The microscope and its application to clinical medicine / by Lionel Beale.

HIGHLEY'S LIBRARY

OF

A.D. 1667.

SCIENCE

AND ART

ANNOUNCEMENT.

Mr. S. HIGHLEY begs to announce that, under the title of

Highley's Library of Science and Art,

ecially
lleges,
e, Art,
lations
well as
, from
vill be
fusely

:—

illus-

trated by the Three Kingdoms of Nature. Numerous Illustrations.

SECTION II.—NATURAL HISTORY.

THE MICROSCOPE IN ITS SPECIAL APPLICATION TO VEGETABLE ANATOMY AND PHYSIOLOGY. By Dr. HERMANN SCHACHT. Translated by FREDERICK CURREY, Esq. Numerous Woodcuts. 5s. being the

BOTANICAL
Dr. B. PAU
published at

OF EDUCATIONAL MINERALOGY. With
Diagrams.

SECTION III.—MEDICAL SCIENCE.

THE MICROSCOPE, AND ITS APPLICATION TO CLINICAL MEDICINE. By LIONEL BEALE, M.B. Lond., Professor of Physisiology and General and Morbid Anatomy in King's Gollege, London. 250 Illustrations. *Now Published.*

CLINICAL HANDBOOK OF AUSCULTATION AND PERCUSSION: an Exposition, from First Principles, of the Method of Investigating Disease of the Respiratory and Circulating Organs. From the German of WEBER. By JOHN COCKLE, A.M., M.D., F.R.C.S. *Now Published.*

MANUAL OF ZOO-CHEMICAL ANALYSIS, QUALITATIVE AND QUANTITATIVE. By E. C. F. VON GORUP-BESANEZ, M.D., Professor of Chemistry at the University of Erlangen. Translated, with the co-operation of the Author, by J. W. SLATER. With numerous beautiful Illustrations of the Microscopical Characters of Animal Products, &c., selected from the Works of Robin and Verdeil, Funke, Donné and Fourcault, &c.

SECTION IV.—ART.

LECTURES ON THE ART-ANATOMY OF THE HUMAN FORM, delivered at the Department of Art, Marlborough House. By JOHN MARSHALL, F.R.C.S.E. With numerous original Illustrations.

SECTION V.—APPLIED SCIENCE.

A MANUAL OF PRACTICAL PHOTOGRAPHY, in its Special Application to Illustrated Literature. Containing the History, Theory, and Practice of Photographic Art—Optics—Construction of Apparatus, Laboratories, Manipulation, and Preparation of Photographic Chemicals—Processes on Metal, Glass, and Paper—Transferring to Metal, Wood, and Stone. With numerous Illustrations.

SAMUEL HIGHLEY, 32, FLEET STREET.

Glasgow University Library

Highley's Library of Science and Art.

SECTION V.—APPLIED SCIENCE.

PHOTOGRAPHY IN ITS APPLICATION TO SCIENCE.

I.—INTRODUCTION.

II.—PHOTOGRAPHY IN ITS APPLICATION TO MEDICAL SCIENCE.

PHYSIOGNOMY

OF

THE TYPES OF INSANITY.

A Series of Photographs from the Life.

WITH BRIEF MEDICAL NOTES.

BY DR. HUGH DIAMOND, F.S.A.

III.—PHOTOGRAPHY IN ITS APPLICATION TO SURGERY.

A STEREOSCOPIC PHOTOGRAPHIC ATLAS

OF

SURGICAL ANATOMY.

IV.—PHOTOGRAPHY IN ITS APPLICATION TO MICROSCOPY.

AN ATLAS

OF

MICROSCOPICAL PHOTOGRAPHS.

BY JOSEPH DELVES, ESQ.

V.—PHOTOGRAPHY IN ITS APPLICATION TO PALÆONTOLOGY.

PHOTOGRAPHS

OF

THE RARER FOSSILS.

MR. S. HIGHLEY'S SCIENTIFIC PUBLICATIONS.

Quarterly Journal
OF
MICROSCOPICAL SCIENCE.
INCLUDING THE
Transactions of the Microscopical Society of London;

EDITED BY

E. LANKESTER, M.D., F.R.S, F.L.S., &c.

AND

G. BUSK, F.R.C.S., F.L.S., &c.

Volume I. 8vo. Cloth, 17*s*.

Containing with Index, numerous Woodcuts, a Photographic and 15 Lithographic Plates.

ORIGINAL COMMUNICATIONS

From Dr. P. B. Ayres—T. E. Amyot, Esq.—G. Busk, Esq., F.R.S.—Dr. Golding Bird F.R.S.—Dr. L. Beale—J. Brightwell, Esq.—Dr. T. S. Cobbold—J. Delves, Esq.—P. H. Gosse, Esq.—J. Gorham, Esq.—Dr. J. E. Gray, F.R.S.—Professor Gregory—T. H. Huxley Esq., F.R.S.—Dr. T. S. Holland—S. Highley, jun., F.G.S.—Dr. Herapath—Arthur Henfrey Esq., F.R.S.—Dr. Inman—George Jackson, Esq.—J. Lister, Esq.—J. B. Mummery, Esq.—Dr. E. A. Parkes—Professor Quekett—G. Rainey, Esq.—Dr. P. Redfern—Professor Riddell—J. B. Simmonds, Esq.—G. Shadbolt, Esq.—Rev. H. Smith—S. J. A. Salter, Esq., M.B.—John Tyrrell, Esq.—Professor Williamson—Professor Wheatstone, F.R.S.—E. G. Wright, Esq

TRANSLATIONS

From the papers of Kolliker—Schacht—Siebold—Leydig—Herbst—Montague.

REVIEWS, NOTES AND MEMORANDA, &c.

CONTINUED IN PARTS, Illustrated with Four Lithographs, &c. 4*s*. each.

A MICROSCOPIC EXAMINATION
OF THE
Water Supplied to the Inhabitants of London and the Suburban Districts.

BY ARTHUR HILL HASSALL, M.B., F.L.S.,

8vo. Coloured Plates. 2/6.

A Treatise on the Germination, Developement, and Fructification
OF
THE HIGHER CRYPTOGAMIA.

BY DR. HOFMEISTER.

Translated, with the co-operation of the Author, by F. CURREY, Esq.

Text 8vo. Plates 4to. 1019 Engravings. 21/.

NOTICE.

This Work, so valuable to BOTANISTS and MICROSCOPISTS, will be published by Subscription; and as only a limited number will be printed, gentlemen are requested to forward their names to Mr. HIGHLEY as early as possible, as the Work, from its costly character, will not be proceeded with till the expense of production can be guaranteed by the requisite number of Subscribers having been obtained.

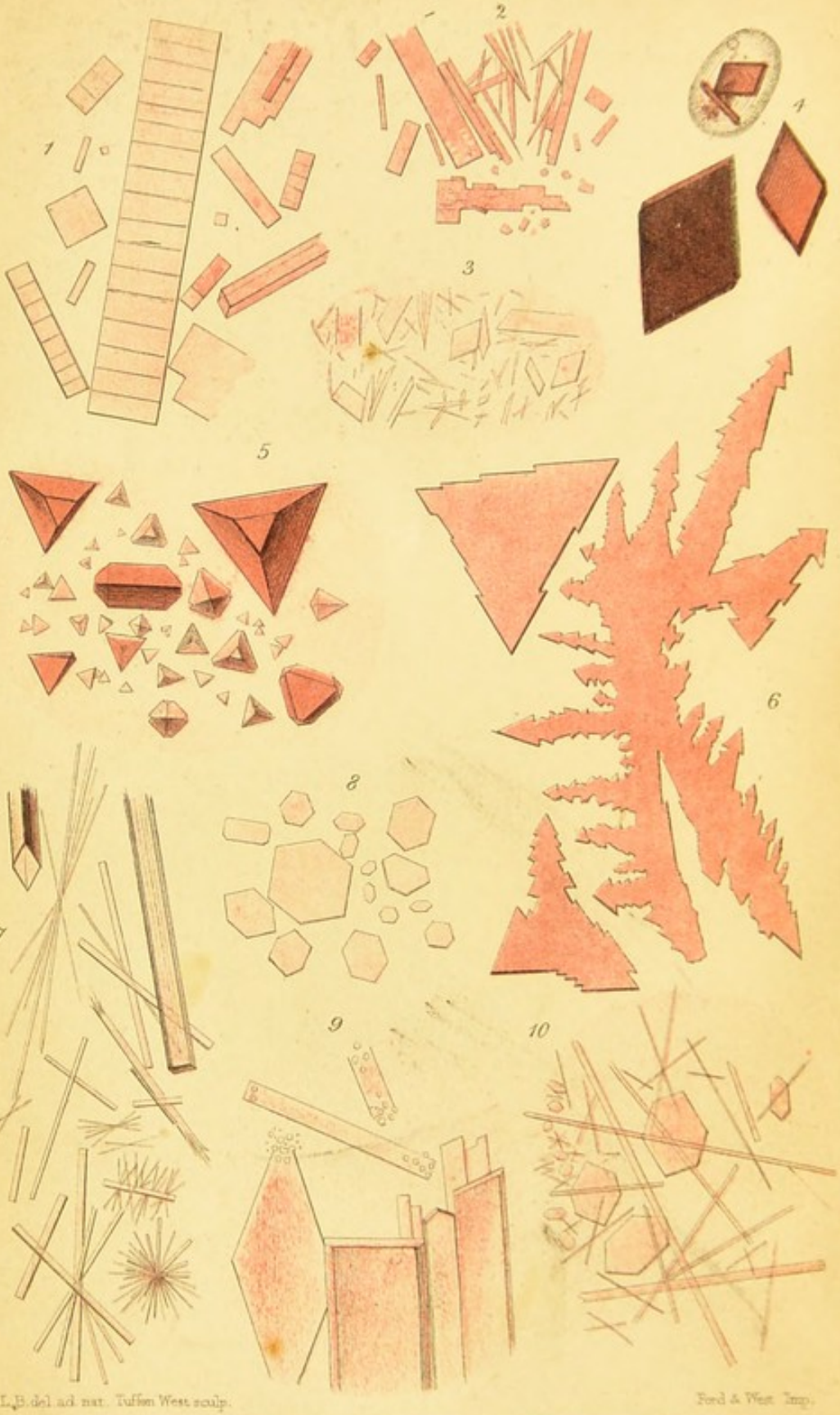
Bigbley's Library of Science and Art.

SECT. III.—MEDICAL SCIENCE.

THE MICROSCOPE,

AND

ITS APPLICATION TO CLINICAL MEDICINE.



BLOOD CRYSTALS.

THE MICROSCOPE,

AND

ITS APPLICATION

TO

CLINICAL MEDICINE.

BY

LIONEL BEALE, M.B. Lond.

PROFESSOR OF PHYSIOLOGY AND GENERAL AND MORBID ANATOMY IN KING'S COLLEGE, LONDON.

LONDON:
SAMUEL HIGHLEY, 32, FLEET STREET.
1854.

LONDON: PRINTED BY WILLIAM CLOWES AND SONS, STAMFORD STREET.

PREFACE.

A SHORT course of lectures, which was given in the spring of last year, forms the basis of the present volume. To the notes which had been prepared, and which the author had originally intended to print for the use of his pupils, much has since been added, and it is hoped that, in its present shape, the work may afford some assistance to practitioners and students in medicine who employ the microscope in clinical investigation, or in physiological and pathological inquiries.

In the present day this branch of investigation is being pursued by all who are most anxious to increase our knowledge of the structural alterations taking place in disease, and of adding to our information with reference to some of those important processes which interfere with the due performance of the healthy functions of different organs—investigations in which all may find ample employment, and may thus contribute to the advancement of the true interests of their profession, and aid in the elucidation of truths which may ultimately promote the interests and welfare of mankind in a degree not less than they will add to the advancement of science.

Except in cases referred to in the text, the woodcuts, which have been carefully executed by Mr. Davies, have been copied from drawings taken by the author from objects actually under observation.

The dimensions of all the drawings which are magnified with one of Powell's quarter of an inch object-glasses, can be readily ascertained by applying to them the scale figured in

page 36, which represents divisions 1-1000th of an inch apart, magnified with the same power as the objects delineated.

In preparing the work, the author has to acknowledge the assistance he has derived from the suggestions of many; and he is very desirous of taking advantage of this opportunity of acknowledging how much he owes to his kind friends Dr. Todd, Mr. Bowman, Dr. Johnson, and Dr. Acland, not only for the valuable assistance and information which he has on all occasions derived from their instruction and advice, but also for the warm encouragement they have constantly afforded him while he was a pupil and ever since.

To his friend, Mr. Conway Evans, the thanks of the author are also due for much kind assistance.

27, *Carey Street.*
4th April, 1854.

The author will feel greatly obliged to any gentleman who will communicate to him the results of any processes for the preservation of animal structures, which have been found to succeed better than those usually followed, or any hints upon points of manipulation, &c., connected with microscopical research.

CONTENTS.

CHAPTER I.

INTRODUCTION.

CHAPTER II.

THE MICROSCOPE.

Simple and Compound Microscopes.

Essential Points in the Construction of a Microscope.

Mechanical Portion of the Instrument.

CHAPTER III.

ACCESSORY APPARATUS.

CHAPTER IV.

APPARATUS NECESSARY FOR MICROSCOPICAL RESEARCH—CEMENTS.

CHAPTER V.

OF MAKING CELLS FOR PRESERVING PREPARATIONS—THE VARIOUS FORMS OF CELLS EMPLOYED.

CHAPTER VI.

EXAMINATION OF OBJECTS BY THE MICROSCOPE—REFLECTED LIGHT—TRANSMITTED LIGHT—LAMPS—SUBSTANCES EXAMINED IN DIFFERENT MEDIA—DISSECTION UNDER THE SURFACE OF FLUID.

CHAPTER VII.

OF PREPARING OBJECTS FOR MICROSCOPICAL EXAMINATION, AND OF PRESERVING THEM. MOUNTING OBJECTS IN A DRY STATE—IN FLUID. PRESERVATIVE SOLUTIONS. MOUNTING OBJECTS IN CANADA BALSAM. ARRANGING PREPARATIONS IN THE CABINET.

CHAPTER VIII.

OF INJECTING. INSTRUMENTS EMPLOYED IN INJECTING. COLOURING MATTERS. OF THE OPERATION OF INJECTING.

CHAPTER IX.

PREPARATION OF OBJECTS FOR EXAMINATION BY TRANSMITTED LIGHT—METHOD OF EXAMINATION—EXAMINATION OF SOFT TISSUES—KIDNEY—LIVER.

CHAPTER X.

EXAMINATION OF THE BRAIN AND NERVES—VESSELS—MUSCULAR FIBRE—LUNG—GLANDULAR STRUCTURES—EPITHELIUM—SKIN—MUCOUS MEMBRANE—VILLI—SEROUS MEMBRANES—ADIPOSE TISSUE—CORNEA—RETINA—CRYSTALLINE LENS.

CHAPTER XI.

MORBID GROWTHS—ENTOZOA—VEGETABLE PARASITIC STRUCTURES.

CHAPTER XII.

OF MAKING SECTIONS OF HARD TISSUES—INSTRUMENTS—GRINDING AND POLISHING THIN SECTIONS—EXAMINATION OF BONE, TEETH, NAILS, HAIR, &c.

CHAPTER XIII.

EXAMINATION OF SUBSTANCES WHICH FORM DEPOSITS FROM FLUIDS, AND OF THEIR PRESERVATION—APPARATUS EMPLOYED—EXAMINATION OF URINE, AND THE METHODS OF DETECTING URINARY DEPOSITS.

CHAPTER XIV.

URINARY DEPOSITS, AND THE METHOD OF PRESERVING THEM AS PERMANENT OBJECTS.

CHAPTER XV.

BLOOD—MILK—SEROUS FLUIDS—SPUTUM—VOMIT—SUBSTANCES PASSED BY THE BOWEL—DISCHARGES FROM THE UTERUS AND VAGINA—PUS.

CHAPTER XVI.

THE APPLICATION OF CHEMICAL ANALYSIS TO MICROSCOPICAL INVESTIGATION—METHOD OF APPLYING TESTS—EFFECTS OF REAGENTS UPON ANIMAL STRUCTURES.

Reagents.

Method of applying Tests to Substances intended for Microscopical Examination.

Effects of Reagents upon Animal Structures.

CHAPTER XVII.

OF OBTAINING CRYSTALLINE SUBSTANCES FROM THE FLUIDS AND TEXTURES OF THE ANIMAL BODY, AND OF THEIR MICROSCOPICAL EXAMINATION.

Examination of Crystalline Substances.

APPENDIX.

EXPLANATION OF THE PLATE.

All the objects, except figs. 6 *and* 10, *are magnified* 220 *diameters.*

1. Blood-crystals from the finger of a healthy man treated with a drop of water.
2. Another specimen of human blood-crystals.
3. Crystals obtained by diluting putrid blood with a drop of water.
4. Crystals found among some clots which had been effused into a large hydated cyst of the liver.* Two are seen to be situated within a large oil globule.
5. Guinea-pig's blood, crystallized, after the addition of a drop of water.
6. Very large crystals obtained fron Guinea-pig's blood,—magnified 40 diameters.
7. Crystals obtained from dog's blood after the addition of a drop of alcohol.
8. Crystals from squirrel's blood.
9. Crystals from cat's blood after adding alcohol.
10. Crystals from mouse's blood after the addition of a drop of ether.

* The liver from which these crystals were taken, was shown at the Pathological Society by Dr. Cotton, March 1854.

THE MICROSCOPE,

AND ITS APPLICATION TO CLINICAL MEDICINE.

CHAPTER I.

INTRODUCTION.

In the following pages it is my wish to direct attention to microscopical examination, more especially in its bearings upon clinical medicine, and the investigation of healthy and morbid structures of the human body; not to describe minutely the appearances observed upon placing a specimen under the microscope, but to consider the practical methods by which the investigation may be most successfully carried out, and the means at our disposal for *demonstrating* the minute structure of those tissues and deposits, with the characters of which it is important for the practitioner to be acquainted.

My aim will therefore be, to endeavour to show *how* the structures are to be submitted to examination, rather than to describe *what* are the appearances observed.

The question often occurs, whether an object is to be examined after having been immersed in some fluid medium, or simply enclosed between two pieces of glass, without any previous preparation, and then placed in the field of the microscope. Again, it will be inquired what liquid is best adapted to display the minute structure of the substance under examination, or in what manner can we hope to preserve most effectually the characters it possessed when recent, in order that it

may be compared with other specimens, at a subsequent period of time. The application of certain reagents may be necessary, either to dissolve matter which is accidentally present, or, perhaps, to render one element of the tissue more transparent, in order that the structure of another constituent may be better observed.

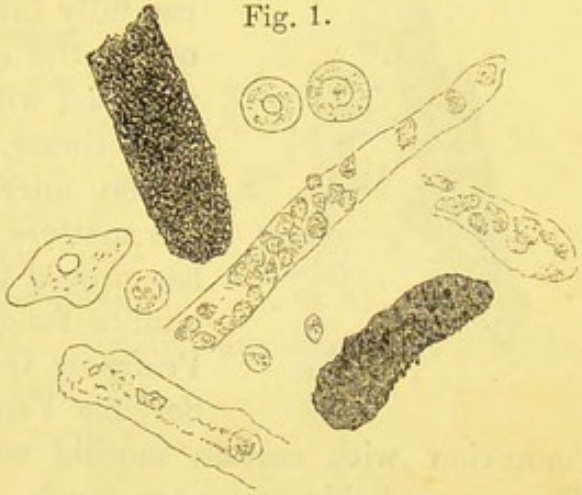
To the practitioner, a familiar acquaintance with the microscopical characters of the elementary tissues, and a knowledge of the way in which their structure is to be demonstrated, are essentially necessary. Without this, how can he expect to be able to investigate successfully the changes which take place in certain textures in disease; or to make out the complicated structure of morbid growths?—In the examination of urine, he may fail to discover the presence of most important deposits, in consequence of not being acquainted with some very simple mechanical contrivance adapted for collecting them; and, in many instances, he may lose the advantage of making a correct diagnosis in an obscure case of disease, in consequence of not being able to resort to microscopical examination.

1. The value of the Microscope as a means of Diagnosis.—It may be well here briefly to refer to a few of those instances in which the microscope is known to have afforded valuable aid to the practitioner in the diagnosis of disease.

Diseases of the Kidney.—There is no class of diseases in which its powers have been more advantageously brought to bear by the practical physician, than in those of the kidney. By a microscopical examination of the urine, we are frequently enabled to ascertain the nature of certain morbid changes which are going on in the kidney, and even to distinguish, during life, the existence of certain well-defined pathological conditions of that organ. The laborious researches of Dr. Johnson have shown us how, by the peculiar character of the casts (fig. 1) of the uriniferous tubes, which are found in the urine, we can ascertain whether the epithelium be desquamating, or, on the other hand, whether it presents no such tendency, but remains firmly attached to the basement membrane of the tube. If the epithelium be undergoing that peculiar change termed fatty degeneration, we shall often be able to

ascertain the fact, by examining a specimen of the deposit from the urine by the microscope. So again, by the presence of certain other deposits, and a knowledge of the symptoms usually associated with them, the physician is enabled to direct his attention, as the case may be, to the existence of local changes, affecting some part of the genito-urinary mucous membrane, or to more general disturbance, in the changes which take place in primary and secondary assimilation.

Fig. 1.



Fatty Degeneration.—Of late years, the remarkable changes which take place, and which have been described under the name of fatty degeneration, in some of the highly complex textures of the body, in consequence of which their properties become changed, and their functions impaired, or altogether destroyed, have been undergoing careful investigation by a vast number of highly talented investigators.

Fig. 2.

The recent discovery of a state of fatty degeneration affecting the arteries of the brain (fig. 2), in the majority of cases of apoplexy, by which the strength of their coats becomes deteriorated, and their elasticity entirely destroyed, would tend to lead us to infer, that this disease is dependent rather upon complicated changes affecting nutrition, than upon the presence of a condition of plethora or hyperæmia, as was formerly supposed, and acted upon.

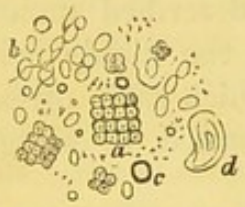
The connexion between fatty degeneration of the margin of

the cornea (arcus senilis), and similar changes taking place in the muscular tissue (fig. 3), of the heart (a subject which has been carefully investigated by Mr. Canton), or in the cerebral vessels, must be regarded with great interest by every practitioner.

Fig. 3.

The microscopical examination of the matters vomited in certain cases, has proved to us that the presence of minute fungi, originally discovered by Professor Goodsir, and named by him Sarcinæ Ventriculi (fig. 4), occurs in connexion with certain morbid conditions of the stomach. These remarkable cases are much more frequently met with than was formerly supposed, and form an exceedingly interesting class of diseases.

Fig. 4.



Tumours and Morbid Growths.—The microscope has many times afforded important aid in the diagnosis of tumours, although it has certainly failed in many instances; which circumstance has been brought forward by some, as an argument against its employment altogether. After careful microscopical examination, the best observers have failed in deciding as to the nature of a particular tumour submitted to examination; and they have been unable to pronounce as to its malignant or non-malignant character.

On the other hand, not unfrequently this question has been positively and correctly answered in the affirmative or negative, and therefore it would surely not be right altogether to discard the use of an instrument which, although eminently useful in many instances, is not infallible; for it would appear to be the opinion of some, that the use of the microscope ought to be altogether abandoned in the diagnosis of tumours. We shall have to return to this important question at a future time.

For the discovery of Imposition, the microscope is invaluable, as it almost necessarily follows that, in consequence of the frequency with which urine is subjected to minute investigation, patients often resort to various expedients to deceive the practitioner. Perhaps flour, starch (fig. 5), sand, and milk, are more frequently employed for this purpose than any other substances. The microscope will obviously enable any one to detect the first three. If milk be added to urine, the mixture may very readily be mistaken for a specimen of the so-called chylous urine. Although a considerable quantity of fatty matter is present, in either case this fatty matter exists in a very different state. In milk, we find the oil-globules (fig. 6) so characteristic of this fluid, while, in true chylous urine not a single oil *globule* can be found, although the specimen may contain a large quantity of fatty matter in a molecular state (fig. 7).

Fig. 5.

Fig. 6.

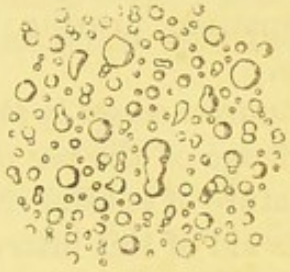


Fig. 7.

Larvæ of the Blow-fly in Urine.—A specimen of urine containing several bodies of about half an inch in length, and of a rounded form, was once sent to Dr. Todd for examination. The bodies in question looked not unlike the larvæ of some large fly, but, as it was confidently affirmed that they were passed by the urethra of a gentleman, the accuracy of this view of their nature was doubtful.

Upon placing a portion of one of them under the microscope,

tracheæ (fig. 8)—(the air-vessels characteristic of the class of insects) were observed in considerable numbers; and this circumstance alone enabled me to say positively that they were not entozoa, and that they could not have been passed in the manner stated. They were afterwards proved to be the larvæ of a fly.

Fig. 8.

The claws of echinococci and portions of hydatid cysts have on several occasions been discovered in the urine, sputa, &c., upon submitting portions of these fluids to microscopical examination, proving beyond a doubt the existence of hydatids.

Substances passed by the Bowels.—If the practitioner have a good knowledge of the use of the microscope, he can often ascertain the nature of substances passed from the alimentary canal; and, by the aid of this instrument, he can often at once decide as to the nature and origin of substances, which, to the unaided eye, only present most doubtful characters. Considerable perplexity has arisen from the presence of bodies in the stools of patients, which afterwards proved to be portions of almonds, gooseberry skins, portions of potato, the testa of the tamarind, husks of wheat, &c.: not many years ago the uredo of wheat was mistaken for, and described as, a peculiar fungus, to which it was supposed the phenomena observed in cases of cholera were due.

Portions of vessels which, unlike the other constituents of the food, have resisted the process of digestion, have been met with in the fæces, and mistaken for small intestinal worms, which they much resemble when examined by the unaided eye. Upon being subjected to microscopical examination their true nature was readily discovered.

In Medico-legal Inquiries the microscope has often afforded valuable aid. The distinction between blood spots and red stains produced by fluids resembling blood in colour,—between

human hair and that of animals,—and the detection of spermatozoa in cases of rape, need only be adduced as examples of the importance of the microscope in such investigations.

For detecting Impurities in Food and Drugs the microscope has afforded important aid, and there are several other purposes to which it may be applied, some of which will come under consideration in a subsequent chapter.

2. **Importance of a knowledge of the methods of Preserving Microscopical Specimens.**—The preservation of certain substances of rare occurrence is often a matter of great moment to the scientific practitioner, either for the purpose of comparison with other specimens, or for obtaining the opinion of different observers as to their nature. This desirable object, it need scarcely be said, cannot be effected without a certain amount of mechanical manipulation, and an acquaintance with the properties of preservative fluids.

3. **Arrangement of the subject.**—The different mechanical operations, the different methods of examining various tissues, their behaviour under the influence of chemical reagents, and other practical points bearing upon microscopical examination, will be especially dwelt upon in the following pages.

A minute description of the instrument, and the various accessory apparatus, with directions for their employment in this branch of investigation, naturally forms the introduction to a work on the use of the microscope; but, as this book is to be looked upon as strictly practical, this part of the subject will be dismissed as soon as possible, and I shall only attempt to bring under notice those instruments and pieces of apparatus that are most essential to the purposes of investigation. So many beautiful contrivances have lately been devised by various observers, that to enter into a short description of each would occupy all the pages which I have proposed to devote to the whole subject. For an excellent description of the different microscopes, and of several beautiful and eminently useful pieces of apparatus, I cannot do better than refer the reader to the treatise of Professor Quekett. After this subject has been briefly noticed, I shall direct attention to the method of making

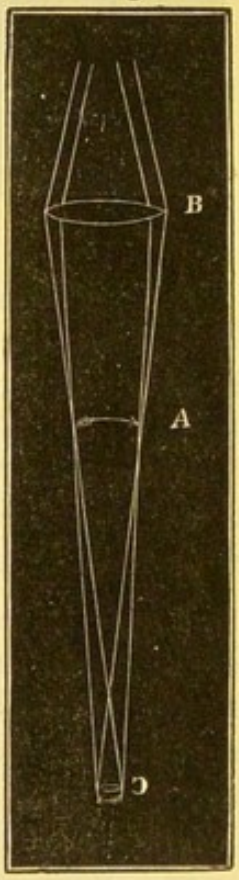
cells to contain microscopical preparations; and then the various processes by which thin sections of different tissues can be obtained, and the mode of injecting will be considered. Afterwards the mode of demonstrating the structure of those tumours and morbid growths, which the physician is most frequently called upon to submit to examination, will be discussed. Next, the method of collecting the deposits which subside from different fluids, such as serum, urine, &c., will be brought under notice; and, in the last chapter, will be considered the great importance of a knowledge of chemistry to the microscopical observer, and the most convenient manner of applying chemical tests to minute quantities of matter, together with the principles upon which the microscopical and chemical examination of different substances are conducted.

CHAPTER II.

THE MICROSCOPE.

I SHALL not enter into a description of the different microscopes made by various makers, but shall content myself with directing attention to the most important points to be looked for in a good practical instrument. In discussing this question, the price of the microscope forms a most important part of the consideration.

Fig. 9.

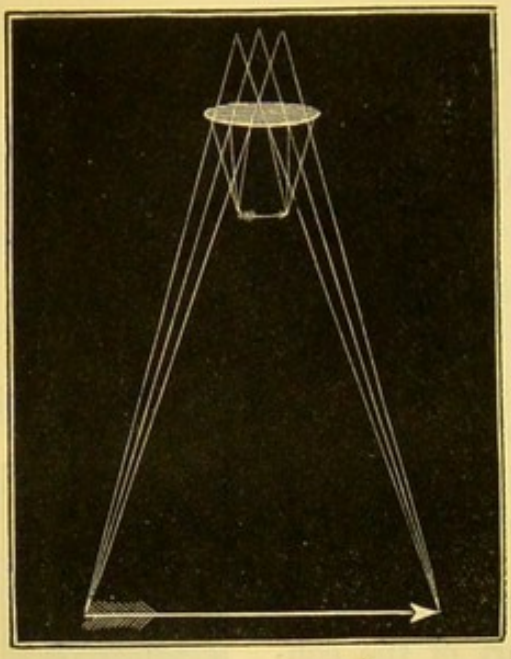


Microscopes may be divided into two classes: the instruments usually termed students' microscopes, below the value of ten pounds, including object-glasses, &c.; and the more elaborate instruments, which cost more than this sum, and, when complete, even attain the price of fifty or sixty pounds.

The microscope in use in the present day for delicate observation is the "compound" microscope (fig. 9), so called because the magnified image produced by the object-glass, C, and brought to a focus at A, is again magnified by a second glass, B, before it enters the eye; while, in the simple microscope, (fig. 10), the object is only magnified by one lens. Figs. 9 and 10 illustrate the construction of the "compound" and "simple" microscope.

4. Students' Microscopes.—These microscopes are made by most of the London makers, and vary in price from five to ten pounds. Of these, the instruments of Ladd and Pillischer, I think, deserve special recommendation. The cheapest instrument, however, that I have seen, and one which is very efficient and well adapted for students, has been made by Mr. Salmon (fig. 11). This microscope has two powers, a small bull's-eye condenser and diaphragm. Packed in an upright case it costs five guineas, or, fitted with a coarse as well as with a fine adjustment screw, six guineas.

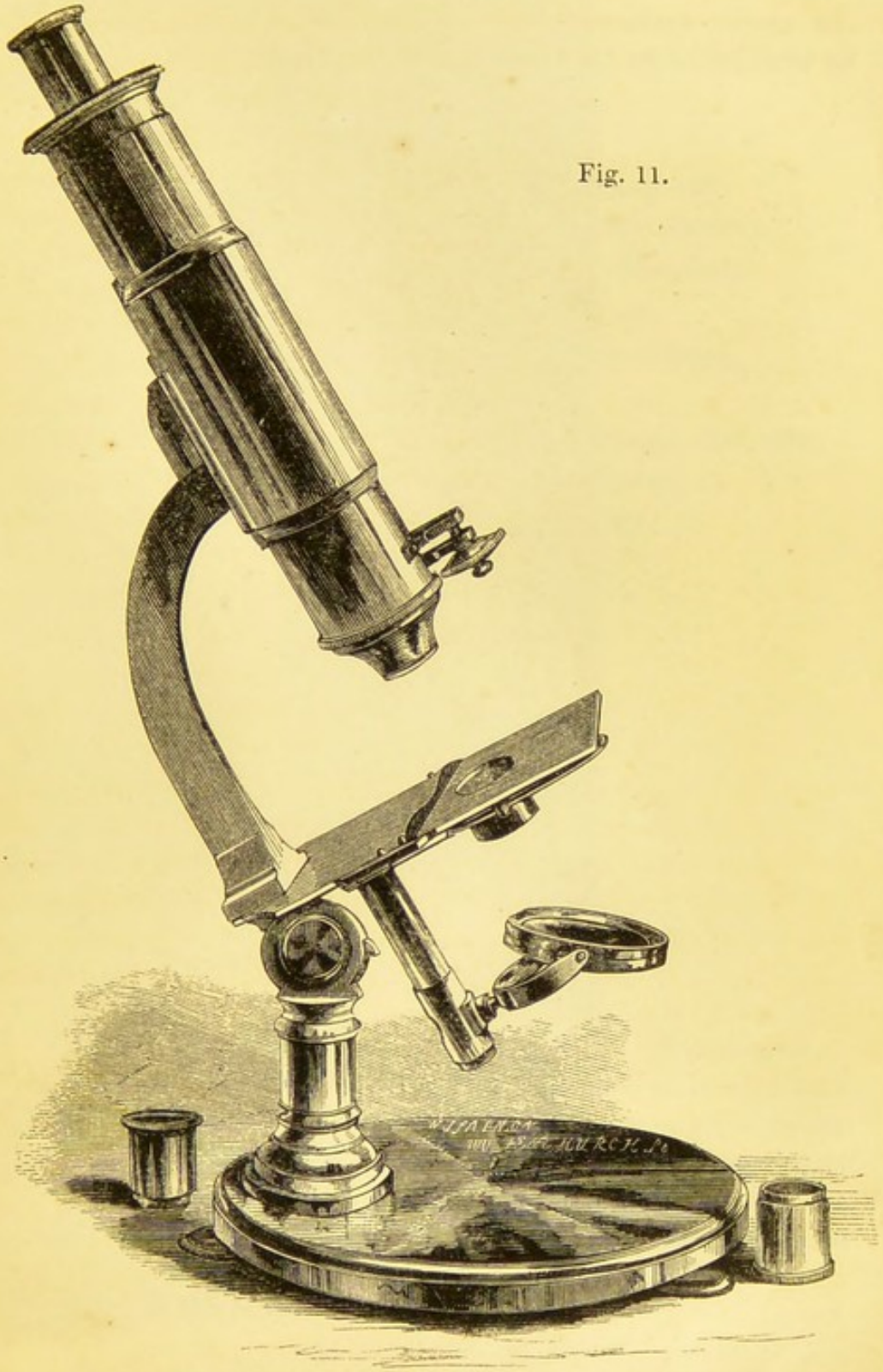
Fig. 10.



Mr. Highley has also brought out an excellent instrument, provided with two powers, for the price of 6*l.* 10*s.* This microscope is mounted upon a double pillar with tripod stand (fig. 12). The foreign microscopes of Nachet, Oberhauser, and others, are many of them very useful instruments for general use, and are very portable. These may be purchased at from five to seven or eight pounds.

5. Large Microscopes.—The larger microscopes are much more elaborate and beautiful instruments, but I do not think that the practical advantages afforded to the student are sufficient to make up for the great increase of price. For those observers who wish for an instrument as perfect in all points as can be obtained in the present day, those made by Powell and Lealand, Ross, and Smith and Beck, may be especially recommended. For an accurate description of these beautiful instruments I must refer to Mr. Quekett's Treatise on the Microscope.

Fig. 11.



STUDENTS' MICROSCOPE, MADE BY MR. SALMON.

6. Microscope-makers. — The microscope-makers whose addresses I have been able to find, are arranged alphabetically as follows:—

English Makers.

Bryson	Princes Street, Edinburgh.
Dancer	43, Cross Street, Manchester.
Ladd	29, Penton Place, Walworth.
Pillischer	88, New Bond Street.
Powell and Lealand .	Seymour Place, New Road.
Pritchard	Fleet Street.
Ross	Featherstone Buildings, Holborn.
Salmon	100, Fenchurch Street.
Smith and Beck . .	Coleman Street, City.

Foreign Makers.

Amici	Modena.
Brunner	Paris.
Chevalier	Paris.
Frauenhofer, Merz .	Munich.
Nachet	16, Rue Serpent, Paris.
Oberhäuser . . .	Paris.
Pistor	Berlin.
Ploesl	Vienna.
Schiek	Berlin.

Essential points in the Construction of a Microscope.— It will be convenient to refer, in the first place, to the mechanical, and secondly, to the optical, portion of the instrument. Under the former head, the stand, the stage, and the apparatus for adjusting or altering the focus will be considered; and in the latter division the mirror, eye-pieces, and object-glasses will be brought under notice.

