circular Commutator.
4 The Galvanoscope or Current-Indicator.
5 Du Bois-Reymond's Induction Apparatus.
6 Principle of the Helmholtz' Modification.
7 Demonstration of the Break Extra-Current.
8 Demonstration of the Make Extra-Current.
9 To cut out the Make or Break Current.
10 Verification of Ohm's Law.
11 Action of Rheostat as a Shunt.
12 The Rheochord; the Monochord.
13 Measurement of Resistance.
14 Kelvin's Reflecting Galvanometers.
15 Measurement of Potential by Compensation.
16 Demonstration of Equipotential Lines.
17 Excitation of Nerve by the Condenser; Minimum Energy of an Electrical Stimulus.
18 Unipolar Stimulation.
19 Unpolarisable Electrodes.
20 Lippmann's Capillary Electrometer.
21 Recording Apparatus.
22 The Chronograph.
23 Photo-galvanometric and Photo-electrometric Records.
24 To Pith a Frog; Decerebration.
25 To Prepare a Muscle or a Nerve-Muscle for Experiment.
26 Action of Curare.
27 Action of Veratrine.
28 A Single Muscular Contraction. Effect of Heat and Cold.
29 Two Successive Contractions.
30 Many Successive Contractions; Clonus; Tetanus.
31 Fatigue.
32 Extensibility of Muscle.
33 Electrotonic Alterations of Excitability. (Frog).
34 Pflüger's Law of Contractions. (Frog.)
35 Law of Contractions. (Man).
36 Electrotonic Alterations of Excitability. (Man).
37 Measurement of the Velocity of Nervous Impulses. (Man.)
38 Influence of Temperature upon the Excitability of Nerve. (Gotch.)
39 Electrotonic Currents.

40 The Paradoxical Contraction.
41-2 Galvani's First and Last Experiments with and without metals.
43 Muscle-Currents.
44 Nerve-Currents: the Current of Injury and its Negative Variation.
45 Action of Anæsthetics upon Isolated Nerve. Carbon Dioxide, Chloroform, and Ether.
46 The Secondary Contraction.
47 Secondary Contraction from the Heart.
48 An Apparent Anomaly due to Secondary Contraction. (Hering.)
49 Secondary Excitation from Nerve to Nerve.
50 Currents of Action of Frog's Heart.
51 Currents of Action of Mammalian Heart.
52 Currents of Action of Human Heart.
53 Retinal Currents. (Frog.)
54 Sound is Produced during Muscular Contraction.
55 Heat is Produced during Muscular Contraction.
56 Tendon-Reflex Time. (Man.)
57 Tendon-Reflex Time. (Rabbit.)
58 Function of Nerve-Roots. (Müller's Experiment.)
59 "Overlap" of Nerve-Supply. (Sherrington.)
60 Reflex Actions of Brainless Frog. (Goltz' Klopf-Versuch.)
61 Inhibitory Action of Superior upon Inferior Centres.
62 Time of Reflex Action. (Frog.)
63 Action of Strychnia. (Frog.)
64 Summation of Stimuli. (Frog.
65 Reflex Winking Time. (Man.)
66 Sensory Reaction-Timing. (Man.
67 Discrimination Time. (Man.)
68 Volition Time. (Man.)

ELECTROPHYSIOLOGICAL INSTRUMENTS AND PRINCIPLES.

(1) PUT up a galvanic cell; you should first amalgamate the zinc and scrape the ends of all wires and terminal screws.

The cells in ordinary use in this laboratory are the Daniell, the bi-chromate, and the Leclanché.

The Leclanché is the most convenient, but must not be used too long in a circuit of low resistance (*e.g.*, primary coil), and must never be left "short-circuited."

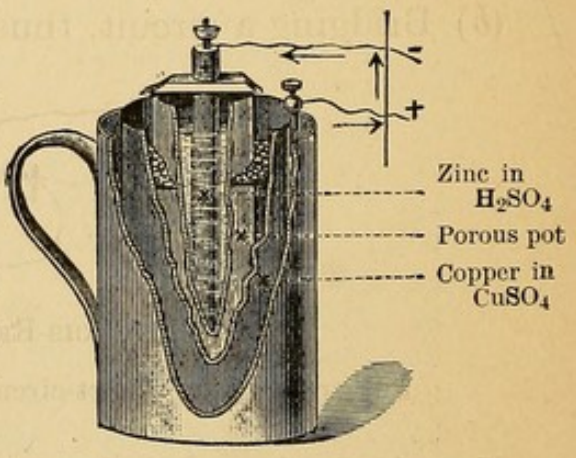


FIG. 1.

Remember that in all these cells the zinc is the positive

element, the end of the wire from it is the negative electrode or kathode, and the direction of current is from anode to kathode; we shall habitually designate any kind of cell by the conventional figure ⊂.

(2) Study the du Bois-Reymond key and Pohl's commutator, in connection with a cell and a current indicator.

The du Bois key can be employed—

(*a*) To interrupt a circuit, the key being interposed in the course of one of the wires of the circuit, which is therefore made by closing the key, broken by opening the key;

(*b*) To bridge a circuit, both wires from the cell being connected with the two sides of the key, and other two wires being connected with the electrodes; the current is therefore made through the electrodes by opening the key, broken by closing the key. When used in the primary circuit of a coil, the key should be put up to break one wire (*a*), when used in the secondary circuit the key should be put up to bridge both wires (*b*).

We shall habitually designate any kind of key by the letter K,

(*a*) Interrupting a circuit, thus—

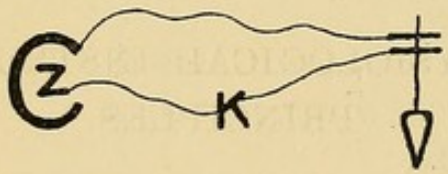


FIG. 2.

(*b*) Bridging a circuit, thus—

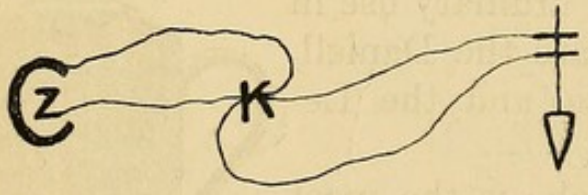


FIG. 3.– DU BOIS-REYMOND'S FRICTION KEY.

Put up so as to "short-circuit" current when it is closed.

For producing a "clean" make and break, this key is not suitable on account of friction, and a simple contact or spring key or a simple mercury key is preferable.

Tumbler key.—A very convenient, cheap, and reliable form of du Bois-Reymond key is afforded by the "tumbler key" used in the electric light trade. As used in this laboratory, the two metal blocks are each provided with a double terminal for convenience of fixing two pairs of wires. The key may be used to interrupt or to bridge a circuit as above described.

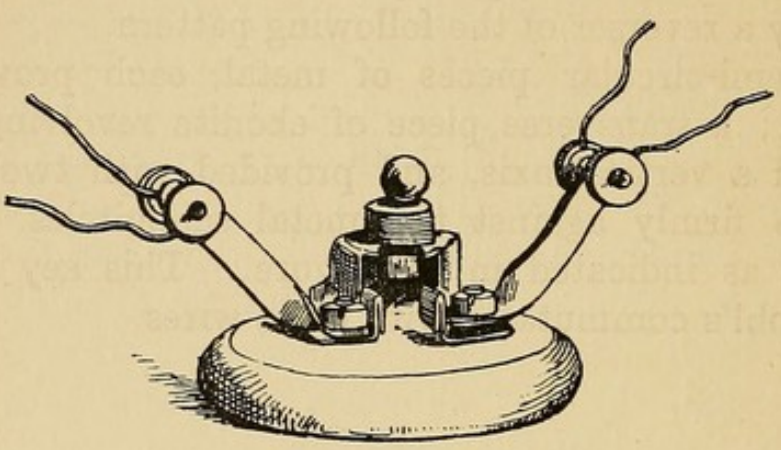


FIG. 4.—"TUMBLER KEY," REPRESENTED AS IF BRIDGING A CIRCUIT.

(3) **Pohl's commutator** can be employed—

(*a*) With cross wires, to reverse the direction of a current;

(*b*) Without cross wires, to turn a current into one or other of two circuits.

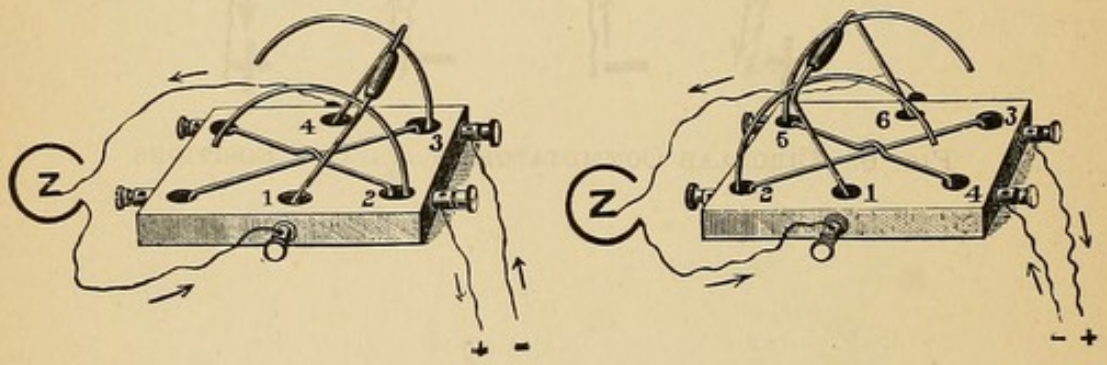


FIG. 5.—POHL'S COMMUTATOR (WITH CROSS WIRES).

The Daniell cell and electrode wires remaining fixed, the direction of current in the latter is reversed by moving the cradle to right or left. Numbers 1, 2, 3, &c., indicate the path of current in the two cases.

In this laboratory mercury apparatus are, as far as may be, replaced by brass instruments. Spring keys are used in preference to mercury keys, and Pohl's commutator is replaced by a reverser of the following pattern.

Two semi-circular pieces of metal, each provided with a terminal; a transverse piece of ebonite revolving horizontally round a vertical axis, and provided with two terminals which rub firmly against the metal semicircles. Reversal of current as indicated in the figure. This key is used in place of Pohl's commutator with cross wires.

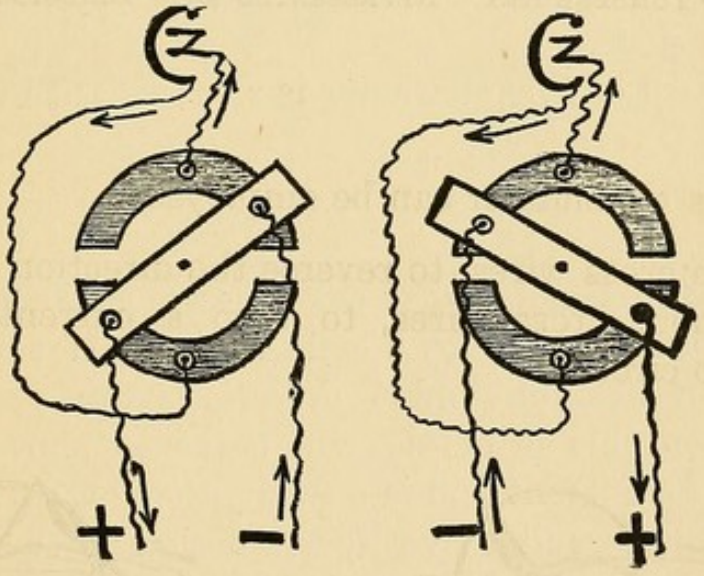


FIG. 6.—CIRCULAR COMMUTATOR IN ITS TWO POSITIONS.

If it is desired to turn a current into one or other of several circuits, or—what amounts to the same thing—to lead off from one or other of several circuits to a galvanometer, or—as when "compensation" is made—to conjoin two currents into one circuit, a convenient and simple apparatus is a keyboard on this principle, composed of a series of tumbler keys arranged as in fig. 7.

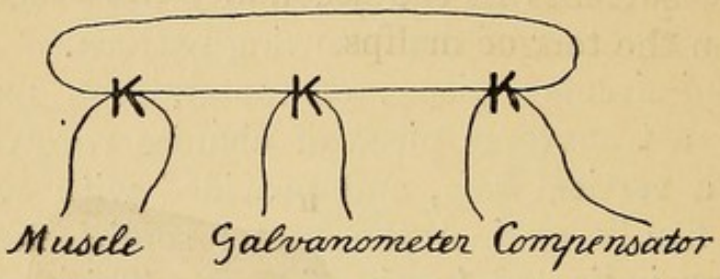


FIG. 7.

When all the keys are closed, current is short-circuited; when any key is opened current passes in the circuit connected with its two sides.

A key-board of this character is represented in fig. 7.

(4) **The current-indicator or galvanoscope** consists of a magnetised needle surrounded by a coil of wire. It serves to show the *passage* of a current and its *direction;* a properly graduated instrument on this principle will serve to show further the *magnitude* of a current and is now a *galvanometer.* (Ex. 14.)

Put up this circuit to verify the action of the commutator in reversing current through the galvanoscope.

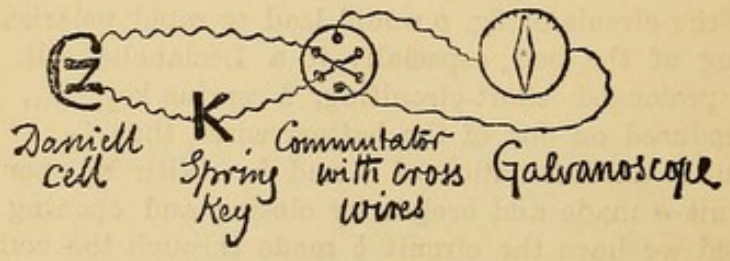


FIG. 8.

(5) **The induction apparatus** of du Bois-Reymond consists of (*a*), a cell supplying current to (*b*), a primary or thick wire coil by which currents are induced in a secondary or thin wire coil. These secondary currents are those employed for excitation, and their strength is altered by altering the distance between secondary and primary coils.

Connect the cell with the primary circuit, with a key to gap one of the wires. Connect the electrodes with the secondary circuit, with a second key to bridge the circuit. Test for the currents in the secondary circuit by placing the electrodes on the tongue or lips.

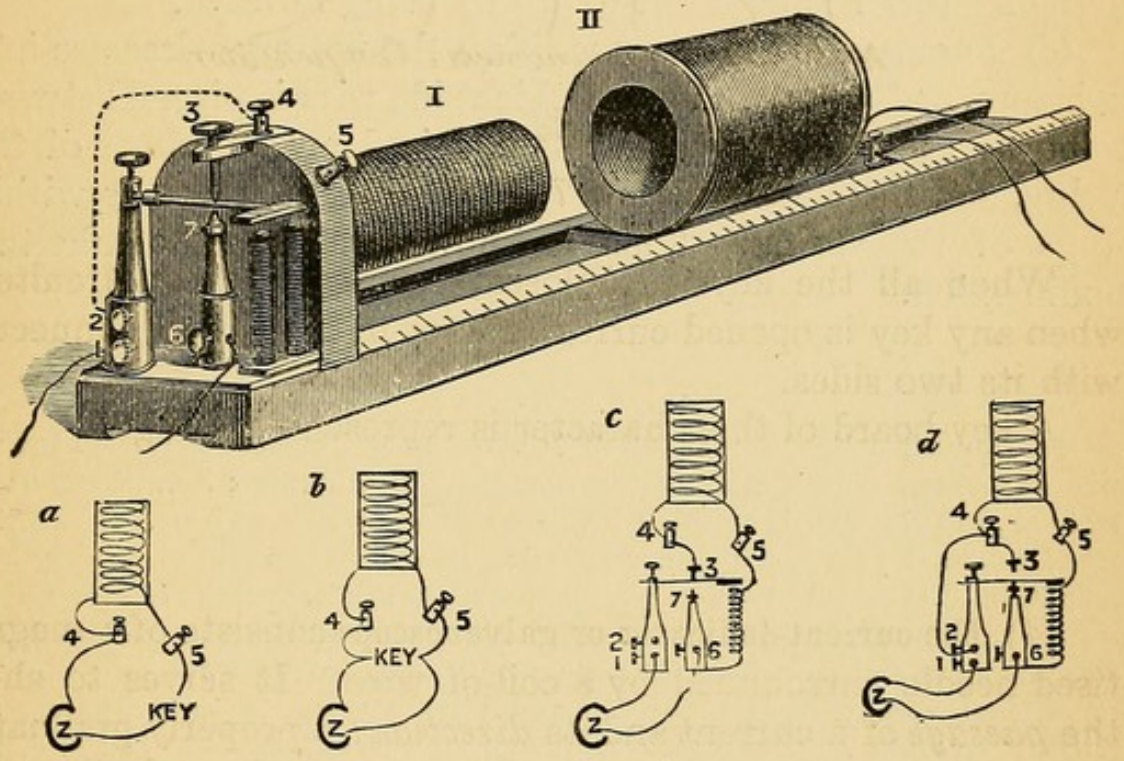
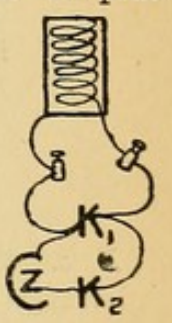


FIG 9.—DU BOIS-REYMOND'S INDUCTION APPARATUS, WITH DIAGRAMS OF THE CONNECTIONS TO BE MADE BETWEEN CELL AND PRIMARY COIL FOR THE FOUR KINDS OF CURRENTS THAT MAY BE OBTAINED, *a*, *b*, *c*, *d*.

N.B.—Figs. *a* and *b* are given for the sake of systematic clearness. In practise, however, the circuit of fig. *b* would lead to rapid polarisation and weakening of the cell, especially if a Leclanché cell. To prevent too prolonged short-circuiting, a spring-key, K_2, had better be introduced on one of the battery wires, thus:—



This circuit is a combination of *a* and *b*. With K_1 open we have the circuit *a* made and broken by closing and opening K_2. With K_2 closed we have the circuit *b* made through the coil by opening K_1, and broken by closing K_1.

The numbers 1 to 7 indicate the terminals and contact screws connected with the primary coil.

For single shocks the two battery wires are to be connected with the terminals 4 and 5, which are at the two ends of the primary wire.

(*a*) *Ordinary shocks* are obtained when a key is used to interrupt one of the wires.

(*b*) *Modified shocks* are obtained when a key is used short-circuiting the primary wire.

(*c*) *For repeated shocks* (*ordinary*) the two battery wires are to be inserted at 1 and 6. The circuit now includes the spring interrupter and the wire of the electro-magnet by which the circuit is made and broken at the contact screw 3; the contact screw 7 is kept out of use by being lowered.

(*d*) *For repeated shocks* (*modified*) the battery wires are left, as before, at 1 and 6. A short thick side wire is placed between 2 and 4. The contact screw 3 is raised out of range of the spring, and the contact screw 7 is raised until it comes within range of the spring. This is known as the "Helmholtz" modification.[1]

[1] In ordinary coils the graduation is given in millimeters only. But mere distance gives no true idea of relative strength of stimulation; *e.g.*, with the coil at 10 cm., the strength is nothing like one half what it is at 5 cm. The better sort of coils are provided with a graduation in units of strength. Failing this graduation, it is useful to draw up a graduation in arbitrary units of strength by means of a reflecting galvanometer. Single induction shocks from the secondary coil at different distances from the primary, give galvanometer deflections nearly proportional with current strength; and from a series of such readings an arbitrary strength scale can be constructed on the coil opposite to the centimeters of the distance scale. This should be done by the demonstrator.

The accompanying table gives an example of such a graduation:

Distance.	Strength.	Distance.	Strength.
15	30	7	255
14	35	6	405
13	45	5	700
12	55	4	1460
11	70	3	4000
10	90	2	10300
9	115	1	18700
8	175	0	25000

Note.—With an ungraduated coil an approximate scale is afforded by taking the strength as varying inversely as the square of the distance.

When you have become familiar with the four modes of connection described in connection with fig. 9, take a series of observations with the electrodes on the tongue and observe—

With (*a*) that the break induction shock is stronger than the make induction shock;

With (*b*) that both shocks are reduced, but especially so the break;

With (*c*) and (*d*) that the effect on the tongue is greater with (*c*) than with (*d*), with (*c*) than with (*a*), with (*d*) than with (*b*).

Note in each case the greatest distance of secondary from primary coil at which you first feel the secondary make and break currents, and fill up the accompanying table with your results.

		Single Ordin. (*a*)	Single Modif. (*b*)	Repeated Ordin. (*c*)	Repeated Modif. (*d*)
Greatest distance at which shock is felt.	At make				
	At break				

[Remember that make and break currents in a secondary coil are altogether different from the make and break of a battery current.]

(6) **Principle of the Helmholtz' modification.**—Not only does a current made or broken in one coil (primary) induce other currents in a second coil, but in any single coil every turn of wire has an inductive influence upon every other turn. This influence is termed self-induction, and the currents thus generated are termed extra-currents. The inequality between the ordinary make and the break shocks from the secondary coil is due to an extra-current in the primary coil; the direction of an extra-current is against the battery current at make, with it at break, thus delaying the rise (but not delaying the fall, inasmuch as it does not pass at all, or, more strictly, inasmuch as it is non-existent, the circuit being broken). Therefore, if means can be provided for the break extra-current to exist, the break shock will be reduced and made more nearly equal to the make shock. This is done by the Helmholtz side-wire between the terminals 2 and 4 (fig. *d*), which keeps the primary circuit complete during variations of its current. Those variations are effected by a key or interrupter cutting out current by "bridge" at the lower screw 7, instead of by "gap" at the upper screw 3 (fig. 9).

As a general rule the Helmholtz modification of the coil should be adopted when induced currents are applied to nerve or muscle. By this precaution, the risk of two possible fallacies is greatly diminished, viz., (1) *an undesirable predominance of currents in one direction,* i.e., *in that of the break;* (2) *unipolar stimulation.*

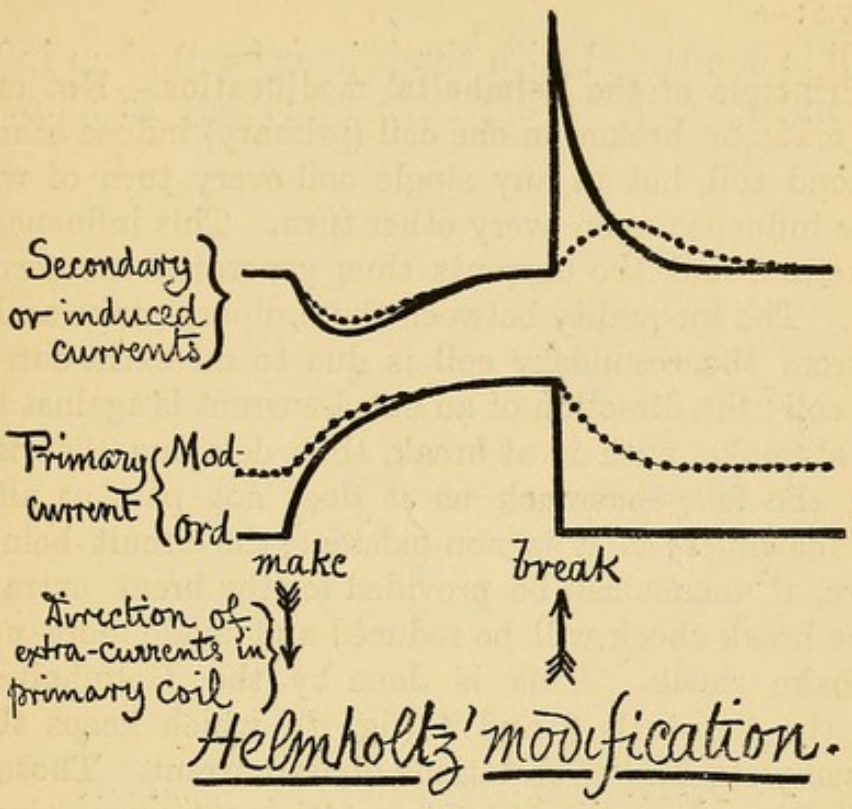


FIG. 10.—TO ILLUSTRATE INDUCTION CURRENTS AND THEIR MODIFICATION BY THE HELMHOLTZ SIDE-WIRE.

In the primary coil at make the current rises gradually to a maximum, being delayed by the opposed extra-current, at break it suddenly falls to zero, undelayed by extra-current, inasmuch as the circuit is now broken. The make and break currents induced in the secondary coil are indicated by the unbroken line; they are of opposite directions, and the break is of greater intensity than the make current.

The several currents, as modified by the side wire, are shown by dotted lines. The total current variation at make and break in the primary circuit is smaller than before, *and the break extra-current can now take effect, inasmuch as a circuit is preserved through the side-wire.* The fall of current in the primary wire is thereby delayed, and the break current in the secondary coil is much reduced. Obviously, as the primary current variation is smaller, the secondary make current must also be slightly reduced.

(7) The existence of a **break extra-current** is demonstrated as follows :—

A cell is connected with the primary coil of an inductorium (the secondary coil having been removed) as shown in the diagram.

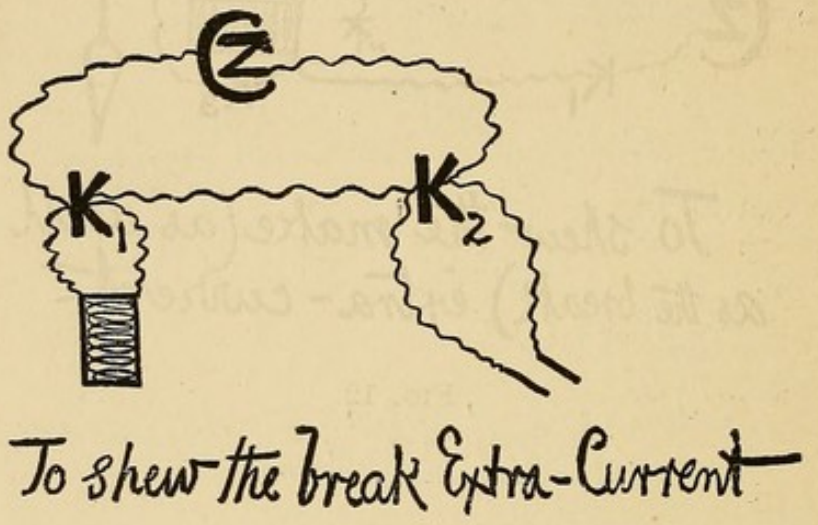


FIG. 11.

One key K_1 bridges the coil, the other K_2 bridges the electrodes, which are applied to the tongue. Close K_1, *i.e.*, cut out the coil, and while K_1 is closed, open and close K_2, *i.e.*, make and break the battery current through the tongue; little or nothing is felt.

Now open K_1 so as to put the coil into circuit, and while K_1 is open, open and close K_2; as K_2 is opened the current through the coil is suddenly reduced, and a smart twinge is felt, which is the effect of a break extra-current. Nothing is felt when K_2 is closed, for the make extra-current then passes in the metallic circuit across the bridge K_2.

(8) *To demonstrate the* **make extra-current,** a nerve-muscle preparation must be used, and a rheostat to diminish the battery current. Connections as per diagram.

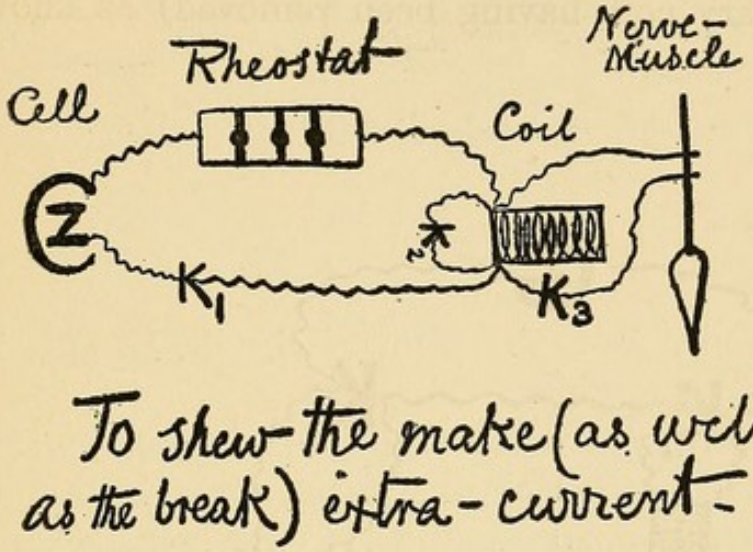


FIG. 12.

With K_2 open, opening and closing either K_1 in the principal circuit or K_3 in the nerve circuit, makes and breaks a branch current in the latter circuit independently of any self-induction in the coil sufficient to excite the nerve. By means of the rheostat the current is now reduced until K_3 gives no effect at make or break.

(1) Opening and closing K_1 (with K_2 open so as to send current through coil, and K_3 closed) cause contractions by the break and the make extra-currents, whereas opening and closing K_3 are ineffectual.

(2) Or (with K_1 and K_3 closed) while closing K_2 has no effect (the break extra-current then produced being bridged through K_2), opening K_2 so as to make current through the coil gives contraction. In the first case we have demonstrated the effect of the make and of the break extra-currents, in the second case that of the make extra-current alone.

(9) **To cut off the Make or Break Shock.**—It is sometimes desirable to use only make shocks[1] to test the excitability of nerve or muscle, in which case the break shock must be cut out. This can be done as follows :—

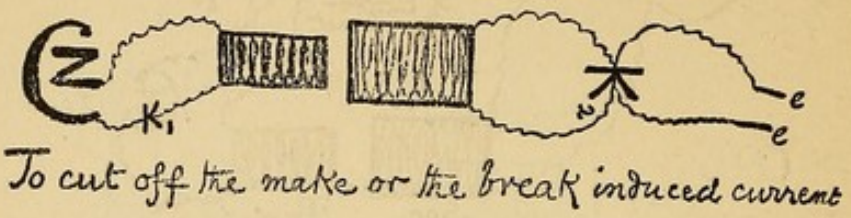


FIG. 13.

K_1 is a gap key in the primary circuit, K_2 is a bridge key in the secondary circuit.

To cut out the make shock, the two keys are worked in this order :—close K_2, then close K_1 (the make is bridged at K_2) ; then successively open K_2 and K_1 (the break passes through the electrodes).

To cut out the break shock, open K_2, close K_1 (the make passes through the electrodes), then successively close K_2 and open K_1 (the break is bridged at K_2).

But to attend to these details during experiment is inconvenient, nor would it be possible to obtain a rapid succession of only one kind of shock.

It is convenient to use an automatic "cutting out" interrupter in the two circuits.

Automatic "cutter out."—The armature of an electromagnet in the primary circuit is attracted at make, tilts the lever *a b*, so as to make contact at the mercury cup *a*, which forms a "bridge" to the secondary and electrode circuit. But before the contact has been made at *a*, the make shock has taken effect. The break induction shock passes while the con-

[1] With the ordinary arrangement of the coil, the make induction shock is far more uniform in strength than the break induction shock. The latter is in reality a double current partly due to the break of the battery current, partly due to the sudden make and break of the extra-current which sparks across at the interrupter in a varying manner.

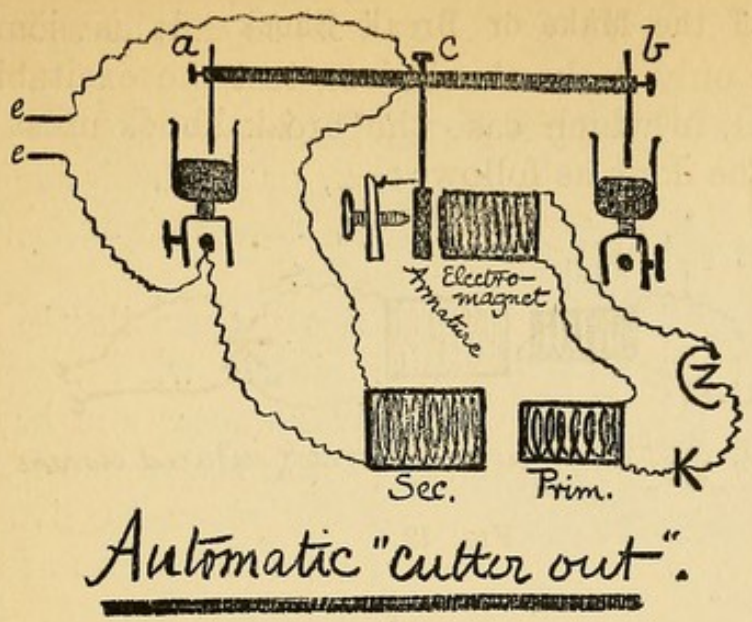


FIG. 14.

tact at *a* is still unbroken. Care must be taken that the wire plunges well into and out of the mercury, so that the secondary coil is bridged at *a* at break in the primary circuit, *i.e.*, the break induction shock is cut off from the electrode circuit. There is no short circuit at *a* at make in the primary circuit, *i.e.*, the make induction shock passes to the electrodes.

The apparatus can be connected up in various other ways, cutting out the make or the break by bridge or by gap at *a* or at *b*.

Double Keys.—It is sometimes desired to simultaneously make or break two currents in two separate circuits (*e.g.*, to simultaneously excite and signal the excitation) and a double key is easily improvised for this purpose. *Helmholtz' Key*, by which a "make" in one circuit is simultaneous with a "break" in another circuit, will occasionally be found useful.

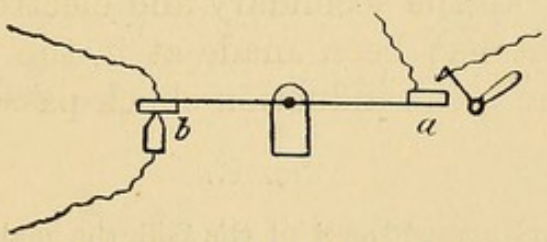


FIG. 15. HELMHOLTZ' KEY.

By depressing the handle at *a*, a circuit is made at *a*, and another circuit is simultaneously broken at *b*.

A rheostat is a set of resistance coils, graduated in ohms, by means of which more or less resistance can, at will, be put into a circuit.

(10) Verify **Ohm's law** ($\text{Current} = \frac{\text{Pressure}}{\text{Resistance}}$) using for the purpose 1, 2, or 3 Leclanché cells, key, rheostat and galvanometer.

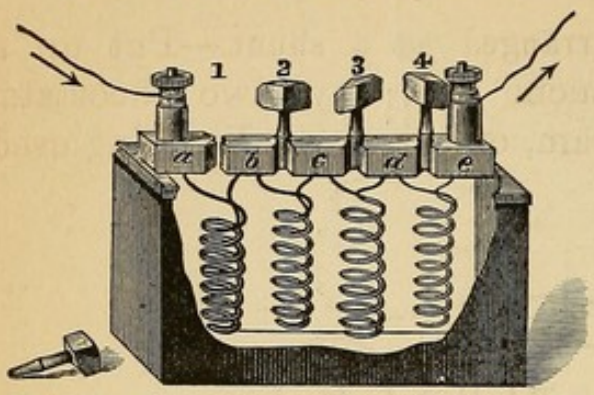


FIG. 16.

Wires of definite resistance unite the metal blocks *a*, *b*, *c*, *d*, *e*, which can be connected and disconnected by inserting or removing metal plugs. If all the plugs are inserted the resistance is practically zero; if a plug is removed (as shown in figure), resistance in a circuit is increased: if the four wires in the box have resistances of 1, 2, 3, and 4 ohms, removal of all the plugs would give a resistance of 10 ohms.

Set up the cells "in series," in circuit with a rheostat, galvanometer and key. Adjust the resistance by means of the plugs to give a convenient deflection of the galvanometer magnet (say 10 milliampères) with the three cells in circuit, as shown in the diagram.

A. Keeping one of the wires (say from the zinc) in position, shift the other wire from the copper of the third cell to that of the second and then to that of the first, and note the deflection obtained with one, two and three cells in circuit. Observe that the current varies with the electromotive pressure.

B. In the preliminary adjustment you have had occasion to observe that the current varies inversely as the resistance. Take readings of the current strength unplugging the rheostat to give resistances between, say, 1,000 and 100 ohms. Plot out the results on millimeter paper.

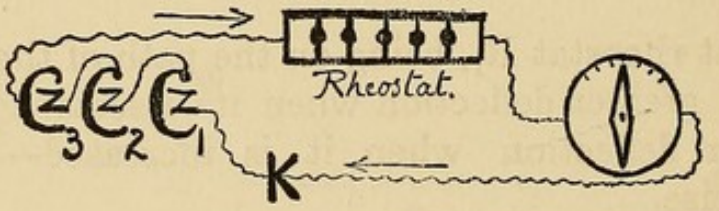


FIG. 17.

Note.—An exact verification of Ohm's law is not sought for in this exercise, but only a verification of the principle. The graduation of the galvanometer is comparatively rough, and the resistance in circuit is not only that of the rheostat, but also the resistance of the galvanometer, and wires, and the internal resistance of the cells.

(11) **Action of a rheostat arranged as a shunt.**—Put up a divided circuit with a Leclanché cell, key, two rheostats, and galvanometer, as per diagram, one rheostat, R_1, being used

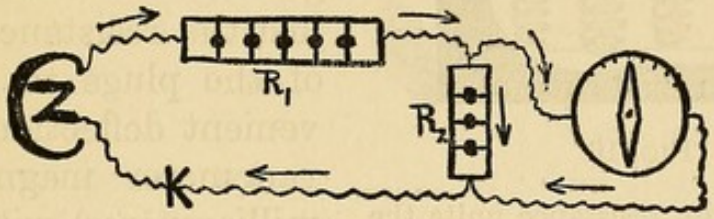


FIG. 18.

to adjust the current to a convenient strength, the other rheostat, R_2, being disposed as a "shunt" or short-circuit to the galvanometer.

Make R_1 = say 100 ohms. Then take readings of the galvanometer with variation of R_2 from 1 to 10 ohms. Observe that as the resistance is increased and diminished in this "shunt" the galvanometer deflection is increased and diminished.

The circuit branches at R_2 current passes through R_2, and through the galvanometer; if R_2 has little resistance a greater fraction of current passes through R_2, and a lesser fraction of current through the galvanometer; as R_2 is increased a greater fraction of current is diverted into the galvanometer.

Notice that rheostat R_1, being on the path of the undivided current, gives greater deflection when its resistance is diminished, smaller deflection when it is increased—as in the previous exercise.

(12) A **rheochord** is a wire and slider so disposed that a very low but variable resistance can be offered as a deriving or "shunt" circuit by the side of a principal circuit of higher resistance. It affords means of dividing a current into two parts, and of thus obtaining any desired small fraction of the current of a single cell, the larger part passing through the rheochord, which is of low resistance, the smaller part passing through the principal circuit, which is of high resistance. If, for instance, the current of a cell is made to branch (*a*) through a rheochord with a resistance of 1 ohm, (*b*) through a nerve with a resistance of 9999 ohms, then the current in the shunting or rheochord circuit will be $\frac{9999}{10000}$, the current in the nerve circuit will be only $\frac{1}{10000}$ of the entire current of the cell. The rheochord, as a deriving circuit, is to the principal circuit what the galvanometer shunt is to a galvanometer (R_2 in fig. 18); by means of a slider the resistance of the deriving circuit, and, consequently, the magnitude of the current diverted into the principal circuit, can be increased or diminished at will.

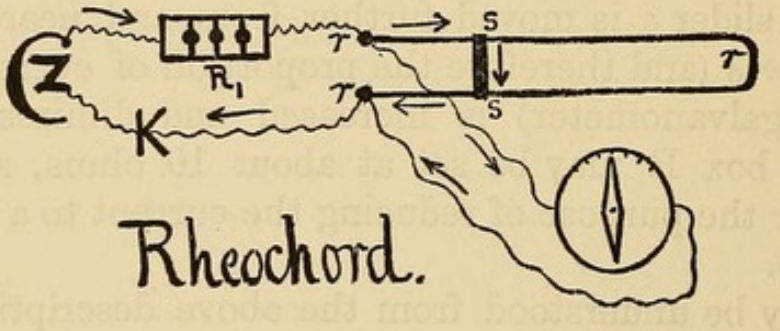


FIG. 19.

Put up a circuit similar to that of Exercise 11, but substituting a rheochord *r r*, for R_2, acting like it as a shunt; and adjusting R_1 to give any convenient deflection with the slider midway between its two extreme positions. Observe that as the metal slider *s s* is moved further from and nearer to the rheochord terminals *r r* (to which both the battery and the galvanometer wires are attached), more and less current is diverted into the galvanometer in accordance with the greater and smaller length (and therefore resistance) of the shunting path *r s s r*.

The *monochord* is in principle a rheochord, but with a slightly different system of connections, as should be verified in accordance with this diagram.

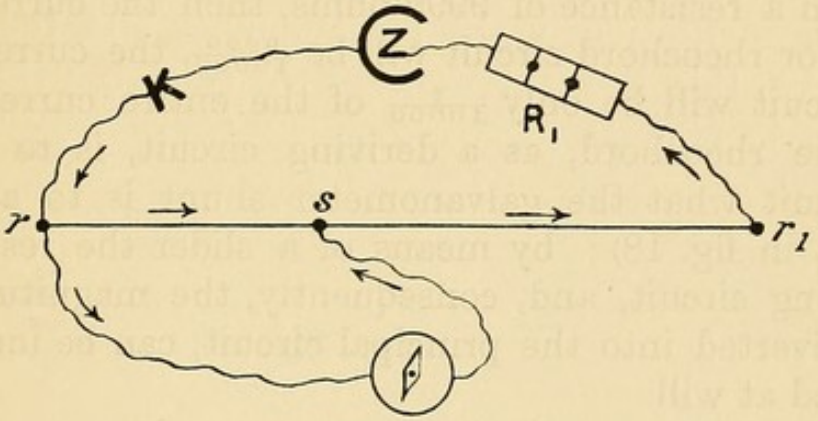


FIG. 20.

As the slider *s* is moved further from and nearer to *r*, the resistance *r s* (and therefore the proportion of current diverted into the galvanometer) is increased and diminished. The resistance box R may be set at about 10 ohms, and is convenient for the purpose of reducing the current to a convenient magnitude.

As may be understood from the above description, a rheochord is a means of obtaining a small electromotive pressure. One of its chief practical applications is to the measurement or neutralisation of "currents of injury" by compensation. (See Exercise 44.) A monochord is easily improvised by stretching a wire between two terminals fixed in a board with a brass spring clip and attached wire to serve as a slider.

(13) **Measurement of Resistance.** **Dem.**—Use a Leclanché cell, two spring keys, Wheatstone bridge and galvanometer.

Principle.—The two points b b_1 are at the same potential, *i.e.*, there is no current through a wire (and galvanometer), joining these points when the resistance A is to the resistance B as the resistance x is to the resistance C; viz., when $\frac{A}{B} = \frac{x}{C}$, or $x = \frac{A\,C}{B}$, that is $= C \times \frac{A}{B}$,

Therefore, with the three known resistances adjusted until there is no deflection of the galvanometer and the unknown resistance x, this last is determined by resolving the equation $x = C \times \frac{A}{B}$.

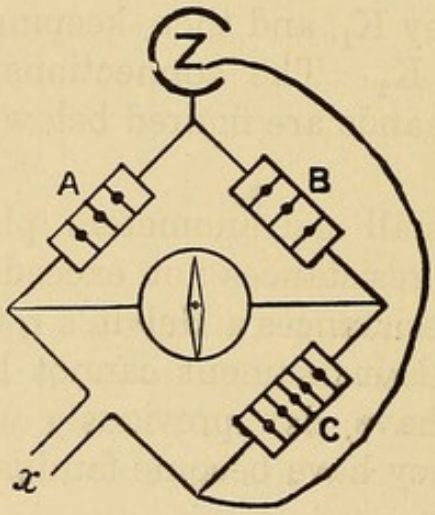


FIG. 21.

If A and B are equal, $x = C$; if $A = \frac{1}{10}$ B, $x = \frac{1}{10}$ C; if $A = 10$ B, $x = 10$ C, &c.

In practice, we shall as nearly as possible have the four resistances of the same order of magnitude, *i.e.*, in the tens, hundreds or thousands of ohms. But with x very large we shall adjust $A = 10\,B$ or $= 100\,B$; and with x very small we shall put $A = \frac{1}{10}$ B, or $= \frac{1}{100}$ B.

The "no current" state will be found by subsequent adjustment of C, the final value of C being taken as the mean between the "just too much" and "just too little," as shown by the slightest possible galvanometer deflection first in one then

in the opposite direction. In the first rough trial we note in which direction the magnet swings when C is evidently too great and evidently too small. In testing for the current first close the battery key K_1, and then keeping K_1 closed, close the galvanometer key K_2. The connections of the Wheatstone box put into your hands are figured below.

NOTE.—The small galvanometers placed in your hands will serve only for resistances not exceeding 1000 ohms. For measuring higher resistances a Kelvin's reflecting galvanometer must be used. This instrument cannot be put into the hands of students who have not previously worked in a physical laboratory, until they have become familiar with the use of the small galvanometer.

(14) **Kelvin's Reflecting Galvanometers.**—A galvanometer is an indicator of the presence, direction and magnitude of a galvanic current. In principle it consists of a coil of wire surrounding a suspended freely-swinging magnet, which becomes deflected from its position of rest when current passes through the wire. In practice (*i.e.*, in Kelvin's reflecting galvanometer), a suspended system of magnets is used, with poles so disposed as to make the system not far from "astatic," *i.e.*, not to set too strongly in a position of rest pointing to the magnetic pole, and further controllable by an independent large magnet, by which the "set," and therefore the sensitiveness, of the suspended system may be modified. The distance of this magnet from (and therefore its effect upon) the suspended system can be altered at will, and its poles may be turned so that the "set" is increased or diminished. In the former case the magnet is said to be "friendly," the set is increased, the suspended system is less sensitive, and bringing the magnet closer increases the set. In the latter case (marked end pointing north) the magnet is said to be "unfriendly," the set is diminished, the sensitiveness is increased, and bringing the magnet closer diminishes the set (up to a certain limit, beyond which the set is reversed). The movements of the suspended system of magnets are shown greatly magnified by means of a light mirror which reflects a spot of light on to a horizontal scale. (If desired, the varying positions of this spot can be recorded photographically. See p. 40.)

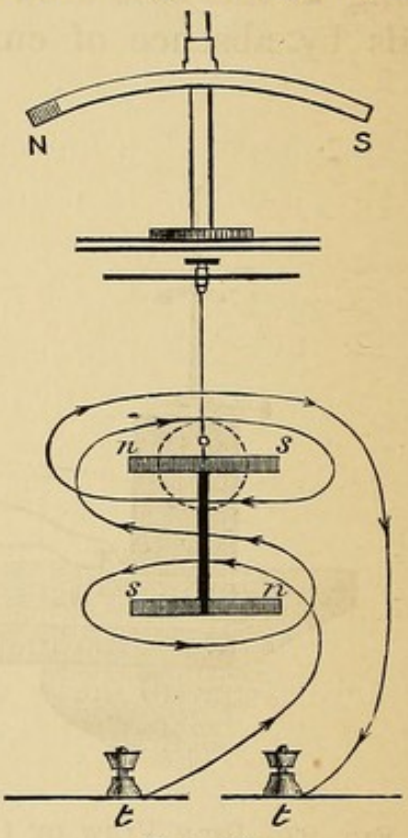


FIG. 22.