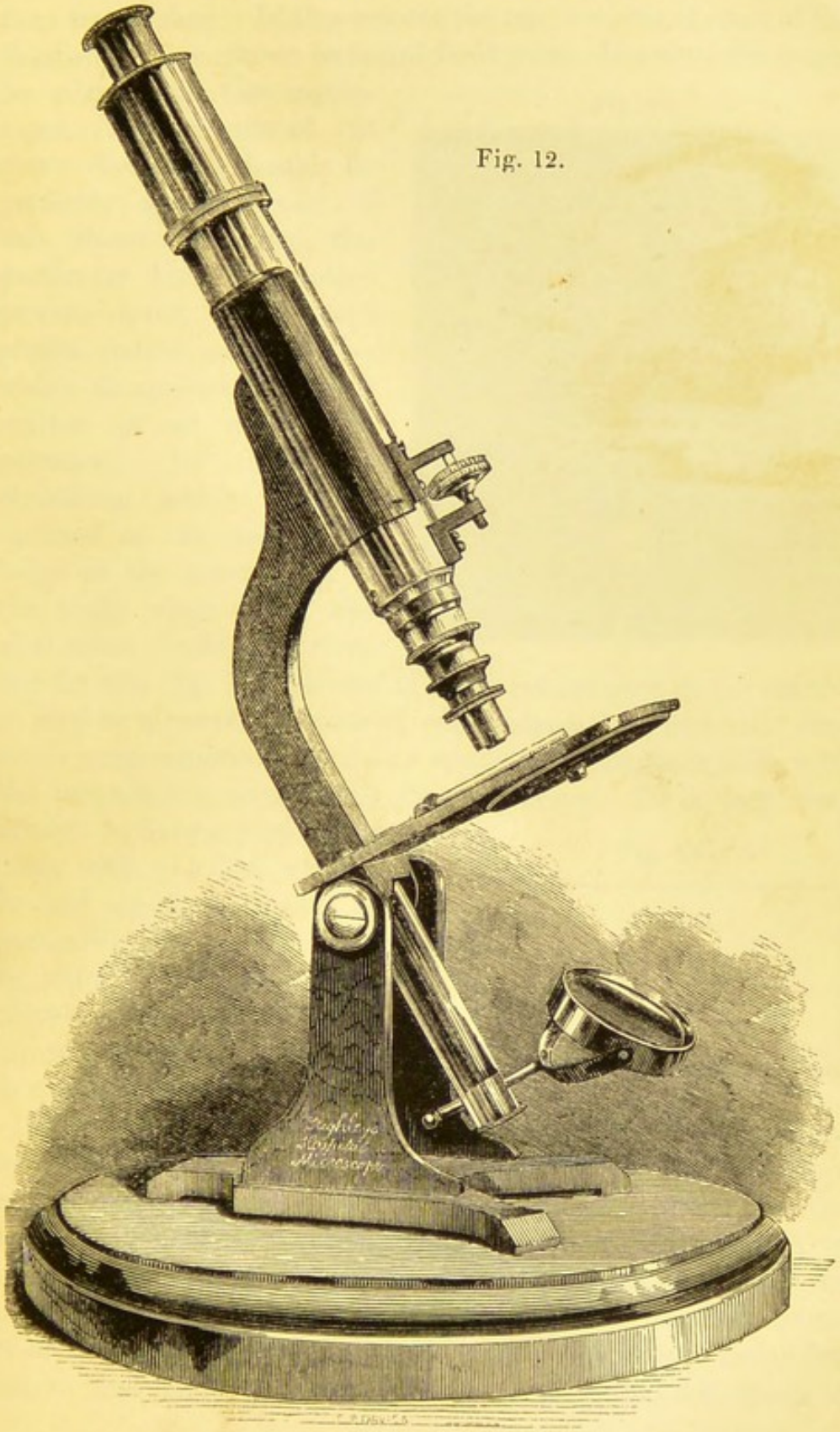
MECHANICAL PORTION OF THE INSTRUMENT.

7. Stand.—The instrument should stand firmly, whether the body be inclined or arranged in a vertical position; and not the slightest lateral movement should be communicated to the body of the microscope when the focus is altered by turning the adjustment screws. The base or foot should be sufficiently heavy to give steadiness, and should either be made of a tripod form, or placed upon three small feet (a simple means of insuring steadiness not attended to in many of the foreign instruments).

8. Arrangement for inclining the instrument.—The body ought to be provided with an arrangement by which it may be inclined or placed in a horizontal position (figs. 11 and 12), which is required when drawings are made with the camera, or when objects are measured by the aid of this instrument. Another advantage which is gained by the microscope being provided with this moveable joint is, that the muscles of the neck do not become so tired when the body is inclined, as when the head has to be bent over an instrument standing upright for several hours at a time. The larger the microscope may be, the more necessary does this joint become for the comfort of the observer; and as it in no way impairs the steadiness of the instrument, and only adds a few shillings to the expense, I recommend every one, in the choice of a microscope, to select an instrument which may be used in a vertical, inclined, or horizontal position. Many of the foreign instruments are deficient in this respect.

9. Stage.—The stage (fig. 13) should be sufficiently large to admit either edge of a glass-slide, two inches in diameter, to be brought under the object-glass. The stage of the microscopes of Nachet, Oberhäuser, and others, are too small, and inconvenience is often felt in consequence of it being impossible to subject every part of a surface little more than an inch in diameter to examination, without removing the slide, and turning its opposite edge towards the pillar of the instrument. The distance from the centre of the object-glass to the upright pillar (*a* to *b*, fig. 13), should therefore not be less

Fig. 12.



HIGHLEY'S HOSPITAL MICROSCOPE. FROM A PHOTOGRAPH.

than two inches. In this respect the microscopes of most of the English makers cannot be found fault with. If a moveable stage be adapted to the instrument, the movement in every direction should be perfectly smooth; and if this object be gained, the particular kind of motive power,—lever, rack, and pinion, endless screw, &c.,—which is applied, is not a matter of so much importance. In those instruments which can be inclined at an angle, the ledge at the lower part of the stage upon which the slide rests, should reach from side to side (fig. 13), instead of being interrupted in the centre, as may be observed in some of our instruments, in which case much inconvenience is found to result from the glass slide, with the preparation, occasionally slipping down. It is very convenient to have a piece of plate-glass, with a ledge, which may be laid upon the brass stage, particularly when objects are to be subjected to the action of chemical reagents which would injure the brass-work if brought in contact with it (fig. 14).

Fig. 13.

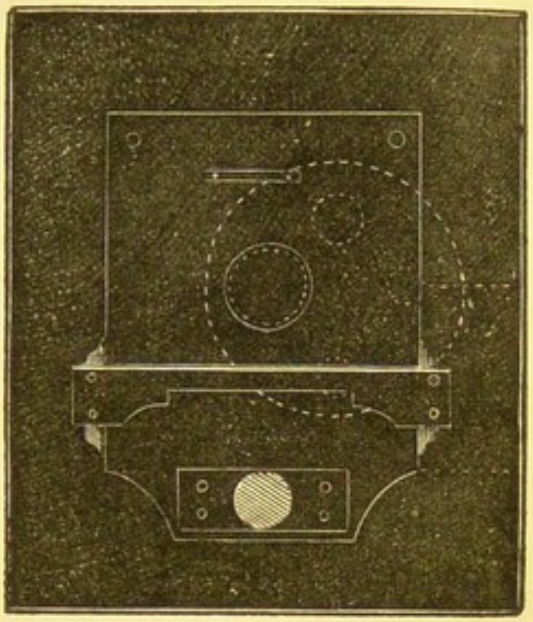
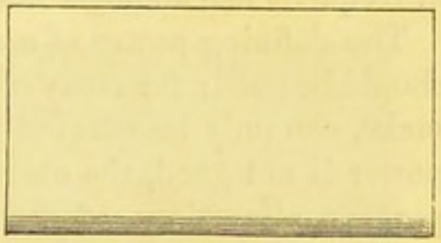


Fig. 14.



10. Arrangement for altering the Focus.—The arrangement by which the focus is altered must be of the best workmanship, as it is very important that this should be effected without the slightest lateral or irregular motion being communicated to the portion of the instrument which bears the glasses. In microscopes of inferior workmanship this will often be found to occur, in consequence of the thread of the screws being too coarse and roughly made. The alteration of the focus is

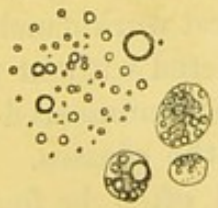
usually effected in a more satisfactory manner, when that part of the tube which bears the object-glass of the instrument is raised or lowered (fig. 12), than when the stage of the microscope, and with it the object, is moved up or down by means of a screw, an arrangement met with in some continental microscopes. Usually, two adjustments are provided, one coarse, for low powers; and the other fine, for higher powers. The cheaper glasses are only supplied with one movement, in consequence of which a certain amount of difficulty is experienced in focussing with the higher powers, as the thread of the screw is often made too coarse. The chain movement of Mr. Ladd supersedes the necessity of a fine adjustment. In the instrument made by Mr. Salmon, the tube is provided with a fine adjustment; but when low powers are used the focus is altered by sliding the tube up and down with the hand, as in Nachet's and Oberhaüser's microscopes.

OPTICAL PORTION OF THE INSTRUMENT.

11. The Object-glass.—I shall not attempt to discuss the causes of the imperfection of object-glasses, or the means by which such imperfection is prevented in the best glasses, further than may be necessary to explain one or two terms in frequent use amongst observers.

The defining power of a microscope used by the practitioner should be good; for many observations which he is called upon to make, can only be effected with a good glass. If the defining power is not good, the observer is liable to overlook many important alterations of structure, such, for instance, as fatty degeneration of certain tissues, in which very minute oil-globules are deposited in the texture (fig. 15). In an inferior instrument it would often happen that the precise state of the tissue could not be ascertained with certainty, in consequence of the defined circular outline of each minute individual oil-globule not being rendered sharp and clear.

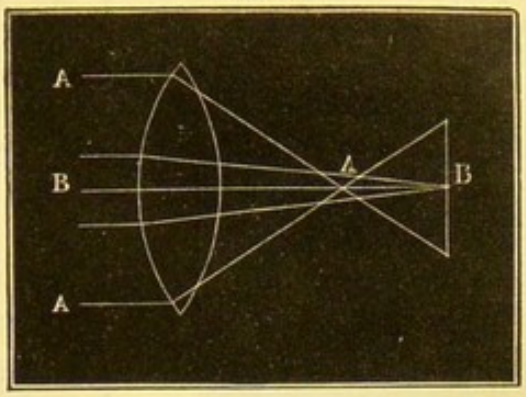
Fig. 15.



12. Spherical Aberration.—By "spherical aberration" is un-

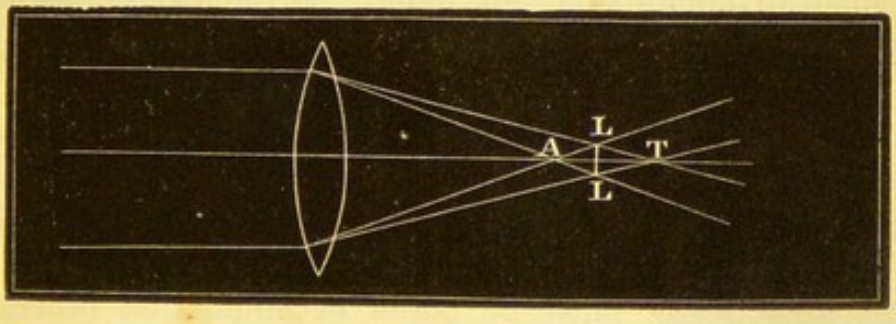
derstood that every part of an image is not equally distinct, or in focus at the same time; and this arises from the different points of focus to which the rays of light passing through the peripheral portion, and those which pass through the more central part, of an uncorrected lens, are collected. The peripheral rays A, being refracted in a greater degree than those which pass through towards the centre B, are brought to a focus much nearer the lens than the latter. Hence only the central or peripheral part of the field of an uncorrected lens can be in focus at one time (fig. 16).

Fig. 16.



13. Chromatic Aberration.—"Chromatic aberration" depends upon the unequal refrangibility of the different colours of the spectrum, the violet and blue rays A being brought to a focus much nearer to the lens than the red rays T; and hence if we examine objects with an uncorrected lens we find them fringed with colour, which varies as we alter the focus (fig. 17).

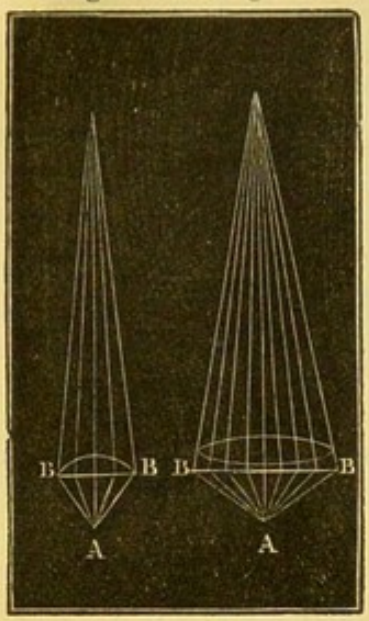
Fig. 17.



14. Angle of Aperture.—The excellency of definition of the modern object-glasses depends in great measure upon the increased "angle of aperture," a term which has been much used of late by microscopists. The angle of aperture is that angle which the most extreme rays that are capable of being transmitted through the object-glass, make with the point of

focus. Thus in figs. 18 and 19, B, A, B, is the angle of aperture.

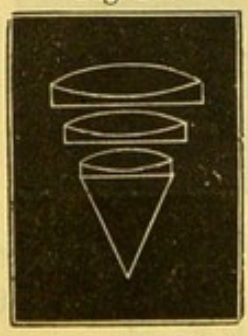
Fig. 18. Fig. 19.



Upon comparing the two figures, both of which represent glasses of similar magnifying power, the angle of aperture is seen to be much greater in fig. 19 than in fig. 18, which represents an uncorrected lens. Consequently, a much larger quantity of light is transmitted by the former than by the latter, when any given point of an object is subjected to examination. In order to see an object at all distinctly with an uncorrected lens, it is necessary to diminish the aperture so much, as to interfere with the transmission of the amount of light required.

Upon subjecting a delicate object to examination with inferior glasses, it may appear confused and indistinct, some points being in focus, and others out of focus, or it may be surrounded by a coloured ring, or the lines may be slightly fringed with colour,—appearances which result from the glasses not being corrected, or only partially corrected for "spherical and chromatic aberration," or from the "angle of aperture" being too small to admit sufficient light. As the corrections necessary to overcome these imperfections involve a great amount of labour and very skilful workmanship, the price of a good achromatic English glass of high power becomes considerable (fig. 20). Many of the foreign object-glasses, however, possess good defining power; but it must be borne in mind, that others are equally bad, although the price may be the same in either case. Foreign glasses are now adapted to many English microscopes; and before purchasing one of these instruments it is well to subject some well-known object to examination. The glasses of the best English makers are almost perfect in this respect.

Fig. 20.



15. **Over-corrected.**—The best object-glasses are what is termed "over-corrected," by which is meant that the dispersive power of the different lenses composing the object-glass is so arranged that the blue rays shall be brought to a focus more distant than the red rays, which it will be remembered is the reverse of what occurs in an ordinary lens (fig. 17).

The object of this over-correction is to reduce the coloured rays to a state favourable for being transmitted colourless to the eye of the observer, by the influence of the eye-piece, presently to be described.

16. **Object-glasses necessary.**—The large microscopes are generally furnished with several object-glasses, the lowest power being a two or three inch, and the highest an eighth or twelfth. The student will only require two or three powers—the most useful being an inch, or a two inch, and a quarter. With these he will be able to demonstrate almost everything he is required to observe.

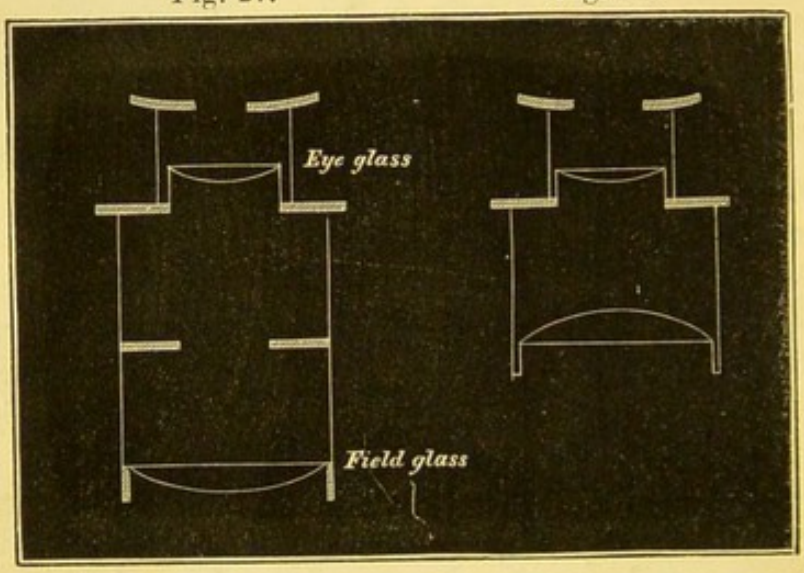
17. **Method of Ascertaining the Magnifying Power.** — The magnifying power of a glass cannot, however, be ascertained by measuring the focal distance: it becomes, therefore, important that the observer should be able to ascertain the magnifying power of the different combinations with which his instrument is provided. The microscope being arranged as for observation, a stage micrometer, divided, say into hundredths, is placed in the field. Two or three inches divided into tenths are then accurately measured out upon a piece of white paper or card-board, the lines being made distinct by drawing them with ink. This measure is next placed on a plane, parallel with the stage of the microscope. Upon examining the lines of the magnified micrometer with one eye, and looking at the measure with the other, we can read off accurately the length of the interval which the magnified image covers. Suppose, for instance, that the magnified 1-100th of an inch covers a space of two inches, the magnifying power of the glass will be two hundred, or two hundred *linear*, or two hundred diameters; for the magnified image of 1-100th of an inch is found to cover a space of two inches. The camera may also be employed for ascertaining the magnifying power of a glass, the same pre-

cautions being observed as recommended in section 37. If, however, the magnifying power be expressed in its superficial extent, it will be as $200 \times 200 = 40{,}000$ times. This mode of expression, however, is only used in the most popular treatises.

18. Eye-pieces.—The eye-piece consists of the eye-glass and the field-glass (fig. 21), the former being the farthest from, and the latter the nearest to, the object-glass.

Eye-pieces are of two kinds, the negative (fig. 21), or Huyghenian, which was the invention of Huyghens, and used by him for telescopes, and the positive eye-piece, invented by Ramsden (fig. 22).

Fig. 21. Fig. 22.



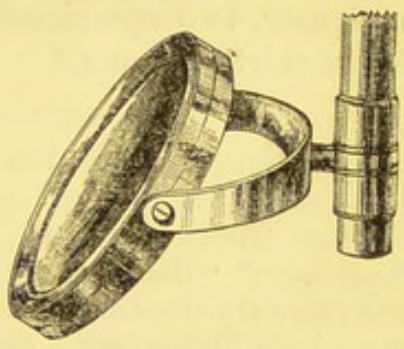
The former is the eye-piece which is now employed in achromatic microscopes for ordinary purposes; but the micrometer is often adapted to the positive eye-piece, in consequence of the divisions being seen more distinctly than with the negative. The magnifying power of the eye-piece may of course be increased to almost any extent, but any imperfection which may exist in the definition of the object-glass, becomes increased in the same degree as the image so produced is magnified, hence no advantage is derived from having eye-pieces of high magnifying power. It is usual to supply two or three eye-pieces with the best instruments, but the student will find that one good eye-piece of not very high magnifying power will be all

that he requires, and will enable him to demonstrate almost every structure with which he should become acquainted.

19. **Defining Power.**—In order to test the defining power of a microscope, we have merely to subject some object, the structure of which is exceedingly minute and delicate, to observation. The objects generally employed for this purpose have been termed "test objects;" and the structure of the most delicate of these can only be clearly made out when the glasses are good, and the illumination of the object is properly managed. Among the most favourite tests are the scales of the podura and some species of navicula; but if minute oil-globules, or the transverse striæ upon muscular fibre, are well defined, or the outline of some delicate epithelial cells, as those from the mouth, is rendered sharp and well defined, the instrument will be well adapted for all the purposes of the medical practitioner, although the glasses may not be sufficiently good to separate distinctly the delicate lines upon the scale of the podura, or define the minute dots upon the navicula angulata. In all cases, however, the objects in different parts of the field should be in focus at the same moment, and the lines should be sharp and free from coloured fringes.

20. **The Mirror.**—The Mirror is placed below the stage, and is so arranged that its position is easily altered, and that it may be inclined at any angle, in order that the rays of light may be reflected through an object placed upon the stage, and their direction varied, according as it is found necessary to examine the preparation by the influence of oblique or direct rays of light. The concave mirror collects the rays to a focus, and in this way we may obtain a much more intense light than by the flat mirror.

Fig. 23.



A white porcelain mirror is also sometimes used, or a piece of opaque white glass, or even writing-paper may be employed; but for general examination, a mirror of this kind is not often

required. Every microscope should, however, be furnished with a concave and a plane mirror, which should not be much less than two inches in diameter.

21. Choice of a Microscope.—With reference to the purchase of a microscope, it may be observed, that the selection of the particular kind of instrument will be affected somewhat by the nature of the research to which it is intended to be applied by the student. If the microscope is required for the examination of urinary deposits, and other substances likely to engage the attention of the medical practitioner, it will, I think, be found that one of the simpler microscopes, without moveable stage, but with good glasses, will be as efficient (figs. 11 and 12) as a more expensive and complicated instrument. On the other hand, if the purchaser wishes for what may be termed a perfect instrument, he must buy one of the beautiful microscopes made by the great English makers, and provided with a moveable stage. By this arrangement some time may be saved in examining objects, and, in using the higher powers, greater facilities for subjecting different parts of the field to examination, are certainly obtained; but a moveable stage is not *necessary* for observation. An efficient microscope for all general purposes, without moveable stage, may now be obtained for five guineas; and a more perfect instrument, provided with a moveable stage, and supplied with two powers, bull's-eye condenser, two eye-pieces, &c., costs from twenty to twenty-five or thirty guineas.*

On the subjects referred to in Chapter II. the following works may be consulted:—

Article Microscope, by Dr. Carpenter, "Cyclopædia of Anatomy and Physiology;" "Hannover on the Microscope," edited by John Goodsir, 1853; several articles in the "Microscopical Journal," 1852-3; "Pritchard's Microscopic Illustrations," quoted by Quekett; "A Practical Treatise on the Microscope," by John Quekett, second edition, 1852; "Du Microscope et des Injections," par le Dr. Ch. Robin, Paris, 1849.

---

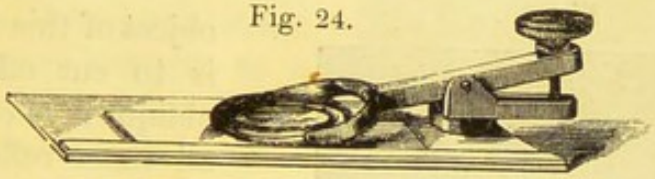
* For list of makers, vide page 13.

CHAPTER III.

ACCESSORY APPARATUS.

22. Compressorium.—Not unfrequently it is required to tear up delicate portions of tissue upon the field of the microscope, or to float them out, as it were, from the general substance under examination. In examining minute living animals we often wish to fix them in a position for careful observation. These objects are effected by an instrument termed a compressorium, (fig. 24), which simply consists of a mechanical arrangement, by which we are enabled to apply a certain amount of pressure upon the thin glass covering an object, which may be regulated at will. The compressorium consists of a flat piece of brass,

Fig. 24.

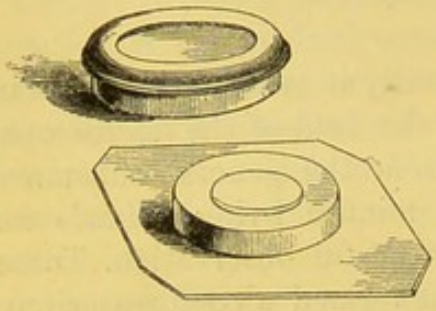


with a small ledge on one side, and a large hole in the centre. To one end is attached a lever, bearing a flat brass ring, about a quarter of an inch in width, which is free to move, and capable of being gradually raised up and down upon the object, by moving a screw, having a fine thread, which passes through the other extremity of the lever. The compressorium I have just described is one of the simplest forms I have seen, and presents this great advantage, that the ordinary glass slide, with the preparation upon it, may be subjected to pressure without the latter being removed, and placed upon the glass, which is, in most compressoriums, fixed in the hole cut out of the brass plate. A compressorium, made at the suggestion of my

friend, Dr. Branson, also possesses this advantage, but the slide is held in its position by springs, an arrangement which is not so simple as the ledge of brass in the instrument just described. This compressorium is made by Messrs. Powell and Lealand.

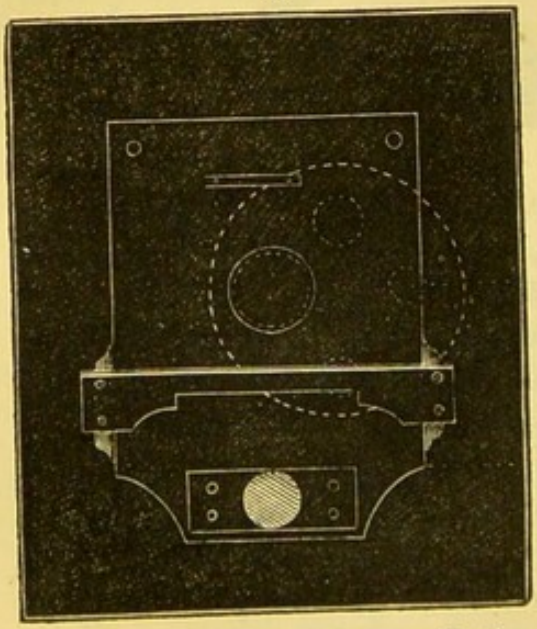
23. Animalcule Cage.—This piece of apparatus, particularly the form shown in fig. 25, may also be used as a compressorium, although the amount of pressure which can be exerted by it is not very much. It will, however, be found an exceedingly useful instrument when the amount of pressure required is not great, and especially, where strata of fluids varying in thickness are to be submitted to examination. These cages are usually supplied with the larger microscopes, but the form represented (made by Messrs. Powell and Lealand) in the adjoining woodcut is the most useful, and does not easily get out of order. If the thin glass should be broken, it is readily replaced by unscrewing the upper ring.

Fig. 25.



24. Diaphragm.—The object of this instrument is to cut off the most oblique rays of light, and all those reflected from the mirror which are not required for the illumination of the object. The clearness of definition is in this way much increased. The holes in the diaphragm are made of four different sizes, adapted to the various magnifying powers that may be used. The instrument fits on underneath the stage (fig. 26). When opaque objects are examined, that portion of the diaphragm not per-

Fig. 26.

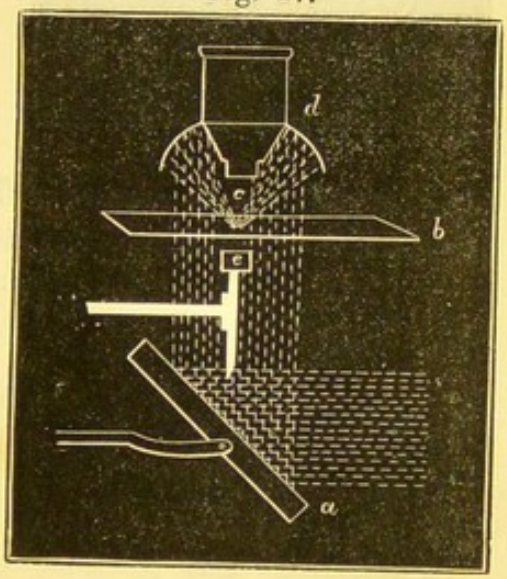


forated, may be placed under the object to cut off any rays of light that may be accidentally reflected from the mirror. The same end is obtained by the stops in those instruments which are provided with them.

25. Lieberkuhns.—These instruments consist of little circular concave metallic reflectors, which are fitted on at the lower part of the object-glasses. The polished surface presents that degree of concavity which is adapted to reflect, and bring any rays of light that may fall upon it, to a focus, coinciding with that of the object-glass itself.

These little reflectors are named from their discoverer. In the present day they are not much employed. Their use will be seen from the accompanying diagram (fig. 27).

Fig. 27.

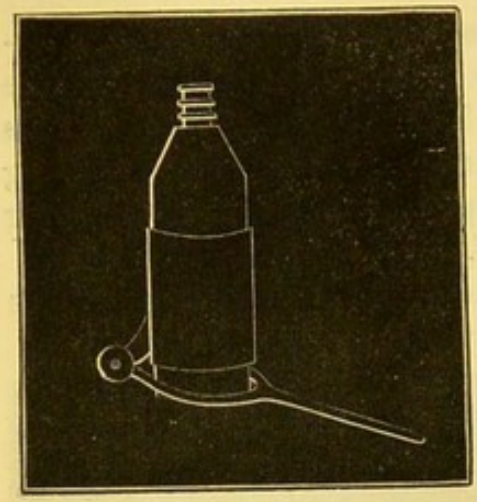


The light reflected from the mirror *a*, passes through that part of the glass slide *b*, not covered with the object, and the rays after having impinged upon the concave metallic reflector, or lieberkuhn *d*, are collected and brought to a focus upon the object *c*. If this be transparent, it becomes necessary to place a *stop e*, or a small round piece of cardboard, underneath, in order to prevent the light from being transmitted through the object.

26. Achromatic Condenser.—There are many delicate structures, the nature of which can only be clearly made out when they are subjected to examination by achromatic light. Minute lines or markings, which cannot be seen at all with common light, become often sharp and well defined when the light is achromatized; an object which is readily effected by transmitting the light through an achromatic combination of lenses, placed beneath the object. The framework supporting the lenses is either provided with a rackwork, or fitted with a sliding tube, which arrangement enables us to alter the in-

tensity of the light as may be desirable. Mr. Quekett has adapted a lever to the condenser fitted in a sliding tube, by which means a very simple and efficient arrangement for altering the focus is obtained (fig. 28). A foreign combination of half-an-inch focus makes an excellent achromatic condenser.

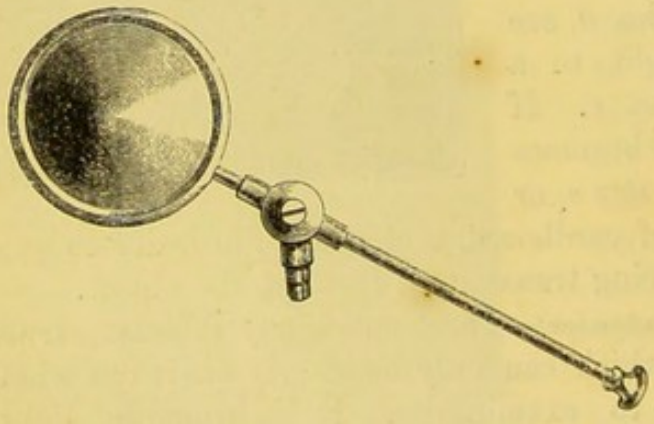
Fig. 28.



All the instruments attached to the larger microscopes are provided with a rackwork. In these the condenser is usually fitted on underneath the stage, with a bayonet joint, or by means of a sliding plate.

27. Bull's-eye Condenser.—The bull's-eye condenser is employed for condensing the light upon anything that is to be examined as an opaque object, or for concentrating the light upon a preparation which requires minute dissection; a large bull's-eye lens, about two inches in diameter, or more, and mounted upon a stand (fig. 30), will be found of great use to the microscopical observer. Those attached to the student's instruments (fig. 29) are inconveniently small, although, with good management, they may be made to answer the purpose for which they are intended, namely, for condensing the light upon opaque objects, for examination with low powers.

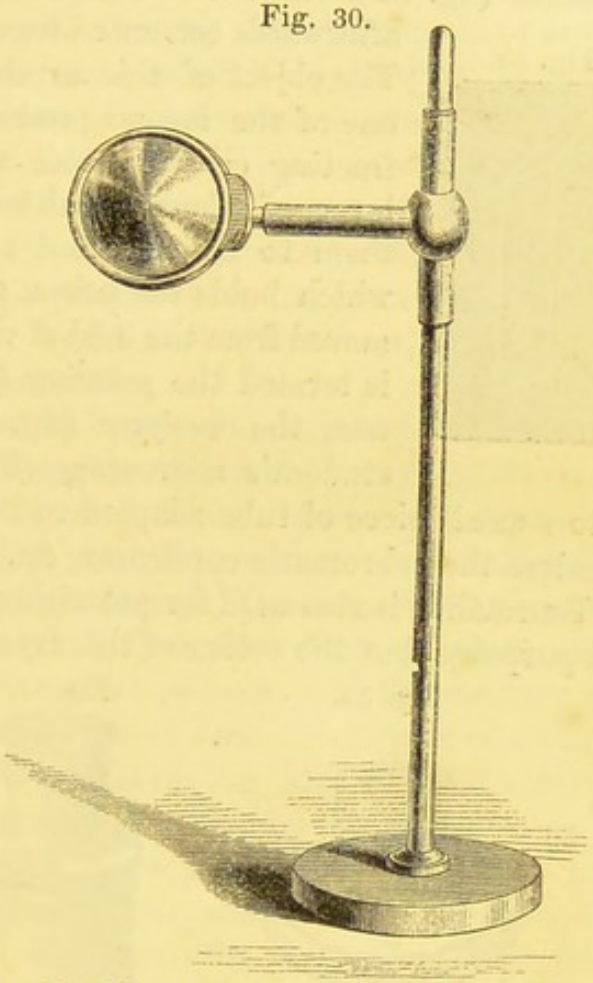
Fig. 29.



28. Stage Forceps.—Many microscopes are provided with

small spring forceps (fig. 31), fitted with a hinge or universal joint, and arranged so that they may be fixed upon one side of the stage. For examining parts of insects, calculi, and other small objects with low powers, they will be found useful, but I do not think that they will often be required by the practitioner. Other forceps will be spoken of in Chapter IV.

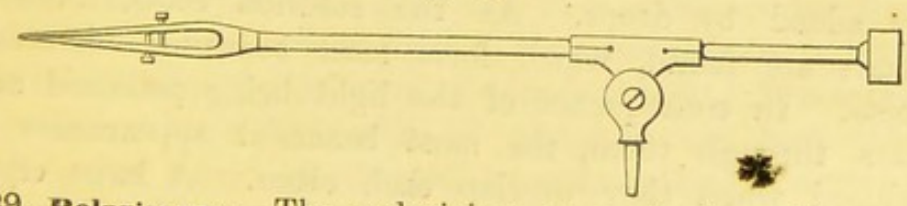
Fig. 30.



In those cases in which small crystals or crystalline fragments, which would be injured by being placed in the forceps, are to be examined as opaque objects, it will be found convenient to lay them upon a small slab of black wax (§ 86), which may be placed upon the stage.

With the aid of needles (§ 52) the object can easily be turned round, and by slight pressure may be readily fixed in any position.

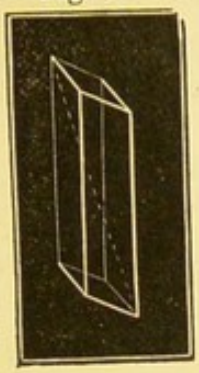
Fig. 31.



29. **Polariscope.**—The polarizing apparatus in ordinary use is that proposed by Mr. Nicol, and consists of two prisms of Iceland spar, one of which is fitted beneath the stage, while the

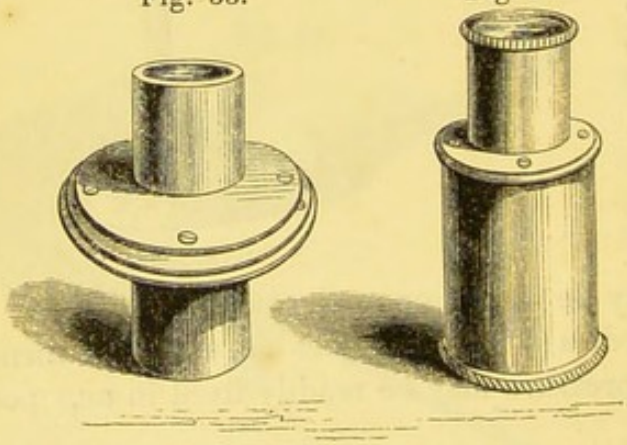
other is attached to the eye-piece. Each prism is divided into two portions, the division being carried from one angle to the opposite (fig. 32). The two surfaces are next polished, and afterwards cemented together by Canada balsam. The object of this arrangement is to get rid of one of the images produced by this doubly-refracting crystal; and this is effected by the layer of Canada balsam which causes one of them to be refracted to the side of the tube which holds the prism, and it is in this way removed from the field of vision. The lower prism is termed the *polarizer* (fig. 33), and the upper one, the *analyzer* (fig. 34). In Mr. Salmon's student's microscope (fig. 11) the polarizer fits into a small piece of tube adapted to the diaphragm, which also receives the achromatic condenser, &c.

Fig. 32.



Tourmaline is also used for polarizing the light for microscopical purposes, but the colour of this crystal usually renders it objectionable. Lately a most beautiful crystal has been applied to this purpose by Dr. Herapath. A solution of disulphate of quinine in acetic acid is raised nearly to the boiling point, and a strong solution of iodine in spirits of wine is carefully added by drops. As the solution cools, numerous crystals are found, which form most beautiful microscopic objects. In consequence of the light being polarized as it passes through them, the most beautiful appearances are produced when they overlap each other. A large crystal of this sort may be mounted, and used for polarizing the light in microscopical examination; but it is, I believe, a difficult matter to obtain one sufficiently large for this purpose.

Fig. 33. Fig. 34.



30. **Polarized Light** is important for displaying the structure of many substances. Objects mounted in Canada balsam, or turpentine, sometimes exhibit their minute structure in a more perfect manner by this than by any other mode of examination. Various crystals form beautiful objects when examined in this way, especially those of a spherical or oval form, as, for instance, the crystals of carbonate of lime, so common in the urine of the horse, or the dumbbells of oxalate (oxalurate?) of lime, occasionally met with in human urine. The influence of polarized light upon the crystalline lens has formed the subject of a most valuable paper by Professor Brewster, in the Edinburgh Philosophical Transactions.