Astatic couple of magnets *n s*, *s n*, suspended by a silk fibre and carrying a mirror (indicated by the dotted circle); the surrounding line and arrows indicate the disposition of the coils; N S is the neutralising or controlling magnet. All these parts are represented as if viewed by an observer standing west, *i.e.*, in the position of the lamp in the next fig.

Reflecting galvanometers in ordinary use in a physiological laboratory are of two kinds, viz., of *high resistance* (5,000 to 20,000 ohms), and of *low resistance* (1 ohm or less). The

former are used for currents of muscle and nerve, the latter for thermo-electric currents.

In connection with a high-resistance galvanometer, a "*shunt*" is frequently employed; this serves to reduce the sensitiveness when desired by carrying off $\frac{9}{10}$ or $\frac{99}{100}$ or $\frac{999}{1000}$ of any given current (the coils in the shunt having respectively $\frac{1}{9}$ or $\frac{1}{99}$ or $\frac{1}{999}$ of the resistance of the galvanometer coils), thus leaving to pass through the galvanometer $\frac{1}{10}$ or $\frac{1}{100}$ or $\frac{1}{1000}$ of the total current. As ordinarily used this galvanometer is an indicator of current, but by adopting the method of compensation it becomes an indicator of potential or pressure, being in this case used to indicate equality of opposite potentials by absence of current. (See next exercise 15).

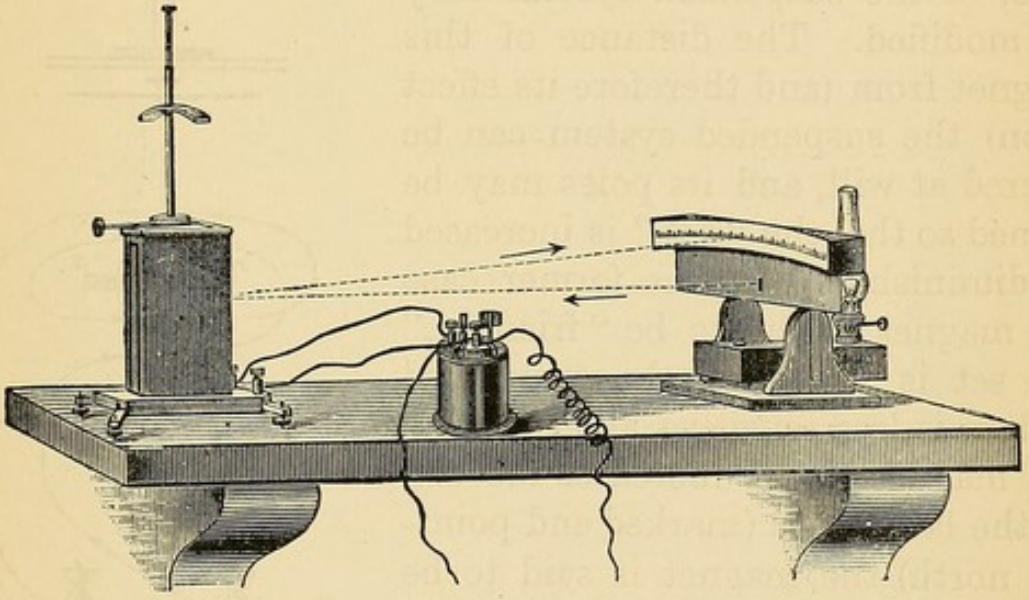


FIG. 23.—SIDE VIEW OF GALVANOMETER AND SHUNT, LAMP AND SCALE.

The galvanometer and scale are placed east and west, and appear as if viewed by an observer standing on the north side; the path of light is indicated by dotted lines. The essential parts concealed by the galvanometer case are diagrammatically given in fig. 22.

The suspended system of magnets of an ordinary galvanometer, after it has been set in movement, comes to rest by a series of diminishing oscillations above and below its position of rest. In such case the instrument is said to be "*undamped*" or "*partially damped*," and an instrument in which the unavoidable damping to the resistance of the air is made as small as possible is called a *ballistic* galvanometer. If, on

the contrary, the damping is increased—as by a light vane turning in a confined air-space—the movement is rendered "*dead-beat*" or "*aperiodic*," *i.e.*, the oscillations are suppressed, so that the magnet (or system of magnets) does not swing beyond its steady deflection by a current, nor beyond its zero with cessation of current. In such case the movement by which the magnet takes up a new position of rest occurs more slowly than is the case with an undamped magnet, and with a gradually increasing slowness; the time occupied by this movement is called the "falling-time" of the magnet. With an undamped magnet the time occupied by one oscillation to and fro is its "*period*," and the relation between the magnitudes of two successive operations is the "*decrement*."

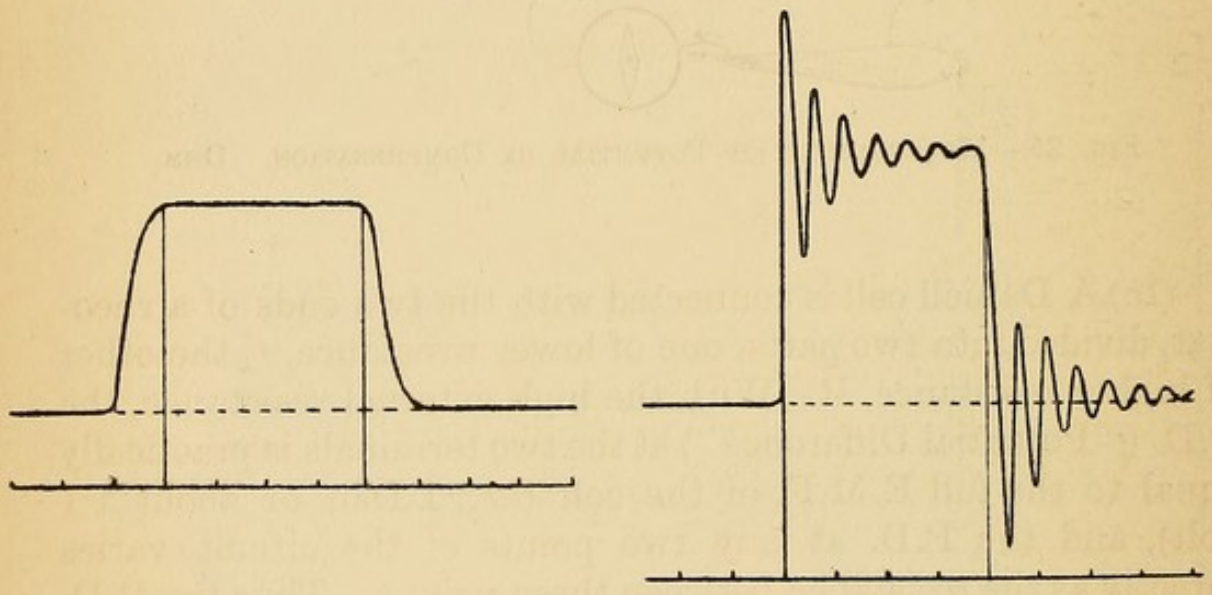


FIG. 2.

Deflection of a dead-beat galvanometer.
Falling time = 15 secs.

Deflection of a partially damped galvanometer.
Period = 8·5 secs.
Decrement = about 2.

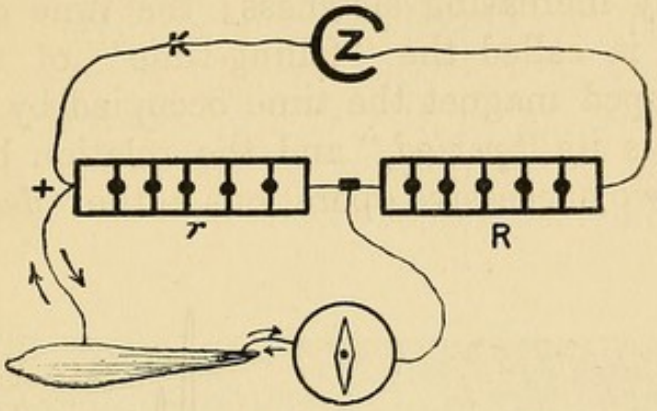


FIG. 25.—MEASUREMENT OF POTENTIAL BY COMPENSATION. DEM.

(15) A Daniell cell is connected with the two ends of a rheostat, divided into two parts, one of lower resistance, r, the other of higher resistance, R. With the high external resistance, the P.D. ("Potential Difference") at the two terminals is practically equal to the full E.M.F. of the cell (*i.e.*, 1 Dan. or about 1·1 volt), and the P.D. at any two points of the circuit varies directly as the resistance between these points. Thus the P.D. at two points of a muscle is ascertained by finding the resistance, r, at which it is balanced (*i.e.*, no current through the galvanometer); it is then equal to $\frac{r}{r + R}$. For example, if the balance is obtained with $r = 250$ ohms, and $r + R = 7500$ ohms, the muscle potential is $\frac{250}{7500}$, or ·033 Dan.

(16) Equipotential Lines. Dem.

A Daniell cell, a du Bois key, galvanometer, a large flat dish of zinc sulphate, two pairs of amalgamated zinc electrodes.

Fix the leading-in electrodes at + —; place one leading-off electrode midway between them.

(1) Determine the equator O O by shifting the other leading-off electrode to positions at which no current passes the galvanometer on closure of the key.

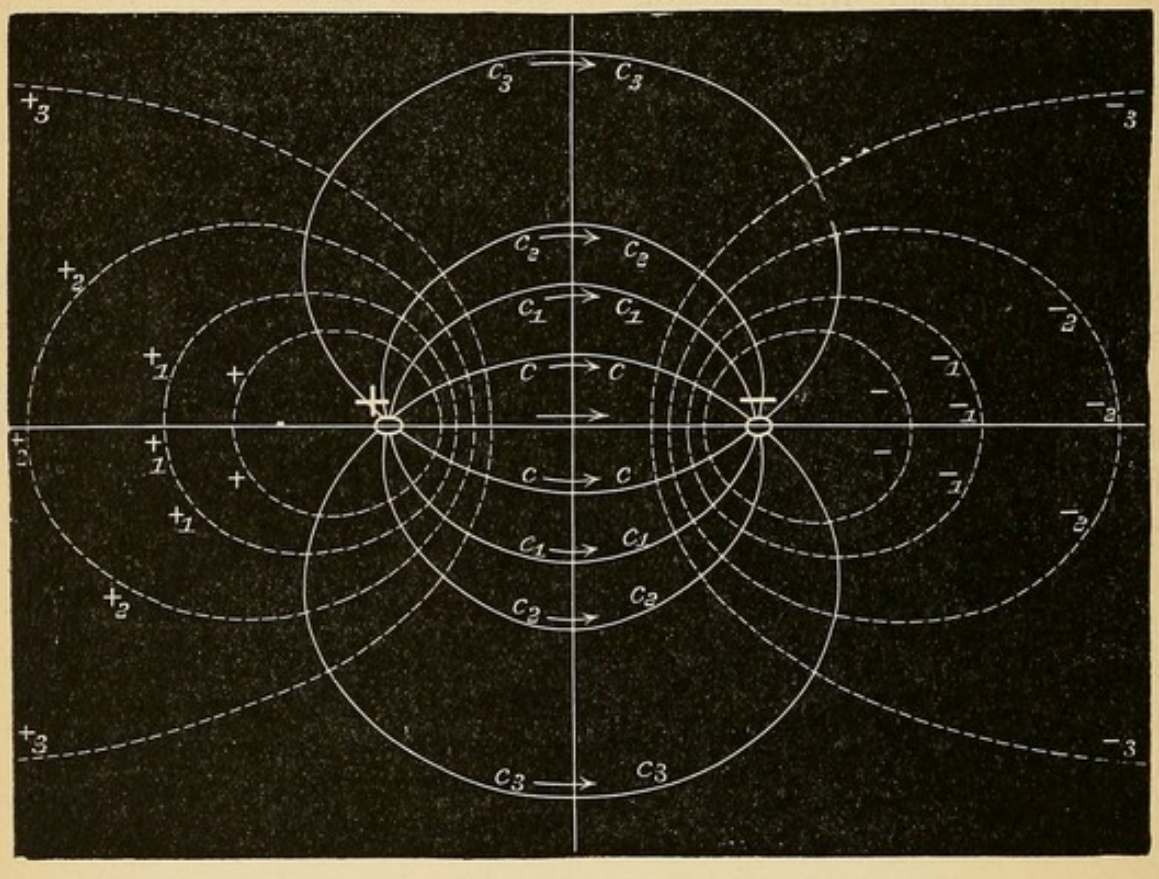


Fig. 26.

(2) Shift the electrode towards +, and on closure of the key observe a current through the galvanometer from S to N, indicating + potential at this point.

(3) Shift the electrode towards —, and on closure of the key, observe a deflection from N to S, indicating — potential at that point.

(4) Shift the first leading-off electrode nearer to —, and by successive trials of shifting the second lead-off, find several equipotential points, from which construct a curve.

(5) Repeat the observation with the first leading-off electrode nearer to +. (*Vide* "Human Physiology," p. 306).

(6) Find equipotential curves near and far from one of the poles, place the leading-off electrodes on any two points of each of these curves, and observe the galvanometer on closure of the key; or, more simply, take a pair of leading-off electrodes at a fixed distance from each other, and find positions in the field at which more or less current passes in one or other direction.

(17) Excitation of nerve by the Condenser.

A condenser in its simplest form is a pair of metallic surfaces separated by a thin layer of air, glass, paraffin, mica, &c., that are charged by being brought into metallic connection with points of different electrical pressure, such as the copper

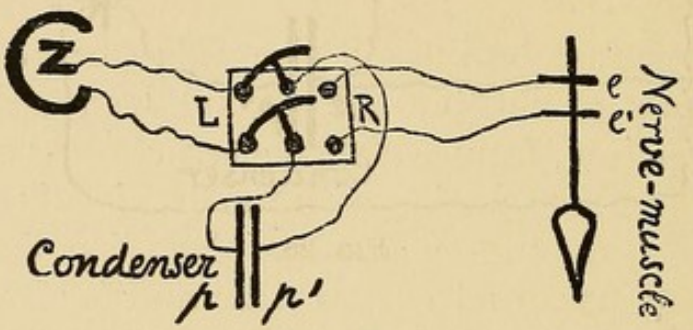


Fig. 27.

and zinc of a Daniell cell, and discharged when connected by a sufficient conductor. Either the charge or the discharge may be made through a nerve.

Connect the middle pair of pools of a commutator without cross wires to the terminals of a "condenser," connect the two lateral pairs with a cell and with the nerve of a nerve-muscle preparation as in fig. 27.

With the cradle of the commutator as shown in the diagram, the condenser is connected with the cell and "charged." When the cradle is turned over, the charged condenser is disconnected from the cell and connected with the nerve through which it is "discharged," provoking a single twitch of the attached muscle.

Minimum energy of stimulus. Dem.

With stimulation by the discharge (or charge) of a condenser we can express in terms of energy the value of the electrical stimulus.

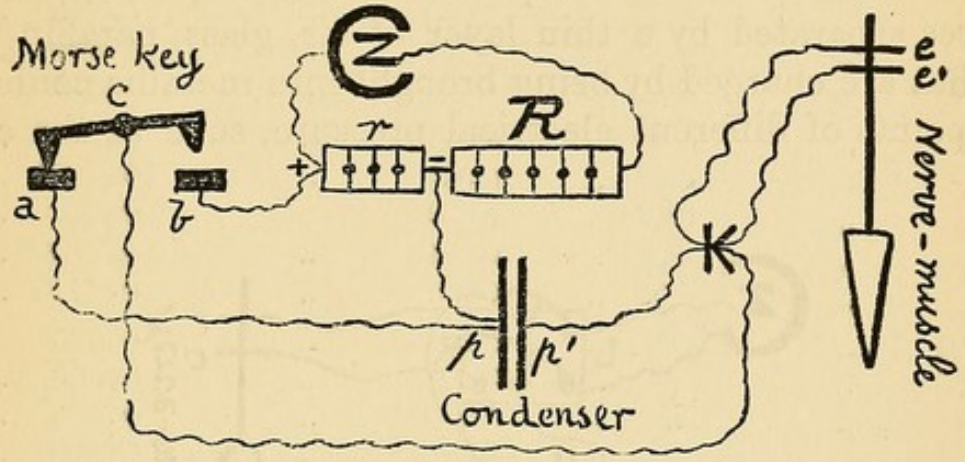


FIG. 28.

Charge from + through *b c e' e p' p* to — when contact is made at *b* by depressing the Morse key (and broken at *a*) viz., ascending in the nerve.

Discharge from *p'* through *e e'c a* to *p* when contact is made at *a* by recoil of the Morse key (and broken at *b*) viz., descending in the nerve.

The bridging key is for the purpose of cutting out from the nerve either the charge or the discharge. If desired this may be effected without the bridging key by the circuits 1 and 2 of fig. 29, substituting the exciting electrodes *e e'* for the galvonometer SN.

Put up connections in accordance with fig. 28, using a condenser of 0·10 microfarad, subdivided into parts = 0·01.

Determine by adjustment of the rheostats *r*, R, the smallest fraction of a volt that will excite the nerve of a nerve-muscle preparation :—

(A) with the condenser at 0·10 microfarad ;

(B) with the condenser at 0·01 microfarad ;

and calculate in the two cases the quantity and the energy of such a minimal electrical stimulus.

(C) with a convenient fraction (say 0·1 of a volt)

find the minimum effective condenser value (between 0·01 and 0·10 microfarad) and calculate as before.

N.B.—You may expect the energy value of a minimal effective stimulus to come out at something like 0·001 erg., viz., 1 millierg.

NOTE.—Recollect the difference between "quantity" and "energy" of an electrical charge (or discharge)—(1) that quantity varies directly as capacity and as pressure; (2) that energy varies directly as capacity and as pressure squared. Identical quantities may be of very different energies; a given energy may be derived from very different quantities, *e.g.* :—

Capacity.	Pressure.	Quantity.	Energy.
0·01 microfarad	0·1 volt	0·001 microcoulomb	0·0005 erg.
,, ,,	0·2 ,,	0·002 ,,	0·0020 ,,
,, ,,	0·3 ,,	0·003 ,,	0·0045 ,,
,, ,,	0·4 ,,	0·004 ,,	0·0080 ,,
,, ,,	0·5 ,,	0·005 ,,	0·0125 ,,
0·1 ,,	0·01 ,,	0·001 ,,	0·00005 ,,
,, ,,	0·02 ,,	0·002 ,,	0·00020 ,,
,, ,,	0·03 ,,	0·003 ,,	0·00045 ,,
,, ,,	0·04 ,,	0·004 ,,	0·00080 ,,
,, ,,	0·05 ,,	0·005 ,,	0·00125 ,,
0·01 ,,	0·1 ,,	0·001 ,,	0·0005 ,,
0·02 ,,	,, ,,	0·002 ,,	0·0010 ,,
0·03 ,,	,, ,,	0·003 ,,	0·0015 ,,
0·04 ,,	,, ,,	0·004 ,,	0·0020 ,,
0·05 ,,	,, ,,	0·005 ,,	0·0025 ,,

&c.

In accordance with the formulæ :—

$$\underset{\text{(in microcoulombs)}}{Q} = \underset{\text{(in microfarads and in volts)}}{FV}$$

$$\underset{\text{(in ergs)}}{E} = \underset{\text{(in microfarads and in volts.)}}{5\,FV^2}$$

A sufficient approximation may be arrived at from the following data :

Taking a fresh Leclanché cell (of which the E.M.F. = 1·47 volt) as the source of pressure, make the total resistance $r + R = 14700$ ohms, when 10, 100, 1000 ohms in r will give at its two ends pressures of 0·001, 0·01, 0·1 volt.

In order to preserve a constant value of the total resistance $r + R$ when r is increased, the simplest plan is to arrange the two rheostats so that for each plug removed in r, an equivalent plug can be inserted in R; resistance is thus diminished in R by as much as it is increased in r, and the total value of $r + R$ is kept constant.

E.g., to obtain a series of pressures from a Leclanché cell in decimal parts of a volt, we may arrange to unplug r and plug R as follows :—

r.	R.	Volt.
0	14700	0
+ 1000	14700-1000	0·1
+ 2000	14700-2000	0·2
+ 3000	14700-3000	0·3
+ 4350	14700-4350	0·435
+ 100	14700-100	0·01
+ 200	14700-200	0·02
+ 300	14700-300	0·03

With a fresh Daniell cell $r + R$ should be made 11000 ohms.

Before putting up the condenser circuit in connection with a nerve-muscle it should be put up in connection with a galvanometer (or a capillary electrometer), and the action of the Morse key verified in the cases 1, 2, and 3 (fig. 29). In case 3 the circuit is identical with that of fig. 29.

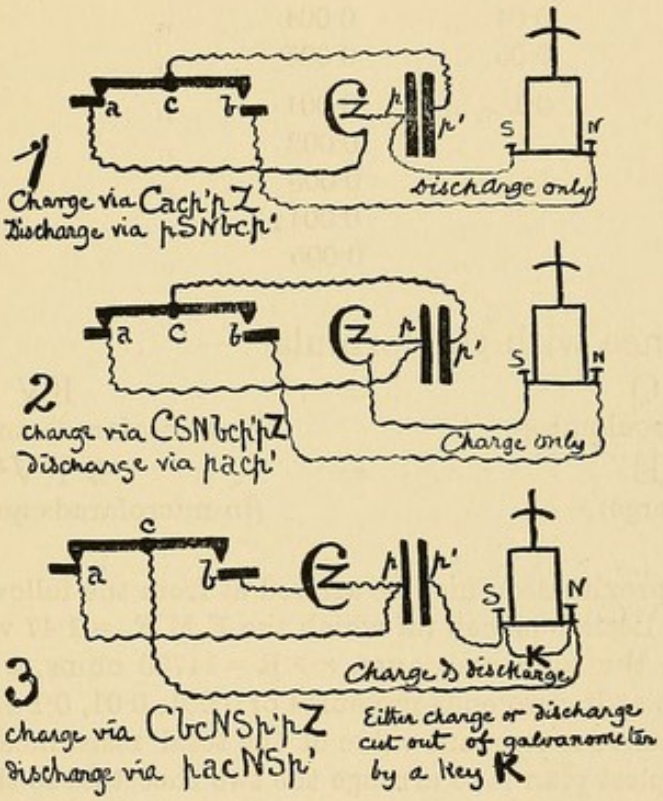


FIG. 29.

(18) **Unipolar stimulation.**—Lay the nerve across a single electrode connected by a single wire with the secondary coil.

Carefully insulate the frog or nerve-muscle preparation on a dry glass plate, also the coil in the same way. If the insulation is perfect, no contraction occurs when the coil is set in action. But if the insulation be destroyed, contractions occur although only one pole is connected with the nerve.

Note that unipolar contraction is most apt to occur at the break shock with the ordinary arrangement of the coil. This is one reason for using the Helmholtz modification; also for using a key as a bridge when it is desired to cut off the secondary current from a nerve.

(19) **Unpolarisable electrodes** are constructed as follows. A carefully amalgamated[1] zinc rod dips into a saturated solution of zinc sulphate, which in turn communicates with a plug of china clay made up into a paste with normal saline; a glass tube shaped according to requirements and fixed in a suitable holder. Such an electrode has the following qualities: it is unpolarisable by weak currents, it is not itself a source of electromotive force, it is of high resistance, it can be applied to living tissues without appreciably injuring them. A pair of unpolarisable electrodes should be tested before use by bringing their plugs into contact while they are connected with the galvanometer; they should then give little or no current. Another form of unpolarisable electrode is that of d'Arsonval; it consists of a silver rod coated with fused silver chloride dipping into a tube filled with normal saline.

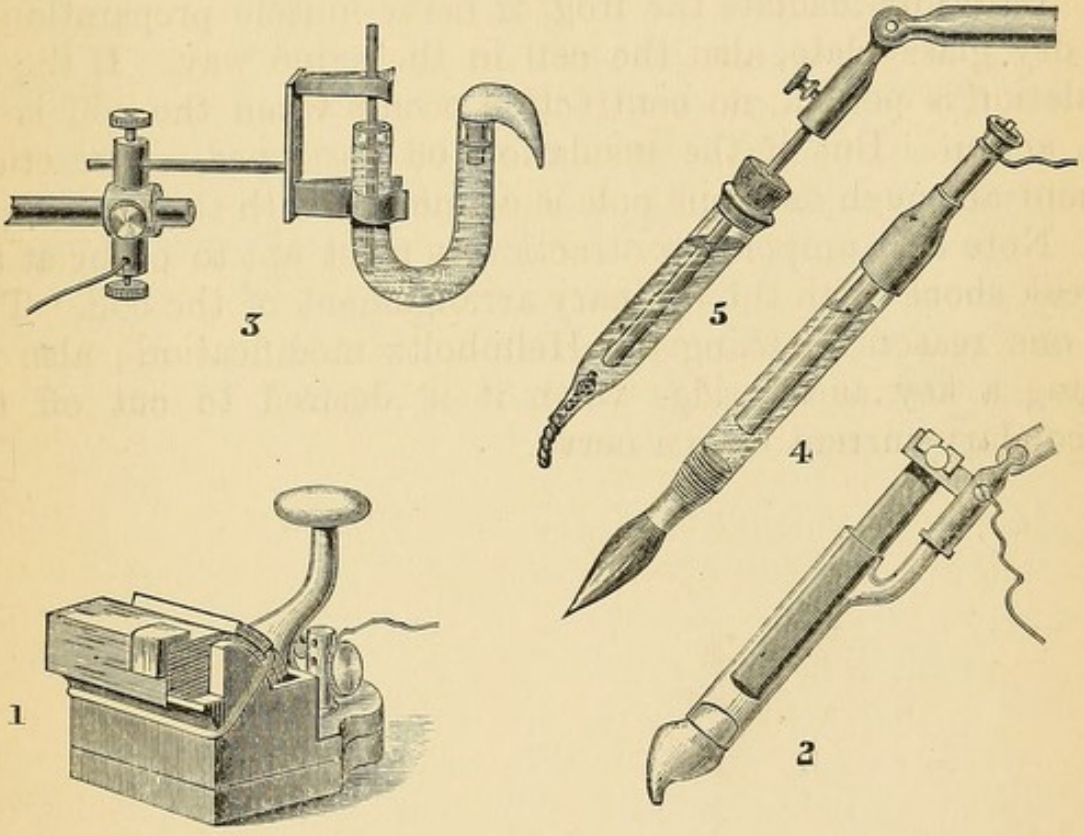


FIG. 30.—SEVERAL MODELS OF UNPOLARISABLE ELECTRODES.

1 and 2, du Bois-Reymond's; 3, Burdon-Sanderson's; 4, von Fleischl's; 5, d'Arsonval's.

In 1, 2, 3, and 4 the component parts are zinc, zinc-sulphate, and saline clay in 5 a silver rod coated with fused silver chloride dipping in normal saline contained in the tube from which a thread projects.

[1] *Amalgamating fluid for unpolarisable electrodes.* Dissolve 3 c.c. of mercury in 50 c.c. HNO_3 + 150 c.c. HCl. Add 10 per cent. HCl. to make up 1000 c.c.

(20) **The capillary electrometer** may be used instead of the galvanometer for the demonstration of muscle and nerve currents; it is more suitable than the galvanometer for the electrical variations of the frog's heart, and it is the only instrument by which the electrical variations of the human heart can be demonstrated. The galvanometer indicates electrical current, the electrometer indicates electrical pressure, being, in fact, an extremely delicate electrical manometer, by which minute differences of potential between any two points of muscle, nerve or other tissue can be exhibited.

The apparatus consists essentially in a glass tube drawn out at one end to a fine bore (20 to 30 μ) filled with clean mercury, and in air connection with a pressure apparatus. The capillary end dips into a tube containing 10 per cent. sulphuric acid, and is viewed through a microscope magnifying 50 to 300 diameters or more.[1] Two platinum wires establish connection with the mercury and the sulphuric acid respectively. By means of the pressure apparatus mercury is forced along the capillary (which tapers slightly towards its end) up to a certain point, according to the pressure used, and the meniscus is adjusted in the field of the microscope. The surface of the mercury meniscus is in a state of tension, which is very easily altered by variations of electrical pressure, such alterations causing the mercury to advance or to recede in the capillary. Advance or retreat of the mercury signifies rise or fall of potential at its electrode.

N.B.—In putting up the instrument for the first time be careful to use clean mercury and filling pipettes; do not allow the mercury in the glass tube to come in contact with india-rubber tubing, nor with mercury from the pressure apparatus.

In testing a new instrument see that the mercury moves freely (1) to slight variations of pressure, (2) to slight electrical variations caused by touching the electrodes, (3) to the electrical variations accompanying the action of your own heart. (Ex. 51). If it responds to this third test, it is more than sufficiently sensitive for the exposed heart of a frog or mammal.

When you have done with the instrument lower the pressure bulb nearly to, but not below zero, and close the short-

[1] With the higher powers the definition of the edge of the meniscus is much improved by using a drop of water between the tube and objective, thus converting the latter into an immersion lens.

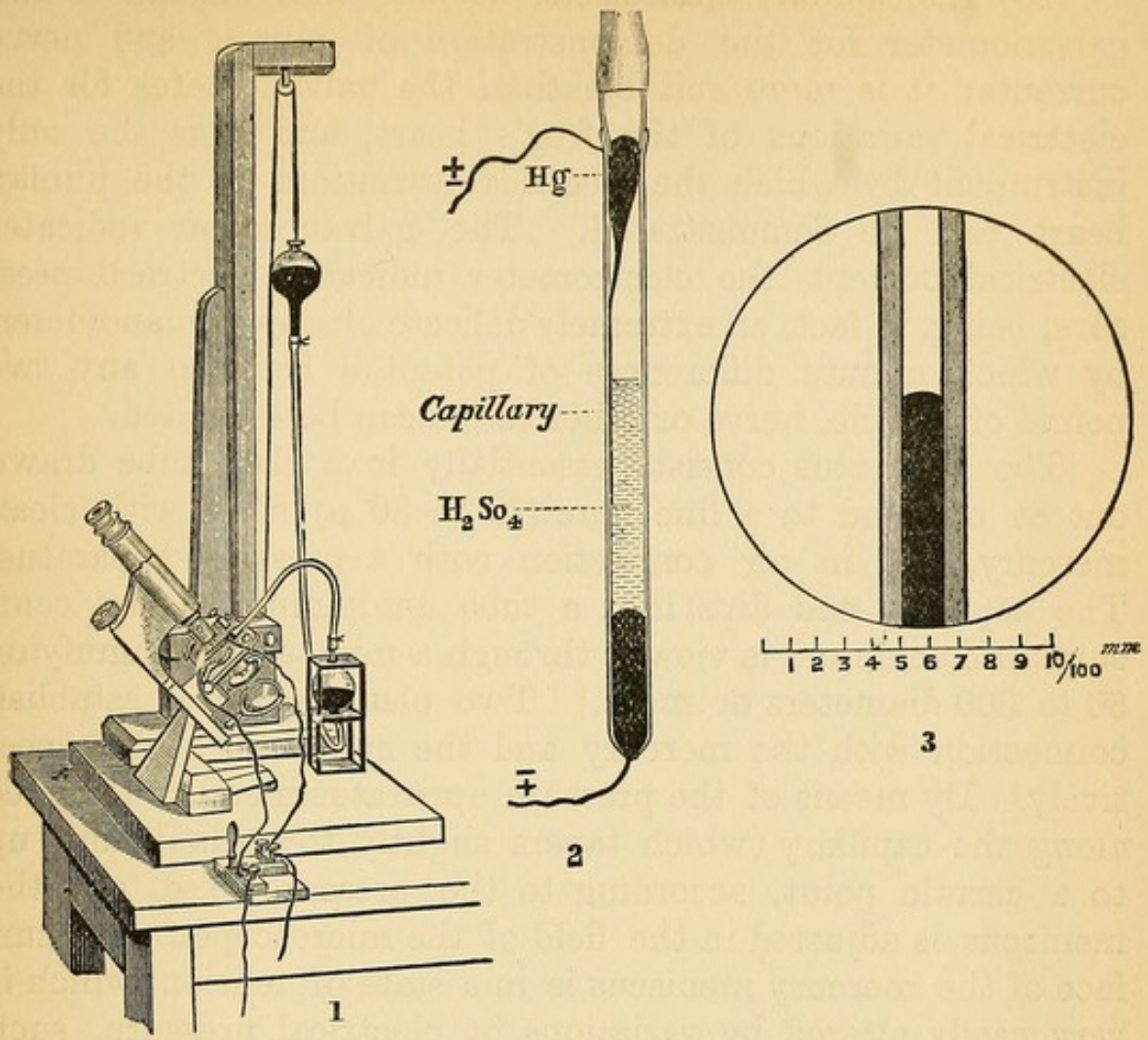


FIG. 31.—LIPPMANN'S CAPILLARY ELECTROMETER.

(1) Pressure apparatus and microscope on the stand of which the capillary tube is fixed.

(2) Capillary tube dipping into H_2SO_4 in a surrounding tube, and in connection with pressure apparatus (the mercury in the lower part of the surrounding tube serves only to establish connection with the platinum wire).

(3) The capillary tube and column of mercury as seen in the field of the microscope. (Scale in $\frac{1}{100}$ths mm.)

circuiting key, to which the electrometer wires are attached. The two defects to which a capillary is most liable are: (1) stickiness of the tube, causing the mercury to move in jerks instead of smoothly with variations of pressure, (2) blocking of the tube by (?) sulphate of mercury. A block may sometimes be got rid of by applying considerable pressure (two metres of mercury); but, in general, it saves time to reject a faulty tube at once and put up a new one. A tube in good working order may be so kept for months or years; it should be kept short-circuited, and the sulphuric acid replenished from time to time so that the capillary is never dry.

(21) Recording Apparatus.

The recording apparatus in use in this laboratory are :—

(1) A cylinder fitting either the hour or the minute axis of an American clock; speed 25 mm., and 300 mm. per hour. Used for *e.g.*, fatigue of muscle, action of drugs on frog's heart, temperature records on man, respiration records on man.

(2) Cylinders driven by a water motor; ordinary speeds between 10 and 100 mm. per second.

(3) The spring myograph.

(4) The pendulum myograph.

(5) The railway myograph.

For all ordinary purposes (sphygmograms, cardiograms, myograms, latent period, rate of nerve-impulse, reaction times, &c.), the cylinder is sufficient. For the higher speed phenomena (latent period, rate of nerve-impulse), the spring or pendulum myograph is more convenient; the railway myograph consists essentially in a vertical smoked plate carried horizontally across the field of a lantern, and is used only for demonstrations.

In the *spring-myograph* a smoked glass plate is fixed in a metal frame which is shot along wire guides by the release of a spring. The speed of movement can be varied by varying the strength of the spring, and it is indicated by means of a vibrating reed (100 per sec.) that is set in movement by the release of the spring. One (or two) trigger keys are set so as to be struck open by the carrier in its passage, and the instant of stimulation is marked in the usual way by bringing the carrier slowly up to each key and then marking the position of the recording lever.

In the *pendulum myograph* a smoked glass is fixed in a frame at the end of a pendulum, which is allowed to swing from one clutch to another clutch, adjusted so that the pendulum, when released from the first, swings so as to be just caught by the second. The frame and plate sweep past the myographic and chronographic levers and strike open one or more trigger keys; the levers are adjusted so as to come into light contact with the plate, and the instant or instants of stimulation are marked in the usual way. The speed of movement can be varied by varying the amplitude of swing with alteration of the position of the clutches; but a convenient speed having once been obtained, it is best to let well alone.

With these two instruments the usual and convenient speeds are such that $\frac{1}{100}$th sec. measures 5 to 10 millimeters.

The "*railway myograph*," consisting of a miniature truck slowly moved along rails, and carrying a smoked glass plate, is very convenient for the demonstration of experiments in which changes are gradually developed, *e.g.*, fatigue, action of drugs on heart. The apparatus is arranged so that the smoked plate, against which the lever is writing, is slowly carried across the field of a lantern by which the magnified tracing is projected on a screen.

The trigger key and peg fixed respectively to the clock, or cylinder or plate-carrier, are for the purpose of obtaining a break induction shock by the revolution of the cylinder at a definite point. The key is placed in the primary circuit, left open until the cylinder is at full speed, then closed so that the next time the peg comes round, the trigger is knocked over and the contact broken. It is used, *e.g.*, to measure the latent period or the rapidity of transmission of a nerve-impulse. The lever indicating muscular contraction begins to rise a small space (*i.e.*, time) after the point corresponding with the knock down of the trigger. To determine this correspondence on the stationary cylinder or plate bring the peg very slowly against the trigger, and mark the point on the cylinder by a touch on the lever; having done this, do not disturb the lever before taking your observation, and verify the correspondence at its conclusion.[1]

The principle upon which the use of these instruments is based will at once be realised by one or two simple experiments; of these the easiest is a determination of the latent period—

(*a*) By a break induction shock applied to an isolated gastrocnemius muscle of the frog.

(*b*) By make of a constant current to muscles of the human forearm.

(*c*) By break of a constant current to muscles of the human forearm.

In *a*, *b* and *c*, the latent periods should come out respectively at about 0·01, 0·02. and 0·05 sec., but, except in the last case, the latent period is more apparent than real.

[1] A slip of flexible metal (*e.g.*, a piece of watch spring), fixed to the cylinder so as to strike against a pin at each revolution, will answer the same purpose as the more complicated trigger key.

(22) The rate of movement of a surface upon which a record is or has been taken, is ascertained by means of some form of time-marker or chronograph.

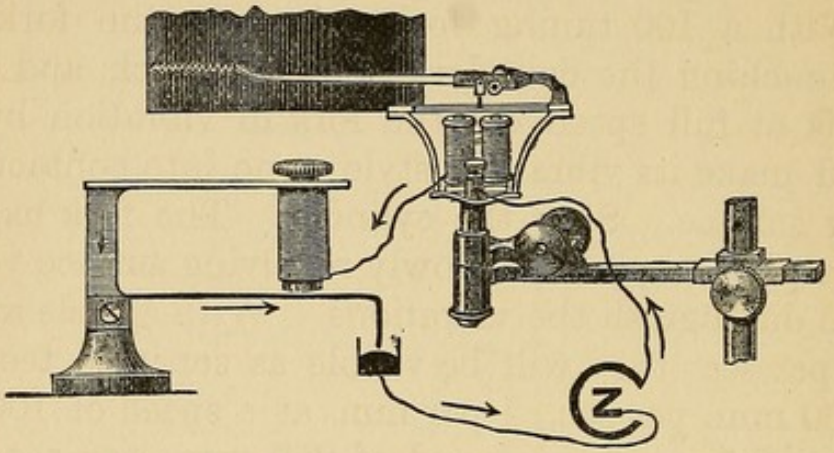


FIG. 32.—CHRONOGRAPH, OR TIME-MARKER.

Composed of battery, vibrating reed (Page's), signal (Pfeil's).

For slowly travelling surfaces, *e.g.*, a cylinder revolving once in twelve hours, or once in one hour, or once in one minute, it will be sufficient to arrange a lever against the smoked surface, and to make a mark with it by hand at each hour, or at each minute, or at each second. The circumference of the cylinders in ordinary use is 300 to 600 millimeters; therefore at once per twelve hours, 25 to 50 mm. represent one hour; at once per hour 5 to 10 mm. represent one minute; at once per minute 5 to 10 mm. represent one second.

In most experiments on muscle and nerve, time has to be measured in small fractions of a second—hundredths or thousandths, and the recording surface is used at higher speeds —50 to 1000 mm. per second.

A tuning-fork vibrating 100 times per second carrying a style that marks the vibrations against the smoked surface is the simplest instrument by which to obtain a time record of hundredths of a second.

For convenience' sake it is more usual to employ some form of electrical chronograph. Its essential components are: a battery, a vibrating reed and a marker. These three portions of apparatus are set up in a single circuit, the reed being

arranged so as to give a succession to interruptions by vibrating in and out of a mercury pool in the circuit. The makes and breaks of current thus produced act as makes and breaks of two electro-magnets A and B; A keeps up the vibrations of the reed, B gives corresponding vibrations of the recording lever.

Take chronograms.

(*a*) With a 100 tuning fork. Arrange the fork close to but not touching the cylinder; start the clock, and when the cylinder is at full speed, set the fork in vibration by a smart blow, and make its vibrating style come into contact with the revolving surface. Stop the cylinder. The fork has vibrated 100 times per sec.; with a slowly revolving surface you will be unable to distinguish the vibrations. With speeds at or above 50 mm. per sec. they will be visible as separate teeth—2 per mm. at 50 mm. per sec., 1 per mm. at a speed of 100 mm. per sec., 1 per 2·5 mm. at a speed of 250 mm. per sec. By this means you ascertain the time values of *distances* traversed by the revolving cylinder.

(*b*) With an electrical chronograph. Set up the chronograph as per diagram. See that the reed-points and mercury surface are clean. By raising or lowering the mercury cup and the two electro-magnets A and B by the two milled head screws (*a*) and (*b*), find such an adjustment of parts that the reed shall continue vibrating automatically when once its vibrations are started by a twinge or tap.

Take as neatly as you can for future reference the following series of chronograms:

20 per sec. reed on fast, medium and slow rates.

50 per sec. reed on fast and medium rates.

100 per sec. reed on fast and medium rates.

100 tuning-fork on fast and medium rates.

Finally, put up a Marey tympanum and closed india rubber tube connected with it, and mark seconds on the three rates by pressing the tube in time with the ticking of a watch.

(23) Photo-Galvanometric and Photo-Electrometric Records.

The value of galvanometric and electrometric indications is greatly increased when they are photographically recorded. This can be done by very simple apparatus, of which the essential part is a sensitive plate moved by clockwork. For most galvanometer experiments it will be found convenient to use "ordinary photographic quarter-plates" let down behind a screen with a horizontal slit, about 0·5 mm. broad, at a speed of 10 to 20 cm. per hour, by means of a wheel fixed to the minute axis of an American clock; the spot of light being formed by an ordinary paraffin lamp at a distance of about 50 cm. from the galvanometer, and focussed upon the slit by a lens of about + 5 D.

For most electrometer experiments, a more sensitive plate and a greater speed of movement, *i.e.*, 1 to 10 cm. per sec., will be found suitable.

The recording apparatus must be used in a dark chamber—which may be a simple box, or preferably a dark room. The photographic plates are developed and fixed by any of the usual methods; in this laboratory the "hydroquinone developer" is usually employed.

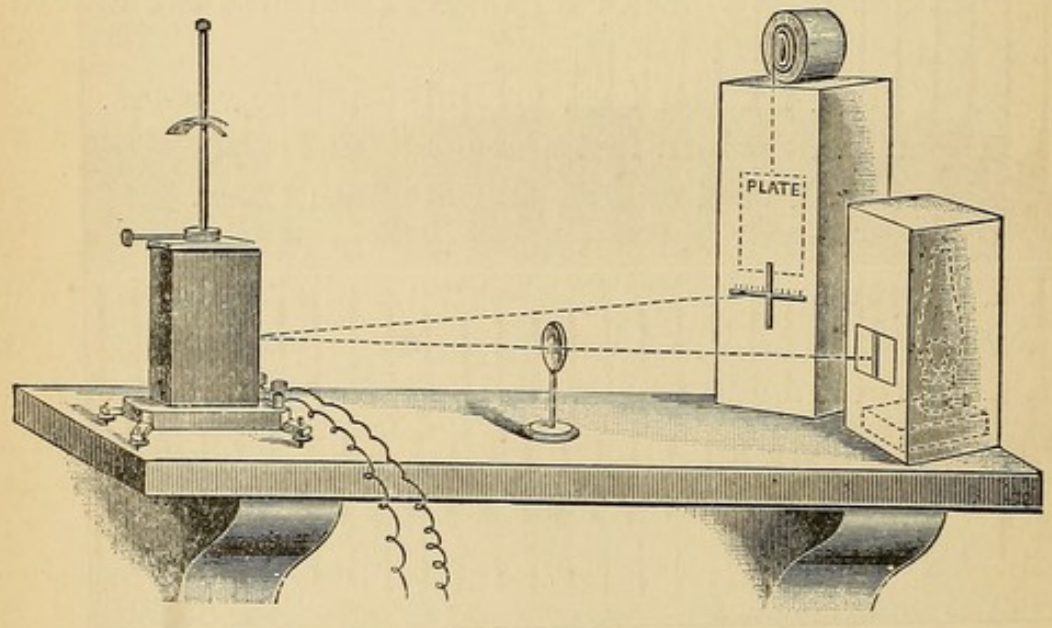
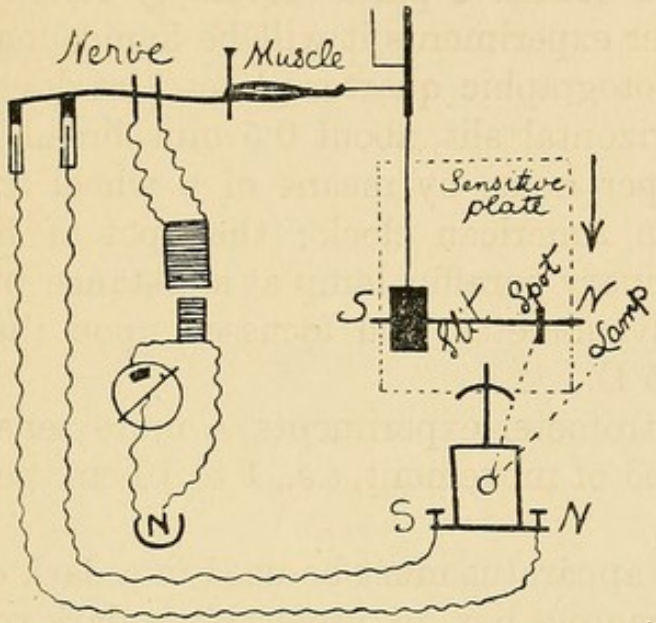


FIG. 33.—RECORDING GALVANOMETER.

GALVANOMETER RECORDS.—The vertical spot of light from the lamp is reflected to cross the horizontal slit S N in the front of a dark box, within which a sensitive plate is let down vertically by clock-work. The spot of light deflected to right and left between S and N yields a black line on the developed plate, and thus records the movements of the galvanometric magnet and mirror.



Plan of apparatus for simultaneously recording excitation effects of Nerve & Muscle

FIG. 34.