The polariscope is not often required in pathological researches, but is occasionally used for ascertaining the particular character of certain crystalline substances which occur ready formed in the animal body, or which are prepared, by various chemical processes, from organic substances. Upon examining certain crystals in this way, we shall find that some polarize, while others are not affected by polarized light. If, for instance, we examine some crystals of oxalate of lime, or chloride of sodium, we shall find that they do not allow the light to pass through them when the upper prism, or analyzer, is rotated so as to render the field dark; while, if we place some crystals of carbonate, or sulphate of lime, under similar circumstances, the light will pass through, and often the crystals will be most beautifully coloured.

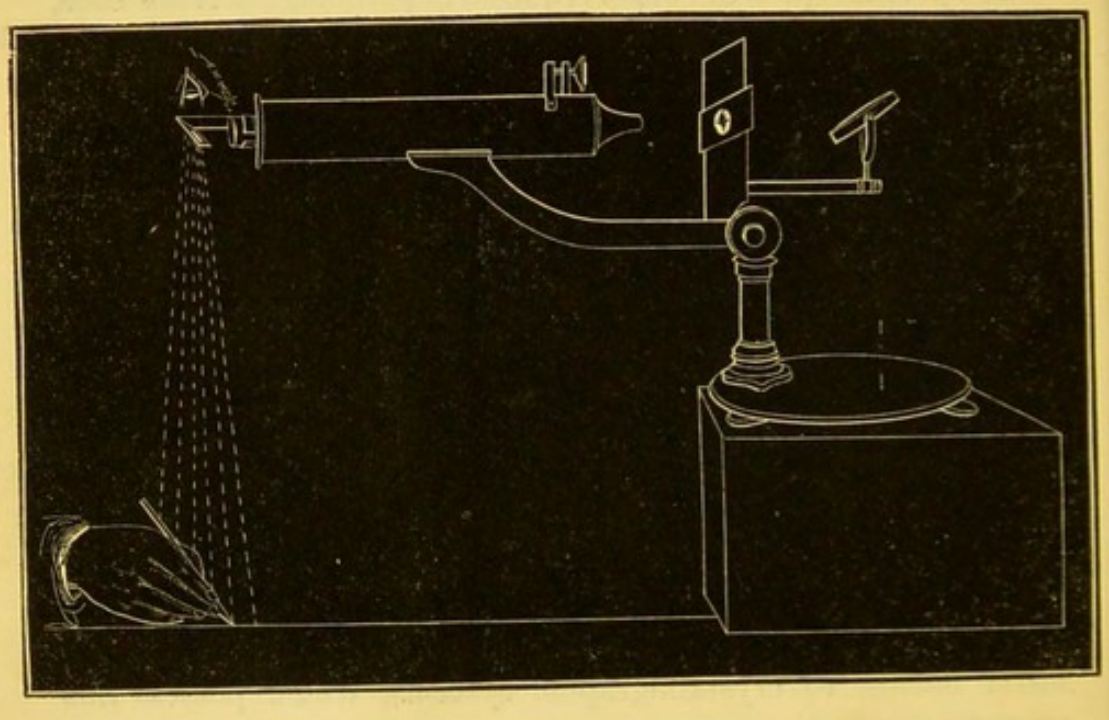
The former crystals belong to the regular, or tessular system, and do not possess the property of depolarizing light; while the latter belong to crystallographic systems, which possess this property.

In testing very minute quantities of a saline residue, for the presence of potassium or sodium, recourse may be had to examination under the influence of polarized light. The solution, to which a little bichloride of platinum has been added, after having been concentrated by evaporation, is allowed to crystallize. The octohedral crystals, composed of the double chlorides of platinum and potassium, do not polarize the light, while the corresponding sodium salt, which crystallizes in long

needles, possesses this property in a great degree. Professor Andrews states, that by employing this method, he has been enabled to detect the presence of the most minute traces of chloride of sodium.*

31. Drawing objects with the aid of the Camera Lucida, &c. —In making drawings of appearances observed in the microscope, much time will be saved, and correctness insured, by drawing the outline of the object with the assistance of the camera, or one of the instruments presently to be described (32). They are only adapted for taking the outline. Whichever instrument be employed, it is used in the same manner. The

Fig. 35.



microscope, with the apparatus placed on the eye-piece, and the object carefully fixed in its place in the field, is arranged in the horizontal position (fig. 35). The height must be carefully regulated in order that the image obtained on the paper may be of the same size as the magnified image: for this will vary according to the distance of the paper from the eye. The

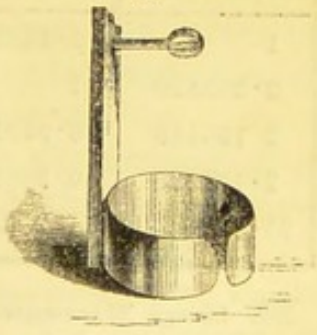
* Report of the British Association, 1852.

proper distance from the camera to the paper will be found to be about the same as that from the eye-piece to the object; but after having made the drawing, it may be compared in size with the object, by placing the paper on a level with the stage, and looking at the object in the microscope with one eye, and at that on the paper with the other. If the two images correspond in size, we may be sure that the microscope is arranged at the proper height. In drawing with one of these instruments it will always be found necessary to throw a stronger light upon the field of the microscope than upon the paper, or the pencil will not be distinctly seen. Frequently it will be found advantageous to shade the paper by placing a thin blind between it and the light. A little practice, however, will soon teach the student these points.

A microscopical drawing should be made with a very fine sharp-pointed pencil, and pains should be taken to make it as true to nature as possible. The drawings may be afterwards tinted, or, if preferred, they may be made with a very fine steel pen and ink. Prout's brown is a very convenient form of ink for making microscopical drawings, as with it exceedingly thin and sharp lines may readily be made. The importance of being able to make accurate delineations of the microscopical characters of tissues, and especially of morbid growths, can hardly be sufficiently dwelt upon, and every student should learn to draw as soon as possible.

32. **Steel Mirror; Neutral Tint Glass Reflector.**—The steel disk, or Soemmering's mirror, is fitted on in the same way as the camera lucida. It consists of a little circular disk of steel, smaller than the pupil of the eye, mounted at an angle of 45°. It is sufficiently small to enable the observer to see his pencil round it as it were. The only objection to this instrument is its liability to rust.

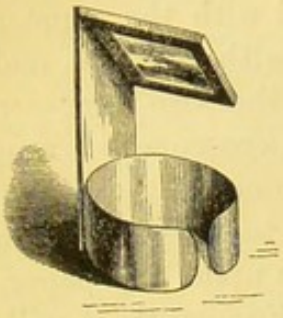
Fig. 36.



The neutral tint glass reflector consists of a small piece of plate glass about an inch in length and half an inch in

breadth, also placed at an angle of 45°. It reflects the image towards the eye, and is at the same time sufficiently transparent to enable the observer to see his pencil through it very readily, particularly if he takes the precaution of not having too much light upon his paper.

Fig. 37.



33. Micrometer and methods of Measuring. — Formerly objects were measured by placing in the field some substance, the diameter of which was known. Leeuwenhoek used small grains of sand; and since his time the sporules of certain plants have been employed. Small portions of thin wire, and hairs, the diameter of which had been previously ascertained, have also been used for this purpose. Blood-corpuscles will be found very useful for rough measurements. A few may be placed with the objects between the glasses, and the size of the latter compared with that of the former. The diameter of the blood-corpuscle may be reckoned to be about 1-3500th of an inch.

In this country we usually take the inch as the standard for measurement, but on the Continent other measures are employed. Some of the most important are represented in the following table from Hannover, which also shows their comparative value:—

Millimetre.	Paris Lines.	Vienna Lines.	Rhenish Lines.	English Inch.
1	0·443296	0·455550	0·458813	0·0393708
2·255829	1	1·027643	1·035003	0·0888138
2·195149	0·973101	1	1·0071625	0·0864248
2·179538	0·966181	0·992888	1	0·0858101
25·39954	.11·25952	11·57076	11·65364	1

34. Stage Micrometer.—The only stage micrometer that is now used, consists of a slip of glass on which very fine lines are scratched with a diamond, at certain distances apart. These slips may be easily obtained with lines separated by a distance

of the 1-100th to the 1-1000th of an inch. This method is only applicable when low powers are employed, because in the examination of objects by higher powers the lines will be out of focus at the time the object is in focus, and no accurate measurement can be obtained.

35. **Eye-piece Micrometer.**—Of late years the micrometer, consisting of a glass divided, as above described, has been placed in the focus of the lens of the eye-piece. An excellent improvement on this plan was proposed by Mr. Jackson about twelve years ago. It consisted in having the glass with the lines ruled upon it, so arranged in a small brass frame, that by turning a screw at one end, the scale could be made to traverse the field for the required distance, until one of the lines was brought accurately to one side of the object; when its diameter could be at once read off, if the value of each degree had been previously ascertained, by placing a stage micrometer in the field, and ascertaining the number of divisions that corresponded to one of the divisions of the latter. This apparatus was placed between the lenses of the eye-piece, and with it a measurement could easily be effected.

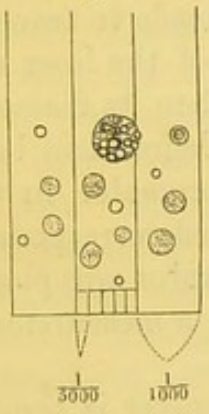
36. **Cobweb Micrometer.**—The cobweb micrometer is the instrument in most common use, and by it, with a little practice and care, tolerably accurate measurements may be taken very quickly. The instrument essentially consists of an eye-piece (§ 18), in the focus of which two cobwebs are placed across the stage, and so arranged that one may be separated from the other by turning a screw, to which a graduated circle is attached. The value of each turn of the screw having been obtained by placing a glass micrometer in the field as just now mentioned. This beautiful little instrument, however, is expensive, and not usually provided with the student's microscopes.

For a further description of it, and for the method of calculating the diameters of an object with this micrometer, I must refer to Mr. Quekett's treatise on the microscope.

37. **Method of Measuring the Size of Objects with the Camera Steel Disc or Neutral Tint Glass Reflector.**—This method was devised by Mr. Lister. It is exceedingly simple, and at the same time accurate. One of the above instruments being attached to the

eye-piece, and the microscope being placed horizontally (fig. 35), a stage micrometer, graduated to hundredths or thousandths of an inch, is to be placed in the focus of the object-glass, and the magnified lines are to be accurately traced upon the piece of paper intended to receive the drawing. The micrometer is next to be removed, and the object to be substituted for it. Upon now tracing the object upon the paper, it is easy to see how many of the intervals between the lines it occupies; and the measurement of each interval being known, the diameter of the object is at once obtained (fig. 38).

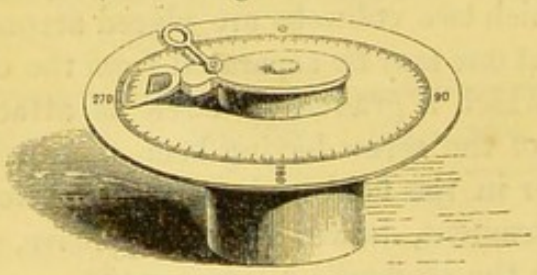
Fig. 38.



The magnified divisions of the micrometer, when examined by different powers, may be scratched upon pieces of slate or ground glass. The value of each division, and the object-glass and eye-piece which was used being also indicated; any object which we wish to measure may be traced with a pencil upon the slate, and its diameter at once read off, care being taken that the distance from the eye-piece to the surface upon which the drawing is made, is always the same.

38. **Goniometer.**—This instrument is employed in the measurement of the angles of crystals. Although not much used in this country at present, it is important briefly to refer to its construction, as by it we are enabled to distinguish the nature of certain crystals met with under various circumstances. Crystals which nearly resemble each other in their general form, and even in size, will be found to exhibit differences in the measurement of their angles.

Fig. 39.



The simplest method of measuring the angles of microscopic crystals is that of Schmidt. The goniometer consists of a positive eye-piece (fig. 22), which is so arranged as to be

easily rotated within a large and accurately-graduated circle. Across the focus of the eye-piece a single cobweb is drawn; and to the upper part is attached a vernier. The crystals being placed in the field of the microscope, and care being taken that they lie perfectly flat (for without great attention to this point it will obviously be impossible to obtain an accurate measurement), the vernier is brought to zero, and then the whole apparatus turned until the line is parallel with one face of the crystal: the framework bearing the cobweb with the vernier is now rotated until the cobweb becomes parallel with the next face of the crystal, and the number of degrees which it has traversed may then be accurately read off (fig. 39).

Dr. Leeson has applied the property of double refraction, possessed by Iceland spar, to the measurement of the angles of crystals under the microscope. A description of his apparatus will be found in Mr. Quekett's treatise; but the cobweb goniometer just referred to, will, I believe, be found to answer all the purposes for which this instrument is required by the physiological or pathological observer. For special crystallometrical investigations, however, a more elaborate apparatus becomes necessary.

39. Application of Photography to the Microscope.—Within the last year or two, great advances have been made in bringing to perfection the application of photography to taking microscopical drawings, and in the hands of Mr. Delves some very beautiful results have been obtained. The third number of the "Microscopical Journal" is illustrated with two photographic pictures taken from negatives made by this gentleman; and although much more perfect representations have since been obtained, these indicate the immense advantage which microscopical science will ere long receive from this beautiful discovery.* The same number of the journal contains a short communication from Mr. Delves, and an excellent paper by Mr. Highley on this subject. These papers contain some sug-

* Since the publication of this Number, I have had an opportunity of seeing a most beautiful photograph of the Navicula angulata taken by this gentleman with a $\frac{1}{12}$ object glass.

gestions of great practical value, and should be read by all interested in this branch of science.

40. With the ordinary Photographic Camera.—Mr. Delves produces photographic pictures of microscopic objects, by removing the lens from an ordinary photographic camera of long range, and substituting for it the tube of the microscope, with the object-glass attached, but from which the eye-piece has been withdrawn. The junction between the microscope tube and camera is made complete by winding a piece of black cloth round them at this point. The direct rays of the sun are then received upon the plane mirror, from which they are reflected up the axis of the arrangement. The object being placed upon the stage of the microscope, and carefully focussed, its image is received upon a plate of ground glass placed at a distance of about twenty-four inches from the object-glass.

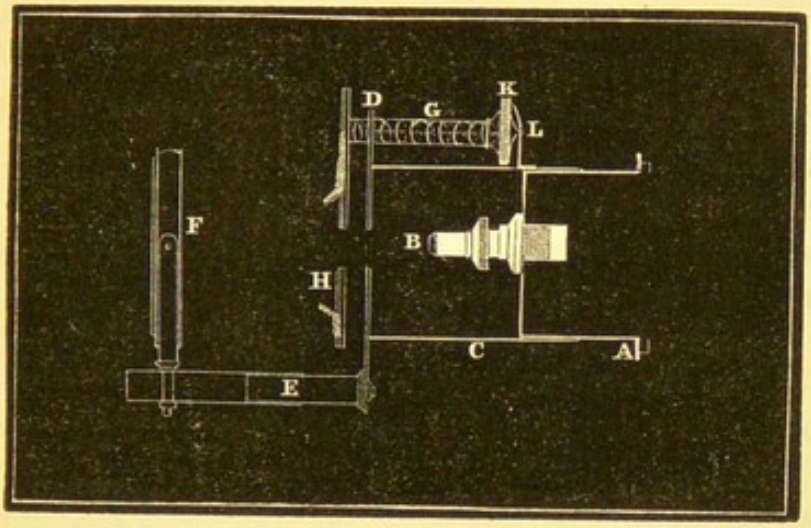
Allowance must now be made for the difference between the focal distance of the chemical, or actinic, and visual rays—a difference which exists in all microscopic object-glasses; these latter not having been yet corrected for this defect, as has been effected in photographic object-glasses. This is effected by increasing or diminishing slightly, the distance between the object and object-glass, by means of the fine-adjustment screw; separate photographic pictures being taken until a satisfactory and well-defined image is obtained.

Everything being carefully arranged, the ground glass upon which the image has been received is removed, and a plate of glass prepared with a sensitive collodion film substituted for it. After a few seconds' exposure, the plate is removed and the latent image developed in the usual way. Mr. Shadbolt has obtained photographs of microscopic objects by the aid of the light derived from a camphine lamp, and Mr. Busk by that of a gas lamp.

In those cases, in which, from the extreme tenuity of certain crystals, the lines are too thin and delicate to be seen distinctly, Mr. Highley has suggested the employment of the polarizing apparatus, with a Darker's selenite stage; by which arrangement the form of the crystals becomes well defined, while their colour differs from that of the ground upon which they are placed.

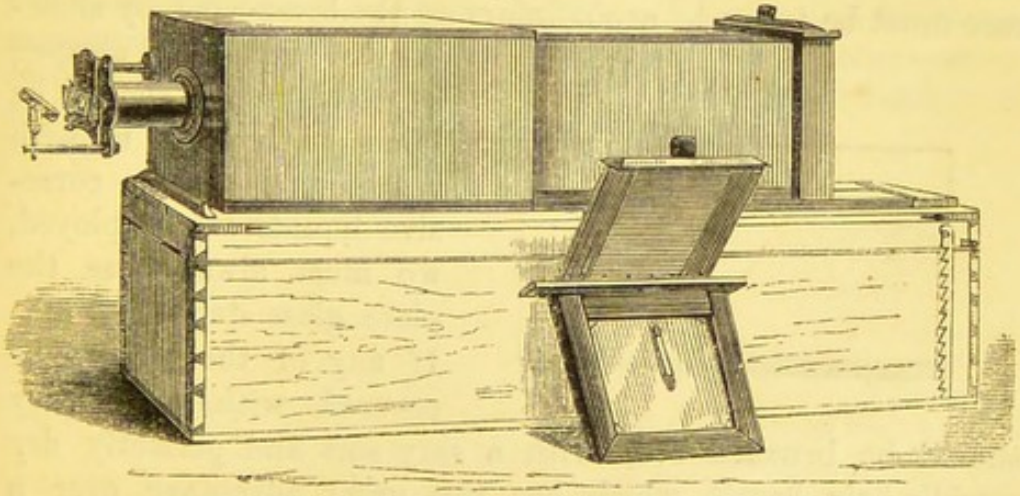
41. **Photographic Camera for taking Microscopical Specimens.**—Mr. Highley has suggested a very convenient arrangement, which possesses some advantages over the plan just described. It is more steady and compact, and is always ready

Fig. 40.



for immediate use. This improvement consists in adapting a stage with adjustment-screws, object-glass, and mirror, to the end of a long camera box. Fig. 40 shows the very simple manner in which this ingenious arrangement has been effected,

Fig 41.



which has been described in No. IV. of the Microscopical Journal. Fig. 41 represents the whole arrangement.

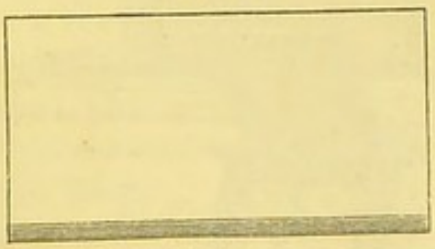
Mr. Highley has also suggested to me the possible use of the photographic camera for class demonstration. The image being received upon the ground glass plate might be readily seen by several persons at a time, while the whole apparatus might be mounted upon a semi-circular tramway, and could thus be very readily moved round the lecture table. I have, however, not yet had the opportunity of trying this experiment, and cannot therefore speak of it from practical experience.

I much regret that I cannot enter more into the details of this beautiful process, which bids fair to advance accurate observation more than almost any discovery of modern times; but must content myself by referring for more extended information to the papers just mentioned.

42. Importance of Cleanliness in Microscopical Investigations.—After the microscope has been used, it should always be placed in its case; or it may be kept constantly under a glass shade, which saves the trouble of putting on the glasses when the instrument is required for use. Mr. Highley's microscope (fig. 12) is placed upon a stand arranged to receive a glass shade. Great inconvenience results if the instrument cannot be placed in its case entire, and much time is lost if we have to unscrew the barrel, and detach other parts, before the microscope is put away; a fault which exists in some instruments.

Whenever anything is examined which is of a fluid nature, care must be taken to avoid injury to the brass-work by allowing the liquid to escape; in which case it should be immediately wiped up with a dry cloth. When corrosive liquids are employed, we must always use the plate glass stage (fig. 42). If any of the glasses be covered with dust, they should be brushed over with a very soft and perfectly dry camel's-hair brush, which has this great advantage over a cloth or leather, that there is no danger of scratching the glass with grit or sand which frequently adheres to a cloth.

Fig. 42.



Schacht recommends a piece of clean and dry elder pith for cleaning the object-glass. It is, I think, a good plan to have the stand of the microscope bronzed, as this does not tarnish like brass-work.

All slips of glass should be washed immediately after use, and returned to their places, and the utmost care should be taken to preserve all the instruments from rust, which will soon render them quite useless for delicate dissections.

The student cannot be too careful to preserve the most scrupulous cleanliness in all his investigations; for, not only does carelessness on this point render it more difficult for him to prepare his objects for examination, but exposes to his view a host of objects, such as filaments of cotton, portions of hair, &c., which he may suppose were derived from the object under his observation; and he also runs the risk of finding some of the objects met with in a former examination in any fresh specimen he may obtain. By carelessness on this point, particularly by not keeping pipettes and glasses clean, much inconvenience and annoyance may be caused, besides which an observation may be rendered useless.

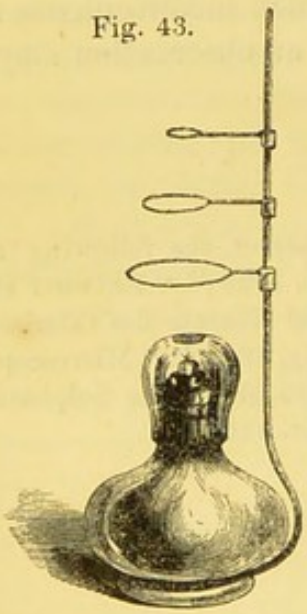
Besides the works referred to in the last chapter, the following may be consulted:—"Leeuwenhoek, Arcana Naturæ, 1722;" "Entwurf einer allgemeinen Untersuchungsmethode der Säfte und Excrete des thierischen organismus von Carl Schmidt, Mitau und Leipzig, 1846;" "Microscopical Journal," Nos. III, V., VI.; Dr. Herapath's paper on the Sulphate of Iodoquinine, "Phil Mag.," March and September, 1852.

CHAPTER IV.

APPARATUS NECESSARY FOR MICROSCOPICAL RESEARCH.—CEMENTS.

Before proceeding to describe the various methods by which the microscopical structure of different textures can be demonstrated, and the manner in which they may be preserved as permanent objects, it will be advantageous to give a short description of those pieces of apparatus which may be necessary for this object. Such instruments as may be required for special investigations will be described when these come under notice. Objects are generally mounted in glass cells, which may be purchased ready for use ;* but microscopists living in the country will find it more economical to be acquainted with a method by which they may prepare for themselves cells of any required size, for otherwise it becomes necessary to keep a large stock always on hand.

Fig. 43.



43. **Spirit Lamp.** — The spirit lamp may be made of brass, tin, or glass, fitted with a ground glass cap. The latter form is readily obtained, and at small cost. The ordinary spirit lamp is very convenient for microscopical purposes. It may be fitted with a stand for holding watch-glasses, as is shown in fig. 43 ; or brass lamps, to which a small retort-stand is fitted, may be purchased.

---

* Glass cells of every variety may be obtained of Mr. Dennis, 1, Charles-street, St. John-street Road ; Mr. Matthews, Portugal-street, Lincoln's-Inn ; Mr. Topping, New Winchester-street, Somers-town.

44. Small Retort Stand.—Simple wire stands, which are fixed to a heavy leaden foot, as represented in fig. 44, will be found exceedingly useful little instruments to the microscopical observer. The rings can be readily raised or lowered at pleasure, and are well adapted to support light objects, such as glass slides over a lamp, test-tubes, flasks, watch-glasses, &c.*

Fig. 44.

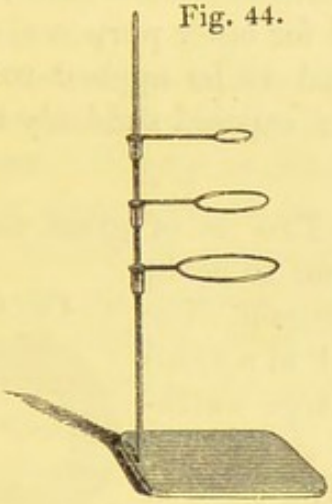
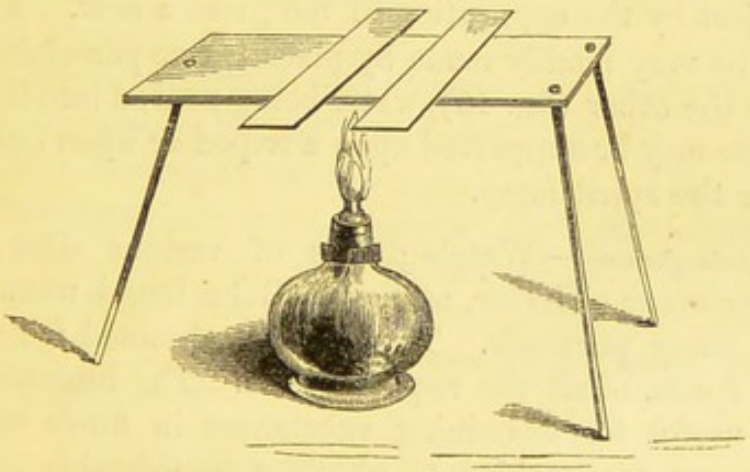


Fig. 45.

45. Tripods are made of thick iron wire, and will be found useful for supporting several pieces of apparatus used in microscopical research. They may be obtained at any philosophical instrument makers (fig. 45).

46. Flat Brass Plate.—The brass plate should be about six

Fig. 46.



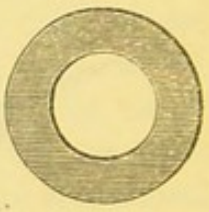
* To be obtained of Mr. Matthews, price 1*s.* 6*d.*, of whom most of the instruments referred to in the text can be purchased.

inches long by two broad, and about the thickness of thin millboard. It should be supported on three or four legs, of a convenient height for the spirit lamp to be placed underneath (fig. 46), or it may be placed upon a small tripod, or supported on one of the rings adapted to Mr. Highley's lamp (fig. 81). The brass plate is used for heating the glass slides, in order to fix on the glass cells by means of marine glue (§ 68), for mounting objects in balsam, and for other purposes, where a uniform degree of heat is required to be applied to plate glass, which is very liable to crack if exposed suddenly to the naked flame.

47. Small Water-Bath (fig. 47).—This is of great use for drying objects previous to being mounted in Canada balsam. The object may be placed in a small porcelain basin or large watch-glass, or it may be simply laid upon a flat plate. The basin or plate is then to be placed over the bath and heat applied. In order that vessels of different sizes may be placed upon the bath, it is very convenient to have a few pieces of thin copper plate, with holes of different sizes cut in them, adapted for watch-glasses (fig. 48). The advantage of drying by a steam heat consists in there being no danger of destroying the texture of the object by the application of too great a heat. A water-bath may be very readily made by placing two porcelain basins one above the other (fig. 45), water being poured into the lower one. These may be supported upon a tripod or upon one of the rings over the spirit lamp.

Fig. 47.

Fig. 48.



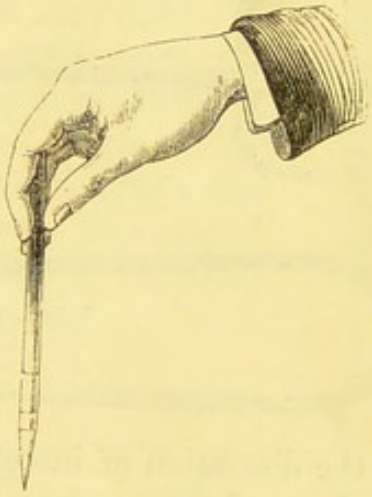
48. Watch-glasses.—Watch-glasses of various sizes should be kept by every observer, as they will be found most convenient for many purposes. They may be obtained for about a shilling a dozen, of all the required sizes. The lunette-glasses are very useful for examining substances in fluids with low powers, as we are enabled to obtain a considerable extent of fluid of nearly uniform depth for observation.

49. Plate Glass Slides, the edges of which are ground and polished, may be obtained ready for use at sixpence or eightpence a dozen, or they may be easily cut out with the diamond, and the edges ground on the grinding-slab (§ 67). The slides now in common use in this country are three inches in length and one in breadth. They should always be made of plate-glass, and pieces as clear as possible should be selected.

50. Thin Glass.—The thin glass now used for microscopical purposes is called cylinder glass, and is manufactured at Birmingham. It may be obtained of different thicknesses. Thin glass in sheets should be kept in fine sawdust, as it is very readily broken, in consequence of being imperfectly annealed. When cut up in small pieces for microscopical purposes, it should be kept in a small box, with a little powdered starch, which prevents the pieces being broken.

51. Writing Diamond.*—This instrument (fig. 49) is used for cutting the thin glass for covering objects; and also for writing the name of the preparation upon the glass slide. Directions for cutting thin glass will be described when the methods of making cells are brought under consideration.

Fig. 49.



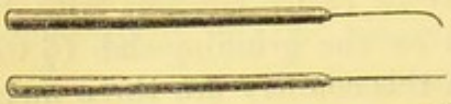
52. Needles, of various sizes, form very useful instruments to the microscopical observer. They are used for making minute dissections; for tearing or unravelling various tissues, in order to display their elementary structure, or for separating any minute object from refuse or extraneous matter, previous to being mounted. Very thin needles may be employed for separating substances under the field of the microscope.

---

* Price from five to seven shillings.

Needles may be mounted in the cedar handles used for sticks of camels'-hair brushes (fig. 50). A very convenient handle for needles, used for dissection, will be found in the holder sold with crochet needles used by ladies.

Fig. 50.



53. Scissors.*—Several pair of scissors are required for microscopical purposes. Besides the ordinary form used for dissection, the anatomist will find a small pair, with curved blades (fig. 51), of great service in dissection, and a pair of very delicate scissors, with blunt points (fig. 52), such as are employed

Fig. 51.

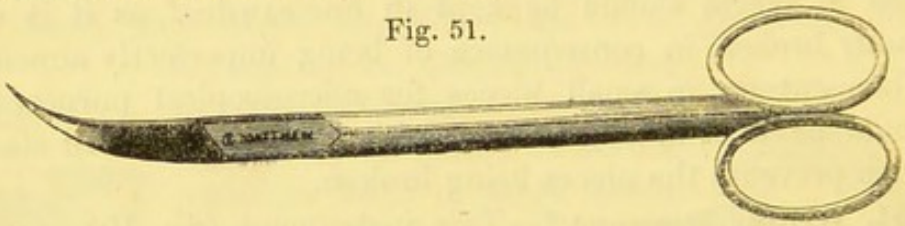


Fig. 52.

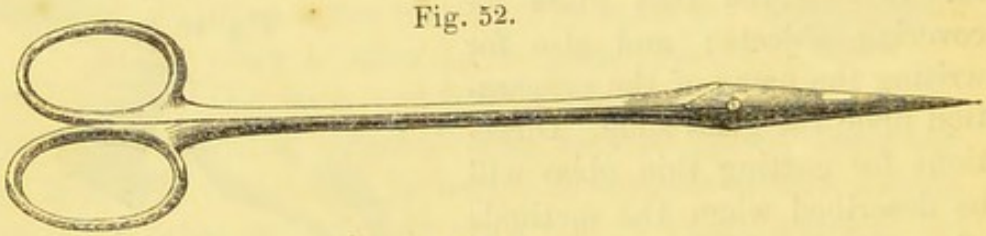
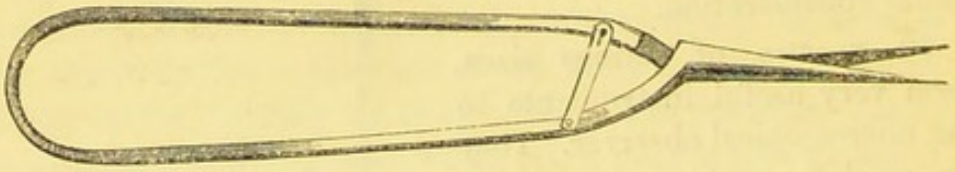


Fig. 53.



for the dissection of insects, will be found of use. Scissors of the form of forceps have been recommended. Another form of spring scissors, somewhat resembling the microtome, which I have been much in the habit of using, and which are adapted more especially for dissecting the nervous systems of insects, for following out the delicate ramifications of nerves

* Scissors of all kinds required by the microscopist may be obtained of Mr. Matthews, Messrs. Smith and Beck, Mr. Weedon, and other instrument makers.

and vessels, or other minute dissection, is represented in fig. 53. The advantages of these scissors is, that the blades can only be opened a very short distance, and that they are under the command of the forefinger and thumb; so that the movements are perfectly under control. Scissors with curved blades will be found very useful in cutting off fine sections of tissue, for examination.

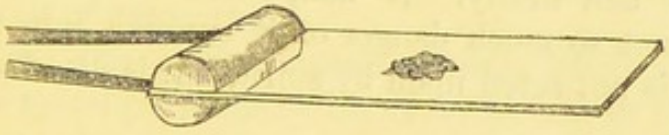
54. **Knives; Scalpels.**—In the dissection of textures for microscopical examination, but few knives are required; a good dissecting scalpel or two; a knife with a thin blade, for cutting sections of soft tissues (fig. 54); and a stronger knife for making sections of horn; are all that will be absolutely necessary. Knives adapted specially for making sections of soft tissues will be described when this subject comes to be considered.

Fig. 54.

55. **Forceps.** A pair of thin brass forceps will be found convenient for laying on the thin glass cover, after the preparation has been placed in the cell. Two pair of dissecting forceps will be found useful. One should be a strong pair with straight limbs, the other pair should be small, with thin curved blades, terminated with slightly rounded rather than pointed ends (fig. 55).

Fig. 55.

Fig. 56.



Wooden forceps made of box-wood, with broad ends, are convenient for holding the glass slides when hot, as, if held with cold metal forceps, they often crack. The same object may be gained by fastening a small piece of cork with a flat surface on the end of each leg of a pair of common brass or steel forceps, as shown in fig. 56.

CEMENTS.

The chief cements in use amongst microscopical observers are gold size, sealing-wax varnish, solution of shell-lac, solution of asphalt, marine glue, Canada balsam, gum, and a French cement composed of lime and India-rubber. These cements are used for fixing the glass cell on the glass slide; for fixing the cover after the preparation has been properly placed in the cell, and for other purposes. The liquid cements should be kept in very wide-mouthed bottles, or in the capped bottle figured in 57, which will be found a most convenient form.

56. **Gold Size** is prepared by melting together gum animi, boiled linseed oil, red lead, litharge, sulphate of zinc, and turpentine.* Gold size adapted for microscopical purposes may be also prepared as follows:— 25 parts of linseed oil are to be boiled with one part of red lead, and a third part as much umber, for three hours. The clear fluid is to be poured off, and mixed with equal parts of white lead and yellow ochre, which have been previously well pounded. This is to be added in small successive portions, and well mixed; the whole is then again to be well boiled, and the clear fluid poured off for use. In this country gold size may be obtained at almost any of the oil-shops, and forms a very useful cement, as it dries slowly and firmly. If lamp-black be rubbed up with it, there is not so much danger of it running into the preparation. It is not acted upon by spirit, but is dissolved by turpentine.

Fig. 57.

57. **Sealing-wax Varnish** is prepared by dissolving small pieces of the best sealing-wax in strong alcohol: sufficient sealing-wax should be employed to make the solution thick enough to use with an ordinary brush. Sealing-wax varnish is

* For proportions and method of making see "Ure's Dictionary of Arts and Manufactures."

apt to dry rather brittle, and should not, therefore, be used in cases where it is of the greatest importance to keep the cell perfectly air-tight. It forms a good varnish for the last coat. Various colours may be kept according to taste.

58. **Solution of Shell-lac** is recommended by Mr. Ralphs. It is made by dissolving shell-lac in spirits of wine. The shell-lac should be broken in small pieces, placed in a bottle with the spirit, and frequently shaken, until a thick solution is obtained. It dries rapidly, and, if put on in thin layers successively, forms a good cement. It is not acted upon by weak spirit.

59. **Brunswick Black.**—Solution of asphalt in turpentine commonly known by the name of Brunswick black, may be obtained at any oil-shop, and forms a most useful cement, both for making very thin cells, and also for fixing on the thin glass covers. If a little solution of India-rubber in mineral naphtha be added to it, there is no danger of cracking when dry; a hint for which I have to thank my friend, Mr. Brooke, of the Westminster Hospital.

Common Brunswick black is prepared by melting one pound of asphaltum, and then adding half a pound of linseed oil, and a quart of oil of turpentine. The best Brunswick black is prepared by boiling together a quarter of a pound of foreign asphaltum, and four and a quarter ounces of linseed oil, which has been previously boiled with half an ounce of litharge until quite stringy; the mass is then mixed with half a pint of oil of turpentine, or as much as may be required to make it of a proper consistence. It is often improved by being thickened with lamp black. It must be remembered that this cement is soluble in oil of turpentine.

60. **Marine Glue.**—This substance was, I believe, first used for microscopical purposes by Dr. Goadby, now of Philadelphia. It is prepared by dissolving, separately, equal parts of shell-lac and India rubber, in coal or mineral naphtha, and afterwards mixing the solutions thoroughly with the application of heat. It may be rendered thinner by the addition of more naphtha. Marine glue is readily dissolved by naphtha, ether, or solution of potash. It is kept best in a tin box.

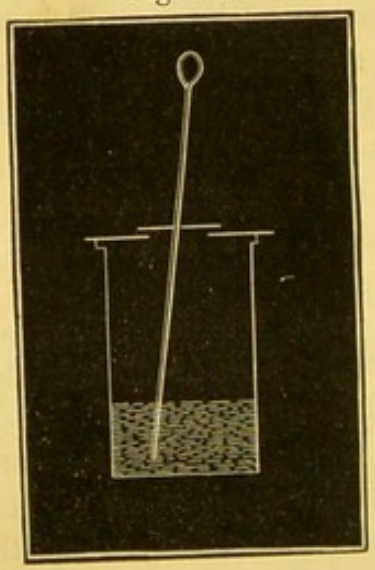
The method of applying the marine glue will be described in speaking of making cells.

A cement for attaching cells of gutta percha or India rubber to the glass slide may be made as follows:—According to Harting, gutta percha is to be cut into very small pieces and stirred, at a gentle heat, with 15 parts of oil of turpentine; the gritty, insoluble matter, which the gutta percha always contains, is to be separated by straining through linen cloth, and then one part of shell-lac is to be added to the solution, kept at a gentle heat, and occasionally stirred. The mixture is to be kept hot until a drop, when allowed to fall upon a cool surface, becomes tolerably hard. When required for use, the mixture is to be heated, and a small quantity placed upon the slide upon which the cell is to be fixed; the slide itself is then to be heated.

61. Canada Balsam is now much employed by microscopical observers: formerly it was used for cementing cells together, but this is now effected more readily by the aid of marine glue.

Canada balsam is a thick viscid oleo-resin, which becomes softer upon the application of a gentle heat. If it be exposed to too high a temperature, the volatile oil is expelled, and a hard brittle resin remains behind. It is chiefly employed for mounting hard dense textures; and, in consequence of its great power of penetrating, and highly-refractive properties, the structure of many substances, which cannot be distinguished in ordinary examinations, is rendered manifest when immersed in this medium. Canada balsam should be preserved in a tin box of the form represented in fig. 58, care being taken to exclude the dust; or in one of the bottles (fig. 57). Care should be taken to keep the balsam very clean, otherwise preparations mounted in it will often be spoiled in consequence of the accidental introduction of foreign bodies with the balsam.

Fig. 58.



It has been frequently recommended that the oldest specimens of balsam should alone be employed for microscopical examination. By exposure to the air, the balsam becomes very thick, and unfit for use: it may be thinned by the addition of turpentine. It is, however, always better to use balsam which has been kept in well-closed vessels.

62. Gum.—Thick gum water will be found very useful for attaching labels to preparations, and also for fixing on the cover when preparations are mounted in the dry way. It is prepared by dissolving common gum-arabic in water, and keeping the bottle in a warm place until the solution has become sufficiently thick. It should always be strained before it is placed in the bottle for use.

Gum water, thickened with powdered starch, will be found a very useful cement for fixing the glass cover on preparations mounted dry. When dry it forms a hard white coating. The addition of a little arsenious acid will prevent the growth of mildew. Another very convenient solution is made by dissolving powdered gum in a weak solution of acetic acid.

63. French Cement, composed of Lime and India-rubber.—This cement is made as follows:—Some common India-rubber, or any refuse pieces (which can be bought by the pound), are placed in an earthen pipkin over a fire, and stirred frequently with an iron rod, until the whole has become a liquid mass. Powdered lime is then thrown in, in small quantities at a time, and the mixture well stirred until it becomes thoroughly incorporated. Lime should be added until the mass becomes very thick and tenacious. It may now be coloured by mixing vermilion with it, or a little Venetian red, which gives a fine rich brown colour.

This operation should always be performed out of doors, as it gives rise to a very offensive smell; and care should be taken that the mixture does not catch light, as, in this case, much would be spoilt, in consequence of the carbonaceous residue which remains rendering it gritty and unfit for use. It may be kept in tin or earthenware pots.

The great advantage which this cement possesses is, that it never becomes hard, so that large quantities of fluid may be kept

in a cell, the cover of which is fixed on with this substance, without danger of the glass cracking in consequence of the alteration of the volume of the fluid by variations of temperature. Another advantage is, that when pressed upon wet glass, the thin layer of fluid is expelled by the pressure, and the cement adheres firmly. In order to fix on the cover of a preparation jar with this cement, all that is necessary is to roll a small piece out between the hands, and lay it all round the top of the jar or cell. By pressing it gently with the finger and thumb, it adheres firmly to the glass; the cell is then filled with the preservative solution, and the cover applied (§ 93). The edge may be covered with any coloured varnish, or paper, or it may be bronzed or gilt, according to taste. This cement will not answer where strong spirit is used, but if only weak spirit be employed, or some aqueous preservative solution, the joint will last for almost any length of time. I have several preparations which have been placed in the creosote and naphtha solution (§ 97) in large cells, and they are now perfectly air-tight, although upwards of five years have elapsed since they were first put up. The lime and India-rubber cement answers well for fixing on the glass tops of large preparation jars, and looks exceedingly neat; but, if spirits be used, a little air must be permitted to remain in the jar, or the cover may be cemented on the cell according to the plan described in § 93.

The apparatus, cements, &c., used in microscopical investigations, may be obtained, arranged in a case, of Mr. Highley, 32, Fleet Street.

Upon subjects treated of in the preceding chapter, the following works may be referred to:—Translations from "Het Mikroskoop," Harting, Utrecht, in the Edinburgh Monthly Journal for March, 1852, New Series; "Anatomical Manipulation," Tulk and Henfrey; Papers by Dr. Goadby, in Silliman's "American Journal of Science;" Dujardin "Nouveau Manuel de l'Observation au Microscope," Paris, 1843; Strausdurkheim "Traité pratique et theorique d'Anatomie Comparative," Paris, 1842.

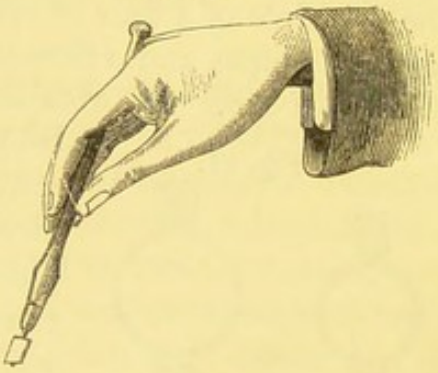
CHAPTER V.

OF MAKING CELLS FOR PRESERVING PREPARATIONS. THE VARIOUS FORMS OF CELLS EMPLOYED.

64. Of Cutting Glass.—In making the cells which are intended to contain anatomical preparations we often require to be able to cut very thick plate glass. For this purpose a large plate-glass diamond is necessary. The use of the diamond is easily learned with a little practice. The most important points to bear in mind are—

1st. To hold the diamond firmly in its proper position, by placing the upper part of the handle between the first and middle fingers, the fore-finger resting on the anterior, and the thumb pressing upon the posterior flat surface (fig. 59).

Fig. 59.



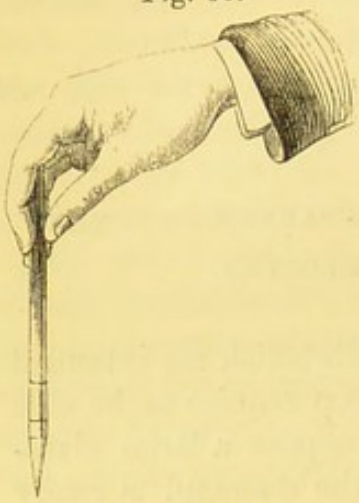
2ndly. To be careful not to bend the wrist, but to allow the entire movement, when the diamond is drawn over the glass, to take place in the elbow and shoulder joints; and,

3rdly. Always to keep the instrument inclined at the same angle. The diamond should not be pressed strongly upon the glass, for in this case it frequently produces only a mere scratch. It should never be attempted to go over any part of the same line twice; as in this way the diamond would soon be injured. For cutting plate glass of ordinary thickness, a common glazier's diamond only will be required.

65. Of cutting the thin Cylinder Glass.—The thin glass which is used for covering preparations is cut with what is termed a writing diamond (fig. 60): a small instrument which

is used for scratching marks on glass. In order to cut the thin glass into small squares, the sheets must be placed upon a very flat surface; as, for instance, a piece of plate glass, or a board planed perfectly smooth. The diamond being held upright, straight lines are marked with the aid of a flat ruler, according to the size of the squares to be cut. When all the lines are made, the pieces of glass can be readily broken off. They should be kept in a box with a little starch powder, to prevent them from being broken in smaller pieces.

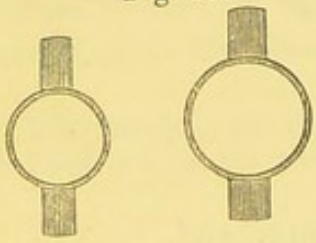
Fig. 60.



A piece of thin glass of any shape can be readily cut with the writing diamond, taking care not to lean too heavily.

66. Method of cutting circular pieces of Thin Glass.—Thin glass circles have been cut with a beautiful instrument on the principle of a pair of compasses; but the following method is very simple and efficacious. Common brass curtain rings of various sizes are obtained; and on each side of a ring is soldered a straight piece of wire, or a round hole is made in a perfectly flat piece of brass, as shown in fig. 61. By placing a finger on each side, the ring can be maintained in a proper position, while the circle is marked out with the diamond. These rings may be obtained of Mr. Matthews; or small pieces of cardboard, or gutta percha, in which circular holes of various sizes have been cut out, may be substituted for them.

Fig. 61.



67. Of grinding Glass for making Cells, &c.—For the purposes of grinding, it is essentially necessary to obtain a perfectly-flat surface. A large flat piece of thick cast iron will answer the purpose very well, or a perfectly-flat stone may be employed, which should be about a foot square, or larger, if cells of considerable size are required.

Perhaps, however, the best substance to make a plate for

grinding glass upon is pewter. A flat slab of pewter can be readily obtained, and as the emery becomes imbedded in the metal, a most efficient grindstone is obtained. This, I believe, was first suggested by Dr. Goadby. Common sand, of a fine quality, or emery powder, will be required. The grey sand, which can be purchased at the oil-shops, answers very well for coarse glass-grinding. Water is poured upon the slab, and the glass rubbed very gently round and round. If rubbed too forcibly, there is danger of breaking it, or of chipping off small pieces from the edge. Care must be taken to keep the sand constantly wet during the operation. After being ground upon a stone with sand, it may be rendered more smooth by being further rubbed down with emery.

Sometimes it is merely required to roughen the surface of the glass, as in making the thin cells. In this case, it is better to use fine emery powder moistened with water or turpentine, and placed upon a small piece of thick plate glass; or a common hone may be used. The surface of glass may also be ground by rubbing it well with a rag covered with emery powder.

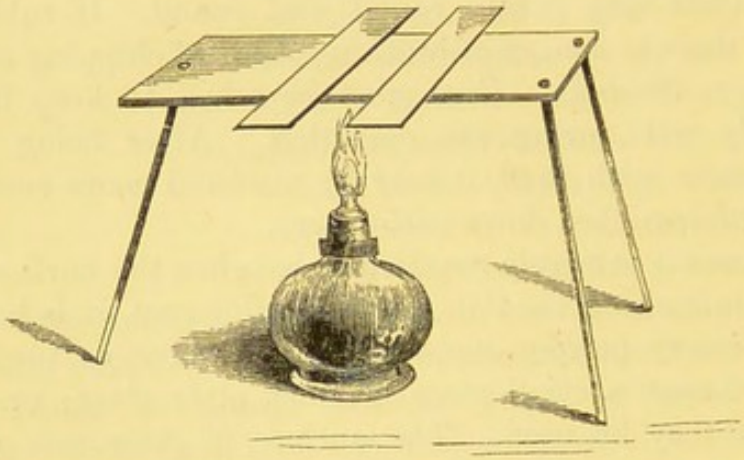
68. On fixing Cells to the Glass Slides.—Before referring to the various kinds of cells, it will be better to describe the method of fixing them to the glass slides. This may be effected by gold size, Canada balsam, or by varnishes of various kinds; but the method which is to be preferred in most instances, and that which is now in constant use, is to cement them with marine glue, which forms a most perfect joint. To effect this, it is necessary that the glass should be heated some degrees above the boiling-point of water, in order that the glue may run upon it freely. When the glue is thoroughly melted upon the slide, the cell is applied, gently pressed down, and the whole allowed to become cool.

The most convenient manner of making the slides sufficiently hot is to place them on a flat brass or iron plate, to which heat may be applied by a lamp placed below (fig. 62).

If a considerable number of cells are to be mounted, it is better to have a large and perfectly-flat cast-iron plate, which can be heated by a gas or oil lamp, upon a proper support, or

by a small stove. The slides may be placed in a row in one part of the plate, and the cells in another part. As soon as the slides are hot, a few small pieces of marine glue may be laid upon the slide in the position in which it is intended to place the cell, or the glue may be first melted in a pot and applied to the warm

Fig. 62.



glass with a stick. When the glue is thoroughly melted, the cell is moved to its place with a pair of forceps, and gently pressed upon the slide until it is found that the glue has wetted it in every part, forming a thin and almost invisible layer between the glass surfaces. It is frequently necessary to press the cell firmly on the glass slide, in order to break down little gritty particles often present in considerable quantity in marine glue. The whole can then be removed from the hot plate, and allowed to cool gradually. In moving the cell or slide, forceps, the ends of which have been protected with small pieces of cork, or covered with thread, pieces of stick, and a cloth will be found of service. The sudden application of the cold finger often causes the glass to crack. The greater part of the glue which has run over the slide may be removed with any small instrument having a sharp square end. A large brad-awl (fig. 63) answers very well. If a little solution of potash be rubbed upon the glass with the aid of a stick, the superfluous glue can easily be removed from the glass, or the

Fig. 63.

cells may be soaked in dilute potash for half an hour, and the softened glue removed with a hard brush and soap and water. After cleaning the slides with potash, they should be well washed in soap and water, and afterwards thoroughly cleaned by being rubbed with a little weak spirit and a soft cloth.

CELLS FOR PRESERVING PREPARATIONS.

In order to preserve a preparation for any length of time, it becomes necessary to place it in a cell or air-tight vessel, more especially if it be preserved in a liquid. In order to effect this object, several precautions must be observed, the nature of which will be considered in the next chapter. In this place I propose to describe the most useful cells to the microscopical observer, and the method of making them.

The forms and thickness of cells for microscopical preparations must of course vary according to the nature and size of the object to be mounted.

Thin cells may be made of various substances. Even paper answers exceedingly well in some cases, and is well adapted for dry preparations. A thin layer of white lead, which has been allowed to dry, has also been employed for the same purpose. White lead, made into a thick liquid with linseed-oil and turpentine, has been recommended by some observers. Various varnishes have likewise been used; but where it is required to keep the specimen in some preservative solution, glass is the substance which in all cases forms the best material for making cells.

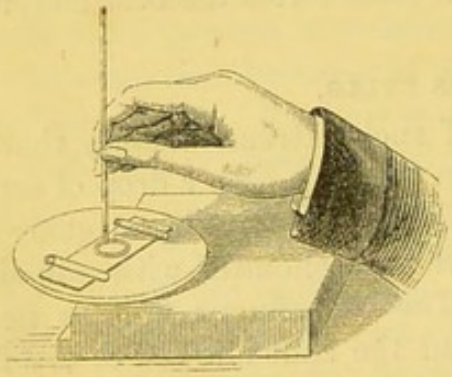
THIN GLASS CELLS.

Sometimes preparations are of such extreme tenuity that it is only necessary to place them on the slide with a drop of some preservative solution, and then to cover them with a square of thin glass, the edges of which have been anointed with gold size or other appropriate cement. The superfluous fluid is next absorbed with bibulous paper, and the slide allowed to dry for a few minutes. A layer of gold size or other cement is then applied round the edges of the thin glass in order to fix it to the slide. In this way an excessively-thin cell may be formed; but preparations mounted in cells made in this manner can

seldom be kept for any length of time without the entrance of air-bubbles. This arises from the outer layers of the gold size drying more rapidly than the more internal layers. By the contraction thus produced the edges of the cement are drawn off from the glass, to which, however, it does not adhere with great tenacity, in consequence of the surface being highly polished. It is, therefore, always better to make very thin cells of glass or other material, which can be cemented to the glass slides with marine glue or other cement; or else to make the cell by painting the slide with a ring of varnish, marine glue, or Brunswick black, and allowing this to dry thoroughly before the preparation is placed in it. In this manner the thinnest cells which can be required are readily made.

69. **Cells made of Brunswick Black.**—Perhaps Brunswick black (§ 59) is, for the purpose just mentioned, the best. It is painted upon a glass slide with a fine camel's-hair brush, and allowed to dry perfectly, when, if the cell be not sufficiently thick, another layer may be applied. If the cell be required immediately, it is better to warm the slide slightly before applying the varnish. If too great a degree of heat, however, be employed, the varnish becomes brittle and the cell unfit for use. Very neat circular cells, composed of Brunswick black, are readily made by the little instrument designed by Mr. Shadbolt, and figured in the margin (fig. 64). The glass slide is placed under the springs on the circular table, which is then made to revolve, while a brush containing the varnish is held about three-eighths of an inch from the centre of the slide. These cells are very useful for mounting the most delicate urinary deposits, such, for instance, as casts of the renal tubes, &c.

Fig. 64.



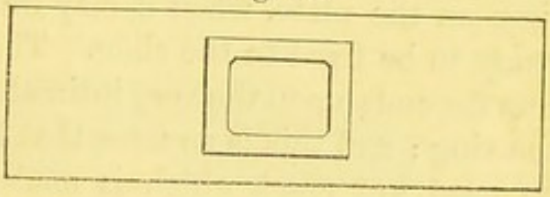
By this method the thinnest cells that can be possibly required may be readily made in a few minutes.

70. **Very thin Cells made of Tinfoil.**—This may be easily accom-

plished by cutting with a pair of scissors a piece of thin tinfoil the size of the cell which it is desired to make. A hole is cut in the centre of the tinfoil sufficiently large to hold the preparation which is to be preserved, and the tinfoil is then attached to the glass slide with marine glue. When cold the cell may be filed perfectly flat with a very fine file, or rubbed with a little emery upon a piece of plate glass, and the marine glue should be afterwards removed from the centre with a little solution of potash. The cover may be fixed on with gold size or varnish, as in other cases. Thin cells have also been made of gutta percha, but there is great difficulty in fixing the cell firmly upon the glass slide. This, however, has been effected by some observers; but in consequence of the difficulty, it is a method not generally employed. Preparations, however, mounted in cells, composed entirely of gutta percha, keep very well for a length of time. For the cement adapted for attaching gutta percha cells to glass, vide § 60.

71. Cells composed of very thin Glass.—These cells are very convenient, and will be found useful for preserving many preparations. They may be obtained of different degrees of thickness, and are made usually by perforating the thin cylinder glass which is used for covering the cells, or by grinding sections of a thick glass bottle to the required tenuity (fig. 65). Round cells of thin glass are made as follows:—A great number of squares of thin glass are cemented firmly together with marine glue, and, when cold, a hole of the required size is drilled through them all. They are next separated from each other by heat, and after being cleaned with potash, may be fixed on the glass slides with marine glue in the usual way, and kept ready for use. It is a good plan to roughen the surface of these cells, which renders the subsequent entry of air less likely, as the gold size adheres much more firmly to a ground, than to a polished, surface. This is readily effected by rubbing the cell, after it has been fixed upon the glass slide, up and down a narrow hone or strip of plate

Fig. 65.



glass, on which some moistened emery powder has been placed. In this way also the thickness of the cell may be reduced if required.

72. New method of making thin Glass Cells.—For some time, however, I have been in the habit of making these thin glass cells as follows :—

One of the thick glass rings (fig. 68) is heated on the brass plate, and one side covered with marine glue. As soon as the glue is thoroughly melted, a small piece of the thin glass is carefully applied, and the whole allowed to cool (fig. 66). When quite cold, the point of a semicircular, or round file is sharply thrust through the centre of the thin glass, which is carefully filed to the size of the interior of the ring, and then taken off by heating it a second time on the plate, when it may be cleaned with potash, and is ready to be fixed to the slide. The success of this simple process depends upon the very intimate adhesion of the thin glass to the ring ; and this is so firm, that however roughly the file may be used, any crack which is made, never runs across that part of the thin glass which is fixed to the ring. In this way, thin glass cells may be made of any shape without the slightest trouble ; and by having many of the rings on the hot plate at the same time, and taking them in rotation, a very short time only is required to make a great number. Very large thin glass cells can thus be readily formed (fig. 67), which could not be made at all, or only with great labour, by any other process.

Fig. 66.

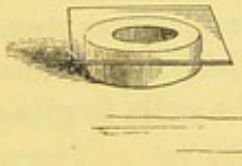
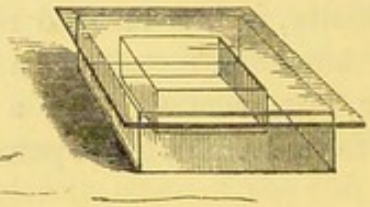


Fig. 67.

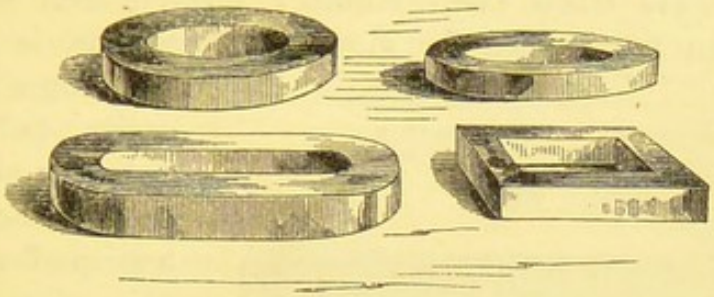


THICK GLASS CELLS.

73. Deep Glass Cells.—The deep glass cells which are used for mounting injections and other preparations of considerable thickness, are made either by cutting sections of thick glass

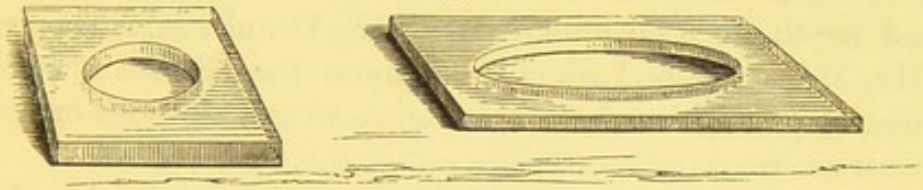
bottles, or round tubing (fig. 68) ; or by drilling holes of the required size, in pieces of plate glass, gutta percha, &c. (fig.

Fig. 68.



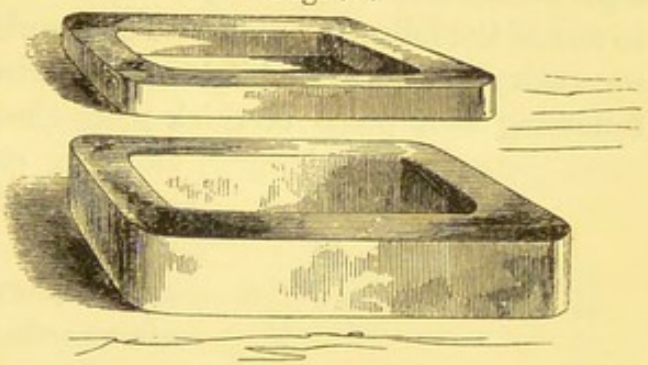
69.) All these of course require fixing to plate-glass slides, as above described.

Fig. 69.



Cells made by these methods may be obtained of various shapes and sizes ;* the small round, or square cells are very convenient for mounting small pieces of injection. When larger preparations are to be preserved, the oval or square-shaped cells, (figs. 69, 70,) may be employed. Mr. Storer has succeeded in making cells on this

Fig. 70.



* The following table gives the prices and dimensions of some of these cells previous to being mounted upon the glass plates—

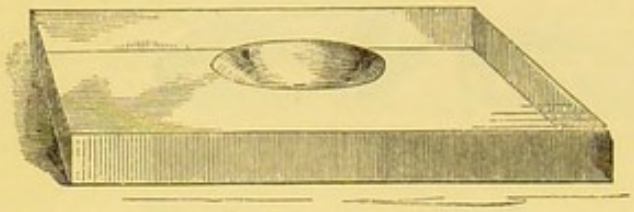
Length. In.	Breadth. In.	Depth. In.	Price.
6	3	½	2*s.* each.
3	3	½	6*d.* each.
1	1	¼	2*d.* or 3*d.* each.

Smaller cells from 1*s.* 6*d.* to 2*s.* per dozen.

plan, as large as six inches by three, and half an inch deep. The glass is cast in a mould, and thin sections are cut off of the required depth.

74. Concave Glass Cell.—Another form of cell which has lately been much used for mounting injections, is made by grinding a deep concavity (which may either be circular or oval), in the centre of a strip of very thick plate glass. (Fig. 71.)

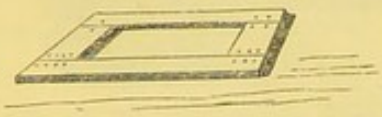
Fig. 71.



Cells of all the forms above mentioned, varying from a quarter of an inch in diameter to six inches, and of any required depth, may be obtained of Mr. Dennis, Mr. Topping, or Mr. Matthews, and when fixed upon the slides and ready for use, vary in price from threepence to two shillings or half-a-crown each.

75. Shallow-built Glass Cells.—Very large glass cells cannot be made by any of the above processes; and when only one cell of a particular size is required, it will be better to make it according to one of the following plans. The simplest method of constructing such a cell, provided it be not required of great depth, is the following:—A piece of thick plate glass, of the proper dimensions, is selected, and portions of equal width are cut off from the four sides, as shown in the annexed diagram (fig. 72); these are afterwards fixed in their proper places on the slide, with marine glue, care being taken to place the surface which has been cut by the diamond downwards, so that the furrows thus made may become filled up with marine glue. The upper surface is then to be ground rough with a little emery powder.

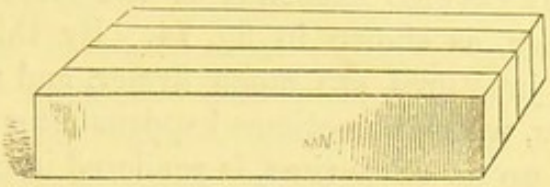
Fig. 72.



76. Deep-built Glass Cells.—If the cell be required of considerable depth, it must be made by joining together four strips of plate glass, the edges and ends of which have been ground perfectly flat and smooth. The following is the method of pro-

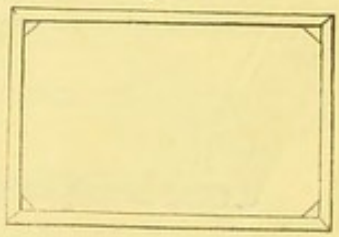
ceeding:—Four strips, of any depth and length required, are carefully cut off a piece of thick plate glass (§ 64); the surfaces of the four pieces are then joined together with marine glue, in the usual manner, and are adjusted as evenly as possible (fig. 73). When cold, both edges are ground perfectly square on a very flat stone, with common sand and water (§. 67). Time, of course, will be saved by using a grindstone, but the above method answers very well.

Fig. 73.



By joining the slips of glass in this manner, we must necessarily obtain the ground surfaces perfectly square, a point of great difficulty if each piece be ground separately. The ends of the slips of glass must now be ground perfectly square; all together, if the sides of the cell are to be equal; but if two are to be longer than the others, they must be separated and ground two and two. When the cut surfaces are ground perfectly even, the glass slips may be separated from each other, and the ends, after being cleaned, joined together with marine glue. The junctures are rendered stronger by fixing a small triangular piece of glass in each corner, as shown in fig. 74. The triangular piece is prepared by grinding down one of the angles of a very narrow strip of plate glass, which may then be cut into the proper lengths with a file. To join the ends we must proceed as follows: the ends of two of the sides are first warmed on the hot plate and joined with marine glue, taking care that they are placed perfectly square, and that the triangular piece is in its place; when cool, a third side is added with the same precautions; and lastly, the fourth is joined to the others: if the slips have been properly ground, this is easily effected, but if the ends be

Fig. 74.



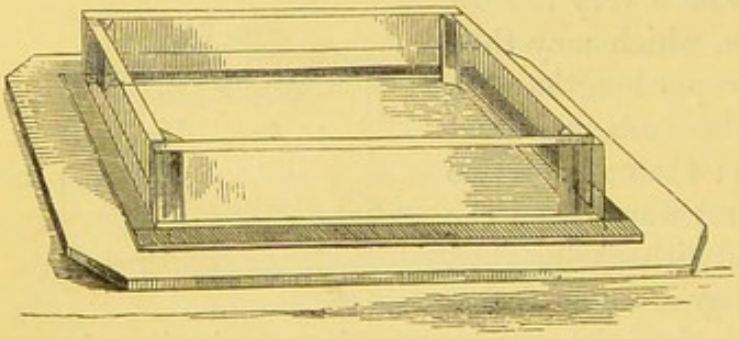
not perfectly square, it is quite impossible to make a good cell.

Mr. Dennis, who makes most excellent cells of this kind, has lately bevilled the ends of the sides before joining them together, as shown in fig. 74. By this excellent arrangement, the junctures are much firmer, and the future collapse of the sides, which sometimes happens after the preparation has been put up for some time, is rendered impossible.

When the angles are all joined, and the glue hard, it becomes necessary to grind both surfaces of the cell again, in order that they may be perfectly flat before it is attempted to fix the cell on the glass slab. This last grinding must be conducted very cautiously, particularly if the cells be large; one surface may be ground, and this fixed to the slab in the usual way, taking care not to raise the temperature too high, for fear of melting the glue by which the corners are joined together. The slab of plate glass should be cut rather larger than the cell, and the edges ground smooth.

When the slab is sufficiently heated, small pieces of glue are to be placed upon it, and, when these are melted, the cell may be applied, by aid of forceps, as described in § 68; narrow strips of glass should then be fixed all round the cell, as shown in the figure, care being taken that every point of the cell is connected with the slab by the glue. After the whole has become quite cold, the other surface of the cell may be ground smooth and the superfluous glue afterwards scraped off, and the cell thoroughly cleaned with potash, naphtha, or ether. (Fig. 75.)

Fig. 75.



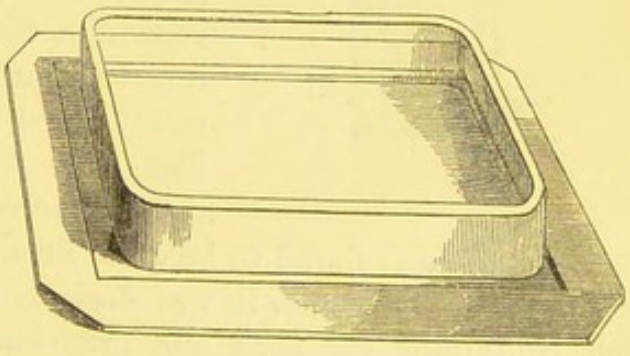
Cells made in this manner occasionally leak after a time, in consequence of the numerous joints which they contain; and

this is more particularly liable to happen if tolerably strong spirit be employed to preserve the preparation, because marine glue is softened by it.

For the method of making the built glass cells, and indeed most of the cells mentioned in the present chapter, we are indebted to Dr. Goadby; to whom also the thanks of practical observers are due, for the many improvements suggested by him in several branches of manipulation, especially in making minute and delicate dissections.

77. New method of making deep Glass Cells.—A method of making these large glass cells, which I have adopted for some time past, to a great extent, obviates some of the defects of the built glass cell, and, at the same time, the cell is made in much less time. Instead of joining the angles of separate pieces of glass together with marine glue, I take one long strip of plate glass of the proper depth, and with spots of ink accurately mark the length of the sides of the cell. At these marks the glass is carefully bent at a right angle in the flame of the blowpipe, taking care to keep each side perfectly square as it is bent; the ends are joined together in the blowpipe flame, and the surfaces are afterwards ground even on the stone. The heat must be applied very gradually, commencing below. In this process there is some difficulty in preventing the glass from cracking as it cools. This may, to a certain extent, be avoided by allowing the cell to cool very gradually, or, what is better, by placing it in an oven for a short time. If flatted flint glass could be obtained, the process would be very easy of execution, and cells of considerable depth might be readily made. When the surfaces have been ground flat, the cell is fixed to a slab in the usual way (fig. 76).

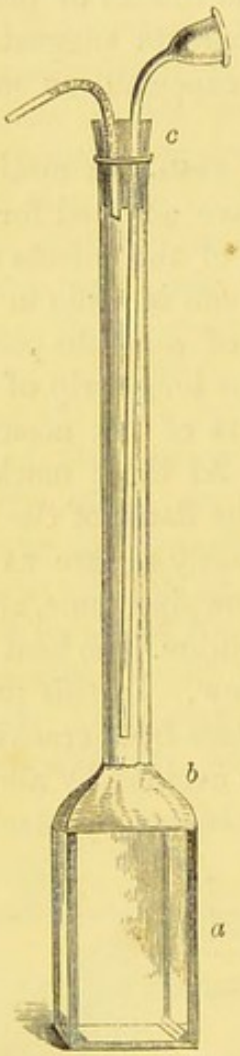
Fig. 76.



78. Cells made with the aid of Gutta Percha.—Occasionally

cells may be required of very peculiar shapes, wide perhaps in one part, and narrow in another; or of a form which it would be very difficult to make with glass only. Some time ago I required a cell of such a form that it would contain a proteus, while the circulation in the branchiæ could be observed under the microscope. The part of the cell containing the branchiæ and head must necessarily be perfectly flat, otherwise the object would not be sufficiently distinct.

Fig. 77.



A cell of a form which would enable the animal to be kept quite steady, and which allowed the water to be frequently changed while he was under observation, was made as follows:—A built cell of the form shown in fig. 77 *a*, was made in the usual manner (§ 67), one end only being left open. A piece of tube, of rather less diameter than the cell in its shortest dimensions, was then fixed to the open end with gutta percha, made soft by soaking in boiling water (fig. 77 *b*). Two pieces of glass tube of the form shown in the figure at *c* were then inserted into a cork, which accurately fitted the mouth of the tube. The total length of this cell was about twelve inches. The gutta percha joint was found to be quite firm, and the cell answered the purpose for which it was designed. The flat part of the cell, containing the head and branchiæ, was placed upon the stage of the microscope, and the animal supplied with fresh water from time to time by the funnel tube. In this way the

proteus could be kept under observation for upwards of two hours. The same plan may be successfully followed in making cells of other shapes.

On the subject of making cells, Papers by Dr. Goadby, in Silliman's "American Journal of Science and Arts," 1852, and "Quekett on the Microscope," may be also consulted with advantage.

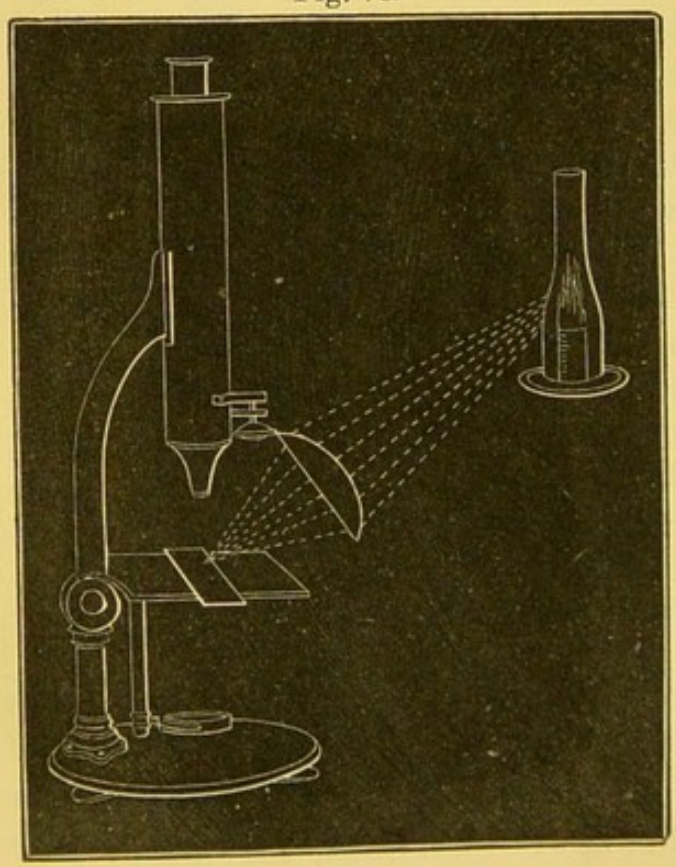
CHAPTER VI.

EXAMINATION OF OBJECTS BY THE MICROSCOPE—REFLECTED LIGHT—TRANSMITTED LIGHT—LAMPS—SUBSTANCES EXAMINED IN DIFFERENT MEDIA—DISSECTION UNDER THE SURFACE OF FLUID.

REFLECTED LIGHT.

79. Examination of Objects by Reflected Light.—Reflected light is employed for the examination of the surface only of substances placed in the field of the microscope, these objects being often too thick to admit the passage of light through them. In those cases in which the surface of transparent objects is to be examined, a piece of black paper, or cloth, or other opaque substance must be placed behind in order to prevent the passage of any rays of light through them. The light is usually rendered more intense by being condensed upon the object by means of a bull's-eye condenser (fig. 79). This is the method usually resorted to for

Fig. 78.