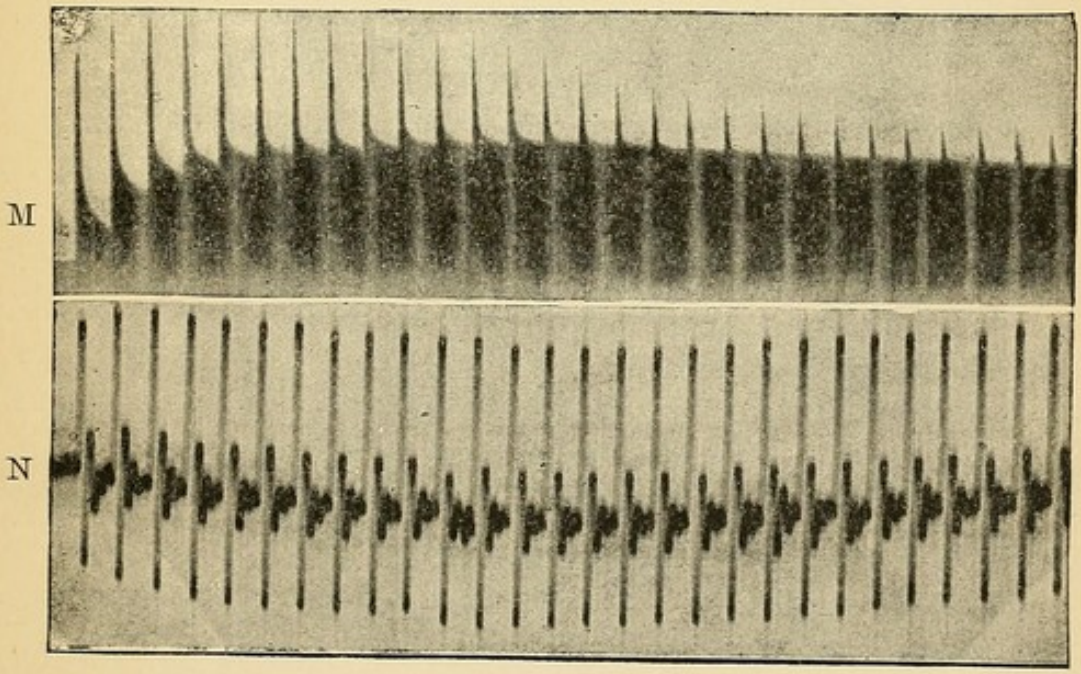


FIG. 35.—Simultaneous record of (M) contractions of muscle and (N) electrical responses of nerve, taken by the apparatus of Fig. 34.

To obtain a simultaneous record of, *e.g.*, the negative variation of nerve and the contraction of the supplied muscle, half the slit (and plate) left obscured is used for the record of the galvanometer spot, and the other half, illuminated by a candle, is used for the record of the muscular contraction, by means of the lever and small screen, as shown in fig. 34.

Electrometer Records.—The image of the vertical column of mercury is made to cover the lower portion of a narrow vertical slit in a screen behind which a sensitive plate travels horizontally, drawn by a falling weight, and restrained

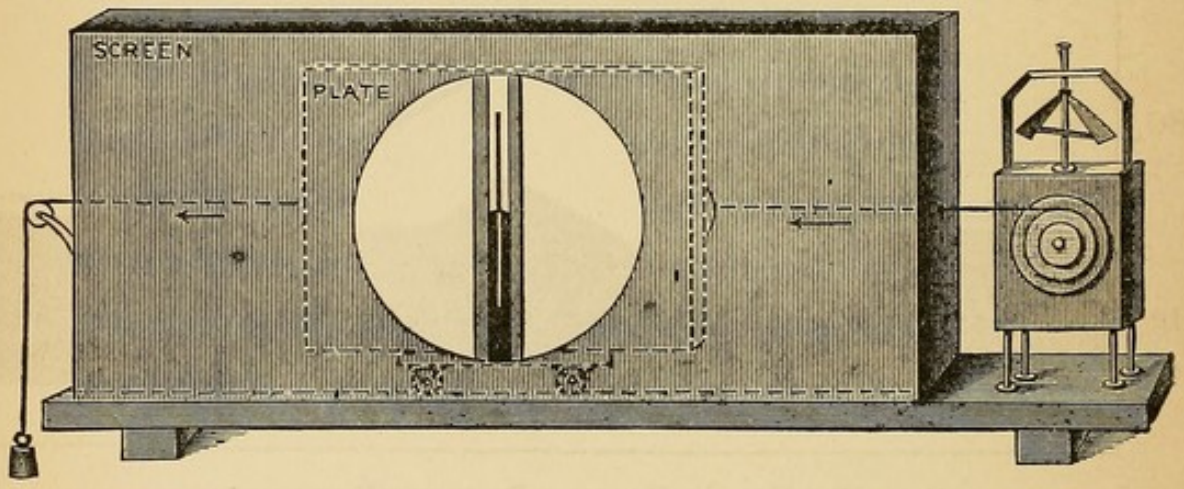


Fig. 36.—Recording Electrometer.

by clockwork. Light passes through the upper portion of the vertical slit, and blackens the upper portion of the developed plate; the lower portion of the slit is screened from light by the mercury, so that the lower portion of the developed plate comes out white or grey, rising and falling with rise and fall of the column. The rate of movement of the plate is recorded by the horizontal shadow of a chronograph lever (not represented in the figure) across the upper part of the vertical slit; on the developed plate the time-record comes out white on black. In a similar manner, *i.e.*, by means of a horizontal lever moved by a muscle or by a heart, a record of mechanical movement (white on black) may be taken above that of electrical change.

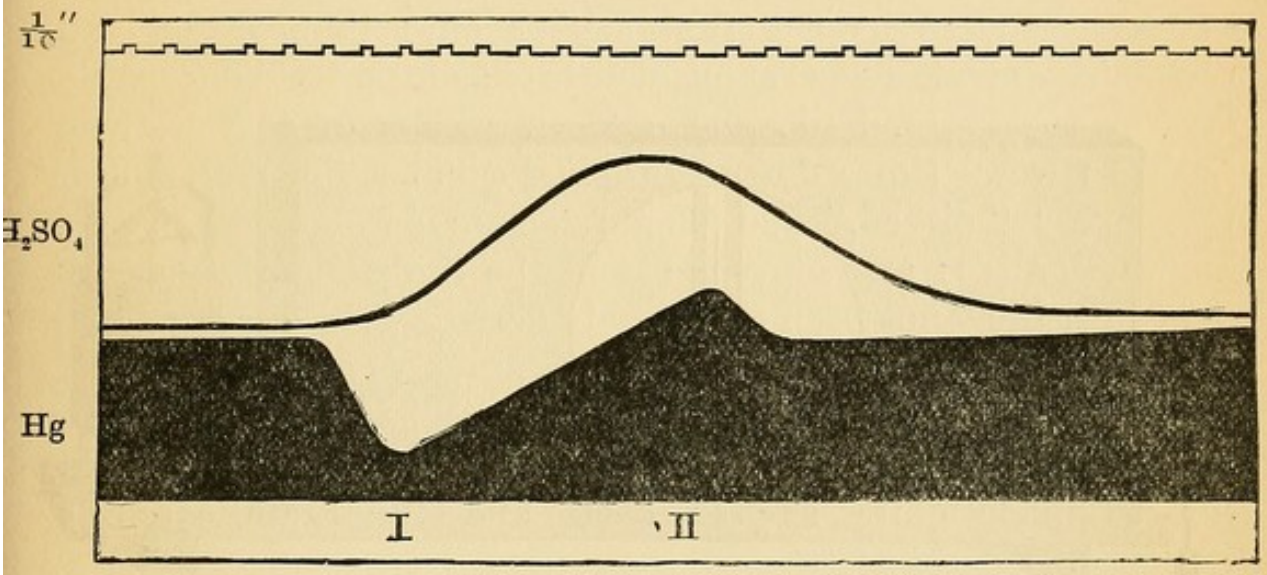


FIG. 37.—FROG'S HEART. DIPHASIC VARIATION.

Simultaneous photogram of a single beat (black line) and of the accompanying electrical change, indicated by the level of the black area, which shows the varying level of mercury in a capillary electrometer. The base of the ventricle is connected with the mercury. I. First phase, base negative to apex. II. Second phase, apex negative to base. (*N.B.*—The figure, as printed, is a positive of the original negative, *i.e.*, black here represents white, and *vice versa*.

(24) To Pith a Frog; Decerebration.

Insert one blade of a strong pair of scissors into the mouth as far back as it will go, and cut off the top of the head. The brain (hemispheres and optic lobes) will thus be removed, or at least exposed at the first scissor cut; in any case, all sensibility is at once abolished by "shock." Thoroughly destroy the brain, bulb, and cord by means of a stout wire pushed several times down the spinal canal. The frog is now thoroughly "pithed," and should remain permanently flaccid and motionless; any movements of the limbs signify that the spinal cord has not been thoroughly destroyed.

For certain experiments it is necessary to only *decerebrate* the frog—*i.e.*, to destroy only the hemispheres or the hemispheres and optic lobes. In this case, the scissor cut must not be made quite so far back, and the brain carefully removed.

In some experiments, again, it is desirable to decerebrate the frog with as little hæmorrhage as possible. In this case the occipito-atlantoid space should be felt for by the finger nail with the frog's head bent forward, opened with the point of a scalpel, and a small piece of wood (a sharpened match) forced through the opening into the cranial cavity so as to destroy the brain. The complete destruction of the brain is best assured by raking out the cranial cavity with a stout wire.

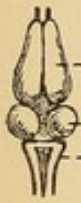


Fig. 38.

(25) To Prepare a Muscle or a Nerve-Muscle for Experiment.

Proceed in the following order:—Pith a frog; expose the tendon of the gastrocnemius muscle and tie a thread or fine wire round it; expose the sciatic nerve, tearing aside the muscles and keeping them open by pins; cut through the ileo-coccygeus and remove the urostyle; cut through the spinal column and use the bit to handle the nerve by; raise the nerve and set it free; clear the lower end of the femur, pass a pin through the joint, or cut the femur if you are about to use a muscle clamp; cut through the tibia below the joint.

Put up the muscle or the nerve-muscle according to what you want to do. For elasticity, single and double contraction, tetanus, you do not need the nerve, and should use direct stimulation.

(26) Action of Curare.

Draw out one or two capillary pipettes in the blow-pipe flame, break the ends and fill a pipette with 1 per cent. solution of curare.

Decerebrate a frog with as little hemorrhage as possible; push one end of the filled pipette beneath the skin of the back and inject the curare solution by blowing into the other end.

(*a*) When the frog lies flaccid and motionless expose the gastrocnemius muscle and the sciatic nerve; cut the nerve and test the muscle and nerve successively. If the curarisation is complete you will get contraction by excitation of muscle, no contraction by excitation of nerve.

(*b*) To prove that curare acts at the periphery, the experiment may be repeated on a frog with one limb protected from the curare by a ligature tied round the thigh, with the exception of the nerve,[1] before the injection of the drug. On the protected side both nerve and muscle respond to excitation, when on the unprotected side only the muscle responds and not the nerve.

(*c*) To prove that curare has no distinct action on afferent nerves or on nerve-centres, proceed as follows: a drop of dilute strychnia solution (1 in 1,000) is applied to the skin, and when absorbed will enhance the excitability of the spinal cord. One limb is protected and curare injected as before. A pinch (*i.e.*, a sensificatory stimulus) of the unprotected motionless limb will cause reflex contraction of the protected limb.

By (*a*) you have learned that after curarisation muscle remains excitable when the excitation of its nerve fails to set it in movement; by (*b*) you have learned that the action of curare is peripheral; by (*c*) you have learned that curare does not appreciably affect sensificatory nerve-ends, afferent fibres, nerve-centres, or efferent fibres. From which you conclude that curare paralyses the junction between efferent fibres and muscle, *i.e.*, the motor end-plates.

N.B.—Obviously if frogs are scarce, the whole series of data can be obtained from a single frog by experiment (*c*), omitting (*a*) and (*b*).

[1] By a thick ligature moderately tight round the intact limb the circulation can be arrested, if desired, without interruption of nerve-conduction.

(27) Action of Veratrine.

Proceed as before (Ex. 26) by using a 1 per cent. solution of veratrine.

On the slowest axis of the cylinder take a record of a normal twitch, and of the twitch of a veratrinised muscle. Make a time tracing (in seconds) below the latter curve. Write a description of the alteration.

(28) Take a tracing of a **single contraction** of frog's gastrocnemius on the cylinder or on the swing, or on the shooter. Mark the latent period. Do not forget to put in a time tracing (100 fork or reed). Take note of the temperature of the room at the time of the experiment, it will probably be between 12° and 18°.

Take a similiar tracing with the muscle put up in the hot and cold air chamber.

(*b*) With raised temperature.

(*c*) With lowered temperature.

Sufficiently well-marked effects will be produced by using for the former a heated poker held not too near the muscle, and for the latter a lump of ice.

(29) **Superposition of two contractions.**—Use either the pendulum or the spring myograph with two coils and two keys. Connections as follows:—

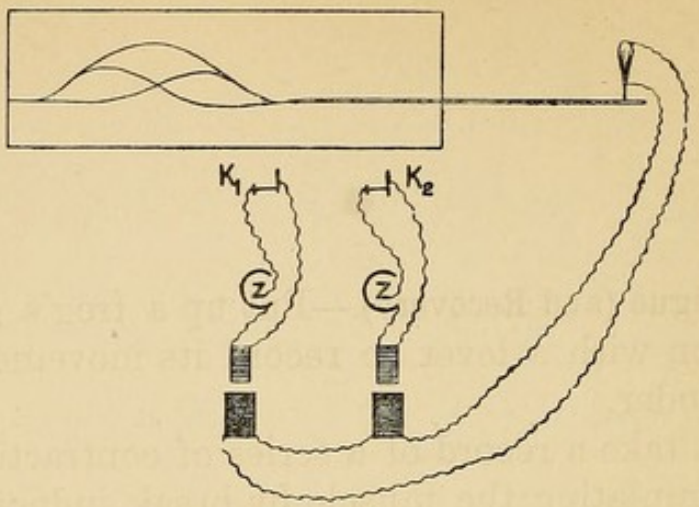


FIG. 39.

Set the keys and myograph lever so that the record will come well on the plate. Adjust the coils to give a stimulus of suitable strength.

Take a single contraction with K_1 only, then with K_2 only, then with K_1 and K_2.

(30) **Composition of Tetanus.**

Arrange a spring to interrupt the primary circuit more or less frequently by vibrating in and out of a mercury pool, according to diagram. The spring is to be held firmly in a clamp, and the vibrating portion taken long or short, according as less or more frequent interruptions are desired. Take a muscle record (*a*), with the spring as long as possible (*b*), with the spring moderately long (*c*), with the spring short, viz., to give less or more frequent interruptions. Observe that with lower frequency tetanus is incomplete (= clonus), and at higher frequency complete.

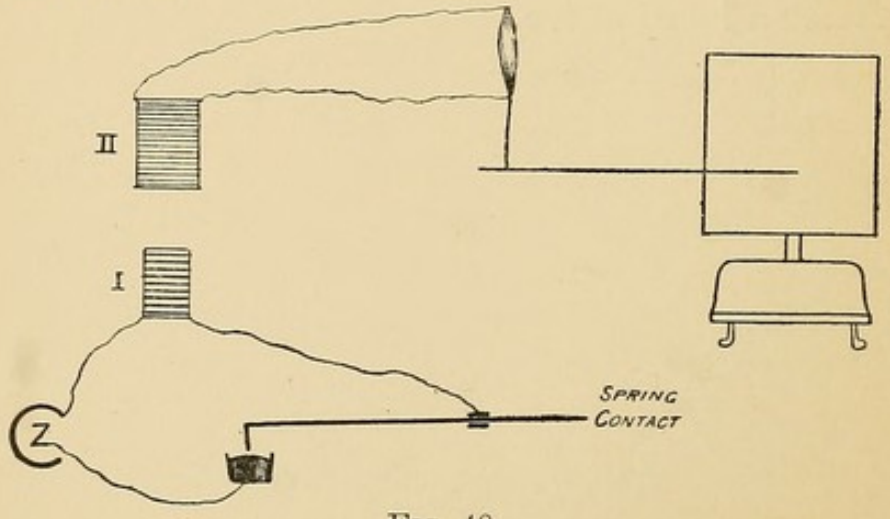


FIG. 40.

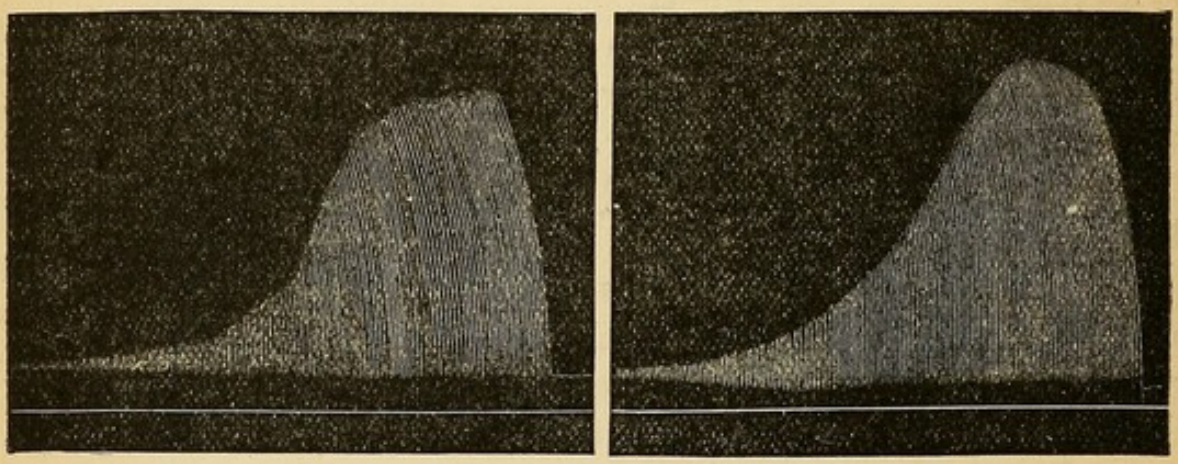
(31) **Fatigue (and Recovery).**—Put up a frog's gastrocnemius in connection with a lever to record its movements, against a smoked cylinder.

(1) First take a record of a series of contractions on a slow cylinder, stimulating the muscle by break induction shocks at 1 sec. intervals, by means of a spring key in the primary circuits, rhythming yourself by aid of the clock sounds, and having found a strength of stim. such that the break gives maximal effects, the make nothing. Take two such records with the two gastrocnemii, one with the ordinary (isotonic) lever, and an after-load of ten grammes, the other with the spring (isometric) lever. Observe in each case the gradual decline of the record, *i.e.*, muscular fatigue. Keep on stimulating until the muscle is quite or nearly exhausted, then pause for two minutes and begin again. Observe that the muscle has recovered power.

(2) With a fresh gastrocnemius take a record of a series of contractions (by either isotonic or isometric method) on a rapid cylinder (250 mm. per sec.), using a trigger-key or other contact fixed to the clock in the primary circuit (p. 37). Each revolution takes about 1·5 sec. Keep on stimulating thus until the muscle is nearly exhausted, then stop the cylinder.

(3) Put up a nerve-muscle preparation, connecting the nerve and the muscle with the coil through a commutator (without cross wires) so that the current can rapidly be turned from the nerve to the muscle.

Stimulate the nerve by single break shocks at regular intervals (or by short periods of tetanisation) until the muscle ceases to respond, and then—still keeping up the stimulation, turn the current to the muscle. Observe that the muscle after having ceased to respond to *indirect* stimulation, does respond to *direct* stimulation. Also that an excised exhausted muscle, allowed to rest for a short period, recovers power.



II. I.

FIG. 41.

Subsequent direct fatigue by subsequent excitation of the muscle.

Indirect fatigue by excitation of the nerve.

Fatigue. (Man.)

A.—Of Voluntary Muscular Efforts.

With the dynamograph and slow clock take series of maximum voluntary grasps of the hand at some regular rhythm, timing yourself by the clock tick. Make the voluntary efforts in some such rhythm as the following:—Each contraction lasting 2 sec.; interval between each two contractions, 2 sec.; a group of 30 such contractions will therefore take 2 minutes. Take two or more such groups of 30 maximal efforts, interposing between each two groups a period of rest of say 1 min. Be as attentive as possible to make the voluntary efforts as regular and as great as possible, and do not look at the tracing until the series is finished. If so inclined, you may further examine the effects of variations of rhythm upon the rate of fatigue.

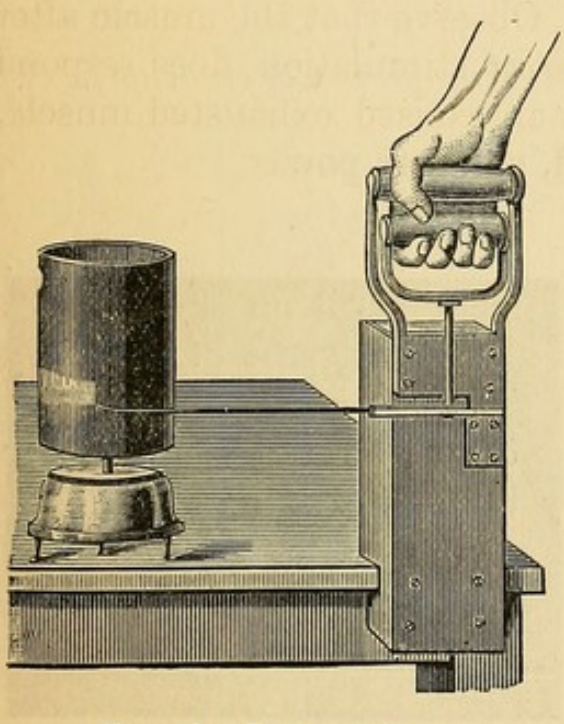


FIG. 42.—DYNAMOGRAPH.

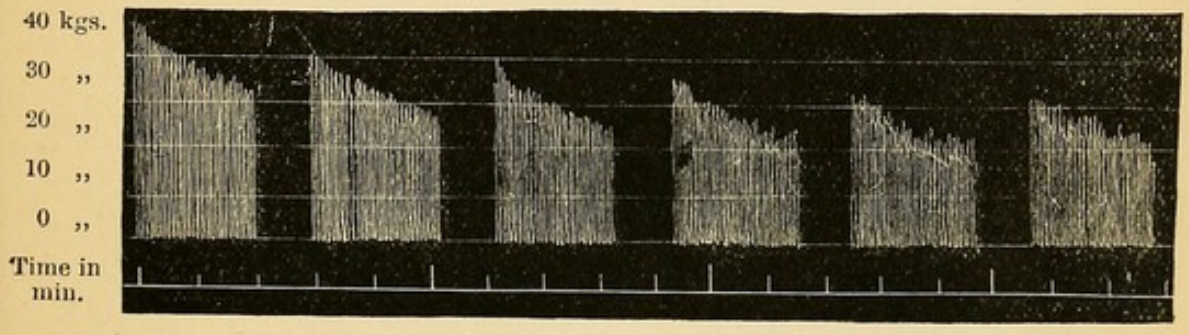


FIG. 43.—DYNAMOGRAPH RECORD.

B.—Of Electrically Excited Muscular Contractions.

With the lighter dynamograph and slow clock take series of muscular tetani of the muscles of the forearm, put into contraction at some regular rhythm by direct excitation, placing one electrode from the induction coil on the muscles of the forearm, the other on any convenient part of the body (*e.g.*, the calf of the leg).

If so inclined, you may test the effect of voluntary muscular action upon the muscle itself, by taking series of electrically excited contractions before and after a series of voluntary muscular contractions.