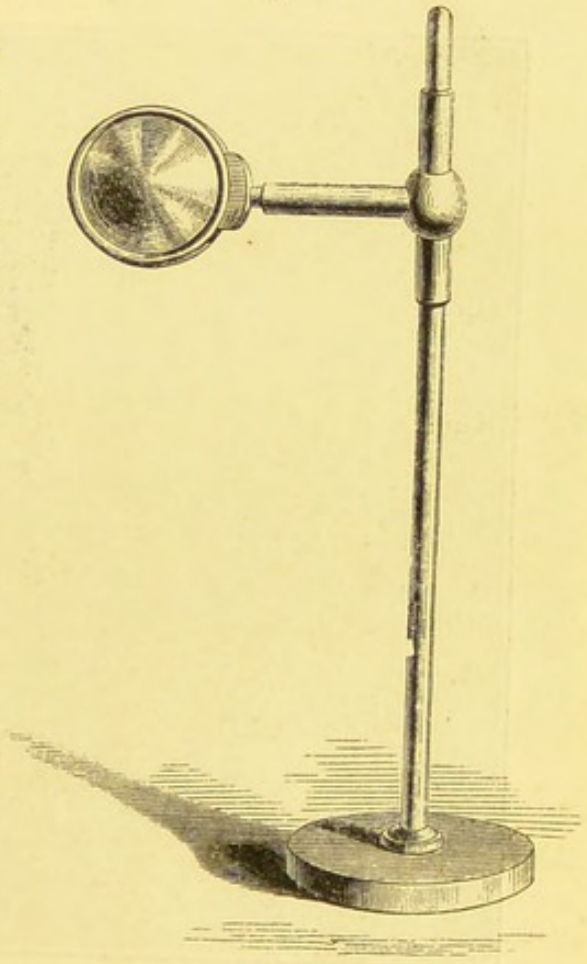


the examination of objects by reflected light. The microscope, light, and condenser are arranged as shown in fig. 78.

TRANSMITTED LIGHT.

80. Examination of Objects by Transmitted Light.—This mode of investigation is by far the most important with which the microscopical observer will be concerned. All transparent objects are examined in this manner, and it is that which more especially concerns the medical practitioner, for it will be according to the facility which he acquires in making thin sections of various tissues which he will subject to examination by transmitted light, that his success in this branch of clinical inquiry will depend.

Fig. 79.

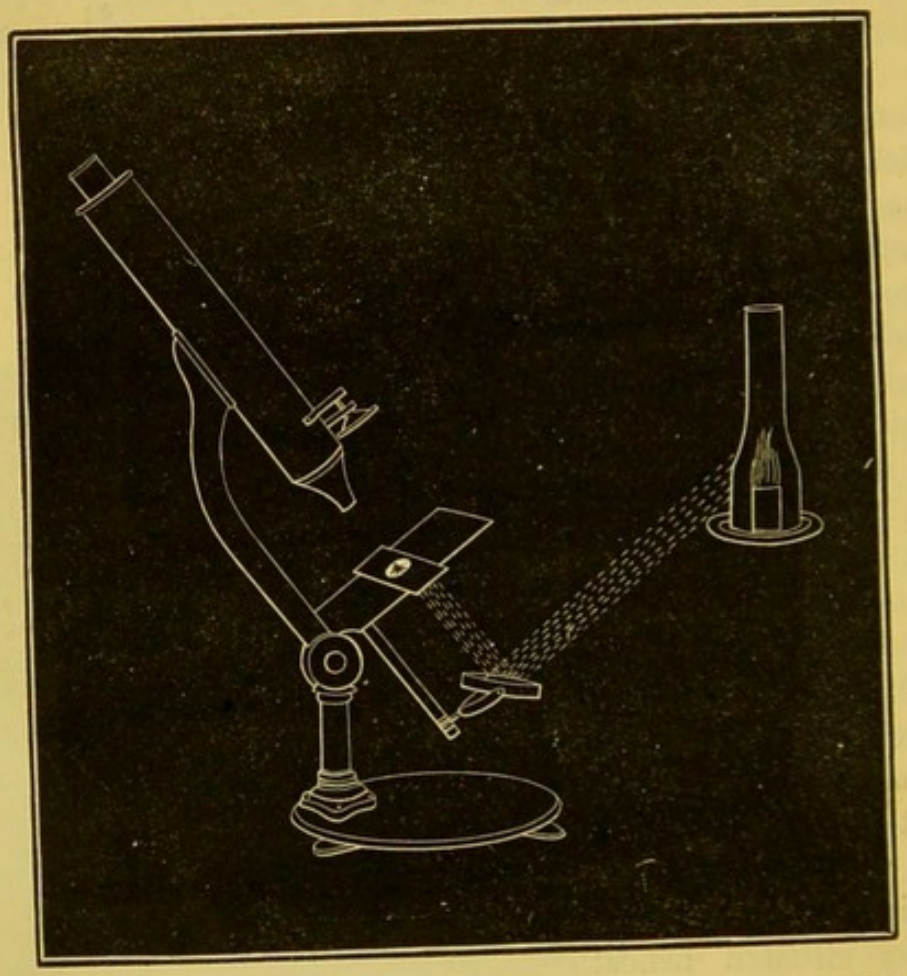


The illumination of objects which are to be examined by transmitted light may be effected in two ways, either by reflecting the light from a mirror situated beneath the stage (fig. 80), or by arranging the instrument in such a manner that the direct light from the lamp may be transmitted through the object. The former plan is the one commonly adopted, and it is the only one that need occupy attention here.

The different appearance of the same objects under the influence of transmitted and reflected light is represented in § 82.

81. Illumininations of Transparent Objects — Lamps. — The best light for microscopical examination is ordinary daylight. If the sun be shining, the light should be obtained from some other part of the sky, as the direct rays of the sun are too dazzling to enable the observer to examine the minute structure of objects, and should never be employed. It has been said that the best light for microscopical examination is to be

Fig. 80.



obtained from a cloud opposite the sun. Of artificial lights, the gas-lamp shown in fig. 81 is to be preferred.

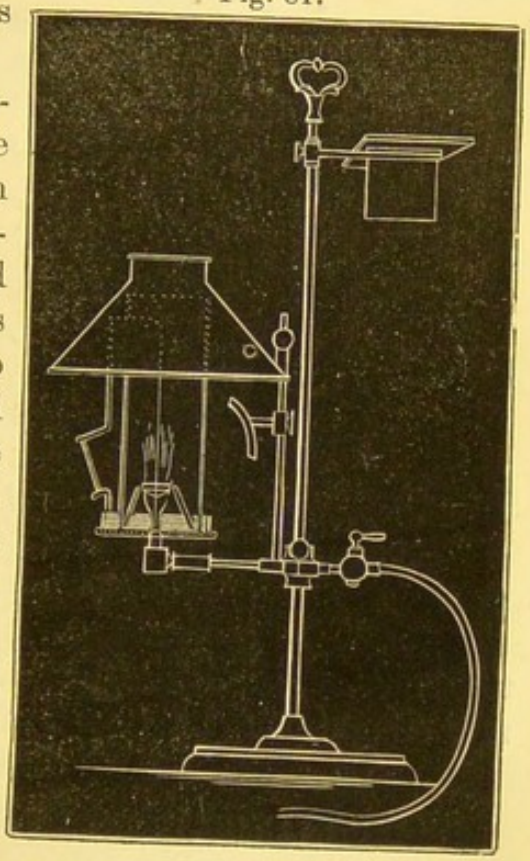
This lamp was devised by Mr. Highley, of Fleet-street, and a description of it will be found in the second number of the "Microscopical Journal," p. 143.

The burner is an Argand, with very small holes. This is covered with a Leblond's blue-glass chimney; and the light

before passing to the mirror is transmitted through a circular piece of neutral-tint glass (which is fixed in the brass shade), as well as through the blue-glass chimney. Thus an unusually white light is obtained, which is very powerful and quite steady.

The lamp is also fitted with a small water-bath, and brass plate for mounting cells, &c.

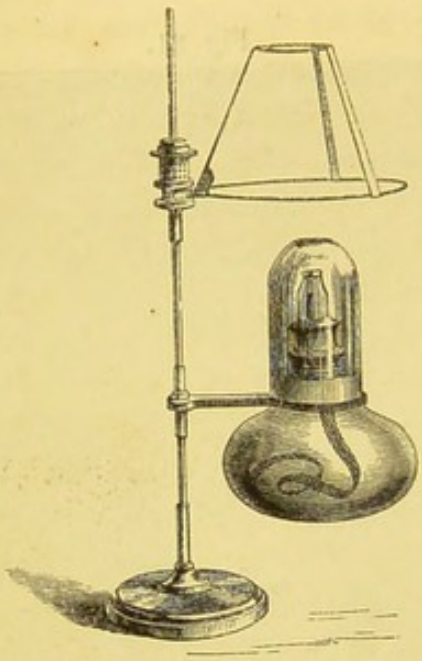
Fig. 81.



An oil-lamp, constructed upon the same principle as the gas-lamp just described, is an excellent substitute. A common fish-tail gas-burner covered with a ground glass answers exceedingly well, and so also does the white light produced from a gas lamp covered with one of the opaque white bell-glasses. The small camphine lamp made by Messrs. Smith and Beck, fig. 82, forms a very convenient, and a most efficient means of illumination to those who do not possess the advantages of gas. The French moderator lamps, which are now coming into general use, are excellent lamps for the microscope. An ordinary Cambridge reading-lamp also answers very well; and where nothing better can be obtained, the light derived from a common wax candle may be employed, more particularly if it be protected with a glass in order to prevent flickering. In all cases the observer will find it a great comfort to protect his eyes from the direct glare, either by covering the lamp with a tin or paper shade, or by placing a piece of thick cardboard around the eye-piece of the microscope, in such a manner as to prevent his eyes being dazzled by the lamp, while at the same time he is enabled to look through the instrument without inconvenience.

It cannot, however, be too much insisted upon, that microscopical examination should be as much as possible avoided at night; and there cannot be a question that artificial illumination, however perfect it may be, is highly injurious to the eyes; and if persisted in for any length of time will lead to serious impairment of vision. Every observer who has had any experience with microscopical examination by candle-light, can testify to the fact that working for a few hours fatigues the eyes greatly, although by daylight no such effect is produced.

Fig. 82.



For the examination of opaque objects, such as injections, &c., artificial light is advantageous, particularly if the bull's-eye condenser be used, in order to concentrate the rays of light more fully upon the object.

EXAMINATION OF TRANSPARENT OBJECTS.

When an object is to be examined by transmitted light, the mirror should be so inclined as to reflect the rays directly through it, except in those cases in which exceedingly delicate structures are subjected to examination; when minute lines or points, which were previously invisible, will be brought into view if the mirror be placed so as to throw the rays obliquely through the object. If sufficient light can be obtained in that way, a plane mirror is the best, but if a powerful light is required, a concave reflector must be substituted. The larger microscopes are furnished with both, but to some of the smaller instruments a slightly concave reflector alone is attached. Mirrors made of porcelain, plaster of Paris, and perfectly white

writing paper, have been used with certain advantages, but they are not commonly employed, and are only necessary for certain special investigations (§ 20).

The well-known appearance of air-bubbles and oil-globules when examined in a fluid medium, is due to the difference of their refracting power, and should be familiar to the eye of every microscopic observer, for in the course of his investigations he will be constantly meeting with these bodies. Figs. 83 and

Fig. 83.

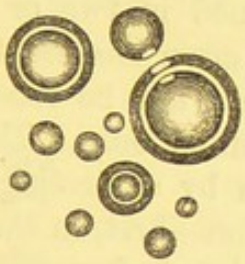
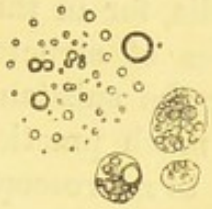


Fig. 84.



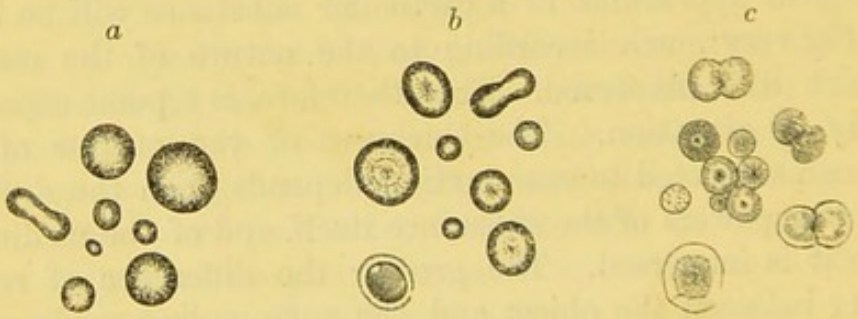
84 show the appearance of air-bubbles and oil-globules of different sizes, in water, when examined with a quarter of an inch object-glass.

82.—**Influence of the medium in which the substance is immersed upon its appearance in the Microscope.**—The microscopical appearance of a particular substance will be found to differ very much according to the nature of the medium in which it is immersed. This, therefore, is a point especially worthy of attention. The thickness of the outline of any specimen subjected to examination depends upon the different refracting powers of the substance itself, and of the medium in which it is immersed. The greater the difference of refrangibility between the object and the surrounding medium, the thicker will the outline appear; but in those cases in which the refracting power is nearly equal, the outline of the specimen will appear as a very thin line. Fig. 87 *c*. If the refracting power of two substances is quite equal, one cannot be distinguished from the other, except by variations in colour, &c.

If we treat blood corpuscles with a drop of acetic acid, or of a solution of an alkaline carbonate, we shall find the membrane of the corpuscle swell up, become more transparent, and appear to dissolve. This solution is rather apparent than real, for if a little solution of iodine be added, the cell wall will become again visible, and it probably depends upon a change taking place in the refracting power of the corpuscle, by which it approximates very nearly to that of the liquid in which it floats. After the addition of strong acetic acid to a drop of blood, the globules will still be faintly visible with a dull light, although by a strong light they are quite invisible.

Many substances should be examined in two or three different media, for in this way we are often enabled to discover peculiarities of structure which could not be recognised by examining the substance in one medium only. The student will gain much practical information by subjecting the same substance to microscopical examination—first, in the dry way; secondly, in water; and, thirdly, dried and afterwards mounted in turpentine, oil, or Canada balsam. The most instructive specimens to examine in this manner are crystalline substances and hard tissues, the intimate structure of which is not destroyed by drying and by being subsequently moistened with different fluids.

Fig. 85.



In fig. 85 is represented the appearance of some crystals of carbonate of lime from horses' urine, examined in different media; *a*, in the dry way; *b*, in water; *c*, in Canada balsam; and under the influence of reflected light (fig. 86 *a*).

In fig. 87 are represented some crystals of oxalate of lime under the same circumstances.

Fig. 86.

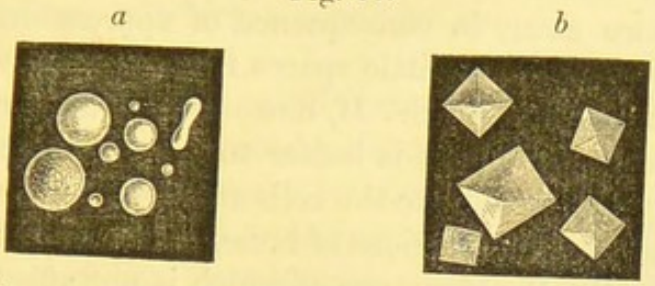
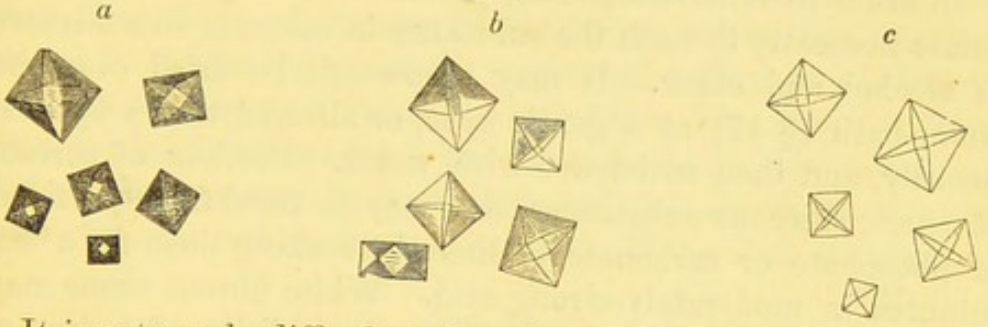


Fig. 86 *b* represents some of the crystals of oxalate of lime viewed by reflected light.

Fig. 87.



It is extremely difficult to lay down rules which will enable the observer to tell which method is best adapted to display the microscopical characters of any particular substance to the greatest advantage.

Substances having naturally a very smooth or polished surface, or to which such a surface can be artificially given, if sufficiently transparent, may be put up as dry preparations.

Thin sections of bone and teeth, specimens of hair and several crystalline substances, may be mounted in this manner.

Substances found moist in their natural state will usually require to be mounted in fluid. In certain cases, however, where outline and general form only are required, the objects may be dried; as, for instance, blood corpuscles or spermatozoa, which exhibit their general characters very well when mounted in this manner. Specimens of epithelium, muscle, nerve, and most of the tissues in a healthy or morbid state, require to be immersed in fluid.

If the substance be very dense and opaque, or contains small

cavities filled with air, which are rendered indistinct by the opacity of the surrounding texture, it will be advantageous to examine it in Canada balsam, which will render the intervening substance more clear, in consequence of approaching it in refracting power, while the little spaces filled with air will appear quite dark and well defined. If, however, the walls of the cavities are to be examined, it is better to use turpentine, or thin balsam, which will penetrate the cells and drive out the included air. Moderately-thick sections of bone, teeth, or shell, and substances generally, the structure of which is not affected by drying, such as hair, nails, horn, &c., may be mounted in Canada balsam.

In order to remove adhering particles of fatty matter, it becomes necessary to wash the substance in ether, or in a mixture of alcohol and ether. It may afterwards be dried over the water-bath (§ 47) at a gentle heat, or allowed to dry spontaneously, and then moistened with water. Portions of certain textures, siliceous substances, &c., may be freed from particles of phosphate or carbonate of lime, by soaking them for a few minutes in moderately-strong acid. White fibrous tissue may be rendered clear and transparent by the addition of a drop of acetic acid. This is an operation which is frequently necessary in examining various textures, particularly if we wish to ascertain the presence of a nucleated structure, or of yellow elastic tissue. These substances having been completely obscured, in consequence of the abundance of the white fibrous tissue, which becomes perfectly transparent after the addition of a drop of acetic acid. Certain other processes are requisite in special cases, which will be more particularly dwelt upon when the methods of examining the different textures and deposits are considered.

83. Importance of cleaning Specimens for Microscopical Examination, and of separating them from other substances with which they may be mixed.—Substances intended for examination should always be carefully separated from impurities, such as dust, &c., which would render their structure indistinct. Washing in water, or other liquid, is often very necessary. In order to effect this object, the substance may be simply held

in forceps, and shaken about in a glass of water, or a small stream of water may be projected upon it from a wash-bottle (fig. 88), or from a common syringe, to which a small jet has been attached.

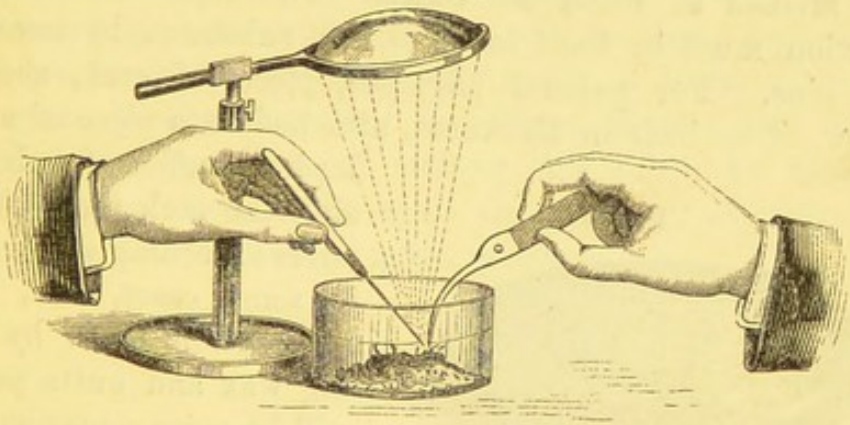
Fig. 88.

Deposits may be separated from dust and lighter particles by treating them with water, and after subsidence, pouring off the supernatant fluid, and replacing it with fresh, as often as may be necessary.

Large particles may be picked out with the aid of forceps or with needles.

84. Dissection under the surface of Fluid.—In making minute dissections of certain tissues, great advantage is often gained by dissecting under the surface of water; or of alcohol, in those instances in which the substance has been for some time previously immersed in the latter fluid. In this way, many delicate tissues may be separated and floated off, as it were, from the adjacent textures (fig. 89). When it is required to

Fig. 89.



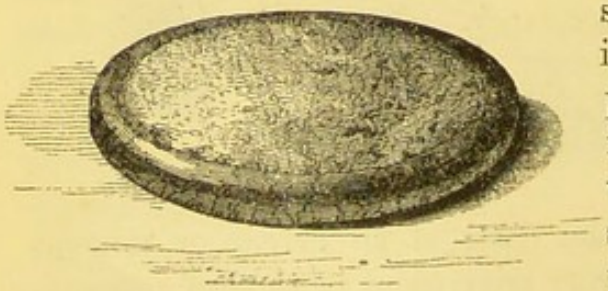
separate from each other the coats of an artery or mucous membrane, this becomes a very convenient method of proceeding. In certain cases, however, in which tissues are intended for microscopical examination, a certain amount of change of structure must be expected; the epithelium will often be abraded, and the cells much distended from endosmosis, so that such tissues should be immersed as short a time

as possible. In tracing sweat, or other minute ducts, or delicate branches of nerves, more especially in the lower animals, dissection under the surface of fluid is the only method by which success can be anticipated.

85. Glasses for dissecting under Water.—The vessel for containing the fluid should be proportioned to the size of the preparation to be dissected, and it should be of sufficient depth for the complete immersion of the object, fixed upon a loaded cork in its proper position; but at the same time the vessel should not be too deep, because greater difficulty would be experienced in following out the delicate branches of nerves, &c. The upper surface of the object should not be more than a quarter of an inch under the surface of the fluid (fig. 89). Vessels for this purpose may be made of glass, zinc, or earthenware. For small dissections, circular glass cups, similar to those sold in water-colour boxes, will be found most convenient; but for larger preparations a square zinc trough may be employed. The square-built glass cells (page 64) used for mounting anatomical preparations will often be found very convenient for dissection in fluid.

86. Method of fixing the Object.—The object intended for dissection must be fixed to some soft substance by means of small pins. For general purposes, pieces of cork, about a quarter of an inch in thickness, attached to a piece of sheet-lead, of sufficient weight to sink the cork (fig. 90), answers exceedingly well; but, in some cases, wax, or a preparation made by mixing wax and gutta percha, will be more convenient. A small plate of this mixture may be placed at the bottom of the cell in which the dissection is to be made, and may easily be fixed in its place by being a little pressed down at the edges, after it has been placed in the cup; or it may be poured while hot into the bottom of the cell, and there allowed to cool.

Fig. 90.



The preparation is to be fixed by means of small pins, or small pieces of thin silver wire, in a convenient position for dissection; and as the fluid becomes turbid from the accumulation of small portions of tissue which have been removed, it is to be poured off and carefully replaced by fresh. From the cell in which the dissection is made, the preparation, intended for mounting in a glass cell, may be removed by allowing it to float with some of the fluid into a watch-glass or tea-spoon, and in this manner it may be transferred.

87. Preparation of Wax and Gutta Percha for fixing Objects to, for Dissection, mounting in Cells, &c.—This preparation is readily made by heating about equal parts of white or yellow wax and gutta percha in a pipkin, over a coke fire, or gas lamp (care being taken to prevent the mixture catching fire, as it is very inflammable), and stirring with an iron rod until an uniform fluid mass is obtained. In order to give a darker colour to the mixture, lamp-black or indigo may be added. Cakes of this substance may be readily made, by pouring it while hot into a tin tray, which has been slightly wetted to prevent the wax adhering too firmly. When cold, this mixture forms a material which will hold a pin firmly, and which is not so brittle as wax. A very thin layer will be found sufficient for most purposes. If required very tough, a larger quantity of the gutta percha than that mentioned in the text may be added. The operation of making this substance should always be performed in the open air, as a very offensive smell is evolved from the melted gutta percha.

CHAPTER VII.

OF PREPARING OBJECTS FOR MICROSCOPICAL EXAMINATION, AND OF PRESERVING THEM—MOUNTING OBJECTS IN A DRY STATE—IN FLUID. PRESERVATIVE SOLUTIONS. MOUNTING OBJECTS IN CANADA BALSAM. ARRANGING PREPARATIONS IN THE CABINET.

Substances require different methods of preparation, in order to display their minute structure to the greatest advantage. As already indicated (page 74), the nature of the medium in which the body is immersed, determines, to a considerable extent, its microscopical appearance. The appearance will also be modified according as the examination is made with transmitted or reflected light (§ 79, 80). In many cases it is important to subject a specimen to examination in two or three different ways, as described in page 74.

The mode of examination and the methods of preserving objects, may be arranged under three heads, each of which will now be separately considered:—

1. Preservation of objects in a dry state.
2. Preservation of objects in aqueous fluids.
3. Preservation of objects in turpentine, oil, Canada balsam, or some highly-refracting medium.

OF MOUNTING OBJECTS IN A DRY STATE.

88. Examination and Preservation of Objects in a dry state.—If it is only required to examine the character of a specimen in a dry state, it may simply be laid upon a glass slide, and placed in the field of the microscope; if, however, the substance be of a very delicate structure, or in a minute state of division, it is

better to place a piece of thin glass over it in the usual manner, in order to protect it.

Dry objects may be mounted in a thin glass cell (§ 71), or in a paper cell, or, if of extreme tenuity, they may simply be placed on a glass slide, and covered with thin glass, which should be fixed to the former by a small piece of gummed paper (rather larger than the glass cover), in the centre of which a hole has been cut of sufficient size to permit the entire object being seen. The paper may of course be of any colour, or ornamented according to the taste of the operator.

When objects are to be examined by reflected light, they may be placed in little glass or cardboard cells, or in pill-boxes; or they may be put up in glass cells. The preparation should be placed upon a dark ground, which may be effected either by cutting a piece of dark blue or black glazed paper, of the exact size of the cell, and placing it within; or the black paper may be fixed on the posterior surface of the slide; or this surface may be covered with black paint or black varnish (§ 59).

OF THE PRESERVATION OF OBJECTS IN AQUEOUS FLUIDS.

89. Examination of Objects in aqueous Fluids.—There are various methods by which preparations may be subjected to examination, and preserved as permanent objects in a moist state, and the different value of the various preservative solutions which are in use, entirely depends upon the nature of the substance to be mounted. Distilled water forms a very good fluid for some objects, while for the preservation of most, it is necessary to immerse them in water impregnated with some antiseptic agent, which is not volatile at ordinary temperatures. Many, again, are best preserved in spirit, or in a solution of some salt. It is very difficult to lay down rules which will enable the observer to choose a preservative fluid for any particular specimen. A little experience, however, will soon enable him to judge which solution is best adapted for the purpose. In those cases in which special solutions are of advantage, it will be stated.

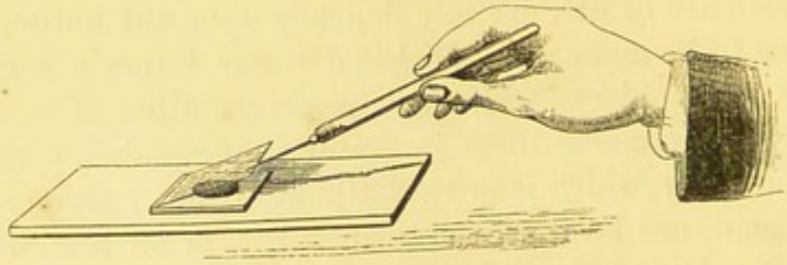
90. Of mounting Objects immersed in fluid in Cells.—Cells

of any form or size may be used in mounting objects in the moist way, according to the size and nature of the preparation. Some objects require to be pinned down to a piece of wax (§ 87), others may be allowed to float in the fluid in which they are placed. Many require a certain amount of pressure between the glasses in order to display them well. The operation, however, is performed in much the same way in each case, and the same general rules will apply to all moist preparations.

After the object has been allowed to soak for some time (a day or two) in some of the fluid in which it is to be preserved, it may be removed by means of forceps, or a pipette, to the cell, which should be previously filled with the solution. After all air-bubbles have been carefully removed by shaking the preparation a little in the fluid, or by conducting them to the surface by means of a camel's-hair brush or a fine wire, the object may be placed in the proper position. The cell should then be quite filled with solution, which may be caused to rise above its walls by pouring it in very carefully, or by letting it gradually flow from a pipette.

91. Of placing on the thin glass cover.—The thin glass cover which should of course be rather less than the external dimensions of the cell, and previously cleaned with a little spirit of wine, may then be taken in the forceps, and, after gently

Fig. 91.



breathing upon the lower surface, one edge is allowed to touch the fluid, and the glass cover may then be permitted to fall gradually, until it floats on the convex surface of the solution; this may be conveniently effected with the aid of a needle, as shown in fig. 91. Gentle pressure is then applied in order to force out the superabundant fluid, which must be sucked up by

a cloth, soft sponge, or piece of blotting paper; or, if in great quantity, it may be removed with a pipette.

The edges of the cell may be allowed to dry for a minute or two, and the thin glass cover may be fixed in its place by putting on a thin layer of gold size, Brunswick black, or solution of shell-lac, with a small camel's-hair brush, or with a small piece of soft wood cut to a blunt point, and hammered in order to separate the fibres a little, which in many instances is more convenient. A very thin layer of the gold size or varnish should be first applied, and this should be allowed to dry thoroughly before a second is put on; if a large quantity be painted on at once, some of it will probably run into the preparation, or the surface of the varnish, in consequence of becoming hard before the inner portion, will contract; the cement being gradually drawn from the surface of the cell, a little of the fluid evaporates, air enters, and the preparation requires remounting. It is a very good plan to put a little gold size round the edges of the thin glass before placing it on the fluid, as by this method air is not so likely to be admitted into the cell while the edges are drying, previous to the application of the varnish externally. The best way of preventing the ingress of air after the preparation has been mounted for some time, is by first applying a few layers of gold size or black carriage varnish, and when these are thoroughly dry, painting them with a solution of shell-lac or sealing-wax in spirit, taking care that each subsequent layer of varnish is a little broader than that previously applied.

92. **Of mounting Preparations in large Glass Cells.**—The method of mounting preparations in the large glass cells is somewhat different to that employed in putting up small microscopical preparations. In consequence of the great size of the cover, it is more difficult to apply than those of the small cells, and gold size or Brunswick black are not very well adapted to fix it to the walls of the cell. After the vessel has been well cleaned with a little weak spirit, the preparation may be placed in it, either floating in the preservative solution, or fixed to a piece of mica, which is cut to fit the cell exactly. Or, perhaps, it may be displayed to greater advantage by being pinned to a tablet of wax, or of the composition described in

§ 87, cut so as to fit the cell exactly. The preparation may also be kept in its proper place by strings attached to loops of thread, fixed in various parts of the cell. The loops may be placed in any situation, by attaching them first to a small piece of glass, which is to be cemented to any part of the cell with a little marine glue, and the aid of a hot iron. This was the method employed by Mr. Goadby in mounting his beautiful dissections of the nervous system of the lower animals, many of which are now in the Museum of the College of Surgeons. When the preparation is placed in its proper position, the cell should be filled with fluid. The cover, after being carefully cleaned, may be put on, by allowing one end first to touch the fluid, and then gradually inclining it until it falls in its proper position, the cell being quite full of fluid, and free from air-bubbles.

93. Methods of fixing the Glass Cover on the large Cell.—The next operation is to fix the cover in its place. All excess of fluid round the edges must first be removed with a soft cloth, piece of sponge, or blotting-paper, and then a layer of thick solution of shell-lac (§ 58) should be immediately applied round the cover, to connect it to the walls of the cell. The edges of the cover may be advantageously anointed with a little of the shell-lac solution before it is applied.

Mr. Goadby fixed on the cover of his large cells with marine glue. This he effected as follows:—The cell being quite clean, and the preparation having been arranged in the proper position, a little of the preservative fluid is to be poured in. The cover, with a hole already drilled in one corner, was applied, and fixed in its place with marine glue, which was melted with a hot iron. When the top was thoroughly fixed with marine glue, the cell was filled with fluid through the hole. After all bubbles had risen to the surface and had been replaced with fresh fluid, the hole was stopped up with a cork, and a small piece of glass was cemented over it to keep the cork in its place. The hole should not be closed for a day or two, in order to permit all the air-bubbles to rise to the surface.

The method of which I have had the greatest experience is

the following:—Some of the French cement (§ 63) is rolled out into thin strings, which are pressed all round the top of the cell with the finger and thumb, care being taken that there is not more cement in one part than in another. This operation must be performed before the preparation is placed in the cell. One small space, about a quarter of an inch wide, is left uncovered with cement at one end of the cell.

The preparation is next put in, and the cell filled with the preservative solution up to the brim. One end of the cover is slightly pressed into the cement at the opposite end of the cell to that in which the vacant space was left; the cover is then allowed to fall gradually, until it everywhere touches the cement. The cell is now quite full of fluid. After the cover has been pushed sufficiently into the cement, and a certain quantity of fluid has escaped, the hole may be stopped up with a small piece of cement, and the preparation should then be allowed to lie still for a time, when it may be wiped dry, all superfluous cement removed with a knife, or smoothed down with a hot iron, and the edges painted with gold size or some kind of varnish. This plan is exceedingly simple and easy of execution, and appears to last quite as well as other methods. The great advantage of it is, that the cement never thoroughly hardens, but always retains sufficient elasticity to allow for change of volume of the fluid, when exposed to the extremes of temperature. If the preparation be preserved in strong spirit, however, the cell will not remain air-tight, as the spirit insinuates itself through the cement and evaporates,—air of course rushing in to supply its place.

The different cements employed for fixing the thin glass cover to the cell have been already described (§ 56-63).

Various other cements have been recommended for the purpose of fixing the cover upon large glass cells. Roman cement has been used by some observers: and my friend Mr. Stewart, who has had considerable experience with it, tells me it answers perfectly well.

PRESERVATIVE SOLUTIONS.

94. Spirit and Water.—Mixtures of spirit and water of various strengths are required for preserving different preparations. In diluting spirit, distilled water only should be employed; for if common water be treated with spirit, a precipitation of some of the salts dissolved in it not unfrequently takes place, rendering the mixture turbid and unfit for use. Proof spirit will be strong enough for all general purposes, except for hardening portions of the brain or nervous system, when stronger spirit must be used. Two parts of rectified spirit, about sp. gr. ·837, mixed with one part of pure water, makes a mixture of sp. gr. ·915-·920, which contains about 49 per cent. of real alcohol, and will therefore be about the strength of proof spirit. One part of alcohol, 60 over proof, to five parts of water, forms a mixture of a sufficient strength for the preservation of many substances.

95. Glycerine.—A solution of glycerine adapted for preserving many structures is prepared by mixing equal parts of glycerine with camphor water. The latter prevents the tendency to mildew. It may be used as other preservative solutions.

Glycerine is obtained by boiling oil with litharge. The oleate of lead remains as an insoluble plaster, while the glycerine is dissolved. It may be rendered free from lead by passing a current of sulphuretted hydrogen through it; and the clear solution, after filtration, may then be evaporated to the consistence of a syrup.

96. Thwaites' Fluid.—This fluid has been much employed by Mr. Thwaites for preserving specimens of desmidiæ; but it is also applicable to the preservation of animal substances.

Water . . . . 16 ounces.
Spirits of wine . 1 ounce.
Creosote, sufficient to saturate the spirit.
Chalk, as much as may be necessary.

Mix the creosote and spirit, stir in the chalk with the aid of a pestle and mortar, and let the water be added gradually.

Next add an equal quantity of water saturated with camphor. Allow the mixture to stand for a few days, and filter. In attempting to preserve large preparations in this fluid, I found it always became turbid, and therefore tried several modifications of it. The solution next to be described was found to answer very satisfactorily. Water may also be impregnated with creosote by distillation. It should be remarked that M. Strausdurkheim has succeeded in preserving preparations in camphor water only.

97. Solution of Naphtha and Creosote.

Creosote . . .	3 drachms.
Wood naphtha .	6 ounces.
Distilled water .	64 ounces.
Chalk, as much as may be necessary.	

Mix first the naphtha and creosote, then add as much prepared chalk as may be sufficient to form a smooth thick paste; afterwards add, very gradually, a small quantity of the water, which must be well mixed in a mortar. Add two or three small lumps of camphor, and allow the mixture to stand in a lightly-covered vessel for a fortnight or three weeks, with occasional stirring. Pour off the almost-clear supernatant fluid, and filter it if necessary. Preserve it in well-corked or stoppered bottles.

I have some large preparations which have been preserved in upwards of a pint of this fluid, for more than five years, and the fluid is now perfectly clear and colourless. Some dissections of the nervous systems of insects have kept excellently; the nerves keeping their colour well, and not becoming at all brittle. Two or three morbid specimens are also in an excellent state of preservation; the colour being to a great extent preserved, and the soft character of the texture remaining. I have one preparation mounted in a large gutta percha cell, containing nearly a gallon of this fluid.*

98. Solution of Chromic Acid.—A solution of chromic acid will be found well adapted for preserving many microscopical

* Mr. Quekett recommends a mixture of one part of naphtha to seven or eight of water as a good preservative solution. Op. cit., p. 281.

specimens. It is particularly useful for hardening portions of the nervous system previous to cutting thin sections. The solution is prepared by dissolving sufficient of the crystallized acid in distilled water, to render the liquid of a pale straw colour.

The crystallized acid may be prepared by decomposing 100 measures of a saturated solution of bichromate of potassa, by the addition of 120 to 150 measures of pure concentrated sulphuric acid. As the mixture becomes cool, crystals of chromic acid are deposited, which should be dried and well pressed on a porous tile, by which means the greater part of the sulphuric acid is removed, and the crystals obtained nearly pure.

99. Preservative Gelatine.*—

Gelatine .	1 ounce.	Spirits of wine	½ ounce.
Honey . .	4 ounces.	Creosote . .	6 drops.

Soak the gelatine in water until soft, and to it add the honey, which has been previously raised to the boiling-point in another vessel. Next, let the mixture be boiled, and after it has cooled somewhat, the creosote dissolved in the spirits of wine is to be added. Lastly, filter through thick flannel to clarify it. When required for use, the bottle containing the mixture must be slightly warmed, and a drop placed on the preparation upon the glass slide, which should also be warmed slightly. Next, the glass cover, after having been breathed upon, is to be laid on with the usual precautions, and the edges covered with a coating of the Brunswick black varnish. Care must be taken that the surface of the drop does not become dry before the application of the glass cover; and the inclusion of air-bubbles must be carefully avoided.

100. Goadby's Solution.—

Bay salt	4 ounces.
Alum	2 ounces.
Corrosive sublimate	4 grains.
Boiling water . .	4 pints.

* Mr. H. Deane, Transactions of the Microscopical Society, quoted in Quekett's Treatise on the Microscope.

Mix and filter. This solution may for most purposes be diluted with an equal bulk of water. For preserving delicate preparations it should be even still more dilute.

101. Burnett's Solution.—This fluid has been patented, and may be obtained in a concentrated form, in bottles of different sizes, 53, King William-street, London-bridge.

Its preservative properties appear to depend upon the chloride of zinc. A strong solution of chloride of zinc forms a very powerful antiseptic, and also possesses the property of absorbing noxious odours, &c.

102. Other saline solutions.— Many other saline solutions have been employed by different observers. Of these, a saturated aqueous solution of chloride of calcium, free from iron, has been much recommended for preserving specimens of bone, hair, teeth, and other hard structures, as well as many vegetable tissues (Schacht). A solution of alum in the proportion of 1 part of alum to 16 of water has been found to answer pretty well for some substances. Gannal's solution, which consists of 1 part of acetate of alumina dissolved in 10 parts of water; solutions of common salt (1 part to 5 of water, with a little camphor), corrosive sublimate, persulphate of iron, arsenious acid, sulphate of zinc, and solutions of several other salts, have been recommended as preservative solutions, but their employment has not been always attended with the most satisfactory results.

Arseniuretted hydrogen gas has also been recommended for the preservation of animal substances, but it is not adapted for microscopical preparations.

The particular preservative fluid adapted for the preservation of the different textures will be indicated when the method of preserving these is brought under consideration, and at the same time any special processes adapted for demonstrating the structure of different textures will be adverted to.

MOUNTING OBJECTS IN CANADA BALSAM.

103. Methods of drying the substance previous to its immersion in the balsam.—It is of the utmost importance that substances which are to be mounted in Canada balsam should be thoroughly dried before being placed in this menstruum, otherwise the preparation will always be covered with little bubbles of steam, which will prevent it from being seen distinctly. Many substances may be dried by leaving them exposed to the air, especially in the sun; but usually it will be better to expose them over the water-bath (fig. 47) for a short time, before putting them up. If a slight elevation of temperature does harm to the structure, it may be thoroughly dried by exposing it for some time over a dish containing strong sulphuric acid, the whole being covered with a bell-jar, or placed under a receiver, which may be exhausted by connecting it with the air-pump.

104. On moving air from the interstices of a tissue.—This may be effected by placing the substance, previously carefully dried in a little fluid balsam, under the receiver of an air-pump; any air in the tissue is then forced out as exhaustion proceeds, and its place becomes occupied with balsam.*

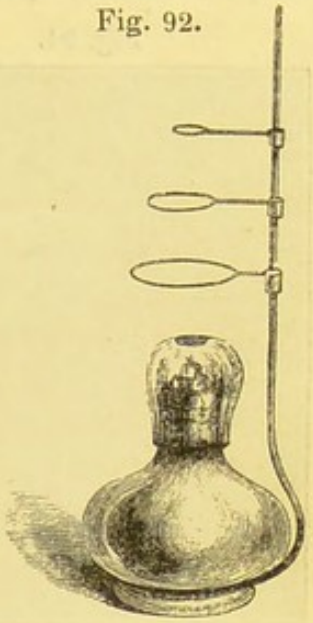
When membranes and thin sections of tissues, such, for instance, as sections of the spinal cord, muscular fibre, &c., are to be preserved in balsam, they must be very slightly washed, and then spread out upon the glass slide with the aid of needles and forceps. They should be allowed to dry spontaneously, and afterwards it is better to expose them for a short time over sulphuric acid, and the section may then be put up in the usual way, but without removing it from the slide. Frequently it will be found advantageous to wet the surface very slightly with turpentine before the balsam is applied. If the sections appear too thick when dry, the surface may be scraped with a sharp knife, or very thin shavings may be removed.

---

* "Dr. Golding Bird has applied this method for mounting the polypi doms of zoophytes with great success."—Microscopical Journal, 1853, p. 85.

105. Precautions to be observed in applying the Balsam.—Canada balsam (§ 61) forms a most useful agent for mounting various substances; and the structure of many can only be clearly made out when they are examined in this menstruum.

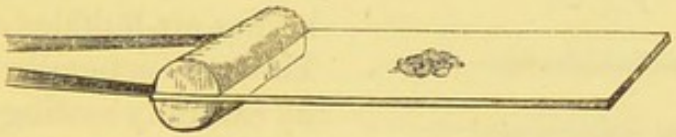
Fig. 92.



In this method of mounting objects no cells whatever are requisite. The balsam should be pale and old. The glass slides must be warmed before the balsam is put on, and for this purpose the glasses may be held in a pair of wooden forceps, or in a pair of common forceps, the legs of which are covered with cork (fig. 93) and heated over the spirit-lamp (fig. 92) or upon the brass plate (§ 46). The latter plan is the most convenient when several preparations are to be mounted at the same time, because they may be arranged in a row along the plate, and the balsam placed upon each slide as it becomes hot.

The Canada balsam may be heated after it is placed upon the slide, or the tin vessel, fig. 94, may be kept warm for some little time before the balsam is taken out, in order to allow the air-bubbles entangled in it to rise to the surface before it is applied.

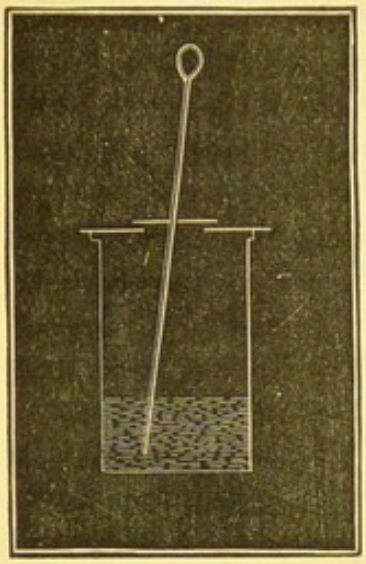
Fig. 93.



The slide being warm, and the small quantity of Canada balsam sufficient to contain the preparation having been placed upon it, it must be gently moved about while the balsam is hot and quite fluid, until all the air-bubbles have floated to the surface and collected together towards one spot. A pointed wire or needle should then be taken, and all the bubbles either drawn out upon the end of it, which may be readily effected, or broken by the wire after it has been heated. In those cases in which

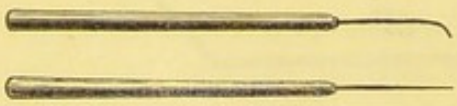
the preparation is not detached from the glass slide upon which it has been allowed to dry, it is only necessary to place the drop of balsam upon it and gently warm it, following the usual precautions; afterwards the thin glass cover may be applied. When the preparation has been dried separately over the water-bath and cleaned, it may be taken in a fine pair of forceps, gently warmed, and carefully placed in the hot and perfectly fluid balsam. After it has been thoroughly wetted by the balsam, and all adhering air-bubbles removed (§ 104), it may be placed in the position it is intended to occupy. The thin glass cover, adapted to the size of the preparation, having been previously cleaned and warmed, may then be taken in a pair of forceps, and, after being held over the warm balsam for a minute, allowed to fall gradually upon the preparation (beginning at one side), until it becomes perfectly wetted with the balsam. The glass may now be slightly pressed in order to force out the superfluous balsam, and the preparation allowed to cool.

Fig. 94.



106. Of removing the Air-bubbles from the Balsam.—The only difficulty of mounting preparations by this method consists in getting rid of all adhering air-bubbles completely. This may generally be effected by carefully heating the balsam on the slide, and the preparation, first separately, and then together, and by removing the bubbles after they have been made to collect at one spot, with the aid of a fine-pointed wire. A needle, which should be stuck into a small wooden handle, is a most convenient instrument for this purpose (fig. 95).

Fig. 95.



If the balsam be heated to too high a temperature it becomes hard and resinous before the preparation can be placed in the proper position; in such a case it is better to clean the slide

and use fresh balsam. Any balsam may be removed from the preparation by soaking it for some time in turpentine. Some preparations require boiling in the balsam, in order to drive out air contained in cavities in their interior. As a general rule, however, care must be taken to prevent the balsam from boiling, because many organic substances curl up and become entirely spoiled at this temperature.

107. Of mounting Preparations of considerable size in Fluid Balsam.—It is sometimes necessary to mount opaque objects of considerable size in liquid Canada balsam, in which case we must either place it in a glass cell or between two pieces of window-glass, which are joined together at the edges with sealing-wax; a plan followed by many continental anatomists. In this case the balsam should be perfectly fluid, but not thinned with turpentine, for Mr. Quekett has observed that if this be done, air-bubbles appear to be developed some days after the preparation has been finished, although when first put up it was perfectly clear. This result is occasioned by the turpentine not being thoroughly mixed with the balsam at first. After some days a complete admixture occurs, and little cavities are formed in consequence of the two bodies occupying a larger bulk when separate, than when intimately mixed with each other.

When the preparation has been placed in its proper position, and the thin glass has been applied, it only remains to remove any superfluous balsam from the slide. This is readily effected by the aid of some rags, an old knife, or the large bradawl, (fig. 96,) and a little turpentine. The greater quantity can be scraped off with the knife, and the remainder may be removed by carefully moistening it with turpentine, and then cleaning it off with a rag. The preparation is now finished, and may be left plain, the edges of the balsam varnished over, or the slide may be covered with ornamental paper, according to the taste of the operator.

Fig. 96.

NAMING PREPARATIONS AND THEIR ARRANGEMENT IN THE CABINET.

108. **Placing the Name on the Glass Slide.**—Every preparation should be named as soon as it is finished. The name is generally written at one end of the slide with the writing diamond, fig. 49, but a small paper label answers every purpose; and it is a good plan, when long descriptions are necessary, to affix a number to each slide corresponding to the number referring to the description of the preparation in a catalogue.

In this catalogue all the particulars having reference to the object should be entered, including a note of the date of mounting, and also describing the fluid in which it was preserved.

Cabinets for Preparations.—Microscopical preparations should be kept in drawers or in boxes prepared for the purpose, in order to preserve them from dust and from the influence of light. Dry preparations and those mounted in Canada balsam may be arranged in vertical grooves cut in the partitions of the drawer. The grooves should be wide enough to allow the slide to fall in easily, and should be about a quarter of an inch apart. Preparations mounted in fluid, however, must be kept in a horizontal position, although they necessarily occupy much space; but otherwise it will be found that the tendency for the cells to leak is much increased. Large preparations can only be kept lying perfectly flat in shallow drawers.

Boxes and cabinets of all kinds for keeping collections of microscopical preparations may be purchased of Mr. Topping, Messrs. Smith and Beck, and of Mr. Matthews.

Upon the subjects treated of in chapters VI. and VII., besides those works referred to in the text, the following have been consulted:—Translations from "Het Mikroskoop," Prof. Harting, Utrecht, in Edinburgh Monthly Journal; "Lectures by Dr. Goadby," in Silliman's Journal; "The Microscope, in its Special Application to Vegetable Anatomy and Physiology," Dr. Hermann Schacht, translated by Currey; "Ralph's British Desmidiæ;" Papers in the "Microscopical Journal," and "Transactions of the Microscopical Society;" "Traité pratique et théorique d'anatomie comparative," Paris, 1842, Strausdurkheim; "Nouveau Manuel de l'observateur au Microscope," Dujardin, Paris, 1843; Quekett, op. cit.

CHAPTER VIII.

ON INJECTING. INSTRUMENTS EMPLOYED IN INJECTING—COLOURING MATTERS—OF THE OPERATION OF INJECTING.

In order to examine the different arrangement of the ultimate divisions of the blood-vessels in various parts of the animal structure, it becomes necessary to fill them with some opaque substance, by which their distribution may be rendered distinct, and their general arrangement readily observed, when subjected to examination by the low powers of the microscope. When the capillaries are empty, they are scarcely visible, and frequently it is quite impossible to distinguish them from the tissues in which they ramify. The process by which these minute vessels are rendered distinct, is called *injecting*, and portions of structure which have been treated in this manner are spoken of as *injections*, or *injected preparations*. Injections are of two kinds,—natural and artificial. Natural injections, as the name implies, are obtained from the dead animal without any preparation whatever, the capillaries being gorged with blood, and thus rendered distinct. Artificial injections are always prepared by forcing some opaque or coloured liquid into the small vessels from a large one.

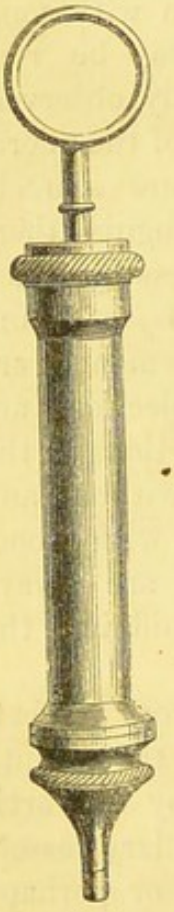
109. Natural Injections are frequently found very perfect, and in some instances show the arrangement of the minute vessels more perfectly than they can be exhibited by any artificial process. This condition results from the capillary vessels being distended with blood at the time of death, or perhaps soon afterwards, and the general redness which is always observed in a tissue in this condition, is said to be due to a state of congestion. Patches of the mucous membrane of the intes-

tines are often found in this state; and if a small portion be transferred to a small saucer of water, and examined with a low power, the vascular arrangement may often be observed very distinctly. Preparations of this description may often be stretched out and carefully dried on a glass slide without heat: when dry, the preparation may be mounted in Canada balsam. The vascular arrangement in some morbid growths may frequently be advantageously examined in this manner, although it is often quite impossible to make an artificial injection of the part.

INSTRUMENTS EMPLOYED IN MAKING ARTIFICIAL INJECTIONS.

Artificial injections require much care for their preparation, and a few instruments are used, of which it will be better to give a short description, before referring to the general arrangements for conducting the process.

Fig. 97.



110. Injecting Syringe.—The syringe should be of brass, and should be adapted to the size of the preparation which is to be injected. Small syringes have the advantage over large ones, as a general rule (fig. 97); and a syringe of an ounce capacity, will be found large enough for all ordinary purposes; but to inject organs of considerable size, or the larger animals, one holding a greater quantity is of course required. In choosing a syringe, care should be taken that the piston be at least half an inch in depth, and that when the finger is placed over the opening, and the piston drawn up so as to exhaust the interior, it readily returns to its former position when let go.

Fig. 98 shows a section of the piston of an injecting syringe. The arrangement is very convenient for re-leathering the syringe, as the buttons represented in the drawing can be unscrewed from the rod, when the old pieces of leather can be removed, and new ones substituted for them.

Syringes of less than two ounces capacity are conveniently made with a ring fixed on each side of the top, in order that the first two fingers may be firmly fixed, while the piston is gradually forced down by the thumb. The syringe must always be kept perfectly clean, and the piston should be well greased with some kind of fat; but oil should never be used for this purpose. Syringes of all kinds may be obtained of most instrument makers. The price varies according to the size; one capable of containing half an ounce costs about 3*s.* 6*d.*; one holding an ounce 4*s.* 6*d.*; and so on. The price of the stopcock is 2*s.*, and each pipe costs about 1*s.*

Fig. 98.

Syringes of this description are made by Mr. Neeve, Broad Street, Holborn.

111. Stopcocks and Injecting-pipes. — Every syringe should have a small stopcock (fig. 99) fitted to it for the convenience of its being refilled without the chance of any of the injection escaping from the vessel into which the pipe is inserted. The most convenient form of pipe is that delineated in fig. 100. The tube is of silver, and there are two projecting wires at the base, to which the thread may be attached after the vessel is firmly tied to the pipe, in order to prevent all possibility of slipping. Each syringe should be furnished with several pipes of various sizes. If the pipes get clogged up, they may be cleaned by passing a very fine wire down them; also, by being boiled in water, or gently heated in the flame of a lamp, they may be made pervious. When a very fine pipe is required, it has been recommended that the opening should be situated at the side, a short distance from the extremity, which may terminate in a blunt point, as this enables us to introduce it into minute vessels very readily.

Fig. 99.

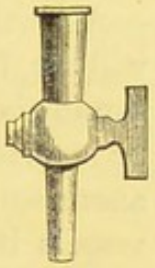


Fig. 100.

112. Syringe for Mercurial Injections.—The mercurial syringe should be made of steel, and of about half-ounce capacity. Perhaps, however, the most convenient and sim-

plest form of apparatus for mercurial injection, consists of a glass tube about half an inch in diameter, and a foot or more in length, to one end of which is fitted a steel stopcock, with pipes of various sizes. The weight of the column of mercury is sufficient to force the fluid into the vessels, and the pressure can be altered by inclining the tube or holding it perpendicularly, while the quantity of mercury which escapes is regulated by the stopcock.

Fig. 101.

113. Forceps of the kind delineated in fig. 101, are very convenient to stop any vessels that may be ruptured; and in injecting parts of animals or small portions of any tissue in which there are several open vessels, they are indispensable. The operator should be provided with several pair of these forceps, so that he can stop the escape of injection at one or several points if necessary.

Fig. 102.

114. Needle for passing Thread round the Vessel.—A small needle like that figured in the margin, will be found very useful for passing the thread round the vessel into which the injecting-pipe is to be inserted. Where the vessels are large, a common aneurism needle answers very well, but for small vessels it is better to use a much smaller instrument. Such an instrument may be readily made by fixing an ordinary needle in a small handle (fig. 102). It should be previously heated to redness and allowed to cool, when it may easily be bent into proper shape. If necessary the eye of the needle can be enlarged by introducing some pointed instrument while it is red hot, and carefully moving it from side to side, so as to dilate it.

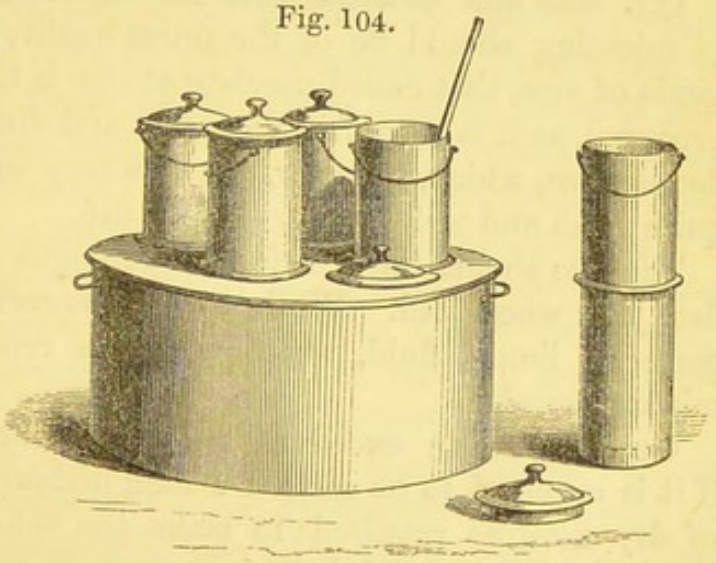
Thread.—The thickness of the thread used for tying vessels, &c., must of course vary according to their size. Silk is best adapted for the purpose, and it should not be too thin, for if so, it is liable to cut through the vessels.

115. Corks for Injecting-pipes. — Where it is necessary to use several pipes, or in those instances in which the use of the stopcock is inconvenient, from its weight or bulk, corks of the form figured in the margin, will be found useful to stop the pipes, when the syringe is being refilled. They may be procured at any cork-cutter's of the proper size to fit the pipes (fig. 103).

Fig. 103.

116. Injecting-cans. — For minute injections melted size is the liquid usually preferred for suspending the colouring matter, and upon the cleanliness and care with which the size is prepared, and mixed with the colouring matter, will the success of the injections mainly depend. As it usually takes some time before the vessels are filled, it is also necessary that the size should be kept at the proper temperature, so that the syringe may be refilled with warm injection as often as required. The size must always be kept hot with the aid of warm water; for, if a naked fire be used, there is danger of burning it. The size may be placed in a vessel which can be heated by standing it in a common tin saucepan of hot water. After it has been strained, great care must be taken to keep the vessel covered over, in order to prevent dust from falling in, and so rendering a second straining necessary. A convenient form of apparatus for melting the size, and keeping it at a proper temperature, is shown in fig. 104. It consists merely of a water-bath, in which the cans, containing the

Fig. 104.



injecting fluid, can be conveniently kept at the proper temperature, and their contents protected from dust, &c., by means of small covers. The bath should be about nine inches in diameter, and four or five in height. It may be made of tin, zinc, or copper. The cans may be about six inches high, and rather less than two and a half wide: if of these dimensions they hold nearly a pint. The bottom of each should be cup-shaped, and perfectly smooth, so that it may be thoroughly cleaned; at the same time the can should be made to stand well, by prolonging the sides a little below the bottom, as shown in the figure. The handles ought to be fixed on the outside of the can, and the cover should be allowed to project a little over the top. When only two or three cans are in the bath, the holes for the others can be covered over with their covers, in order to prevent the unnecessary escape of steam. A little above the middle of each can there is a wide rim, by which it is made to rest upon the top of the water-bath, as it is better that the cans should not quite touch the bottom.

An apparatus of the size above mentioned is quite large enough for all ordinary purposes, and can be conveniently heated over a gas or oil lamp; but if a great amount of injection is required, the cans should of course be much larger.

117. Size and Gelatine used for Injecting.—The size used in injecting should be of the finest quality. Of the different kinds of size, that called parchment size is the best that can be procured, as it is more free from grit and dirt than the ordinary double size, which, however, answers very well if it be obtained quite fresh and well strained before use.

The size should be of sufficient strength to form a tolerably firm jelly when cold. While warm, however, it should form a perfectly limpid fluid, which will flow readily into the most minute vessels.

Gelatine is an excellent substitute for size, particularly if it is required to be carried a long distance. The proportion of dry gelatine required to make the solution of the proper strength varies somewhat according to the temperature. In winter about seven, and in summer twelve parts of gelatine to one hundred parts of water, will be found about the right pro-

portion. The gelatine should be soaked in cold water for some hours until it has become swollen and softened, when the vessel in which it is may be placed in a basin containing hot water, or in the injecting-can (fig. 104), and the mixture soon becomes perfectly fluid. If sufficient gelatine has been dissolved in the water, a drop of the solution placed upon a cold surface will become solid in a few minutes.

Size, or the cold jelly made from gelatine, may be preserved by covering the surface with a thin stratum of alcohol, which can be poured off when it is required to be melted for injection.

COLOURING MATTERS USED FOR INJECTION.

Various colouring matters have been used for injecting. They are usually obtained in very fine powder, and suspended in some fluid. Carmine dissolved in ammonia forms a very fine red liquid, and has lately been very successfully employed by Mr. Smee for injecting the capillaries of the brain. The objection to this, and all other fluids which hold the colouring matter in solution, instead of in suspension, is that the mixture frequently permeates the coats of the vessels, and colours the surrounding tissue in such a way, that the outline of the capillaries frequently cannot be distinguished from the surrounding tissues.

When a fine powder is suspended in size, it is better in all cases, in which it is possible, to prepare the colouring matter immediately before use; for by proceeding in this manner the particles are in a much more minute state of division, than when some time has been allowed to elapse before injecting, in which case numerous particles adhere together, forming small collections too large to penetrate the minute vessels.

118. Size of the Particles of the different Colouring Matters used for Injection.—The comparative size of the granules, of which the colouring matters most commonly used for injection consist, is shown in the annexed woodcut.

Upon comparing the dimensions of the particles obtained from the dried powder with those of the freshly-prepared precipitate, the importance of using colouring matters which have been recently precipitated will at once become evident.

This is especially shown in fig. 105, which represents the

microscopical characters of the dried powder, and freshly-precipitated deposit of several of the substances commonly used for injection.

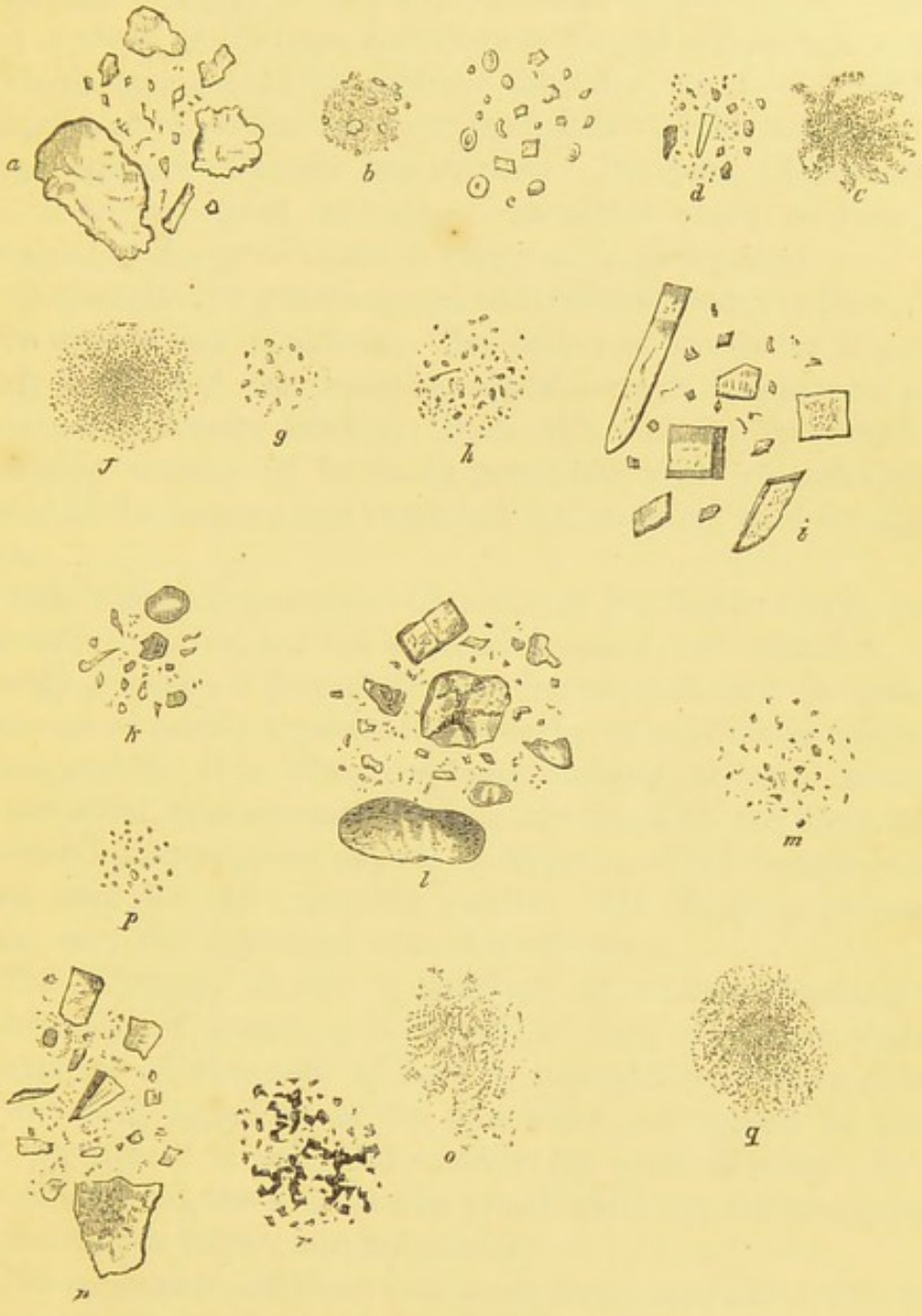
The colouring matters represented are as follows:—*a*, precipitated chalk (purchased in a dry state); *b*, recently-precipitated chalk; *c*, whitening; *d*, Prussian blue; *e*, recently-precipitated Prussian blue; *f*, freshly-precipitated carbonate of lead; *g*, dried carbonate of lead; *h*, freshly-precipitated biniodide of mercury; *i*, dried biniodide of mercury; *k*, indigo; *l*, vermilion (as purchased); *m*, levigated vermilion; *n*, dried chromate of lead; *o*, freshly-precipitated chromate of lead (cold solutions); *r*, freshly-precipitated chromate of lead (hot solutions); *p*, pure carmine; *q*, lampblack.

All these drawings were taken with the aid of the neutral tint glass reflector (§ 32), and were all magnified with one of Powell's quarters with the shallow eyepiece, which is equal to about two hundred diameters. A scale of thousandths of an inch is represented at the lower part of the figure, magnified with the same power.

119. Red Injections.— *Vermilion* is generally considered one of the colouring matters best adapted for fine injections, and is employed more frequently than any other. It may be obtained in exceedingly-fine powder, and runs readily into the smallest vessels, while at the same time there is no chance of its passing through their coats.

Some vermilion is very gritty, and such should not be used. Numerous small crystalline particles may frequently be detected in the common vermilion of the shops upon microscopical examination (fig. 105 *l*). This is quite unfit for use; and in order to render it free from grit, it should be mixed with about eight times as much water, and then well shaken: after allowing a few seconds to elapse for the coarser grit to settle, the mixture holding the finer particles in suspension, may be nearly all poured off into another vessel, and allowed to subside. The gritty residue may be treated a second time with water (or oftener if necessary), and the fine particles poured off, and allowed to subside with the previous washings. When all the powder has settled, the clear water may be poured off, and

Fig. 105.



the vermilion after being dried, without the application of heat, may be kept ready for use (fig. 105 *m*). About two ounces of vermilion to a pint of size will be found to give a good colour; and in these proportions a mixture is formed which runs well.

Great care should be taken in the selection of vermilion for purposes of injection, for upon the quality of this substance in great measure depends our success. The best Chinese vermilion is very good, but dear. Excellent vermilion may be obtained at the price of six or seven shillings a pound.

Golden sulphuret of mercury and the *biniodide of mercury* have also been used as red injections. The colour of the latter is peculiarly bright, but the precipitate is of a crystalline nature, and does not penetrate readily. Both this and the sulphuret of antimony require to be finely powdered, and levigated with water in the manner just described, before being used for injection.

120. **Yellow Injections.**—*Chromate of lead* forms a very good colouring matter, and has some advantages over vermilion. If freshly prepared it frequently runs better, and, as it is lighter, does not so readily separate from the size or solution in which it is suspended. It is, moreover, much cheaper; although it must be admitted, that chromate of lead injections do not form such beautiful microscopical objects as those in which vermilion has been used for the colouring matter. Mr. Topping prepares many very fine injections with this substance.

This colouring matter is obtained by mixing solutions of bichromate of potash and acetate of lead, when the yellow precipitate of chromate of lead is immediately thrown down. If the cold solutions of the two salts just named be used, the precipitate is pale, and contains one atom of water; if, on the other hand, the solutions be mixed while hot, the precipitate is of a deeper colour, and anhydrous.

The appearance of these two precipitates, magnified with the same power, is shown in fig. 105; *o*, represents the precipitate obtained by mixing cold, and *r*, that which results from mixing hot solutions of similar strength.

Chromate of lead which has been dried, should never be used for injecting, as it does not run well, in consequence of the

particles becoming aggregated together, and forming small lumps.

Harting gives the following directions for making this injection, which he highly recommends :—

Two solutions are prepared; the first consisting of 2000 grains of acetate of lead, dissolved in a pint of water. The second is prepared by dissolving 1048 grains of chromate of potash in two pints of water.

When it is required to prepare the injection, one measure of the first solution is mixed with two measures of the second, and to this mixture, after it has been well stirred, two measures of a strong solution of gelatine, consisting of one part of dry gelatine to four of water are to be added. This injection should be used when freshly prepared. It is better, however, to allow the chromate of lead to settle, and pour off the solution of acetate of potash before adding the size, as this salt is apt to corrode the walls of the vessels in those cases in which the preparations are preserved moist.

Injections of chromate of lead may also be made by allowing decomposition of the solutions to take place in the vessels.* The vessels are first injected with one solution, and afterwards the other is slowly forced in. Very fine injections may be readily made in this manner with little trouble, and for dry preparations which are to be mounted in Canada balsam, this plan answers admirably; but if the injections are to be preserved moist, the former method should be employed, as the acetate of potash is apt to destroy the vessels after the preparation has been preserved for some time.

Dr. Goadby uses gelatine in preparing this injection, and suggests the employment of nitrate of lead instead of the acetate, because the resulting salt of nitrate of potash would not exert any injurious action upon the vessels, but, on the contrary, would assist in their preservation. His proportions of bichromate of potash and acetate of lead, are as follows:— Saturated solution of bichromate of potash, 8 fluid ounces;

---

* M. Doyère, "Comptes Rendus," 1841. Bowman, "Phil. Trans." 1842, p. 58.

water, 8 ounces; gelatine, 2 ounces. Saturated solution of acetate of lead, 8 fluid ounces; water, 8 ounces; gelatine, 2 ounces.* The syringe should always be very carefully cleaned after it has been used for injecting these salts.

121. Blue Injections.—*Indigo* used for injections should be of the best quality that can be procured. After it has been well soaked in water for some time, the coarser particles (fig. 105 *k*) may be removed by treating it with a large quantity of water, when the finer particles alone will be held in suspension, and may be separated in the manner described when speaking of vermilion.

Prussian blue may also be employed as a blue injection. It is softer and more easily powdered than indigo. If injected pure, the colour will be too intense, but it may be rendered lighter by mixing it with a certain proportion of white injection. Prussian blue forms one of the finest powders possible, especially if freshly prepared (fig. 105 *e*).

Schrœder van der Kolk uses freshly-precipitated Prussian blue, prepared by mixing solutions of the persulphate of iron and ferrocyanide of potassium together.

As the alkali of the blood tends to destroy the colour of Prussian blue, it is necessary to mix with the solution a small quantity of dilute sulphuric, acetic, or tartaric acids, in order to neutralize this alkaline reaction.

Prussian blue can be obtained in a state of very minute division if mixed with a solution of oxalic acid, and in this case the colour is not affected by the free alkali of the blood. Equal parts of oxalic acid and Prussian blue are to be finely powdered in a mortar, then water is to be very gradually added, and afterwards the strong size solution. Harting strongly recommends this injection, when it is wished to examine injected vessels by transmitted light, and particularly in injecting the capillaries of the lungs.

122. White Injections.—*White lead.* Finely-powdered carbonate of lead has been used as a fine injection. It may be prepared by mixing a solution of acetate of lead, consisting of

---

* Quoted by Dr. Wythes, "The Microscope," Philadelphia, 1852.

2000 grains of acetate of lead to a pint of water, with an equal quantity of a solution of carbonate of soda, containing 1520 grains in a pint of water. A measure of each of these solutions is to be mixed with two measures of a strong size solution.

Precipitated Chalk also forms a good white injection, if carefully prepared; but it does not mix so well as the former.

The microscopical appearances of precipitated chalk which has been dried, freshly-precipitated chalk, whitening, and white lead, are shown in fig. 105 *a*, *b*, *c*, *f*.

Freshly-precipitated chalk is readily prepared by adding a solution of carbonate of soda to a solution of chloride of calcium.

Carbonate of lead is made by mixing together cold solutions of acetate of lead (sugar of lead) and carbonate of soda, as above.

123. Injecting different Systems of Vessels with different Colours.—In those cases in which we desire to examine the arrangement of the different systems of vessels in the same animal or in the same tissue, it becomes necessary to use a distinct colouring matter for each system. It is also a matter of importance when two colours meet in the capillaries, to employ those which, in combination, will produce a tint that reflects the light well, and shows distinctly the outline of the vessels. If red be used for the arteries, and blue for the veins, a dark brownish-red colour is produced in the capillaries, which, it has been observed, renders the vessels indistinct when subjected to microscopical examination. On the other hand, if blue and yellow be allowed to mix in the capillary system, a fine green is produced which does not present this inconvenience. M. Robin therefore recommends blue for the arteries, yellow for the veins, red for the portal veins, and white for the bile ducts or renal tubes.*

* Du Microscope et des Injections, p. 16.

OF INJECTING.

124. General Rules to be observed in Injecting.— Great attention should be paid to the cleanliness of all the instruments to be used in injecting. The syringe should always be kept scrupulously clean and in good order, and the injecting-cans should be carefully covered, to prevent the ingress of dust. Before commencing the operation, plenty of warm water should be at hand; and the subject should be allowed to soak for some time in a basin of hot water, before it is attempted to inject it, in order that it may be thoroughly warmed through. The temperature of the water must vary according to the degree to which the injection is required to be heated: if size and vermilion be used, the water need only be warm; but if melted wax be employed, the water must be so hot that the hand can scarcely be borne in it. The length of time which the preparation is allowed to soak must depend upon its bulk; and the water should be changed as soon as it becomes at all cool.

Time at which the Injection should be made.—With respect to the length of time after death that is most favourable for this operation, no absolute rules can be given.

Generally, it may be remarked that we should not attempt to inject while the *rigor mortis* lasts. Many days may in some cases with advantage be allowed to elapse, particularly if the weather is cold, while in warm weather we are compelled to inject soon after death. As a general rule, the more delicate the tissue, and the thinner the vessels, the sooner should the injection be performed. Many of the lower animals, annelids, mollusca, &c., and fishes, should be injected soon after death. In making minute injections of the brain, only a short time should be allowed to elapse after the death of the animal, before the injection is commenced. Injections of the alimentary canal of the higher animals should be performed early, —not more than a day or two after death.