(32) **Extensibility of Muscle.** — Isolate the gastrocnemius muscle of a pithed frog: tie a strong thread securely to the tendon, divide the femur, and fix the lower end firmly in a clamp. Tie the end of the thread from the tendon to a light, long lever, and to the same thread attach a light scale-pan to receive a succession of weights (pennies, which weigh about 10 grammes each, are convenient for the purpose of the experiment).

Make the point of the lever touch a smoked cylinder or plate, which is to be moved on by hand by an equal space as each equal increment of weight has been made. Carefully place in the scale-pan 1, 2, 3, &c., pennies, and move the recording surface as just directed. Observe that the successive increments of length of muscle, caused by successive equal increments of extending weight, form a diminishing series, forming a curve convex towards the abscissa. Remove the weights one by one, and observe the converse series of elastic shortenings of the progressively unloaded muscle. Repeat a similar experiment with a rather strong piece of elastic substituted for the muscle, and observe that successive equal increments of weight produce successive equal increments of length.

(33) **Electrotonic Alterations of Excitability.** (Frog.)

Apparatus.—Battery, cell and coil, wires, rheochord, 2 unpolarisable electrodes, moist chamber, myograph, 2 keys, commutator.

First prepare the unpolarisable electrodes and arrange them close to a pair of ordinary electrodes in the moist chamber, ready for the nerve to be laid upon them, and so that the ordinary electrodes shall be nearer to the muscle. Connect the unpolarisable electrodes with the battery, for the "polarising current"; and the ordinary electrodes with the coil for the testing current. Place an interrupting key and a commutator in the polarising circuit, and mark what direction the current has in the nerve according as the cradle is turned right or left. Arrange a key in the testing current for single shocks.

Then make a nerve-muscle preparation (Ex. 25); lay the nerve across the electrodes, and attach the tendon of the muscle to the lever of the myograph.

(*A*) Find a distance of secondary from primary coil such that a break induction shock just fails to cause contraction.

Now let the polarising current pass through the nerve in the descending direction, *i.e.*, so that the pole near the testing electrodes is its kathode, and observe that the previously ineffectual stimulus now causes a contraction, proving that the excitability is increased near the kathode.

(*B*) Find a distance of secondary from primary coil such that a break induction shock is rather more than sufficient to give a contraction.

Let the polarising current pass in an ascending direction so that the pole near the testing electrodes is its anode, and

observe that the previously effectual stimulus is now ineffectual, proving that the excitability is diminished in the neighbourhood of the anode.

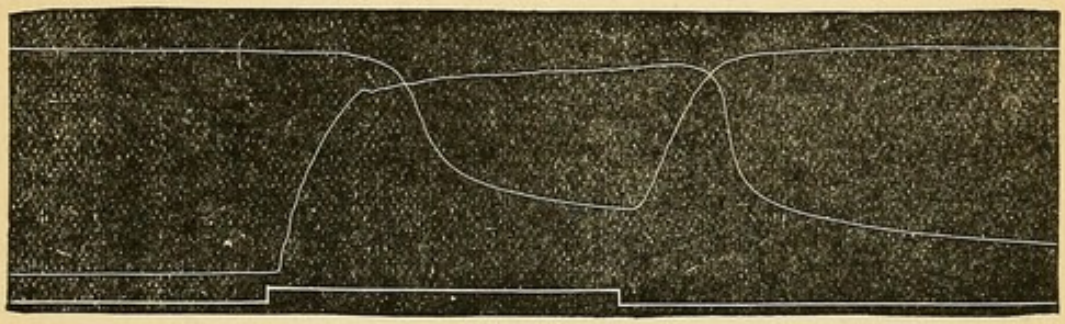


FIG. 44.

(*C*) Arrange the coil for tetanising currents, and repeat experiments *A* and *B* with this test, finding just ineffectual stimuli rendered effectual near a polarising kathode, *i.e.*, giving a tetanus, and effectual stimuli rendered ineffectual near a polarising anode, *i.e.*, giving a remission of tetanus.

(34) **Law of Contractions.** (Frog.)

Apparatus.—As in preceding experiments, omitting the coil and testing electrodes, and using a rheochord to get a "weak" current.

Prepare the unpolarisable electrodes, and arrange them in the moist chamber. Trace the current through the commutator, and mark what the direction will be in the nerve with the two positions of the cradle. Put up a single cell of the battery in connection with the rheochord, key, and commutator, using the rheochord as a shunt, as shown in fig. 19.

Make a nerve-muscle preparation and lay the nerve across the electrodes.

(1) By moving the rheochord slider to or from the terminals, divert a smaller or greater fraction of current into the nerve

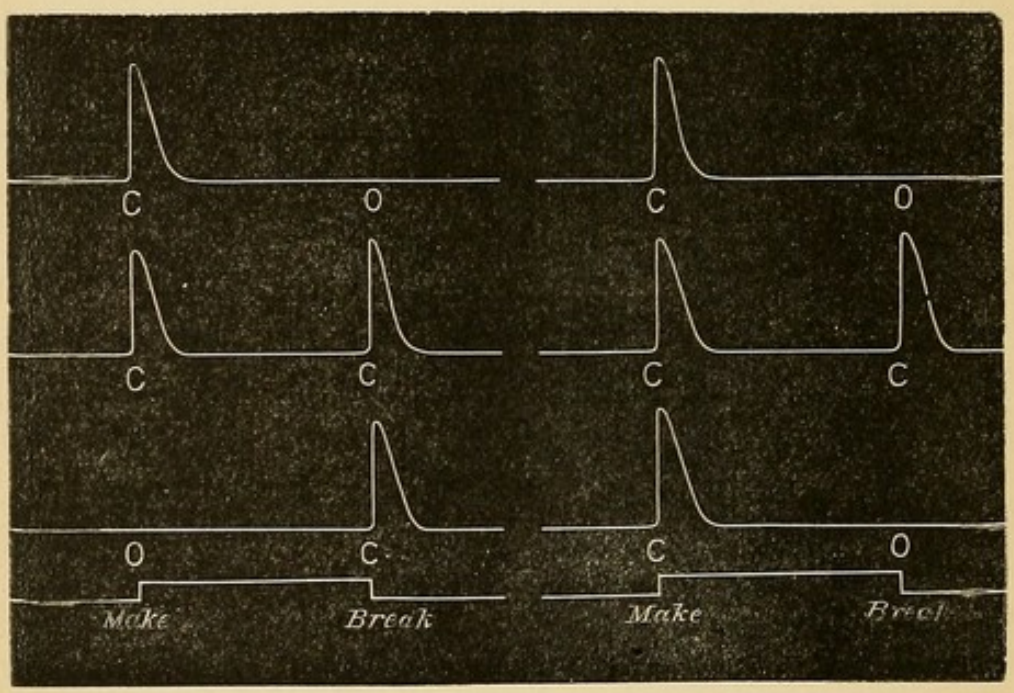


Fig. 45.

circuit. Test by closure and opening of the key. Observe that with this weak current a contraction occurs at make of the ascending and of the descending current, and no contraction at break of either current. Usually the make contraction appears with a weaker current if ascending than if descending.

(2) Remove the rheochord, bringing the battery wires straight to the commutator, but keeping the key in.

Test as before, and observe with this stronger current that a contraction occurs at make and at break of the ascending and of the descending current.

(3) Use a stronger electromotive force, *i.e.*, more cells. Test as before, and find such a strength of current that contraction shall occur at break of the ascending and at make of the descending current, but no contraction at make of the ascending nor at break of the descending current.

You have now verified Pflüger's law of contractions :—

	Asc.		Desc.	
	Make.	Break.	Make.	Break.
I.—Weak current ..	C	O	C	O
II.—Medium current ..	C	C	C	C
III.—Strong current ..	O	C	C	O

From the previous exercise you have learned that excitability is increased at and near the kathode, diminished at and near the anode during the passage of a current. Taking this into account, together with the facts (1) that the immediate after-effect of a current is diminished excitability at and near the kathode, increased excitability at and near the anode, and (2) that the kathodic increase is more efficacious than the after anodic increase, you may understand the meaning of the results you have obtained. Considering the position of kathode and anode in the nerve rather than actual direction of current, you see (1) that C.O. C.O. illustrates the greater efficacy of the kathode as compared with the after anode; (2) that C.C.C.C. illustrates the efficacy of both kathode and after-anode in that O.C. illustrates blocking of a kathodic (make) stimulus by an anodic diminution lower down the nerve, and C.O. blocking of an after-anodic (break) stimulus by an after-kathodic diminution lower down the nerve.

(35) **Law of Contractions.** (Man.)

Apply one electrode of a galvanic battery (of say thirty Leclanché cells) to any indifferent part of the body. Use the other as a testing electrode, applying it as close as possible to some superficial nerve such as the ulnar or median at the elbow, or the peroneal at the head of the fibula. Test on such nerve the effect as regards muscular contraction of make and of break of the kathode and anode, gradually increasing the number of cells in use by means of the collecting dial on the battery.

Observe (1) that the first effect is obtained with kathodic closure; (2) that the next to appear is the anodic closure, or the anodic opening contraction; and (3) that (unless an excessively strong current is used) kathodic opening produces no effect. Note in each case the smallest number of cells with which an effect is visible, or *preferably* put into the circuit the galvanoscope mentioned on p. 6, and note in each case the smallest deflection with which an effect is visible. [N.B.—The graduation of the galvanoscope is supposed to indicate milliamperes.]

(36) **Electrotonic Alterations of Excitability.** (Man).—(A) *Tested by Break Induction Shocks.*

Put up a circuit in accordance with the accompanying diagram, so that an exciting current from the secondary coil of a du Bois' apparatus can be applied in the absence or in the presence of a polarising current from a galvanic battery (of say 30 Leclanché cells). In the latter case, *i.e.*, when the exciting current is superposed upon a polarising current, their coincidence is secured, inasmuch as both currents enter or leave the body by the same eiectrodes. Apply the testing electrode to the median nerve at the bend of the elbow, or to the peroneal nerve at the head of the fibula. The commutators, as shown

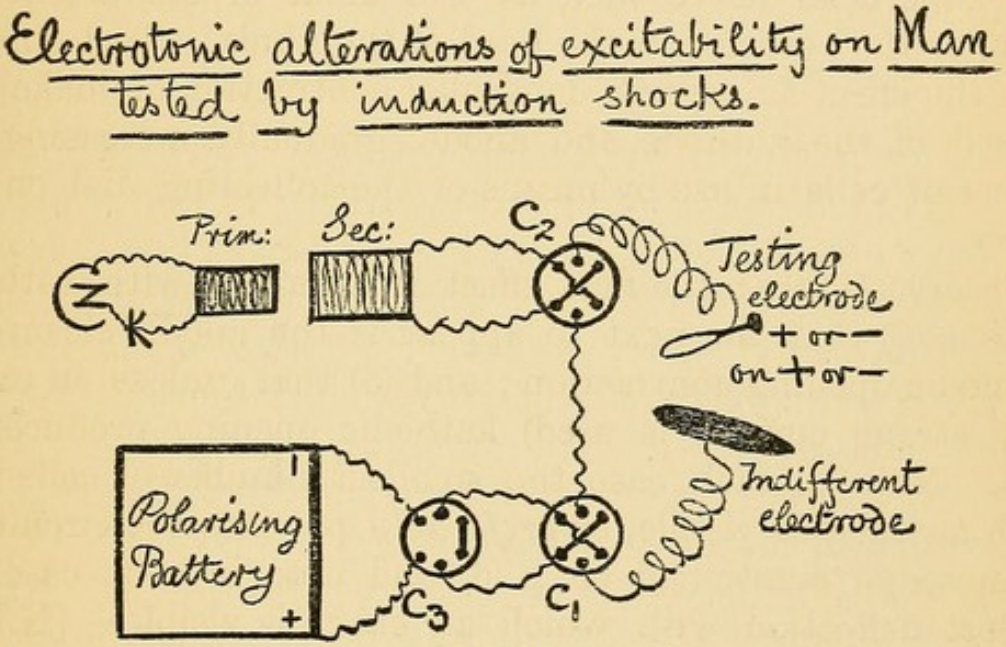


FIG. 46.

The commutators C_1 C_2 with cross wires serve to reverse the direction of current from the battery and from the coil independently of each other. The commutator C_3 without cross wires, but with a junctional wire as shown in diagram, is used for cutting out the battery without short-circuiting it, and without breaking the secondary circuit. (N.B.—The cradles of the three commutators are omitted for the sake of distinction.)

in diagram, enable you to make the testing electrode either kathode or anode of the make or break induction current, with or without either kathode or anode of the galvanic polarising current. Leaving out of account the make induction current by cutting it out, or by taking such a distance of coil that it has no effect) you may now proceed to compare the effects of—

(1) The coil Kathode with and without the battery Kathode.
(2) ,, Anode ,, ,, ,, Anode.
(3) ,, Kathode ,, ,, ,, Anode.
(4) ,, Anode ,, ,, ,, Kathode.

You will find that in cases (1) and (2) the effect of the excitation is increased during polarisation, in cases (3) and (4) diminished during polarisation. The increase in (1) and the diminution in (3) (being effected in the polar region) are respectively more considerable than the increase in (2) and the diminution in (4) (being effected in the peripolar region). In case (4) you may notice that the make induction shock becomes effective during polarisation, unless means have been taken to cut it out. (*I. H. P.*, pp. 364, 372; *Phil. Trans. R. S.*, 1882, p, 961.)

(B) *Tested by Make and Break of a Constant Current.*

Put up a circuit in accordance with the accompanying diagram, so that an exciting current from the testing battery, T.b., can be made and broken in the absence or in the presence of a polarising current from a second battery, P.b. Both currents have, as before, the same electrodes to and from the body; the testing electrode may be applied to the median or to the peroneal nerve. The switches, C_1, C_2, C_3, serve the same purpose as in the previous exercise. Recording apparatus as there described.

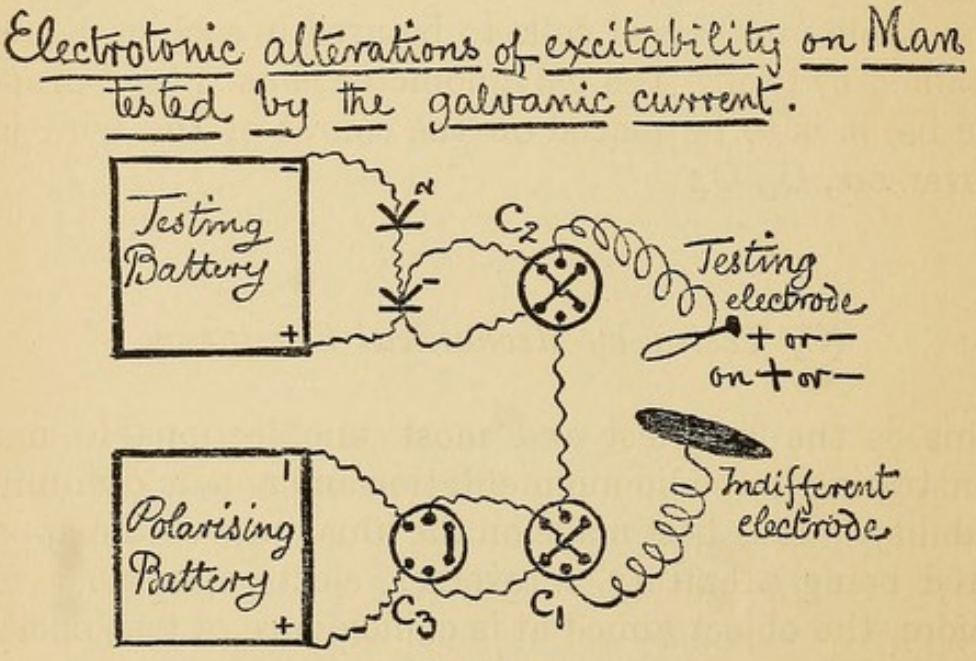


FIG. 47.

The keys, K_1, K_2, are introduced in order to allow the testing current from T.b. to be made and broken without affecting the circuit of the polarising current from P.b., K_1 being used as a short-circuiting key that breaks the test current when closed, and makes it, when opened; K_2 being used as a guard key to save the battery from remaining short-circuited when K_1 is closed; to this end K_2 must be opened directly after K_1 is closed, for a test break; K_2 must be closed directly before K_1 is opened, for a test make.

The comparisons that are to be made with this disposition of apparatus are the effects of—

(1) K.C.C. alone and K.C.C. upon a Kathodic current.
(2) A.C.C. alone and A.C.C. upon an Anodic current.
(3) A.O.C. alone and A.O.C. from an Anodic current.
(1) K.C.C. alone and K.C.C. during Kathodic polarisation.
(2) A.C.C. alone and A.C.C. during Anodic polarisation.
(3) A.O.C. alone and during Anodic polarisation.

Observe that—

(1) K.C.C. is increased during Kathodic polarisation.
(2) A.C.C. is increased during Anodic polarisation.
(3) The increase of K.C.C. in (1) is greater than the increase of A.C.C. in (2); *i.e.*, in (1) the increase is effected in the polar region, in (2) it is effected in the peripolar region.
(4) A.O.C. is diminished during Anodic polarisation; in this case the diminution is effected in the peripolar region.

With regard to the strengths of current to be used for testing and for polarising, no numerical directions are given; the most suitable numbers of cells to be used in each case are to be ascertained by trial. If a galvanometer is used (and properly it should be) it is to be placed on the course of the wire joining the reversers, C_1, C_2.

(C) *Tested by Mechanical Excitation.*

This is the simplest and most unobjectionable mode of demonstrating kathodic augmentation and anodic diminution of excitability. It is best made on the ulnar nerve; the apparatus required being a battery, a reverser, electrodes, and a mallet. As before, the object aimed at is coincidence of test excitation, and of polarisation in a nerve.

Applying the testing-electrode against the ulnar nerve, find, by tapping it with the mallet, a force of blow sufficient to make the fingers twitch slightly. While taps are being applied at regular intervals, giving regular finger twitches, make a polarising current, kathodic, through the test-electrode, and observe that the twitches are strengthened; then make a polarising current, anodic, through the test-electrode, and observe that the twitches are weakened or abolished. The augmentation and diminution are effected in the polar region of the current passing through the nerve.

(37) Measurement of the Velocity of Transmission of Nerve-Impulses in the Motor Nerves of Man.

Use the pendulum myograph, or a spring-myograph. Prepare also (1) a small, thick-walled india-rubber bag, connected by tubing with a Marey's tympanum; (2) a cell, induction coil, and electrodes (one large flat electrode, and one small electrode, fixed to a handle); (3) a tuning-fork (100 per sec.)

Put up the circuit as follows: primary circuit to include a fixed "snap" key, which is struck open by the plate-carrier of the myograph; secondary circuit in connection with yourself, by strapping the flat electrode to one leg, and using the small electrode to excite the brachial nerves. Assure yourself that the contact at the snap key is not loose when the key is closed.

Hold the india-rubber bag between the middle finger and thumb of the left hand, and taking the small electrode in the right hand, press it against the nerves (A), above the left clavicle (B), at the bend of the forearm. Find the best points of application by trial stimuli (these should be made by an assistant, by a simple key in the primary circuit.)

All being ready (*i.e.*, plate ready to let off, electrodes applied, primary circuit closed, india-rubber ball in hand, lever of tympanum just touching the recording surface) the plate is let off. In its passage the plate-carrier strikes open the key, giving a break induction shock, by which the nerves at A are excited, the flexor muscles pinch the bag, and cause a sharp rise of the tympanum-lever.

A second observation is taken with the small electrode applied at B, *great care being taken in re-setting the plate-carrier not to disturb the position of the tympanum.*

It will be found that the contraction caused by distal excitation (at A) commences to rise from the base-line a little earlier than that caused by proximal excitation (at B). For measuring purposes a pair of contractions should be selected which are as equal as possible.

The time-value of the interval between the two rises is ascertained by the tuning-fork.

The velocity of impulse is calculated from this time-value, and from the length of nerve between A and B. It will be found to be about 50 metres per second.

(38) **Influence of Temperature upon the Excitability of Nerve. Dem.** Tested (*a*) by induction currents; (*b*) by make of the constant current. (Gotch.)

Preliminaries.—Arrange two beakers, tubing and clips, to deliver a flow of hot, or of cold water through a thin glass tube across which the nerve is to be laid.

Prepare a cell and rheochord to obtain a weak constant current by a small fraction of a volt. Wire rheostats, as shown in fig. 16, had better not be used, on account of the possibility of induction; a short zinc rheostat, as figured below, is preferable.

Put up the induction apparatus, using a spring key K_1 in the primary circuit, and a short-circuiting key K_2 in the secondary circuit.

Connect the coil and the rheochord respectively with the two sides of a commutator without cross-wires, and lead off the exciting (unpolarisable) electrodes from the two middle pools (or in place of the commutator use two short-circuiting keys, connected as in fig. 7); make the connections so as to have current descending in the nerve.

Put up the nerve-muscle preparation, laying the nerve across the water-tube (and upon short, rather stiff cords), moistened with saline, projecting from the clay ends of the exciting electrodes. Let the water-tube be as close as possible to the electrode nearest the muscle.

Experiments.—Find the minimum stimulus to:

(*a*) A descending induced current	with warm water passing through the tube.
Do. Do.	with cold water.
(*b*) Make of descending constant current	with warm water.
Do. Do.	with cold water.

or, having found a moderately effective strength of stimulation at ordinary room-temperature (about 15°), make stimuli at regular intervals (conveniently so by a metronome) and test the effect of warming and cooling upon:—

(*a*) the effects of induction shocks;
(*b*) the effects of make of the constant current.

The general tenour of your results will be :—

(*a*) Augmented and diminished excitability of the nerve tested by induction shocks at high and low temperatures respectively.

(*b*) Augmented and diminished excitability of the nerve tested by make of a constant current, at low and high temperatures respectively.

(N.B.—The electrical conductivity of nerve, and therefore the strength of exciting current, increase and diminish with rising and falling temperature; this disturbing influence may be diminished by placing large resistance of 100,000 ohms in the exciting circuit. Drying of the nerve and of the electrodes augment the resistance. Strictly speaking, the current-strength should be observed by a galvanometer, but in an ordinary demonstration this precaution must be neglected.)