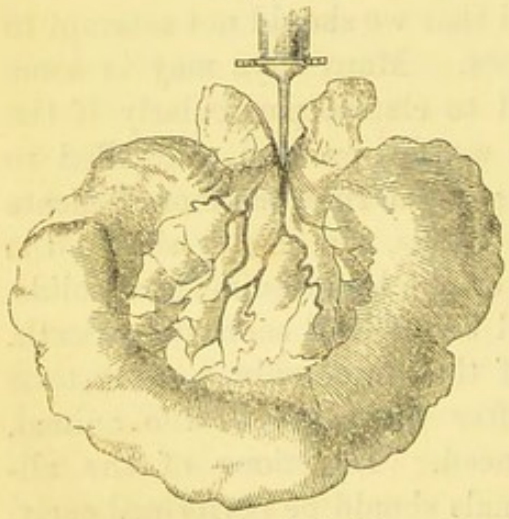
Minute injections of the papillæ of skin, particularly of the fingers and toes, cannot be successfully made until the cuticle has become somewhat softened by allowing the preparation to remain in a damp cloth, or to soak in water for

some days. In these situations the vessels are strong, and in their ordinary state the injection will not traverse them, in consequence of the cuticle preventing their gradual distension by the injecting fluid. A similar plan must be followed in making injections of the tongue, and other parts where the epithelial covering is unusually dense, and firmly adherent to the vascular surface beneath.

125. Of fixing the Pipe in the Vessel.—When everything is properly prepared, we may proceed carefully to fix the pipe in the vessel through which we intend to inject; and this operation will vary slightly in different cases, according to the nature of the preparation to be injected. If the subject be a small animal, it is better to take out part of the sternum, and fix the pipe in the aorta. If only part of an animal is to be injected, the largest artery supplying the part should be selected, and all the other open vessels may be tied or stopped with the small forceps shown in fig. 101.

A small portion of intestine can be injected by cutting out the corresponding portion of mesentery attached to it; and after searching for a large vessel, all the others may be tied together with the open ends of the alimentary tube. This arrangement is roughly represented in the diagram (fig. 106). In each case the proceeding is conducted in a similar manner. A pipe of somewhat smaller diameter than the vessel should be selected, and an opening may then be made in the vessel of sufficient size to admit the pipe, which can now be inserted. The needle (fig. 102), charged with thread or silk, is then carefully passed round the vessel, the thread seized with forceps, and the needle withdrawn over the thread. This operation is sufficiently simple where the vessel is large and strong; but where thin and easily torn, it requires great care. The thread

Fig. 106.



is now tied tightly round the vessel close to the extremity of the pipe, and then attached to the two projecting wires, to prevent the possibility of slipping.

In injecting from veins a similar method is pursued, taking care to choose a vein in which the valves are not numerous, or in which they are altogether absent. The portal vein can be reached by opening the abdominal cavity, care being taken not to tear any of the branches below the point where the pipe is inserted.*

126. Of preparing the Fluid for Injection.—It is very important that the size and vermilion, or other injection which is to be thrown into the vessels, should be thoroughly mixed and well strained before being used. The colouring matter, properly powdered, should be placed in a small earthenware mortar, and the melted size or other fluid carefully added by degrees, the whole being constantly stirred until well mixed. When the proper colour has been obtained, the whole must be strained through muslin, or through a fine perforated strainer, into another vessel, which should be kept warm. The injection

---

* *Injecting the Vascular System of Fishes, Mollusca, and Insects.*—Greater care is required to fix the pipe in the vessels of fish in consequence of their being so readily torn. Excellent injections of fish may frequently be made as follows:—The tail is cut off with a sharp knife at a short distance posterior to the anus, and if the cut surface be examined, the ventral artery may be easily found situated immediately beneath the bodies of the vertebræ. A pipe is carefully introduced and pushed down some distance, so as to prevent the injection from coming out, or the end of the vessel may sometimes be separated from the surrounding parts and tied in the usual way. By this simple proceeding capital injections can often be made very easily.

Minute injections of the branchiæ of some of the mollusca may often be made by very carefully placing the pipe in the largest vessel that can be found, and slowly injecting. The extreme delicacy of the vessels prevents any attempt being made to tie them to the pipe, and of course much injection will be lost. From the large size of the vessels, however, much will often run into the capillaries. In this way I have easily succeeded in injecting the branchiæ of the Pinna ingens and fresh-water mussel (Anodon), both of which form beautiful microscopical objects.

In order to inject the smaller gasteropods (slugs, snails, &c.), we must pursue a different method. In the muscular foot of these are situated many

should be well stirred with a wooden stick previous to filling the syringe.

We can judge of the intensity of the colour by removing a drop of the solution with a stirring-rod, and allowing it to fall on a white plate so as to form a thin stratum, which should have a pretty deep colour. It is always better to have too large a quantity of the colouring matter rather than too little.

Of vermilion, about two ounces will be sufficient for a pint of size; but it is better in all cases to regulate the quantity by examining the intensity of the colour in the manner just mentioned.

127. Of the operation of Injecting.—When the preparation is warmed through, the injection properly strained, and the pipe fixed in the vessel, we may proceed carefully to inject, taking care that the injection is kept at a proper temperature, by allowing it to remain in the warm water-bath during the operation.

The air should be first withdrawn from the upper part of the vessel by means of the syringe, after which the stopcock is turned off and left attached to the pipe. The syringe is then disconnected, and after being washed out once or twice with warm water, is nearly filled with injection, which must be well stirred up immediately before it is taken. The syringe should

---

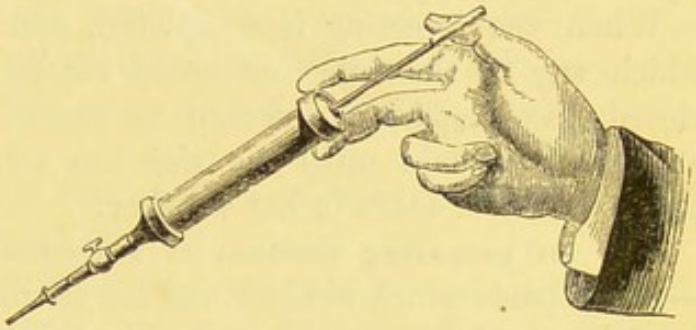
large lacunæ, or cavities, which communicate with the vascular system, or, in fact, form the vessels which are distributed to this organ. If the injection can be forced into any of these lacunæ, it may be made to traverse the whole vascular system. To introduce the pipe, a small hole is made obliquely in the foot, taking care not to force the instrument too far. A small pipe is next inserted, and when the preparation is warm enough, the injection of size and vermilion is very slowly and carefully forced in, and the progress which is made can be seen by observing the vessels distributed to the respiratory organs. When a sufficient quantity of injection has been introduced, the pipe may be withdrawn, and the hole plugged with a piece of wood cut to the proper size, to prevent the injection again escaping before the size has had time to set.

The vascular system of insects may sometimes be partially injected by forcing injection into the abdominal cavity, from which it finds entrance into the dorsal vessel, and from thence is distributed to various parts of the body. The injection in the cavity of the abdomen is then allowed to escape.

not be quite filled, in order that the air in the pipe may be made to rise into the syringe through the injection, by the ascent of the piston, before any of the latter is forced into the vessel. The end of the syringe is then to be pressed firmly into the upper part of the stopcock with a slightly screwing movement.

The piston is now very gently forced down by the thumb, until the syringe has been nearly emptied, when the stopcock must be turned off, and the syringe refilled with warm injection as before.

Fig. 107.



The corks described in § 115 may be substituted for the stopcock. Care must always be taken to keep the syringe in the position shown in the engraving, fig. 107, so that any air which may be in it, may remain in the upper part; and for the same reason, all the injection should not be forced out, for fear of the enclosed air entering the vessels, in which case all chance of obtaining a successful injection would be destroyed.

After a certain quantity of fluid has been injected, it will be necessary to use a greater amount of force, which, however, must be increased very gradually, and should only be sufficient to depress the piston very slowly. If too great force be employed, extravasation will be produced before the capillaries are half filled. Gentle and very gradually-increased pressure, kept up for a considerable time, will cause the minute vessels to become slowly distended without giving way to any great extent. At the same time it must be borne in mind that extravasation frequently occurs at various points in a successful injection; but the longer this event can be kept off, the more likely are we to succeed.

When the injection begins to flow from the large veins mixed with the blood contained in these vessels, and the surface

of the injected preparation looks of a red colour, and has a somewhat velvety appearance, we may infer that the injection has been completed. This occurs at different periods. Sometimes the first or second syringeful causes a general redness of surface, while in other instances a considerable time will elapse before more than a slight blush appears. As a general rule, it is better to proceed slowly and cautiously, and to use as little force as possible, which should not be more than sufficient to produce an observable depression on the piston. Many minute injections will require an hour or more to complete.

When the injecting is completed, and all the openings by which any of the injection could escape during cooling are closed, the preparation should be placed in cold water, and allowed to remain until the size has set, which will require twelve hours or more in hot weather.

128. Of preparing portions of Injection for the Microscope.—Those parts which are required for microscopical examination must be carefully cut out and placed in a saucer of water, in which they may be examined with the microscope, and the best parts selected to be put up as permanent objects.

If a mucous membrane be the subject of examination it will be necessary to brush off the adhering mucus with a camel's-hair brush, the preparation being kept under water while this operation is performed. If the injection be considered sufficiently good, it may be preserved in spirit or other preservative solution, Chapter VII. Portions of mucous membrane should be pinned out perfectly flat upon a piece of cork or wax (§ 86), and allowed to harden for a short time in spirit. When hardened, the edges may be cut square with a sharp knife or pair of scissors, and the preparation may be mounted in a cell (§ 90).

Gland structures should be examined both on the external surface and also in section. After hardening in spirit or other preservative solution, clean sections can readily be made with a sharp knife.

As an invariable rule the preparation should be allowed to soak for some time in the fluid in which it is to be preserved, previous to placing on the thin glass cover, in order to allow time for all air-bubbles to rise to the surface.

Many injections are shown to the greatest advantage by being dried, and mounted in Canada balsam as opaque preparations. Injections of the liver and kidney are easily prepared in this way:—A thin section is cut with a sharp thin knife, slightly washed with clean water, spread smoothly on a clean glass slide, and allowed to dry thoroughly without the aid of heat. When quite dry the surface can be scraped smooth with a knife, and the preparation mounted in Canada balsam in the usual way (page 90). A small quantity of the balsam need only be used if the surface has been scraped perfectly smooth; the thin glass may be carefully pressed down on the injection. Often, before applying the balsam, it will be found advantageous to wet the surface of the injection with a drop of turpentine.

Preparations of this kind look neater when covered with circular pieces of glass (§ 66).

Injections of thin membrane are merely to be stretched out and dried on the slide, when they may be covered with Canada balsam and thin glass. In this way specimens of the iris, serous membranes, &c., may be prepared, and some will be shown off to greater advantage if placed upon a dark ground.

At the same time we must remember, that in the process of drying, a considerable alteration may take place in the appearance of the parts. Thus, for instance, the arrangement of the vessels in the villi of the intestines, and of the mucous membrane generally, cannot be properly demonstrated upon dried specimens. It is always better to examine injected preparations previous to drying them, when, from the arrangement of the vessels generally, some idea may be formed of the change in appearance which will probably take place from this process.*

---

* On the process of injecting, the following authors may be referred to:—Tulk and Henfrey, "Anatomical Manipulation;" Robin, "Du Microscope et des Injections;" Harting, "Het Mikroskoop;" Translations in the Edinburgh Monthly Journal, 1852; Dujardin, op. cit.; Strausdurkheim, op. cit.

CHAPTER IX.

PREPARATION OF OBJECTS FOR EXAMINATION BY TRANSMITTED LIGHT—METHOD OF EXAMINATION—EXAMINATION OF SOFT TISSUES—KIDNEY—LIVER.

129. Methods of Examination. — The most advantageous method of examining particular tissues, and the best manner of preserving them, can only be learnt by long practical experience. It will only be attempted here to introduce some of the methods which are most frequently employed to demonstrate the minute anatomy of those textures which most frequently come under the notice of the practitioner.

In order to examine the structure of many tissues, it is necessary to obtain a section sufficiently thin to permit the transmission of the light readily, and so evenly cut, that the minute structure of the tissue may be submitted to examination in every part of the section. The difficulty of making thin sections of many textures is often very great, and, to effect this object satisfactorily, a knowledge of certain mechanical operations becomes necessary. Sometimes we require to cut a thin section of a soft pulpy texture, which can scarcely be touched without injuring its delicate structure, and altering the position of its constituents; while, in other instances, we must obtain a very thin transparent section of a substance so hard, that steel tools will scarcely scratch it, such as the enamel of teeth, fossil teeth, &c.

The method of making sections of soft tissues will first be briefly described, and afterwards the processes adapted for cutting hard textures will be alluded to. Other mechanical operations, such as tearing up the tissue with fine needles,

pressing it between glasses, removing soft pulpy portions by washing, &c., are often necessary, and will also require to be considered.

Chemical reagents are frequently very useful in elucidating the nature of minute structures, either by destroying or altering some of the component parts of the tissue, or by simply rendering the substance more transparent. Much may frequently be learnt concerning the nature of the inorganic portion of the tissue by ignition upon platinum foil, and burning off the carbonaceous portion of the residue by exposing the specimen for a length of time to the action of a red-heat.

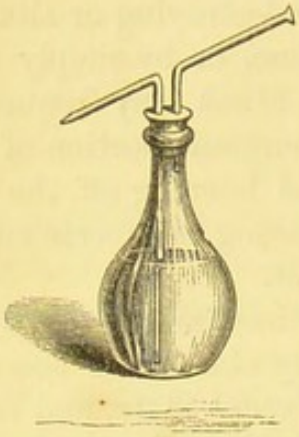
By simply drying a tissue we are sometimes able to make out a point in its structure, which had entirely eluded our observation when the tissue was examined in a recent state, and there are other processes of practical importance in the demonstration of minute structures, which should also be considered.

130. Boiling the Tissue previous to Examination. — This operation is often of great service in enabling us to demonstrate the structure of a tissue. For instance, the fibres of which the crystalline lens is composed, are best shown after boiling the lens in water. The branched muscular fibres in the tongue of the frog, and in other situations, may be made out very readily by boiling the organ in water for a few moments, and then tearing up small portions with fine needles. Beautiful sections of muscular fibre can often be obtained after the texture has been boiled in water. Various glands and other textures often require to be boiled for some time in water, in order to harden them sufficiently to enable us to cut thin sections; but in all cases the microscopical characters of the recent texture should be examined, as well as that which has been hardened by boiling. Small portions of tissue can be readily boiled in a test-tube over the spirit-lamp.

131. Washing, soaking, or pressing the Tissue.—Not unfrequently we wish to get rid of the soft and more pulpy part of a tissue, in order to subject the more dense and fibrous portion to examination. This object is usually effected by soaking the tissue in water for some little time, and then placing it under a running stream of water, by which means the softer portions

are gradually washed away. Soaking in water frequently enables us to tear up a tissue very readily with the aid of needles, and thus to demonstrate its structure. Occasionally it is found necessary to press the tissue, and rub parts of it together, before the soft pulpy portions can be got rid of. In this way we may demonstrate the supporting or trabecular tissue of the spleen, and the areolar and vascular tissue of the liver, &c. Thin sections of kidney, liver, and other glandular organs, may be thus treated when the matrix is to be subjected to examination separately.

Fig. 108.



In these operations the wash-bottle (fig. 108) will be found useful. Generally it will be better to make a thin section of the tissue first, and then soak and wash carefully, when the parts may be seen *in situ*.

132. Drying the Tissue previous to Examination.—Thin sections of various tissues can frequently be obtained only by first drying the substance thoroughly, and then cutting off a thin shaving with a sharp knife. In this way specimens of skin, mucous membrane, and many other tissues, are often most advantageously prepared. The tissue is stretched on a board with pins, and then allowed to dry, when a very thin section can be cut off and examined in Canada balsam; or it may be placed in water for a short time, in which case, when subjected to examination, it will often be found to have regained its first appearance. Portions of muscular fibre, the tongue, skin, and many other tissues, may be allowed to dry in this manner, and then we may with a sharp knife readily obtain exceedingly thin sections, which could not be procured in any other manner. The drying may be effected in a warm room, or in a current of air. A high degree of artificial heat should be avoided.

133. Application of Chemical Reagents. — In the examination of various substances, much information may frequently be

gained with respect to their nature by exposing them to the action of various chemical agents. The action exerted by alcohol, chromic acid, corrosive sublimate, &c., is often very useful in enabling us to cut thin sections for microscopical examination. When we require to make a tissue very hard, we need only soak it in alcohol or some other chemical reagent which has the property of coagulating albumen. By resorting to the use of chemical reagents we are frequently enabled to make out the true nature of a body, which has entirely baffled our powers of observation, when subjected to microscopical examination only. Chemical reagents assist us in microscopical investigation, by rendering parts of the texture transparent, so that we are enabled to see structures which were previously obscured, or by making the tissue itself darker, or by causing certain alterations in its general characters which are not easily explained. The method of applying chemical reagents, and a general description of the changes which ensue, will be found in Chapter XVI.

In describing the methods of demonstrating the minute anatomy of the most important animal tissues in a healthy or morbid state, I shall have frequently to refer to the assistance which is derived from the application of chemical tests.

134. Igniting the Substance in order to remove Organic Matter.—When the inorganic portion of a tissue which we wish to examine is not altered by exposure to a red-heat, recourse may be had to ignition, in order to get rid of the animal matter. In this way crystals of carbonate and phosphate of lime, and granules of siliceous matter, may be separated from the organic material with which they were combined. The beautiful siliceous shells of the diatomaceæ may be separated from organic matter by a similar process. The ignition should be performed in a small platinum capsule, or upon a small piece of platinum foil. The carbonaceous residue must be exposed to the dull red-heat of a spirit-lamp (§ 43) for some time, until only a pure white ash remains, which will be found to contain the objects of our search in a very perfect state. If the siliceous matter only is wanted, the ash should be treated with strong nitric acid, which will dissolve any carbonate or phosphate. The insoluble

residue may then be washed and dried, and subjected to microscopical examination while immersed in turpentine or Canada balsam. In many cases, this method is superior to that of boiling in nitric acid, in order to remove the organic matter. Both processes may, however, be employed where only the siliceous residue is wanted, but if we require the salts of lime, ignition at a dull red-heat is alone applicable.

135. Of cutting thin sections of Soft Tissues. — There is no more important operation in microscopical investigation than the present. The student is continually requiring thin sections of different textures, and whether he pursues the study of vegetable or animal physiology, or morbid anatomy, it will often be necessary to make a very thin section of the tissue which is to be examined; and upon the amount of skill he displays in cutting these sections, will the success which attends his investigation mainly depend. The darker and more complicated the tissue may be, the more important does it become to obtain a section of extreme tenuity, for otherwise sufficient light cannot be transmitted through the tissue to enable us to see its structure; moreover, in a thick section, the objects occupying different planes so much interfere with each other as to prevent the possibility of any one being defined clearly.

Cutting a thin section of a soft tissue may at first sight appear a very simple process, but it will be found to require considerable skill on the part of the operator. Sections of the large glands, and other soft tissues, may be made with an ordinary knife which should be very sharp. A clean surface is first cut, and then a thin slice is removed with a slow sawing motion of the knife, which is much facilitated by the application of a drop of water; indeed, whenever we require a very thin section of a soft tissue, the blade of the knife should always be well wetted with water.

The most important instruments for making thin sections of soft tissues are the following: scissors of different sizes (§ 53), Valentin's knife, double-edged scalpels, or lancets mounted in handles, and a few other instruments, such as forceps (§ 55), and needles of different sizes (§ 52), mounted in handles, are often required in demonstrating minute structure.

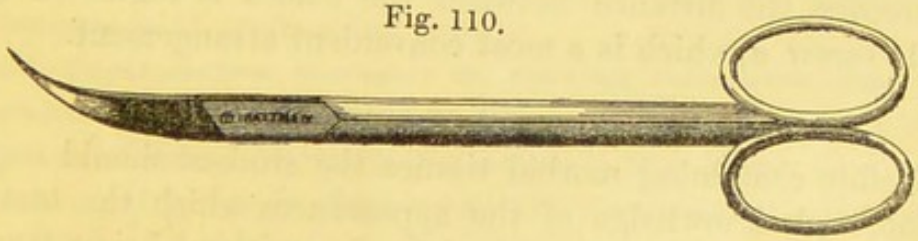
136. Scalpel specially adapted for Cutting thin sections of Soft Tissues.—*Double-edged Scalpel.*—For cutting thin sections, a knife of the form represented in fig. 109, will be found very useful, and, where only sections of small dimensions are required, this will answer all the purposes of Valentin's knife. In cases however, where a section is wanted of considerable size, the latter instrument must be used. The double-edged scalpel is made after the fashion of a common lancet; it is not so wide, but should be quite as thin. When employed for making a section (after cutting a clean surface), the point is made to perforate the surface, and carried along at a proper depth, so as to cut its way out. The width of the section may then be increased by carrying the knife first to the right, and then to the left, until a section of the desired width is obtained.

Fig. 109.

Common Lancets mounted in handles will be found convenient for cutting thin sections, but each side of the blade should be sharpened down to the point of insertion into the handle.

Scissors are also very useful instruments for cutting small thin sections of different tissues. The most convenient form for this purpose is that shown in fig. 110. When only very small portions of a tissue are required for examination, they will be more readily removed with the scissors than with any other instrument.

Fig. 110.



137. Valentin's Knife.—This instrument is of the greatest value in making thin sections of soft tissues, but it requires care

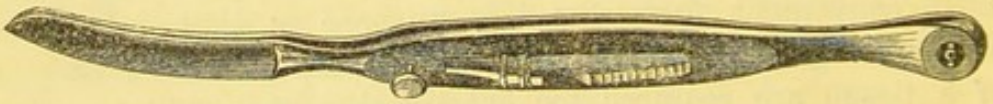
to keep it in good order. It is very easily made blunt if used for cutting fibrous or cartilaginous textures. By its aid most beautiful sections of the kidney, liver, and other soft glandular organs may be obtained with the greatest facility. The blades

Fig. 111.

should always be dipped in water just before use, for, if wet, the operation of cutting is much facilitated, and the section is more easily removed from between the blades. Immediately after use the blades should be washed in water, and dried with a soft cloth or a piece of wash-leather. If a drop of water gets into the upper part of the knife where the blades meet, the screw must be taken out, and each blade cleaned separately. With care the knife may be kept in use a long time.

Two forms of Valentin's knife are used; in one of these the blades are sharp on both edges and of a lancet-shape, and in the other, which I much prefer, of the form represented in the figure (fig. 111). The best form of Valentin's knife that I have used is that represented in fig. 112, which has lately

Fig. 112.



been made by Mr. Matthews. The blades of this knife can be completely separated from each other and easily cleaned. Moreover, the distance between the blades is regulated by a little screw *a*, which is a most convenient arrangement.

EXAMINATION OF SOFT TISSUES.

Before examining morbid tissues the student should acquire a thorough knowledge of the appearances which the textures present when in a state of health; for, without being familiar with the minute anatomy of healthy tissues, he will hardly be in a position to appreciate the changes which have been brought

about by disease. In this work it will be quite impossible to discuss, even in the most cursory manner, the healthy and morbid condition of the various tissues, and the methods of subjecting them to examination; nor can I attempt to describe the morbid alteration which may have taken place in any one gland or texture. At the same time it is my desire briefly to refer to the mode of investigating the healthy and morbid appearances of those textures which most frequently engage the attention of the physician. Where any particular method of investigation is required to demonstrate the minute anatomy of a tissue, it will be my endeavour to give an illustration of it, so that the student will, I hope, be enabled without much difficulty to fill up for himself at leisure, by reference to standard works, those deficiencies which limited space will not permit me to supply.

138. **Method of submitting a portion of Tissue to Microscopical Examination.**—In order to subject a portion of tissue or other substance to examination by transmitted light, we usually proceed as follows:—One of the glass slides (§ 49) is carefully cleaned, and the thin section of tissue which has been removed by the aid of forceps and scissors, or a scalpel, placed in the centre; a drop of clean water is then added, and the whole covered with a square of thin glass (§ 50), also perfectly clean. If the under surface of the thin glass be gently breathed upon it becomes wetted more easily. The substance may be unravelled with needles, or, if necessary, any other operation performed before covering it with the thin glass. If the substance be covered with too much soft pulpy matter, it may be slightly washed in water before being placed upon the slide, or a jet of water from the wash-bottle (fig. 108), may be forced upon it. Thin sections will require to be laid flat upon the slide, with the assistance of needles and forceps.

139. **Great caution necessary in drawing inferences from Microscopical Appearances.**—The difficulty of making out the structure of many organs and tissues is very great, and considerable experience is often required to demonstrate distinctly the anatomical characters of a healthy texture. These difficulties are much increased in the examination of morbid growths. When chemical reagents are applied, the effects

must be very carefully observed, otherwise there is danger of mistaking the change of character produced by the application of the reagent for a morbid alteration. Even the addition of a drop of water often materially alters the microscopical characters of a tissue. It is only by very frequent and careful examination of morbid growths that the observer can hope to recognize and interpret their characteristic appearances, and it should only be with the utmost caution, and after long familiarity with microscopical examination generally, that he should attempt to pronounce an opinion with reference to the nature of a morbid growth; for without extensive observation and great care, he will run the risk of bringing discredit not only upon himself as an observer, but also upon microscopical investigation generally.

Every observation should be carefully recorded in a note-book at the time it is made, and drawings added, if necessary; and it is very important that the student should take every opportunity of describing, in the simplest manner possible, the appearances which he has observed under the microscope.

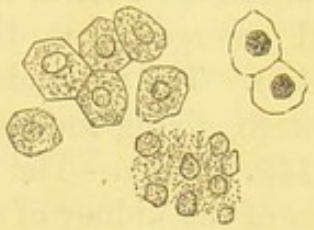
The examples of tissues and morbid growths which will be brought forward have been introduced more especially for the purpose of referring to some particular process of manipulation which is important in the investigation.

As examples of glandular organs the kidney and liver may be referred to, because these are very frequently the subjects of investigation in cases of disease, and the changes which they undergo in structure has received a large share of attention. For a detailed description of the anatomy of these organs in a state of health I must refer to the different treatises on physiology and physiological anatomy, and I shall only here mention such points as should be especially taken notice of in a microscopical examination.

140. Kidney.—The kidney may be examined by making very thin sections (§ 135), or by simply scraping a freshly-cut surface with a knife; in which case small portions of the tubes, isolated Malpighian tufts, and cells of epithelium will be obtained, but the relative position of the structures will of course be lost. A thin section may be obtained either with a sharp

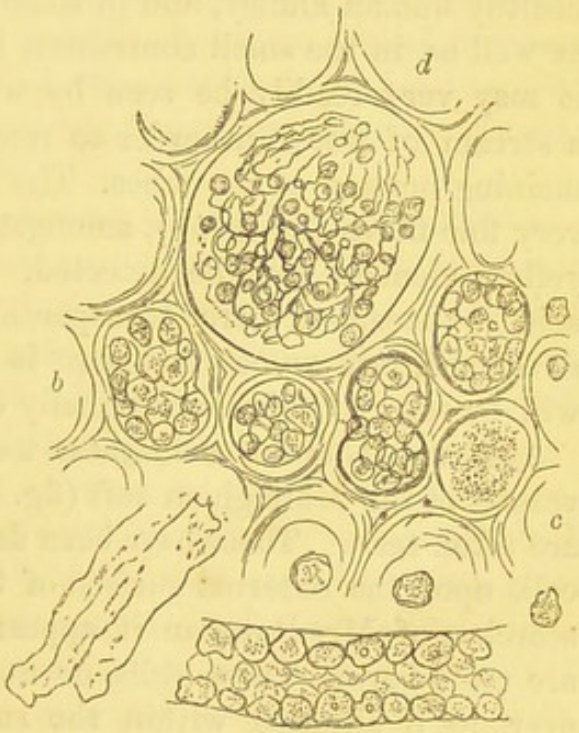
knife, or more advantageously with a Valentin's knife (§ 137), by which means a section including both the cortical and medullary portion of the organ may be made. After washing the section very slightly, it may be placed with a drop of water between two pieces of glass, and examined in the microscope, first using a low power (an inch glass), by which the general arrangement of the tubes will be seen, and afterwards a quarter of an inch object-glass, by the aid of which the different characters of the epithelium in the straight and convoluted portions of the tubes may be demonstrated.*

Fig. 113.



141. Basement Membrane and Epithelium.—Just at the edge of the specimen, a portion of a tube stripped of epithelium, and exhibiting the basement membrane (fig. 114*a*) very distinctly, may often be observed. The thick glandular epithelium of the convoluted portion of the tubes (fig. 113) should be compared with the more scaly form of that which occupies the straight part. It will be found that in the latter the central channel is wider than in the former position, although the total diameter of the tube is less. This arises from the

Fig. 114



* In this, and in the following chapters, unless stated to the contrary, the appearances described are observed with a quarter-of-an-inch object-glass (about 200 diameters); but, as already indicated, it is always important to subject specimens to examination with low as well as with high powers.

greater thickness of the secreting epithelium in the convoluted portion. In looking at knuckles of tubes, an appearance is often produced as of a circumscribed cyst, arising, as Dr. Johnson has shown, from the bending of a tube as it enters and emerges from the different meshes of the matrix (fig. 114 *b*). The cut ends of the tubes often appear curled over, as it were, giving the appearance of a thick ring.

142. Matrix.—The matrix may be seen very clearly in a section of the kidney of a mouse, or in that of many other rodents. Of late, much discussion has arisen with reference to the presence or absence of a fibrous matrix in the healthy human kidney, and observers are not agreed as to which is really the case. It was first described by Goodsir ; and both Kölliker and Dr. Johnson have given drawings representing it very distinctly. With care, I believe, it may be always demonstrated in the healthy human kidney, and in some specimens of fatty kidney, as well as in the small contracted kidney of chronic nephritis, it may very readily be seen by washing a thin section with a stream of water, in order to remove the epithelium and remaining portion of the tubes. The matrix appears to consist of very fine fibres (fig. 114 *c*), amongst which no indications of the yellow element can be detected. By the addition of acetic acid it becomes more transparent, and a few granules are developed, but no other change is produced. Large intervals will be seen which were originally occupied by the tubes.

143. Vessels of the Malpighian Tuft.—Here and there on the vessels of the Malpighian tuft (fig. 114 *d*), a few cell-like bodies are often seen. These have been described by some as epithelial cells upon the external surface of the vessel, but from the researches of Mr. Bowman it appears probable that the vessels are quite bare. These cells, or nuclei, frequently appear, however, as if situated within the substance of the wall of the vessel.

144. Bright's Kidney.—By this term has been understood any of those morbid conditions of the kidney which are accompanied with the secretion of albuminous urine. Of late, however, some important characters have been made out, which have enabled us to distinguish several essentially distinct pathological

conditions of kidney, which result from special morbid changes having taken place in those organs. Some physicians, however, still insist that the pathological states of kidney which have been described as distinct diseases, are really different stages of the same disease. We cannot, however, from a careful consideration of the facts at present known, be brought to the conclusion that the small contracted kidney so commonly met with in old drunkards, with its rough tuberculated surface, and diminished cortical portion, is a different stage of the fatty kidney, or that it results from the occurrence of morbid changes, at all resembling those which end in the production of the large, smooth, and pale kidney of fatty degeneration. Microscopically, as well as chemically, these forms of disease are distinguished by well-marked characters. Dr. Johnson distinguishes acute and chronic nephritis from fatty degeneration, and he gives cases of three other forms of disease which have not been accurately described by any previous writer; these are "waxy degeneration" (corresponding to, and often found in conjunction with, waxy degeneration of the liver), "non-desquamative disease," and "suppurative nephritis." *

145. Microscopical Examination of the Kidney in Disease.—Portions of diseased kidney are subjected to examination in the same manner as sections of the healthy organ. Sometimes a thinner section will be required, in consequence of the opacity resulting from deposition of fatty matter, or a morbid quantity of epithelium, &c., within the tubes; or from thickening or hypertrophy of the matrix external to them. In the chronic nephritic kidney, the condition of the minute vessels should be especially taken notice of, as their walls are often much thickened. The addition of a little acetic acid, or weak solution of caustic soda, often renders the vessels very distinct; and if the preparation be well washed in water previous to examination, in order to wash away the epithelium and granular matter from the tubes, the matrix and vessels will be more distinctly shown. The kidneys of cats are usually found to have the convoluted portion of the tubes loaded with oil, and, in many instances,

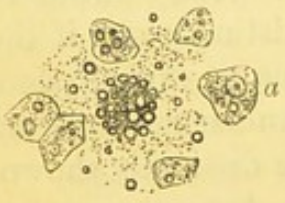
* "Diseases of the Kidney," 1852.

much oil is found in the Malpighian tuft. The fatty matter is frequently so abundant as to give the tubes the appearance of being injected with some white material when examined by reflected light. Sections of kidney may be preserved by immersion in some preservative solution, such as weak spirit and water (§ 93), or in the creosote solution (§ 96). They should be placed in large thin glass cells made after the method described in § 72.

146. Liver.—For the examination of the liver, the same general directions as were given when considering the kidney will suffice. If the cells alone are to be examined, a freshly-cut surface may be scraped with a sharp knife, and the matter thus removed placed in a drop of water or serum, and covered with the thin glass. In order to demonstrate the relation which the different elements bear to each other, the best way is to cut a very thin section by means of Valentin's knife, from the fresh liver of the pig; or thin sections may be taken from portions of liver, which have been hardened in alcohol, chromic acid, &c. The vessels of the liver which, with condensed cellular tissue, form a sort of matrix or network, in the meshes of which the cells are situated, may be readily demonstrated by washing the cells away from a thin section with a stream of water, and then treating it with a little dilute caustic soda.

147. Liver Cells.—The hepatic cells even in a state of health, generally contain a few oil-globules, which vary a good deal in size, but which are for the most part very minute (fig. 115*a*). In disease the cells may become wasted and shrunk in appearance; they may be filled with granular matter, or gorged with fat, as shown in the cell situated in the centre of the figure; or the fatty matter may have increased so enormously in quantity as to cause the obliteration of the cell wall altogether, in which case a thin section of the liver will be found to present, under the microscope, an appearance not to be distinguished from ordinary fatty tissue. Not unfrequently the cells of the liver will be found to contain granules of yellow colouring matter. The cells of a fatty liver contrast

Fig. 115.



remarkably with the starved state of the cells of a scrofulous liver, or with the pale granular cells which are often met with in the liver of patients who have died of diabetes. Chemically, the amount of fat is found to vary much, and the balance shows the enormous increase in a more striking point of view than the microscope. From a fatty liver which I analyzed some time ago for Dr. Budd, I obtained as much as 65·19 per cent. of fatty matter; and upon comparing this with the quantity obtained from a scrofulous liver a remarkable difference was noticed, for in the latter only ·57 grs. per cent. of fatty matter were obtained.*

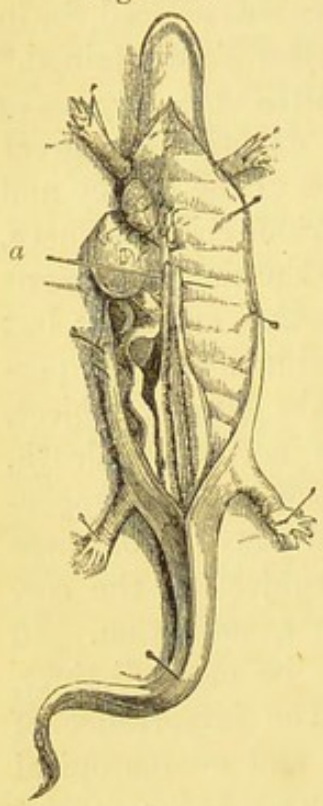
148. **Advantages derived from the Examination of the Organs of the lower Animals.**—The student will often experience a great difficulty in making out clearly the structure of the kidney and liver in a healthy state in man: owing to various circumstances, particularly to post-mortem alterations, or to the commencement of morbid changes, the microscopical characters are often but ill defined. Great difficulty again is found in obtaining perfectly healthy specimens of these organs in the human subject, and it is only in the occasional cases of accidental death, occurring to young healthy persons, that we have an opportunity of examining these glands in a normal state. For these reasons, great advantage will be gained by subjecting the corresponding organs in the lower animals to examination. In these, the structures will often be found to be more distinct, and may be more readily demonstrated. The importance of being thoroughly familiar with the structure and microscopical characters of any particular organ in a healthy condition, cannot be sufficiently dwelt upon, and it is to a want of this knowledge, that many erroneous descriptions of morbid appearances may be attributed. All who wish to use the microscope successfully, with reference to the examinations of organs in disease, will do well to become acquainted with minute anatomy generally, not only of the human subject, but of animals; and without such knowledge it will be found impossible to prosecute pathological inquiries with any degree of success.

149. **Kidney of Frog and Newt.**—The general arrangement

* "Diseases of the Liver," second edition, pp. 284, 313.

and structure of the kidney may be beautifully seen by examining a portion from a recently-dead frog or newt. The tube in the case of the frog will be found ciliated for a short distance from the Malpighian tuft; but in the newt the uriniferous tube in its whole length is lined with ciliated epithelium, which continues in active motion for some time after the removal of the organ from the body of the animal. In order to display this beautiful object, we must proceed as follows: After destroying the newt by cutting off the head, the abdominal cavity is laid open, and by turning the viscera to one or other side the kidneys may be exposed. Towards the pelvis the kidney presents much the same appearance as that of the frog: but, upon tracing it upwards, it will be found to become gradually thinner, and to extend quite into the thoracic portion of the animal. It is this upper thin part of the kidney which shows the ciliary motion to the greatest advantage (fig. 116 *a*). A probe is represented in the cut underneath that portion of the kidney which should be examined. Here the secreting tubes lie upon one plane, so that an individual tube may often be seen at one time with active ciliary motion throughout its whole length. A more beautiful object under a half-inch object-glass, can scarcely be conceived. The portion of the kidney, above described, is to be very carefully removed from the body by delicate manipulation with fine forceps and a pair of scissors, moistened with a little water, or, what is still better, with some of the serum of the animal, and placed in a large thin glass cell, and carefully covered with thin glass. The cell should be sufficiently thick to prevent any pressure upon the preparation. After ciliary action has stopped, the cilia are with great difficulty distinguished. In examining the frog's kidney a thin section must be made with a very sharp knife, taking care to disturb the

Fig. 116.



structure as little as possible. The section may be moistened with a little of the serum, and examined in a glass cell. After examination by the low powers, both these preparations may be examined by a power of 200 diameters or upwards.