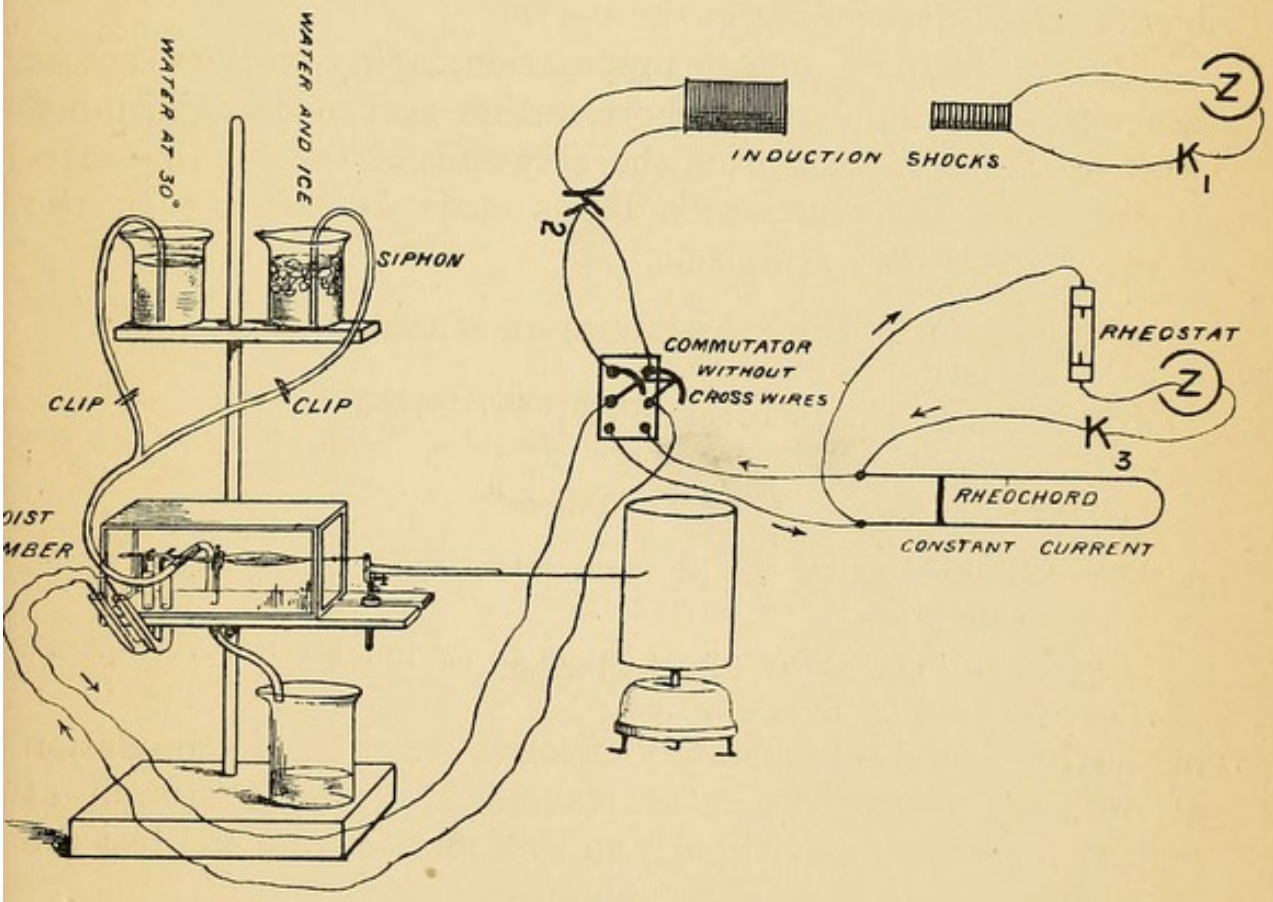


FIG. 48.

(39) **Electrotonic Currents** (extra-polar).

Use d'Arsonval's galvanometer to witness the extra-polar currents produced by the current of a Daniell led into Hermann's model.

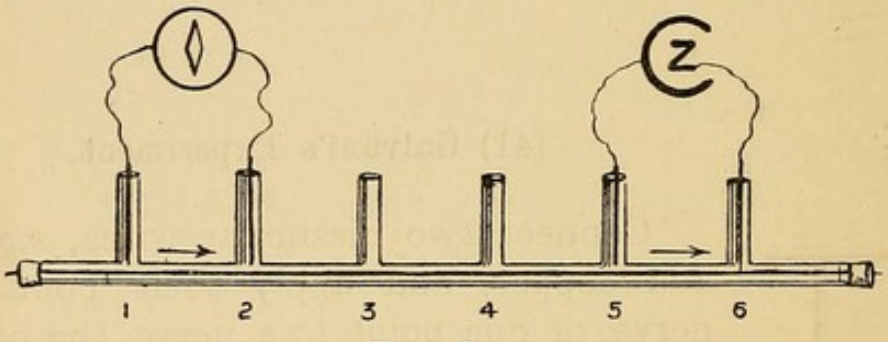


FIG. 49.

Glass tube with short vertical branches, filled with saturated solution of zinc sulphate. Platinum wire in centre stretched between two corks. Amalgamated zinc wires inserted in branches, bent so as to dip into fluid, but not touch central wire, one pair for leading in polarising current, one pair for leading off the extra-polar current.

Observe :—

(1) Direction of extra-polar current.

(2) Direction with reversal of polarising current.

(3) Strength of extra-polar current near and far from polarising current.

(4) Modifications of extra-polar current with various strengths of polarising current.

The results are similar to those obtained on nerve, but are *not* suppressed by anæsthetics.

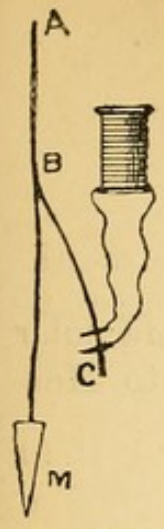


FIG. 50.

(40) The Paradoxical Contraction.

Dissect out one of the two main divisions of the sciatic, and divide it as far as possible from its origin. Galvanic excitation of the central end of the divided branch gives rise to contraction of the muscles supplied by the other branch. The second nerve is stimulated by the electrotonic alteration of the first nerve. Apply the ligature test for current diffusion.

(41) Galvani's Experiment.

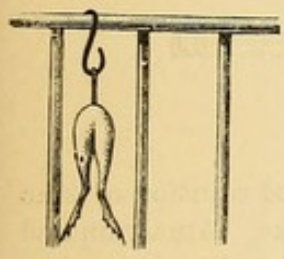


FIG. 51.—GALVANI'S EXPERIMENT WITH METALS.

Connect two dissimilar wires, *e.g.*, zinc and copper, and apply their points to a nerve, or one point to a nerve, the other to any part of the frog; contraction occurs at each application (make current), or if the preparation is very excitable, at each application and removal (make and break currents).

(42) The Contraction without Metals.

Prepare a nerve-muscle preparation, choosing a vigorous and lively frog. Allow the nerve to fall on the muscles of the lower limb stripped of its skin. The muscle of the preparation contracts because different parts of the surface with which the nerve is suddenly brought into contact are at different potentials.

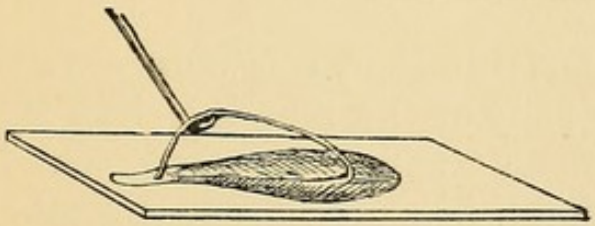


FIG. 52.—GALVANI'S EXPERIMENT WITHOUT METALS.

(43) Current of Injury of Muscle. (*A*) Its Measurement. (*B*) Its "Negative Variation." Dem.

Set up the galvanometer, shunt, lamp, and scale; see that the suspended system of magnets swings freely; adjust it by means of the controlling magnet until the spot of light, when at rest, is at the middle of the scale.

Put up the circuit containing: Daniell cell, low resistance rheostat *r*, high resistance rheostat R, galvanometer G, shunt *s* (and muscle), as per diagram—

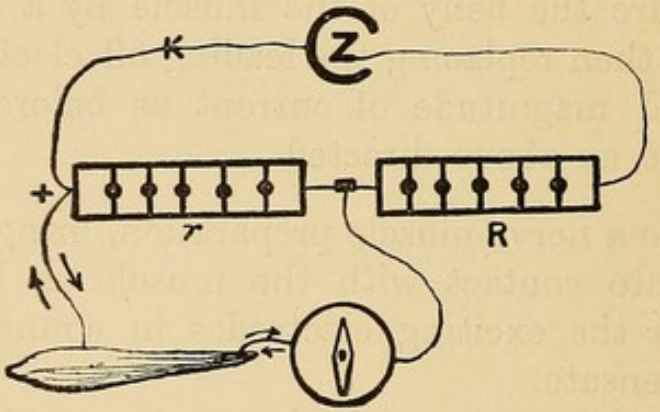


Fig. 53.

Arrange a cell and coil for tetanisation (fig. 40); assure yourself that current passes through the exciting electrodes.

Prepare unpolarisable electrodes for leading off. Expose and isolate a gastrocnemius muscle; tie one thread to the tendon and another to the femur, fixing the two threads to two large pins, between which the muscle is slightly stretched on, but not touching, a sheet of cork.

(*A*) Bring the leading off electrodes into contact with the tendon and the belly of the muscle; close K_2 to see whether in this comparatively "uninjured" state there is any deflection of the spot of light, *i.e.*, any muscle-current. Determine its direction, and, if need be, take only a fraction of it by using the shunt.

Compensate this current. Take R = 1,000 ohms., and find the value of r at which, with the Daniell current *against* the muscle current, the spot of light is brought back to zero, *i.e.*, the two opposed currents balance. Calculate the value of the muscle current. The latter is equal to the following fraction of a Daniell, viz: $\frac{r}{r+R}$. If, *e.g.*, the muscle current is balanced with $r = 50$, its value in terms of a Daniell = $\frac{50}{1050}$.

Now injure the belly of the muscle by a scissor cut, removing and then replacing the leading off electrode. Observe direction and magnitude of current as before. Compensate and calculate as above directed.

(*B*) Make a nerve-muscle preparation, bring the leading off electrodes into contact with the muscle as before; lay the nerve across the exciting electrodes in connection with the coil. Compensate.

Now tetanise the muscle by sending induction currents through the exciting electrodes; observe the deflection of the spot of light; this is in an opposite direction to that of the current of injury, *i.e.*, a negative variation.

N.B.—To be sure that the deflection is really by the muscle, and not a fallacy due to coil magnetism, see that the starting the coil alone does not produce it. In any case, use long wires, placing the coil several yards distant from the galvanometer.

(44) Nerve-currents; the Current of Injury and its Negative Variation. Dem.

Same apparatus and preparations as for the analogous experiments on muscle. Connections as in fig. 53, an excised sciatic nerve enclosed in a moist chamber (fig. 48) taking the place of the muscle.

Having assured yourself that all parts of the apparatus are in working order, isolate and excise a frog's sciatic, and lay it upon the exciting and leading-off electrodes; let the cut end of the nerve rest upon one of the latter.

Close the galvanometer key; observe the deflection, and trace the current from longitudinal to transverse section through the galvanometer, viz., transverse section or injured part negative to longitudinal surface.

Measure this current of injury by compensation, as in the case of muscle.

Now tetanise the nerve for five to ten seconds by sending induction currents through the exciting electrodes. Observe the deflection, which is opposed to that caused by the current of injury, *i.e.*, a negative variation.

Repeat the tetanisation with the direction of the exciting currents reversed, and observe that the negative variation is unchanged in direction.

[N.B.—You may repeat this experiment an indefinite number of times without any diminution of the variation becoming apparent; you will be tired out before the nerve shows any signs of fatigue, provided you do not injure it by currents of excessive strength. The fallacies of current-escape and of electrotonic current have been met by the reversal of the exciting current; that of unipolar excitation can be tested by detaching one of the wires from the secondary coil; any magnetic effect of the coil has been tested for at the outset.]

(45) Action of Anæsthetics upon Isolated Nerve. Carbon Dioxide, Chloroform, and Ether. Dem.

Tetanise the nerve during regular periods at regular intervals and observe that the negative deflections are of regular magnitude. To do this, time yourself by the seconds' hand of a watch, tetanising during say five or ten seconds at minute intervals.[1]

(*a*) Having assured yourself of the regularity of the deflections, blow a little strong ether vapour into the nerve chamber, and continue to tetanise at minute intervals; observe that the deflection is abolished. Clear out the ether vapour, and observe that the deflection returns.

(*b*) Repeat the experiment with strong chloroform vapour. The results are similar; the difference, if any, being to the effect that the abolition is more prompt, the return more delayed.

(*c*) Send a stream of CO_2 gas through the nerve chamber for two or three minutes, then blow it out. The deflection is diminished or abolished during the passage of CO_2, and subsequently greatly augmented.

(*d*, *e*, *f*) If the three preceding experiments are made with weaker reagents—with, *e.g.*, a very slow stream of CO_2, or with one per cent. ether or one per cent. chloroform in the wash bottle, a primary stimulant instead of a primary depressant effect will be obtained, *i.e.*, the deflection will be augmented while the nerve is under the influence of the reagent.

[N.B.—Strong chloroform and ether vapour clear off very slowly, and should be used last if it is intended to show each of the six experiments above described. The best order then is *d*, *e*, *f*, *c*, *a*, *b*.]

[1] It is convenient, but not indispensable, to stimulate automatically. The simplest means to this end is an ordinary clock with a platinum wire soldered to the seconds' wheel, arranged to complete a circuit once a minute for say seven and a half seconds by passing through a mercury pool.

(46) **The Secondary Contraction.** — Pith a frog. Make two nerve-muscle preparations. Lay the nerve of II. across the muscle of I. Tetanise the nerve of I., and observe that the muscle of II., as well as that of I., enters into contraction.

Test for current escape by crushing or ligaturing the nerve of II. If tetanisation of nerve I. still gives a contraction of muscle II., there has been current escape. A true secondary contraction (abolished by ligature) is due to excitation of nerve II. by a negative variation of the current of muscle I.

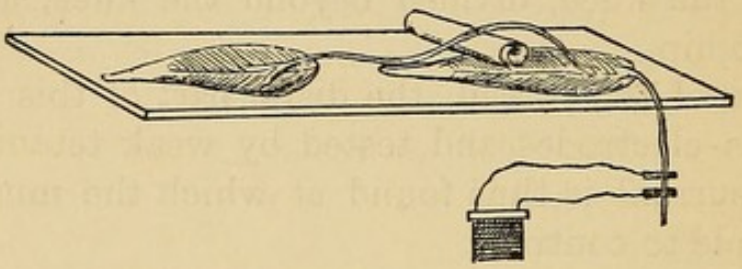


FIG. 54.—THE SECONDARY CONTRACTION.

(47) **Secondary Contraction from the Heart.**

Excise the heart; lay the nerve of a fresh nerve-muscle preparation upon it as per diagram. The muscle contracts at each beat of the heart, being excited by the electrical current which accompanies each beat.

FIG. 55.

(48) **An Apparent Anomaly due to Secondary Contraction.** (Hering.)—After the foregoing experiments have been performed, the following simple and striking experiment by Hering may be undertaken without arousing misconception.

A frog is pithed, and the urostyle removed, exposing on both sides the long nerve roots lying on the ileo-coccygeal muscle, and which form the sciatic nerve. These are to be cleanly divided by scissors on both sides, at or about the middle of their exposed length. The motor nerve roots above are now exposed, or a pair of stiff wire electrodes are pushed down the spinal canal so as to come in contact with them.

On weak tetanisation of these roots the limbs are tetanised.

The result is due to contraction of the coccygeal muscles (supplied by nerves arising above the point of division) which excites the peripheral ends of the divided sciatic roots.

[Superficially regarded, the result appears as if division of nerve has not interfered with the conduction of impulses; but equally obviously (1896) this is absurd.]

(49) **Secondary Excitation from Nerve to Nerve.** (Hering.)—A frog is pithed, a sciatic nerve is exposed from hip to knee, ligatured at the knee, divided beyond the knee, and isolated from knee to hip.

By means of the thread, the distal part of this central end is laid across electrodes and tested by weak tetanisation. A strength of current is thus found at which the muscles of the thigh are made to contract.

This contraction is not augmented, but, on the contrary, disappears when the electrodes are slipped higher up the nerve, *i.e.*, nearer to rest of the frog. Therefore the contraction is not due to current-escape. For the same reason it cannot be a "paradoxical contraction"—an excitation of centrifugal nerve-fibres to thigh muscles by electrotonic currents aroused in adjacent nerve-fibres. Therefore, by exclusion, it is due to the excitation of nerve-fibres to the thigh by the action-currents of adjacent nerve-fibres, *i.e.*, a true secondary excitation of nerve by nerve.

[N.B.—This experiment goes best with "cold frogs," *i.e.*, frogs kept in the cold—0° to 2°—and left for a few hours before use at room temperature, about 15°. On beginning to test the distal end of the nerve, vary the direction of excitation by turning the electrodes one way or the other, and continue to test with the electrodes in the more favourable direction (which will be such that the kathode of the break shock is distal to the anode.)

The "paradoxical contraction" itself is, in certain cases, attributable to action-currents; in such case it diminishes as the electrodes are slipped upwards from the distal end; when it increases with this changed situation of the electrodes, it is of electrotonic origin (or, it may be by reason of current-escape).]

(50) Electrical Variations of the Frog's Heart.

Arrange the capillary electrometer under a low power of the microscope, and assure yourself that the mercury moves freely (Ex. 20). Connect a pair of silver electrodes with the two sides of its short-circuiting key.

(1) Pith a frog and expose the heart. Bring the electrodes in contact with apex and base of the beating ventricle, using bits of moist wool tied to the ends of the electrodes, as the connecting links between electrodes and ventricle, so as to avoid shifting of contacts. Open the key, and, if necessary, readjust the pressure apparatus so as to bring the mercury into field. See whether the mercury pulsates with the pulsation of the heart, and if it does, note the order and direction of its movements, and interpret their significance.[1]

(2) Arrest the heart by a Stannius' ligature. Excise it, and place it on a moist slip of wood. Bring the wool ends of the electrodes in contact with the apex and base of the ventricle. Observe the effect of a beat provoked by exciting the ventricle with a needle-touch (*a*) near the base, (*b*) near the apex.

(3) Separate the ventricle from the rest of the heart by a scissor cut through the auriculo-ventricular junction. Connect the electrodes with base and apex of ventricle, and note the effects on electrometer (*a*) of spontaneous beats, if such occur; (*b*) of beats provoked by a needle-touch, if the ventricle is not beating spontaneously.

[1] With the heart *in situ*, the movements of the mercury are not always easy to interpret; they may apparently be mono-, di-, tri-, or poly-phasic, according to various circumstances, and in consequence of auricle, or sinus, or bulb contributing to the main effect which is of the ventricle.

With a stanniused heart, the effect of a beat excited by a needle touch is normally diphasic, being $\begin{cases} 1. \textit{ base neg.} \\ 2. \textit{ apex neg.} \end{cases}$ when excitation is applied to the base, $\begin{cases} 1. \textit{ apex neg.} \\ 2. \textit{ base neg.} \end{cases}$ when excitation is applied to the apex. But here again the variation may be complicated by an effect from the bulbus arteriosus.

With a ventricle preparation, it will be noted, that the injured base becomes strongly negative, and that the main, or, it may be, the sole variation with each beat, whether spontaneous or provoked, is such as to indicate *apex neg.*

(51*a*) Electrical Variations of the Mammalian Heart. Dem. (Cat.)

Decapitate a cat or kitten. Rapidly expose the heart, and connect it with the electrometer, as in the previous exercise. Note the variations that accompany the spontaneous beat of the heart *in situ*.

Excise the heart, connect again with the electrometer, and note the variations (*a*) of the spontaneous beat, (*b*) of beats excited by needle-pricks applied to the base and to the apex.

(51*b*) Electrical Variations of the Heart of an Uninjured Mammal. Dem. (Cat, Dog, or Horse.)

Silver electrodes of the following shape, fixed by elastic garters to the limbs of a large dog or of a horse, serve as leads-off to a capillary electrometer. The mercury pulsates or does not pulsate, according as the electrodes are in "favourable" or in "unfavourable" combination. The two anterior extremities or the two posterior extremities form an unfavourable combination, the two leads-off being then on the same side of the equator. An anterior and a posterior extremity form a favourable combination, the two leads-off being then on opposite sides of the equator. Large animals are most suitable to this demonstration on account of their low pulse frequency.

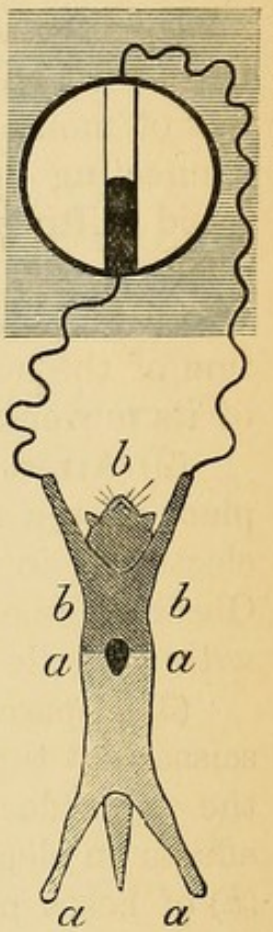


FIG. 56.

Quadruped (Cat). — Nearly transverse equator. The two anterior extremities form therefore an "unfavourable" combination.

(52) Electrical Variations of the Human Heart. Dem.

Flat electrodes, fixed by elastic garters, as in the previous experiment, may be used as leads-off. Or, in order to quickly contrast a favourable with an unfavourable combination, one electrode dips into a dish of salt solution, into which the fingers of the right or of the left hand are plunged, while the other electrode is held in the mouth or fixed to the forehead. "Favourable" combinations, *i.e.*, with the two electrodes on opposite sides of the equator, are :—

Head and Left Arm.	Left Arm and Right Arm.
Head and Left Leg.	Right Arm and Left Leg.
Head and Right Leg.	Right Arm and Right Leg.

"Unfavourable" combinations, *i.e.*, with the two electrodes on the same side of the equator, are :—

Head and Right Arm.	Left Arm and Right Leg.
Left Arm and Left Leg.	Left Leg and Right Leg.

Verify these several statements.

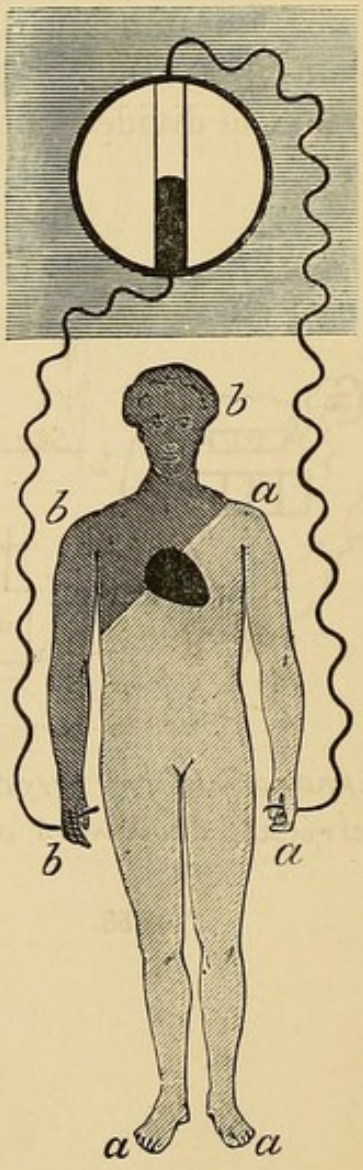


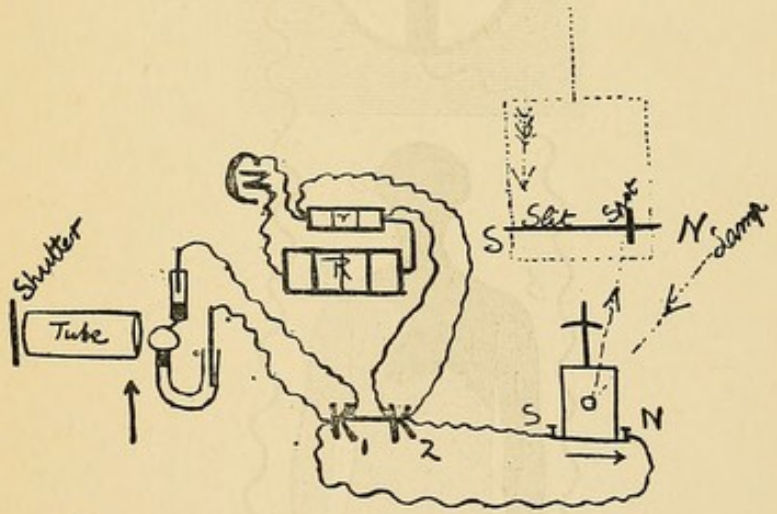
Fig. 57.

(53) Retinal Currents. Dem. (Frog.)

Make unpolarisable electrodes: one U shaped to receive the eyeball; the other straight, with a thread projecting from it, to be brought into contact with it from above. Connect the electrodes with the galvanometer (and compensator, if you wish to measure the E.M.F.).

Remove the eyeball of a pithed frog with as little injury as possible, using scissors to remove the surrounding skin and bone. Place it upon an unpolarisable electrode, with the cornea upwards, and bring the moist thread of the second electrode in contact with the cornea.

Close the galvanometer circuit, and observe a deflection indicating current in that circuit from cornea to fundus, *i.e.*, that the fundus is negative to the cornea. This is an ordinary injury current, owing to the divided optic nerve.



Plan of apparatus for recording photo-electrical currents of frog's eye

FIG 58.

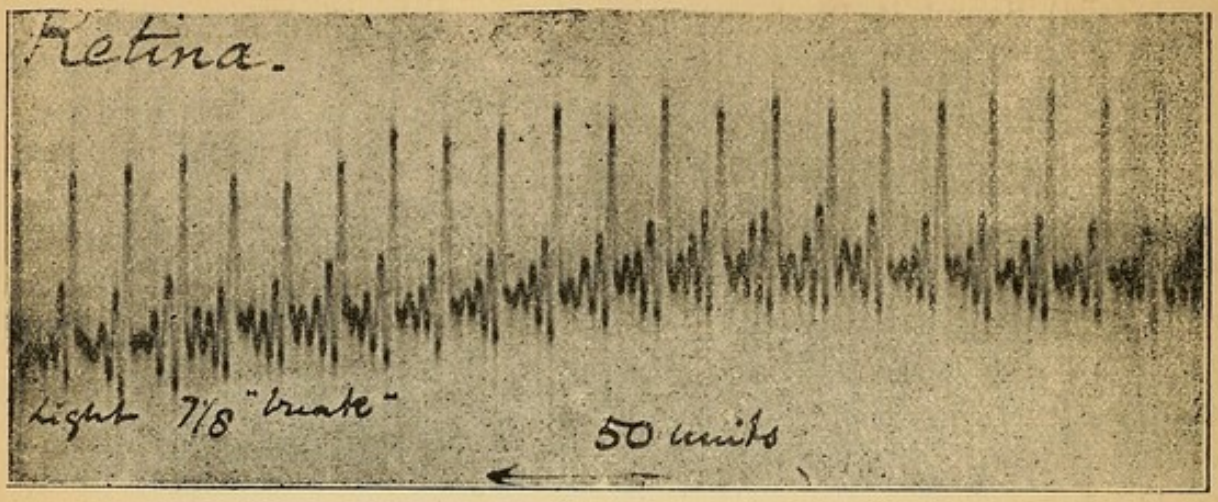


FIG. 59.

Having brought the spot to mid-scale, either by the compensator or by the controlling magnet, cover the electrodes with an opaque box, taking all care not to shift wires or shake the electrode stand.

Observe a deflection in the same direction as the current of injury, *i.e.*, a positive variation.

Remove the box, observe again a positive variation.

Leaving the box on or off for a few minutes, observe that the spot occupies a more positive position during illumination, a less positive position during obscurity.

N.B.—It will save ambiguity to adopt the following disposition :—

Cornea to south terminal of galvanometer.

Nerve to north terminal of galvanometer.

The current of injury will deflect the spot north, the variations at the beginning and end of illumination will be north, and the position of the spot will be further north during illumination than during obscurity.

(54) Sound is Produced during Muscular Contraction.

(*A*) *With Voluntary Contraction.*—Place the junction of the hand and wrist against the ear, and alternately clench and relax the fist; a low rumbling sound is heard throughout, which is much intensified during contraction. The less intense rumbling heard while the fist is relaxed is due to muscles by which the arm is raised to the ear. During the more intense rumbling heard while the fist is clenched, an overtone an octave above the rumbling sound may become audible.

With an ordinary stethoscope auscultate the flexor muscles in the forearm of a second person, who alternately clenches and relaxes the fist.

(*B*) *With Contraction by Faradisation.*—Arrange a battery, induction coil, and interrupter in one room, and connect the secondary coil by long wires with a Du Bois key in another room in which the vibrations of the interrupter are not audible. Use Page's reeds as the interrupter, and tetanise the muscles of the forearm at frequencies of 20, 50, and 100 per second, auscultating as before. A note of corresponding pitch will be plainly audible.

N.B.—You may compare this muscular note with that of the interrupter by means of a telephone introduced into the secondary circuit.

(*C*) *During Galvanotonus.*—Use a strong galvanic current to tetanise the muscles, applying the exciting electrode over the median nerve at the bend of the elbow, and auscultate as before. The pitch of the muscle-sound heard during galvanotonus is identical with that of the sound heard with voluntary contraction.

Note.—You may with reason doubt whether the pitch of the sounds heard in experiments *A* and *C* is due to muscular vibrations, and not simply a resonation tone of your own ear.

(55) Heat is produced during Muscular Contraction.

A.—With the Excised Muscles of a Frog. Dem.

Apparatus and preparations.—Low resistance galvanometer. Two thermo-electric needles. Cell, coil, keys, and two pairs ordinary electrodes. Commutator without cross wires, arranged to turn the secondary current to one or other pair of electrodes. See that the apparatus is in good working order before proceeding further. Note in what direction the spot of light moves when one or other of the two thermo-electric needles is touched.

Pith the frog, expose and isolate both sciatic nerves, and both gastrocnemii; insert each of the two needles longitudinally into each of the two muscles, and lay each of the two nerves across a pair of electrodes. Wait till the galvanometer spot is steady.

Now tetanise one of the muscles for, say one minute, and watch the spot for the next few minutes; it should move in a direction indicating greater heat of the contracting muscle.

After a short interval, turn the commutator so that the other nerve shall be excited when the key in the secondary circuit is raised. Repeat the excitation on that side and watch the spot, which should move in the opposite direction to its movement in the first experiment, indicating however, as before, greater heat of contracting muscle.

N.B.—The galvanometer, and all other terminals, should be protected from draughts of air by wadding, to guard against accidental effects of temperature changes at adventitious junctions of dissimilar metals.

B.—With the Normal Muscles of a Man. Dem.

Apparatus and preparations.—Air thermometer or thermograph. Dynamograph. Cell, coil, and keys.

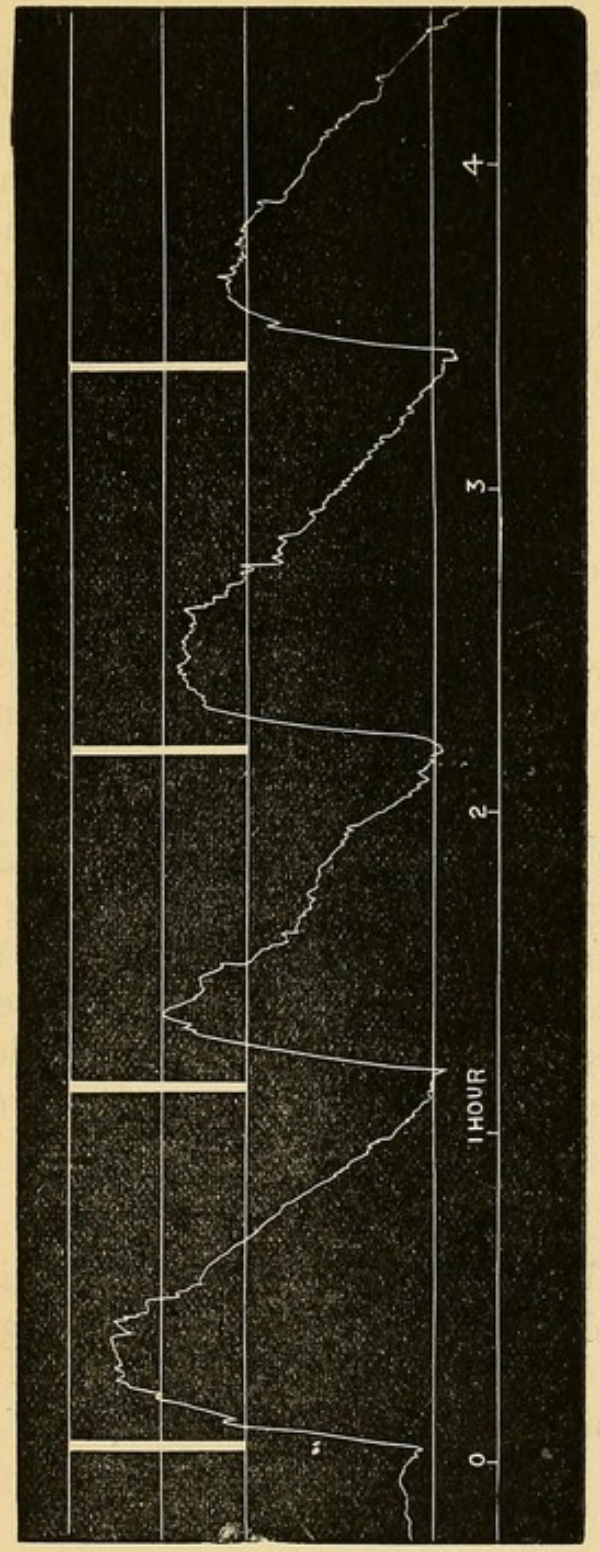


FIG. 60.

Four groups at intervals of about 1 hour, each of 30 grasps at 20 kilogrammes of the dynamograph. Surface temperature of the forearm recorded by an air-thermograph.

The essential part of the apparatus is a hollow metal capsule, to be strapped to the muscles of the forearm or other part, in air-communication through a flexible tube with an indicator. Two forms of indicator may be used:—

(1) For the purpose of demonstration, a small water-manometer in which the level of water can be watched directly, or projected on a screen, by placing the manometer in the magic lantern.

(2) For the purpose of recording the variations, a piston-recorder, the lever of which is adjusted above that of the dynamograph, on the same cylinder.

Whichever form of indicator be adopted, it should be graduated in tenths of a degree, by immersing the capsule in a vessel of cooling water by the side of a mercury thermometer.

When the apparatus is first applied to the skin it should be left *in situ*, with the side tap open until it is in temperature equilibrium. This is known by the lever or manometer remaining stationary with the side tap closed. This occurs with less delay, *i.e.*, in about say five min., if the capsule is warmed between the hands or kept in a pocket against the body before use.

While an observation is in progress, the side-tap is kept shut, the object being to record variations of temperature, and not absolute values. These variations should not exceed 1·5°, if they do, the indicator should be zeroed, by opening the tap.

Using the piston-recorder on the forearm, and the dynamograph, take a record of—

(1) The effect of, say 30 grasps of 20 kilos, of 2 sec. duration, with 2 sec. intervals.

(*a*) With normal circulation.

(*b*) With the circulation arrested by an elastic bandage applied tightly round the arm.

Notice the effect in (*a*), *i.e.*, that the temperature of the forearm is raised, and the much smaller effect in (*b*), *i.e.*, that the rise is in greatest part due to an augmented circulation of blood.

(2) Compare the effect of 30 with that of 15 similar grasps of 20 kilos at 2″—2″ rhythm.

(3) Compare the effect of 30 grasps of 20 kilos at 2″—2″ rhythm with that of 30 grasps of 10 kilos at the same rhythm.

(4) Compare the effect of 30 *voluntary* grasps of 10 kilos at 2″—2″ rhythm with that of 30 similar grasps provoked by faradisation of the muscles. Notice that the temperature effect is greater in the former case than in the latter.

(56) Tendon-Reflex Time. (Man.)

The subject of experiment is seated on a table, so that the leg hangs freely. An elastic bag, fixed by a strap round the middle of the thigh, serves as the explorer; the explorer is connected by tubing with a Marey's tympanum, the lever of which marks against the smoked cylinder (speed of revolution about 250 mm. per sec).

(*a*) Start the clock, and when the cylinder has reached its full speed, smartly tap the ligamentum patellæ with the back of a thin book or a round ruler. Stop the clock, and examine the line; if the ligament has been struck rightly, so that the muscles of the thigh have responded sharply, the line will present two elevations—(1) A small preliminary wavelet, which was caused by the mechanical jar of the blow on the ligament, and serves therefore to signal the moment of excitation; (2) a more prolonged elevation, which was caused by the contraction of the muscles of the thigh. Put a time-tracing of a 100 per sec. tuning-fork under the best of your tracings, and take as the time of response the interval between the middle of the wavelet and the beginning of the main elevation.

(*b*) This time should be compared with the lost time between excitation and contraction of the same muscles under the same conditions of observation, but with a single break induction shock as the stimulus. To this end put up an induction coil and electrodes, using one electrode as the indifferent electrode (ex. 35). Arrange the trigger-key of the clock in the primary circuit. Find, by preliminary trial, a point of application of the testing electrode, and a distance of coil such that the muscles of the thigh contract sharply when the trigger-key is opened. Having done this, start the clock, and when the cylinder is at full speed close the key. After the key has been struck over, stop the clock, reclose the key, and find the time between excitation and contraction in the usual way (ex. 28).

N.B.—The time in this experiment may appear to be excessively long for a latent period by direct excitation. The length is due in part to lost time in the transmitting apparatus, in part to the inertia of the large muscular mass explored.

The lost time in the transmitting apparatus can be determined as follows:—The explorer is arranged on a firm base under a lever, the point of which is adjusted against the cylinder under the point of the tympanum lever. With the cylinder at full speed, the lower lever is tapped; both levers record the movement, the tympanum lever beginning to move slightly after the explorer lever. The difference between the initial points of the two elevations gives the lost time in the transmission of movement from explorer to recorder. To measure this difference at all accurately, these initial points should be measured from "corresponding points," *i.e.*, pairs of points on the abscissæ of the two levers marked by tapping the explorer lever with the cylinder stationary.

(57) Tendon-Reflex Time. Dem. (Rabbit.)

A rabbit laid on its back in a shallow wooden trough, unrestrained by any ligature, will remain quiet for an indefinite time in an attitude that is convenient for the study of "tendon-reflex" and its comparison with other movements.

(*a*) Connect the leg of a rabbit so disposed by a fine thread to a light lever touching the smoked cylinder. Start the clock, and when the cylinder is at full speed tap the lig. patellæ with the edge of a paper-knife. A record is obtained, on which the tap and the response can be identified, and the interval of time between them measured.

(*b*) This time should be compared with the time of a true reflex action of the same muscles under the same conditions of observation. To this end it is sufficient to smartly tap the table or rabbit trough with a slip of wood, while the cylinder revolves past the recording lever. A rabbit in this state of slight hypnosis gives at each tap a slight reflex start without otherwise agitating itself. The excitatory tap and the muscular response are easily identified on the tracing, and the interval of time between them measured as before.

(*c*) (If desired, the lost time of direct excitation of the same muscles by a break induction shock may be taken, the electrical apparatus being arranged as in exp. 56 *b*).

(58) **Function of Nerve-roots.** (Müller's experiment.)

The spinal column of a decerebrated frog is exposed by a median incision and cleared of the muscles on each side of the spinous processes; the cord is then exposed by a pair of saw-cuts on each side, fine bone-forceps or stout scissors being used to complete the exposure of the nerve-roots.

The three posterior roots of the 7th, 8th, and 9th nerves, lying upon the corresponding anterior roots, are now to be isolated by a fine seeker. A posterior root is ligatured and divided as far as possible from the cord; electrical or mechanical excitation of its central end gives rise to general (reflex) movement. Another posterior root is ligatured and divided as near the cord as possible; excitation of its peripheral end produces no effect.

A similar pair of experiments is then made upon two of the underlying anterior roots. Excitation of a central end produces no effect; excitation of a peripheral end produces movement of the corresponding limb.

With a little care, the demonstration may be made as follows:—The three posterior roots are divided on one side (say the left); on the other side the posterior roots are carefully turned aside and the three anterior roots divided. On touching the skin of the left leg with a drop of acid, nothing happens; on touching the right leg with a drop of acid, general (reflex) movements are elicited, with the exception of that leg. The left leg is deprived of sensation, the right leg of motion. (Müller.)

(59) Overlap of Distribution of Spinal Nerves in Skin and in Muscles. Dem. (Sherrington.)

Exercise.—A frog, freshly killed; the brain and the upper part of the spinal cord have been destroyed 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). By raising the lips of the opening thus made each sciatic plexus is brought in view. This plexus consists of the seventh, eighth and ninth spinal nerves joining to form the sciatic trunk. 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.

With fine forceps pinch a toe of one foot. A movement is provoked; this is "reflex;" it 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.

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.

Take the preparation down and pith it now completely. Fix the lower end of each tibia with a pin to the cork of 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 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.

(60) Reflex Actions of the Brainless Frog. (Goltz.)

Expose the cranial cavity of a frog, by a scissor cut, with one blade in the mouth as far back as it will go, and the other blade over the top of the heart.

Remove the brain[1] (hemispheres and optic lobes) and observe that the frog placed at liberty assumes a normal attitude, springs when stimulated, resumes its normal attitude if turned over, avoids obstacles in springing.

Hang the frog up and touch the side of the skin with a glass rod dipped in strong acid; the frog raises the leg of the same side and wipes the irritated spot, and if that leg be held it repeats the manœuvre with the other leg. All these highly co-ordinated movements are reflex movements effected by the bulbo-spinal centres, and not involving sensation. Open the abdomen and draw out a loop of intestine. Expose the heart, and while its movements are under observation, strike or pinch the intestine; the heart is temporarily arrested (Goltz's "Klopfversuch"). If you divide the vagi or destroy the spinal medulla and repeat the stimulus, the arrest does not occur.

Or instead of the intestine you may employ strong stimulation of a limb by the sudden tightening of a string round it. The heart will stop, and the body of the frog will become inert and flaccid, and will not respond to cutaneous stimuli. The bulbo-spinal axis is in a state of "*shock*."

(61) Inhibitory Action of Superior upon Inferior Centres. (Frog.)

Prepare a couple of beakers, one containing one per cent. H_2SO_4, the other saline solution.

Prepare a frog as before, removing only the hemispheres. Hang the frog up by the lower maxilla, and when it has come to rest raise the vessel of acid until the longest toe of one limb just touches the surface. Count the number of seconds elapsing between the stimulus and the retractation of the limb. Place a crystal of sodium chloride upon the exposed optic lobes, and measure the stimulus-to-reaction interval as before. The interval is longer in the second than in the first instance, because the excitability of the spinal cord upon which the reaction depends has been interfered with—"inhibited"—by the excitation of the optic lobes (Setschenow).

[1] You will probably have removed the brain at the first scissor cut.

(62) Time of Reflex Action. (Frog.)

The stimulus-to-reaction interval obtained by the above-described acid method (Türck's) is not a true "time of reflex action"; the former is many seconds, the latter is only a small fraction of a second.

To measure it you must employ recording apparatus, myograph, and electric signal, measuring the interval of time between the application of a single induction shock to the skin and the response of the gastrocnemius muscle.

Use a decerebrated frog as above, adjust electrodes from a secondary coil to touch a toe, expose a gastrocnemius muscle, and connect its tendon with a bell-crank myograph, arrange a chronograph or a trigger-key in the primary circuit. You may measure the reflex on the same side as the stimulus, or on the opposite side (crossed reflex).

(63) Action of Strychnine on Spinal Cord.

(*a*) Proceed as in experiment on curare, using a 1 per 1,000 solution of strychnia. Note the gradual incidence of the effects, and write out a description of them.

(*b*) Take a tracing (on the slow axis) of a strychnia convulsion.

(*c*) Measure the time of reflex action, using the muscle of one limb as your indicator, and stimulating the central end of the other limb by a single shock through the trigger-key fixed to the clock (p. 37).

(*d*) Pass electrodes under the exposed, but undivided, nerves of a frog before and after the intoxication by strychnia. Note in each case the minimum distance at which direct and reflex effects are obtained.

(64) Summation.

Take the same occasion to recognise the effects of Summation of stimuli. Excite with single shocks, and with a series of shocks, the central end of one nerve before and after strychninisation, and note the minimum distance at which reflex effects are observed in the opposite limb. (No need to use recording apparatus.)

Demonstration.—Comparison of the reflex times of strychninised frog in limb excited opposite limb, and limb above; by means of the double myograph.

(65) Reflex Winking Time. (Man.)

Fix a fine thread to the eyelid and to the lever of a bell crank myograph.

From the secondary coil take a wire to a large electrode fixed to any convenient part of the body, and for the second electrode take a silver chloride silver wire covered with chamois leather and moistened with salt solution.

Use the cylinder on the quickest axis, or else use a shooting myograph, placing the trigger-key in the primary circuit.

Press the silver electrode against the conjunctiva of the lower lid; select by trial a suitable strength of shock; see that the thread from the upper eyelid is kept taut, and that the trigger-key is shut; let off the apparatus, and measure the interval between the moment when the conjunctiva is stimulated and the moment when the upper eyelid moves.

(66) Sensory Reaction-Timing. (Man.)

Two persons co-operate in such experiments—(*a*) the examiner, (*b*) the examinee.

The simple reaction-timer consists of a wooden lever resting across a closed india-rubber tube connected with a Marey's tympanum, marking against a smoked cylinder on the middle axis of the ordinary physiological clock. (Speed = something under 50 mm. per sec., but must be carefully measured before beginning). The butt-end of the lever is painted white to serve as a visual stimulus.

(*a*) *Touch.*—The examinee, blind-folded, rests a finger lightly on a lever of the "timer." The examiner taps the finger. The examinee responds by pressing the lever down as soon as he feels the tap. The instant of the tap, and that of the response, are thus marked on the revolving cylinder, and the interval between them gives the reaction time to a *tactile stimulus*, *e.g.*, with the speed given above, an interval of 10 mm. indicates a reaction time of 20 hundredths of a second.

(*b*) *Hearing.*—The examinee, blindfolded, holds the hand ready to press down the lever. The examiner strikes the lever so as to make a sharp sound, and signal it at the same instant. The examinee responds by pressing his lever down as soon as he hears the sound. The interval measured as before gives the reaction-time to an *auditory* stimulus. (The examinee

must be careful not to have his hand in contact with the lever when the sounding tap is made, as in this case there would be nothing to show whether the reaction is auditory or tactile.)

(*c*) *Sight.*—A screen through which the white end of the lever protrudes is placed so as to conceal the rest of the apparatus, and the examiner from the examinee, who watches the white end, with his hand ready to strike it as soon as he sees it move. Reaction-time determined as before.

Take an average of 10 observations in each of the three preceding cases.

(67) The Discrimination Time.

A timer with a double lever is now used; the end of one lever is painted white. Both levers rest across the same tube, so that the question and the answer signals are made by one tympanum.

(*a*) *Touch.*—The examinee places a finger of each hand on each lever, it being agreed that he is to react to a touch on one side, but not to a touch on the other. Sometimes one and sometimes the other finger is tapped by the examiner.

Take the averages of the responses made in succession without mistake.

(*b*) *Hearing.*—The signal is struck either with a bell or with a ruler. The examinee, blind-folded, has to answer only to one or other of these sounds. Take average as before.

(*c*) *Sight.*—With a screen arranged as in ex. 66 *c*, the subject has to signal when he sees one of the levers move, but not when he sees the other. Average as before.

The result in these three cases = the sensory reaction-time + the discrimination-time; the latter is therefore roughly known by subtracting the results of ex. 66 from those of ex. 67.

(68) The Volition or Choice-time.

Repeat the previous trio of observations with the double "timer," but with the understanding that the left hand is to be used to signal touch, sound, or sight connected with the left hand lever, and the right hand for the stimuli connected with the right hand lever. Take averages as before.

The result = sensory reaction-time + discrimination-time + volition-time; the latter is therefore roughly known by subtracting the results of 67 from those of 68.

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