150. Kidney of the Horse and other Animals.—The kidney of the horse is well adapted for displaying the Malpighian tufts, as in this animal they are unusually large, and, when injected, show the arrangement of the vessels in the tufts very distinctly. A section of the straight portion of the tubes cut at right angles from a fresh kidney will be found to show well the general minute anatomy of this part of the organ. A section may be made with the double-edged scalpel (§ 136), or with Valentin's knife (§ 137), at right angles to the course of the tubes.

The kidneys of mice and other rodents, as above mentioned, will be found to display the matrix well. All that is required for this object is to cut a thin section and wash it well previous to examination.

The kidneys of small fish and snakes are well worth careful examination. The tubes of the kidney of the common field snake (*coluber natrix*) will be found to be ciliated.

151. Liver of the Pig and other Animals.—The liver may also be examined in different animals. The anatomy of the liver in the Crustacea (crabs, lobsters), Mollusca (snails), and the cæcal tubes which appear to represent the hepatic organs of insects, are especially worthy of study. The liver of the pig is the most favourable for demonstrating the relation of the constituent parts of the organ to each other; that of the frog, when injected, shows well the arrangement of the minute vessels. If injected from the portal vein with one colour, and from the hepatic artery or vein with another, the injections may often be made to meet, and in this manner a good illustrative specimen obtained (§ 123).

Besides those mentioned in the text, the following works may be referred to upon the subjects treated of in the present chapter:—"Physiological Anatomy and Physiology of Man," Todd and Bowman; "Kölliker's Handbuch der Gewebelehre des Menschen," translated for the Sydenham Society, by Busk and Huxley; Strausdurkheim, op. cit.; Dujardin, "Observateur au Microscope," Paris, 1843.

CHAPTER X.

EXAMINATION OF BRAIN AND NERVES—VESSELS—MUSCULAR FIBRE—LUNG—GLANDULAR STRUCTURES—EPITHELIUM—SKIN—MUCOUS MEMBRANE—VILLI—SEROUS MEMBRANES—ADIPOSE TISSUE—CORNEA—RETINA—CRYSTALLINE LENS.

152. Examination of the Brain.—The brain should be subjected to examination as soon as possible after death. In examining the fresh brain small portions may be removed on the end of a knife, placed upon the glass slide, and moistened with a little serum, or weak solution of sugar. For examining the arrangement and distribution of the nerve fibres, portions of brain should be hardened in the chromic acid solution, when very thin sections can be obtained with a Valentin's knife. Dilute solution of caustic soda is also exceedingly useful for rendering the nerve tubes more distinct. The minute anatomy of the brain may be studied in man and in the higher animals.

The examination of the dura mater and arachnoid is conducted according to the general plan already laid down. Very small pieces are removed, carefully torn up with needles, moistened with water, and covered with thin glass. The gritty substances (*corpora amylacea*) in the pineal body, and those which are not unfrequently met with in other parts of the brain, may be separated from the brain substance by washing them in a glass of water, in which they will sink to the bottom; the supernatant fluid may then be poured off, and replaced by fresh water. After this process has been repeated a few times the bodies in question will become quite clean. They may then be examined in water, or dried and mounted in Canada balsam.

The vessels of the brain may be readily examined if the white or grey cerebral matter be first removed by washing a thin section with water, and occasionally applying gentle pressure

(§ 157). The addition of a little very dilute caustic soda renders the outline more distinct.

If a portion of white cerebral matter be treated with water, the nerve fibres soon become changed in character, apparently in consequence of the partial separation of the oily from the albuminous constituents which are contained within the tubular sheath. The oily matter forms distinct and separate globules, often of considerable size, or it tends to collect in quantity in different parts of the fibre, which produces a beaded appearance. A similar change takes place in nerve fibres generally, if they are not examined very recently, or if they have been soaked for a short time in water. Fig. 117 represents some of these changes.*

Fig. 117.

153. Examination of the Spinal Cord.—Different parts of the cord may be examined in the fresh state, but in order to demonstrate the beautiful structure described and figured in modern works we must have recourse to certain methods of preparation. A solution of chromic acid is invaluable for investigating the structure of the cord. Segments of different parts are placed in the solution and allowed to harden, when very thin sections may be readily obtained and examined.

The method of preparation adopted by Mr. J. Lockhart Clarke, in his beautiful and highly important investigations on the structure of the spinal cord, was the following:—

"A perfectly fresh cord was hardened in spirits of wine, so that extremely thin sections, in various directions, could be made by means of a very sharp knife. A section so made was placed on a glass slide, and treated with a mixture composed of one part of acetic acid and three of spirits of wine, which not only makes the nerves and fibrous portion more distinct and conspicuous, but renders also the grey substance much more transparent. The section was then covered with thin glass, and viewed first by reflected light with low magnifying powers, and then by transmitted light with higher ones.

---

* See also Todd and Bowman's "Physiology," vol. i. fig. 252.

"According to the second method, the section is first macerated for an hour or two in the mixture of acetic acid and spirit. It is then removed into pure spirit, and allowed to remain there for about the same space of time. From the spirit it is transferred to oil of turpentine, which expels the spirit in the form of opaque globules, and shortly (sometimes immediately) renders the section perfectly transparent. The preparation is then put up in Canada balsam, and covered with thin glass. By this means the nerve fibrils and vesicles become so beautifully distinct, that they may be clearly seen with the highest powers of the microscope. If the section be removed from the turpentine when it is only semi-transparent, we sometimes obtain a good view of the arrangement of the blood-vessels. This mode of preparation succeeds best in cold weather; for in summer, the cord, however fresh when immersed in the spirit, remains more or less spongy, instead of becoming firm and dense in the course of five or six days. The spirit should be diluted with an equal quantity of water during the first day, after which it should be used pure. Certain modifications of this mode of preparation may be sometimes employed with advantage by a practised hand."*

All Mr. Clarke's observations were, however, verified on perfectly fresh sections of the cord unchanged by the addition of any chemical reagent.

154. Examination of Nerves.—Nerve fibres may be examined from various situations in the body. A portion of a thick trunk may be removed and torn up with needles; or we may subject some of the fibres nearer the point of distribution to examination. The mesentery of small animals, particularly that of the newt, is very favourable for examining nerve fibres, but the investigation of their general anatomy and arrangement in different parts of the body and in different animals is not attended with great difficulty. The ultimate distribution, however, is very difficult to demonstrate.

Nerve fibres which are not quite fresh will be found to exhibit numerous varicosities, and are disposed to break up, forming twisted or oval masses, or even large globules (Fig. 117).

* "Philosophical Transactions," 1851, Part II.

Phosphoric acid and solutions of iodine have been used by some investigators in researches upon the structure of nerves. Doubtless there are many other chemical reagents which experience will show to be useful in investigations of the nervous system. For examining the arrangement of nerve fibres in papillæ, those of the tongue of the frog will be found advantageous.* A transverse section is made with a very sharp knife, moistened with very dilute caustic soda, and subjected to examination. Preparations of nerves are usually preserved moist, but they may be put up in Canada balsam.

In some cases in which nerves have been subjected to long stretching or pressure during life, certain changes in their minute structure will be observed. Perhaps the nerve fibres have become entirely converted into fibrous tissue, and in a few rare instances that change termed fatty degeneration will be found to have occurred. Of this latter condition I met with a most beautiful example not long ago, in a case of Dr. Todd's, in which one of the pneumogastric nerves had become incorporated with, and stretched over, the walls of a large aortic aneurism.

155. Examination of Vessels.—The smaller vessels may be examined entire, and present beautiful objects for microscopical observation. A portion of the mesentery of a child or of one of the lower animals, or a small piece of the pia mater may be selected; or one of the smaller arteries of the brain may be freed from cerebral matter by gentle washing in water, and placed in the microscope, with the usual precautions. If the specimen be treated with a drop of acetic acid the nuclei of the contractile fibre cells, and also those of the epithelial cells, on the lining membrane are brought into view.

In order to make out the fibre cells of the contractile coat, described by Kölliker, a little care is necessary. To effect this object it is better to take an artery of moderate size, which is not quite fresh, but at the same time in which decomposition has not commenced. The artery is to be slit up, and its lining membrane removed by careful scraping. Small portions of the subjacent elastic tissue are then to be removed, carefully torn

* Dr. Waller, "Phil. Trans." 1849.

up by the aid of fine needles upon a glass slide, moistened with a little water, and placed in the microscope with the usual precautions. According to Kölliker the spindle-shaped or muscular fibre cells are also to be obtained from the veins. The vein from which they may be most readily procured is the renal vein. Many of the fibre-cells contain distinct nuclei, which are rendered very clear upon the addition of a little acetic acid.

156. Capillary Vessels of the Kidney.—A thin section of the cortical substance of the kidney often displays the minute vessels very well. The examination of the vessels in this organ is of especial interest, because they are known to undergo alterations which could not be detected unless the observer were previously well acquainted with the appearance of these vessels in health. If a section of a healthy kidney is to be examined, with the view of observing the characters of the minute vessels, it will be better to wash the preparation previously in water, or with the wash-bottle (§ 131), in order to remove as much as possible of the epithelium of the renal tubes. Upon the addition of a drop of acetic acid the vessels are at once brought into view. It will now be noticed that the coats of the veins everywhere appear to be very thin, being represented only by a defined line on each side of the vessel, while the arteries are at once recognized by the greater thickness of their walls, and by the distinct arrangement of the nuclei of the circular and longitudinal fibres.

The coat of a healthy Malpighian artery appears to be about the fifth or sixth part of the total diameter of the vessel, but in disease the vascular wall may have increased in thickness to such an extent as even to equal in width the canal itself, and to be as much as one-third of the diameter of the vessel. The most perfect specimens of this morbid condition are to be obtained from the small contracted kidneys of intemperate persons. This change is described by Dr. Johnson,* to whom I am indebted for the advantage of having seen many excellent examples of it. Fig. 118 represents the appearance of a portion of a Malpighian artery considerably thickened, but not to such a degree as often occurs. The

Fig. 118.

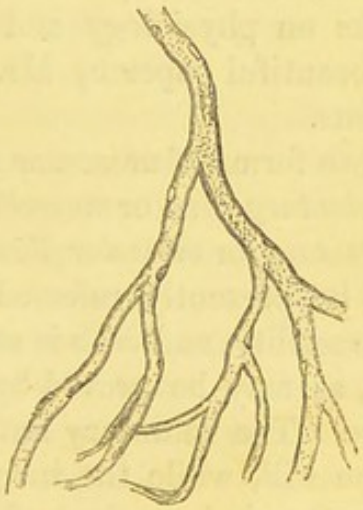
* "Diseases of the Kidney," 1852, fig. 229.

thickening of the walls is sometimes well shown in injected specimens.

157. Minute Arteries of the Brain.—A knowledge of the appearance of the minute arteries of the brain in a state of health is especially important to the morbid anatomist, because in disease these vessels are not unfrequently found to have undergone a very important change, which must necessarily much interfere with the discharge of their functions. In cases of white softening of the brain, this change is very common; indeed in such instances it is, I believe, rarely absent. If we tear away some of the smaller vessels from the softened portion, and after washing them in water, place them in the microscope, we shall often find at short intervals collections of minute oil-globules, easily distinguished by their high refracting power (fig. 119). These may form small aggregations of globules at short intervals, or may extend entirely round the vessel for some distance. Frequently small collections are found on opposite sides of the tubes, alternating with each other, and appearing to occupy the position of the nuclei of the development cells of

Fig. 119.

Fig. 120.



the vessels. This condition has been termed fatty degeneration of the vessels, and is probably intimately connected with the corresponding change in muscular fibre. Fig. 120 represents the

characters of an artery from a healthy brain, magnified in the same degree as fig. 119; the different diameter of the vessel in various points is due to the unequal pressure of the thin glass cover.

158. Atheromatous and Bony Deposits in Arteries.—The atheromatous deposit, so common in the larger vessels in disease, usually contains numerous oil-globules, with granular matter and much cholesterine (fig. 121), which appears to have gradually crystallized out, having been originally deposited in a state of solution in the oil.* This crystallization of cholesterine having taken place may possibly exert an influence on the still further deposition of atheromatous matter, and so lead to the accumulation of the large quantity often met with. Besides atheromatous deposit, plates of bone are often found in the coats of arteries, sometimes forming an osseous ring, completely surrounding the vessel. Thin sections may be obtained and subjected to microscopical examination, according to the method described in Chapter XII.

Fig. 121.

159. Examination of Muscular Fibre.—For a description of the minute anatomy of muscular fibre, I must refer to the various works on physiology and minute anatomy; and especially to the beautiful paper by Mr. Bowman in the Philosophical Transactions.

Two forms of muscular fibre have been described, the *striped* or *voluntary fibre*, or *muscular fibre of animal life*, and the *unstriped*, *involuntary*, or *muscular fibre of organic life*, the characters of which will be presently referred to. Both forms possess inherent contractility, and each is stimulated to contract by simple irritation, as may be proved by direct experiment under the microscope. The voluntary muscle alone is under the direct control of the will, while the involuntary fibre performs its functions altogether independent of volition.

Striped muscular fibre may be obtained from the voluntary muscles of man or any animals. If specimens be taken from

---

* Vide § 243, note.

the different vertebrate kingdoms, certain characteristic peculiarities will be met with, and the muscular fibre of the crustacea, mollusca, and insects, differ from the muscles of the higher animals in many important particulars.

In order to subject a portion of muscular fibre to microscopical examination, it is only necessary to remove a small piece with a sharp knife or a pair of scissors, and after tearing it up with needles, and moistening it with a drop of water, the thin glass cover may be placed on it, and the specimen examined with different powers.

The general arrangement and form of the fibres in voluntary muscles is well shown in a transverse section of the pectoral muscle of a teal (*Querquedula crecca*), which has been put upon the stretch, and allowed to become perfectly dry. A section cut as thin as possible, may be remoistened with water, and examined in the usual manner.

160. Sarcolemma.—The fibre of the skate, as Mr. Bowman has shown, is remarkably well adapted for showing the sarcolemma, as the sarcous matter may be ruptured while the investing membrane remains entire, and may be thus easily demonstrated. A few of the long fibres from the fin may be spread out upon a piece of glass with the aid of needles, and in this operation it will often be found that the rupture of the sarcous matter in the interior has taken place.

161. Branched Muscular Fibres.—Branched muscular fibres have been found in the heart, but they are not very easily demonstrated. Fibres of this nature may, however, be shown to exist in great abundance in the tongue of the frog (as was pointed out by Kölliker), from which organ they may generally be obtained as follows: the tongue is to be separated from the animal, and boiled for a few moments in water; the mucous membrane is cautiously dissected off from a small portion, and a few minute pieces are to be carefully snipped off with scissors, from the edge of the tongue, just beneath the mucous membrane. These are to be torn with very delicate needles, and then examined with a quarter-of-an-inch object-glass. In this manner these beautiful fibres may be generally found but care must be taken not to boil the tongue for too long a time, in

which case the fibres become too brittle to admit of separation. Branched fibres also exist in the upper lip of the rat.*

162. Preparation of Muscular Fibre for Microscopical Examination.—The transverse striæ may usually be demonstrated upon a piece of fresh muscular fibre, and are often seen very distinctly in a portion of ordinary voluntary muscle that has been boiled. I have often obtained most beautiful specimens of portions of fibre from the back of the tongue, a few hours after a meal, of which meat has formed a portion. The fibrillæ often separate readily from each other in a portion of muscle which has been macerated in a solution of chromic acid.

Amongst the matters vomited by patients suffering from certain affections of the stomach, beautiful specimens of striped muscular fibre may often be found; and in the evacuations of cholera patients, such specimens were almost constantly observed. In the stomach the fibres sometimes break up into discs (Frerichs).

The thin, narrow muscular bands, immediately under the skin of frogs and other small animals, will be found to exhibit well the general anatomy of voluntary muscle. The muscular fibre of the eel will be found to split up readily into its ultimate fibrillæ; and beautiful preparations, exhibiting the fibrillæ, have been obtained by Mr. Lealand from the pig. Sections of muscle in various directions may be made from muscles which have been boiled, or hardened in spirit, bichloride of mercury, or chromic acid. The reagents of the greatest use in investigating the structure of muscular fibre are a dilute solution of caustic soda and acetic acid, which are employed more particularly in investigating the arrangement of the nuclei. Preparations of muscular fibre may be preserved moist in glycerine, chromic acid, or solution of creosote, or they may be dried and mounted in Canada balsam.

163. Examination of Muscular Fibre in a state of Fatty Degeneration.—This most interesting subject has lately received the attention of many observers, both in England and also on the

---

* Huxley; "British and Foreign Med. Chir. Review," 1853, No. xxiv., p. 313.

Continent. The paper of Dr. Quain, in the Medico Chirurgical Transactions (Vol. XXXIII.), contains a most excellent account of fatty degeneration of muscular fibre.

The muscular fibres of the heart are found very commonly to have undergone this change, and it is frequently well marked in the musculi papillares, to which the tendinous cords of the mitral valve are attached. Almost any muscles which have long been out of use, as happens in many cases of paralysis, and in numerous forms of club-foot, will also be found to exhibit it.

Sometimes there is not the slightest appearance of transverse striæ on the fibre, which appears to be composed of rows of minute, and highly refracting little globules of oil. In other specimens, no distinct globules can be seen, but the whole fibre appears made up of granular matter. Fig. 122 represents two elementary fibres in a state of fatty degeneration from one of the papillary muscles of the mitral valve. It must, however, be borne in mind, that fibres in a state of commencing decomposition exhibit, in a slight degree, this granular appearance.

Fig. 122.

The examination is conducted in a similar manner to that of healthy muscle; very small pieces may be cut off with scissors, and torn up carefully with very fine needles. The addition of acetic acid causes the oil-globules to become more distinct. The oily nature, both of the globules and granules, may be proved by the addition of a drop of ether, which dissolves them; and upon the evaporation of this ethereal solution, a globule of oil remains behind, which will be found to leave a greasy stain when rubbed upon a piece of clean glass. This reagent also enables us to distinguish between globules of oil and globules of phosphate of lime, which often much resemble the former in general form, and indeed have been occasionally mistaken for them.

Not unfrequently fibres of muscles become converted into fibrous tissue, a change which is particularly common in the

small papillary muscles of the heart. By carefully tearing up with needles, the fibrous structure can be clearly made out.

164. Examination of Non-striated Muscular Fibre.—Involuntary, smooth, or non-striated muscular fibre may be obtained from various situations, both in man and also in the lower animals. These fibres are most abundant in the alimentary canal, the uterus, the bladder, and large vessels, but they are also found dispersed amongst fibrous tissue in certain situations, particularly in the skin. The elongated cells, of which they are composed, are also to be demonstrated in the small arteries, veins, and lymphatics, as well as in the trabecular tissue of the spleen, and corpora cavernosa penis, the urethra, &c. The muscular fibres, which have hitherto been described as consisting of flattened bands, have been demonstrated by Professor Kölliker to consist of the elongated cells just referred to. The contractile fibre cells have been arranged in three classes :—

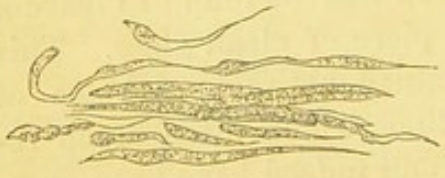
1. Short rounded or flattened cells, somewhat resembling epithelium.

2. Flattened bands, with fringed edges.

3. Long rounded or fusiform fibres, slightly wavy, and terminating at each end in a point.

The first two varieties are obtained from the blood-vessels. The last form is met with in the intestinal canal, uterus, &c. These cells may be readily isolated by macerating small pieces of the muscular coat of the alimentary canal, &c., in dilute nitric acid, containing about 20 per cent. of strong acid. By a little tearing, with the aid of fine needles, separate cells may be readily obtained. Fig. 123 represents some of the contractile fibre cells from the ileum. These cells may also be demonstrated in most of the lower animals; but it is worthy of remark that a portion only of the alimentary canal of some fish is surrounded by involuntary muscle, while it has been shown that the whole of the muscular fibre of the intestine of the common tench is of the striped variety.

Fig. 123.



165. Examination of the Muscular Structure of the Heart.—The muscular fibres of the heart will be found to exhibit the transverse striæ characteristic of voluntary muscle; but they are arranged in long bands, and upon carefully examining a well-prepared specimen, taken either from the heart of man or of most animals, frequent and distinct anastomoses and branchings of the fibres may be observed. The sarcolemma is of such extreme tenuity that it is exceedingly difficult to demonstrate; indeed its existence is questioned by many observers.

In order to exhibit these fibres, the heart of man, or that of any small animal may be taken, and after boiling it for a short time in water, small pieces may be cut off, and carefully torn up with needles. The length of time which the boiling should be continued varies in different cases. Half a minute is sufficient for the hearts of very small animals; sheep's hearts may be boiled for a quarter of an hour.* Fatty degeneration of the muscular fibres of the heart has already been alluded to.

166. Examination of the Lung.—There is not much difficulty in demonstrating the different tissues of which the lung is composed. Small pieces of the pulmonary tissue may be cut off, and spread out upon the glass slide in the usual way; the preparation may be moistened with water. The addition of a little acetic acid causes the yellow elastic tissue to become very distinct. The boundaries and arrangement of the air-cells may also be readily shown. In order to examine the distribution of the vessels, it becomes necessary to look at injected specimens.

A most instructive preparation of the lung, however, is made by injecting the vessels with tolerably thick transparent gelatine, which transudes through their walls, and fills the air-cells. After the lung has been thoroughly injected, it is set aside to get cool. Thin slices may be examined, and the vessels will be seen *in situ* apparently bare, and uncovered by epithelium.†

---

* For the method of demonstrating the arrangement of the bands of muscular fibres of the heart, see Mr. Searle's article in the "Cyclopædia of Anatomy and Physiology," vol. ii. p. 619.

† "Physiological Anatomy," Todd and Bowman, p. 393.

The mucous membrane of the trachea and bronchial tubes must be examined in the recent state by cutting thin sections with a very sharp knife.

In examining the ciliated epithelium of the air-passages it is only necessary to scrape the surface gently, and, if necessary, the preparation may be moistened with a little serum, as water would very soon stop the motion.

The breathing apparatus in various animals may be examined with great advantage.

Tracheæ, characteristic of the class Insecta, may be readily obtained by removing the viscera of a common fly or other small insect, and tearing carefully with needles. They may be examined after the addition of a drop of water, or they may be dried, and moistened with a drop of turpentine, or mounted in Canada balsam. The branchiæ of many mollusca (oyster, mussel) exhibit ciliary motion very beautifully.

The gills of fish, the lungs of frogs, newts, and serpents, will also furnish many very instructive specimens.

167. Examination of the Lung in a Morbid state.—The same general method of procedure is employed here. The lungs of emphysematous persons are particularly worthy of study. According to the observations of Mr. Rainey, the pulmonary membrane is found perforated with many minute holes, and here and there numerous oil-globules may be detected; the fibres of yellow elastic tissue are stretched, and have lost their elasticity; the vessels are much elongated, and the interspaces between them much enlarged.

Different parts of a tuberculous lung should be examined. If we find a cavity, its contents, the surface of the walls, and a section of the immediately subjacent tissue should be separately examined. If the tubercles have not broken down, small portions may be removed upon the point of a knife, moistened with a drop of water, and examined in the microscope.

The figure in the margin represents the general appearance of the small cells (tubercle corpuscles) met with in tubercular deposit, before it has become softened and broken down. These cells are usually found with

a considerable quantity of granular matter, and many minute oil-globules.

Crystals of cholesterine are occasionally met with amongst the cheesy matter which makes up the greater part of some tuberculous masses. If, however, no crystals can be detected, a portion of the mass, placed in a watch-glass, may be treated with a few drops of alcohol. As the alcohol gradually evaporates, beautiful crystals of cholesterine form, and may be subjected to microscopical examination in the usual manner.

The small white, calcareous masses, which are not unfrequently met with in the lungs of phthisical patients, and which are from time to time present in sputum, may be examined as opaque objects, with low powers; or fragments may be broken off, and subjected to examination. After having been dried, they may be placed in turpentine or Canada balsam.

If we test them with a drop of acetic acid, we shall find that they dissolve with effervescence, showing the presence of carbonate. One part of the acetic acid solution may then be treated with excess of ammonia, when a precipitate of phosphate of lime will be immediately thrown down. The presence of lime may be detected in the other portion of the acid solution, by adding to it a little solution of oxalate of ammonia.

The relation of the tuberculous matter to the walls of the air-cells, may often be beautifully seen in an injected specimen of tuberculous lung.

168. Examination of the Thymus and Thyroid, &c.—These glands should be examined in the perfectly fresh state. Thin sections, obtained by a Valentin's knife, will afford the best specimens; the section will generally require washing slightly, in consequence of being covered with the softer and pulpy portion of the gland, which renders the arrangement of the tissue obscure. The glands may be hardened in a solution of chromic acid, in spirit, or by boiling in water—but by these processes the cellular portion of the gland becomes somewhat modified. For making out the relation of the lobules and other structures to each other, hardened specimens are, perhaps, to be preferred.

In many of the smaller animals the thyroid gland is almost membranous, and this renders it very favourable for microscopical examination.

169. **Examination of Lymphatic Glands.**—In the examination of lymphatic glands, a little of the thick fluid which is poured out from a freshly-cut surface, should be placed between glasses, without the addition of water or any other preparation, and examined in the microscope. The student should become very familiar with the character of the cells obtained from lymphatic glands, and should carefully observe their behaviour upon the addition of acetic acid, dilute solution of caustic soda and other reagents, and he should compare the changes which take place with those which occur when pus globules, and white blood corpuscles are treated in a similar manner. The minute structure of lymphatic glands is an exceedingly difficult subject to investigate.

170. **Salivary Glands.**—The salivary glands should be examined in the recent state. A very thin section is obtained, which is slightly washed, and then treated with a little acetic acid or weak caustic soda. The structure may frequently be more clearly defined if we treat the section first with acetic acid, and afterwards with excess of caustic soda. The epithelium of the ultimate lobules is sometimes well seen after the preparation has been soaking for some time in acetic acid, which for this purpose must not be too strong.

Healthy saliva contains a great many round granular cells, which are about the 1-2000th of an inch in diameter; acetic acid renders them more transparent, and the nucleus more distinct. Occasionally the salivary ducts have been found to contain a considerable number of small white granular masses, which consist of cells filled with large oil-globules.

171. **Examination of Epithelium.**—The microscopical examination of epithelium does not usually present much difficulty. The surface from which the epithelium is to be taken is gently scraped with a knife, and a small portion removed upon the blade. If necessary, it may be moistened with a drop of water, or with a solution of sugar, or serum if the cells are delicate, and there is danger of rupture from endosmosis. Generally,

however, the addition of fluid will not be necessary. The chief reagents which will be found of use in the examination of epithelium are acetic and nitric acids, strong and weak solutions of potash and soda, and tincture of iodine. Epithelium is not soluble in boiling water, alcohol, ether, ammonia, or dilute mineral acids; it is for the most part soluble in strong solutions of caustic soda and potash, and in strong acetic acid. Most forms of epithelium will be found to keep very well in the naphtha and creosote solution (§ 97), or in a dilute solution of chromic acid.

172. **Scaly Epithelium** can be readily obtained from the cavity of the mouth, and from several other situations. The nuclei of the epithelial cells from the cavity of the mouth are very distinct, and can always be demonstrated without difficulty. If the cells be placed in a solution of potash for a short time endosmosis takes place, they become somewhat globular, and ultimately the cell-wall dissolves. The addition of acetic acid causes the granules in the interior of the cell to become less distinct, an effect, the reverse of that which usually occurs upon treating many cells with this reagent.

The scaly epithelium from the vagina is composed of very large, irregular, and often ragged cells (fig. 124). In consequence of the flattened character of the cells of scaly epithelium, portions of them will often be found folded upon each other, and creased, as it were, in various directions. The cells of the epidermis, as well as those of nail and hair, present modifications of scaly epithelium.

Fig. 124.

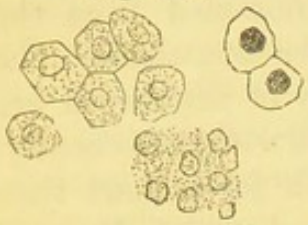
173. **Tesselated or Pavement Epithelium.**—This term has been applied to the cells of epithelium which form an even layer of uniform thickness, each individual cell being placed in juxtaposition with its neighbours, but not overlapping or exhibiting the imbricated arrangement often met with in the variety of epithelium just referred to. The epidermis of the

frog presents a beautiful example of this variety of epithelium; the choroidal coat of the eye, the epithelium of serous membranes, of the lining membrane of the heart, arteries, and veins, and that of the pelvis of the kidney (fig. 125), also present more or less of this character. The nucleus of the cell is usually distinct and well-developed.

Fig. 125.

174. Glandular, or Spheroidal Epithelium.—The cells are of a more or less rounded form, although in many instances, from mutual pressure, they become polyhedral. It is this form of epithelium which takes part in the process of secretion in most glandular organs. It may be readily demonstrated in the convoluted portion of the tubes of the kidney (fig. 126), in the sweat glands, in the secreting tubes of the stomach, in the follicles of the pancreas, in the liver, &c. The nucleus is usually well developed, and frequently surrounded by a considerable number of minute granules, and, in many instances, small oil-globules are also present.

Fig. 126.



175. Columnar, Prismatic, or Cylindrical Epithelium.—The general characters of this variety of epithelium may be well demonstrated by the examination of the intestinal villi, or Lieberkühn's follicles. The epithelium of the gall-bladder, of the ureters, and of the urethra (fig. 127), is of this variety. In the evacuations of cholera, the sheaths of the villi will often be found entire, and afford an excellent opportunity for the examination of the arrangement of this variety of epithelium.

Fig. 127.

176. Ciliated Epithelium.—There are two principal varieties of ciliated epithelium, the one consisting of small cells of nearly the same length and breadth, and the other, of the prismatic or cylindrical form. Ciliated epithelium may always be obtained for demonstration from the back part of the frog's mouth, or from the branchiæ of an oyster or mussel. The cells must be moistened with some of the mucus taken from the same surface, or with some of the juice from the animal,

or with a little clear serum. If water be added the movement soon stops, in consequence of endosmosis taking place. In examining ciliary movement, it is often advantageous to place the smallest quantity of lampblack or carmine with the cells, so that the direction of the current produced by the cilia can be clearly demonstrated by the movement communicated to the insoluble particles.

In the human subject, ciliated epithelium is found in the following situations:—on the surface of the ventricles of the brain and on the choroid plexuses; on the mucous membrane of the nose and its sinuses; on the upper and posterior part of the soft palate, and in the Eustachian tube; in the cavity of the tympanum; on the membrane lining the frontal and sphenoidal sinuses; on the inner surface of the lachrymal sac and lachrymal canal; on the mucous membrane of the larynx, trachea, and bronchial tubes; upon the os uteri; within the cavity of the uterus; throughout the whole length of the Fallopian tubes, and upon their fimbriated extremities.

177. **Examination of Skin**—*Cuticle.*—The cuticle may be subjected to examination either by scraping the surface with a knife, in which case only the most superficial cells, which are often not well defined, will be obtained; or by making a thin section of dried cuticle with a sharp knife. If a portion of skin be allowed to remain for some days in a moist atmosphere, it will be found that large flakes of cuticle can be readily detached, small fragments of which may be moistened with water in the usual way, and subjected to examination.

The epithelium will be found to vary in character according as it is taken from the deep or the most superficial layers of cuticle. In the former situation the epidermic cells are more or less rounded in form, while on the surface they are flattened and adhere to each other, forming small scales, in which the original form of the cell is with difficulty made out. The deepest layer of the cuticle appears to consist chiefly of minute granules, with a few small cells. It is in the cells in this situation that the colouring matter is deposited, and it was to this portion of the cuticle that the term *rete mucosum* was applied. As the cells approach the surface, the colouring-matter appears to

diminish in quantity, owing probably to changes taking place in the chemical nature of their contents. The cells composing the deeper layers of the cuticle are soluble in acetic acid, while those on the surface are unaffected by this reagent.

Upon examining the under surface of the cuticle, which has been removed as above directed, it will be found to present several depressions, in which the tactile papillæ of the cutis are lodged; and upon removing the cuticle by maceration, from some situations, such as the palm of the hand, or heel, or from the anterior surface of the leg, the epithelial lining of the sweat-ducts as they pass through the cutis will often be found adhering firmly to it.

178. **Pigment Cells.**—The cells containing pigment are very readily demonstrated in the skin of the negro, in that of several of the lower animals, or in the freckles which may often be obtained from different parts of the body of some subjects.

A preparation of the cuticle of the negro may be preserved in Canada balsam. The cuticle may be separated as above described, dried flat between folds of clean paper, or between two plates of glass, and mounted in the usual way (§ 104). The method of preparing a vertical section of the cuticle is described in § 180.

The vessels of the peritoneum, lungs, &c., and the skin of the frog, exhibit beautiful varieties of very dark pigment cells, which consist of several branches of irregular form radiating from the central part of the cell.

179. **Papillæ.**—The papillæ may be shown in two ways, either by making a vertical section of the skin previous to the removal of the cuticle; or the latter may be taken off in the manner described in § 177, and a section of the true skin only made.

The best situations from which to take the skin for the purpose of examining the papillæ, are the palmar surface of the hand, and the sole of the foot. After the cuticle has been removed, a transverse section of the skin with the papillæ may be made as follows: a gentle stream of water is to be allowed to flow over the papillæ, in order to make them all fall in one direction, which is readily effected by inclining the piece

of skin while the water is running. After being drained, a cut is made with a very sharp knife across the piece of skin in its upper part, in a direction at right angles to that which the papillæ have been caused to assume. Upon now turning the preparation, so that the freshly-cut surface is in the most dependent position, and allowing the jet of water to flow, the direction of the papillæ will be reversed, and it is obvious that a very thin section off the freshly-cut surface will contain one or more rows of entire papillæ. The section may then be examined, and may be preserved in liquid; or it may be placed upon a glass slide, gently dried, and mounted in Canada balsam. The papillæ of the skin of the foot of the dog are large and well-marked.

In order to examine the structure of the papillæ, a tolerably fresh specimen of skin should be chosen, and as thin a section as possible should be made. The specimen may now be treated with weak caustic soda, or with a little acetic acid, and subjected to examination. It is in this way that the nerves may occasionally be demonstrated in the papillæ, and frequently the vascular loop may be thus rendered distinct; but the arrangement of the vessels is always better shown in an injected specimen.

The "axis-corpuscles," or touch-bodies, may be shown in the papillæ situated at the tips of the fingers, or in the palm of the hand, by treating the specimen with acetic acid. A papilla may be met with in this situation terminating in two or three points. One of these will perhaps contain a touch-corpuscle, while in the others only a vascular loop can be seen, and no nerve fibre whatever can be distinguished. For detailed information on the subject of the axis-corpuscles, I must refer to Kölliker's "Gewebelehre," translated for the Sydenham Society.

180. **Method of making a Vertical Section of Skin.**—In a section of this kind all the structures entering into the formation of skin can be seen, and the arrangement of the hair-bulbs and sebaceous follicles may also be demonstrated if the skin be taken from a part in which these structures abound. The disposition of the sweat-ducts and the arrangement of the

glands may be well shown in such a preparation. It is exceedingly difficult to cut a section of skin in the recent state sufficiently thin for observation; hence, a modification of the method usually employed must be resorted to.

The skin should be perfectly fresh, and a piece about two inches square, or rather less, is to be stretched, with the outer surface downwards, upon a thick deal board, by means of numerous pins. If the sudoriferous glands are to be included in the preparation, care must be taken to leave sufficient of the cellular tissue adhering to the skin. The piece of skin is allowed to dry by exposure to the air. Several small pieces, taken from various parts of the body, may be pinned out on the same board, care being taken to attach a label to each. Specimens may be taken from the scalp, eyelids, chin, mamma, axilla, arm or leg, palm of the hand, tips of the fingers, scrotum, and sole of the foot. With these, the varying thickness of the epidermis and other peculiarities in the different regions may be demonstrated.

The portion of skin being quite dry, it is to be removed from the board, and, after cutting off the edge, several thin sections may be made, by the aid of a very sharp knife, through the whole thickness. In order to obtain a good specimen of the spiral portion of the sweat-ducts, the skin of the heel should be selected, and the section should be made parallel with the furrows, and in a slightly slanting direction, instead of at a right angle with the surface.

The sections may next be placed in a watch-glass with a few drops of clean water; and in the course of a short time it will be found that they have again attained the original thickness of the skin, in consequence of the absorption of water. They may now be submitted to examination, and after selecting a satisfactory specimen, it may be mounted in weak spirit and water, Goadby's solution, or other preservative fluid; or the specimen may be washed in water, placed upon a slide, and allowed to dry slowly by spontaneous evaporation (when it will be found to have adhered tightly to the glass), and mounted in Canada balsam with the usual precautions.

If the section appear opaque when examined in aqueous

fluids, it may be treated with a little weak potash or caustic soda, and carefully washed again before being mounted. If the skin contains very much fat, this may be removed by soaking the section for a short time in ether previous to moistening it with water. In this way a most beautiful section, showing all the structures, including the sweat-ducts, may be sometimes procured. Carbonate of potash has been employed for rendering the section transparent. The skin may be macerated in dilute nitric acid (one of acid to two of water) for twenty-four hours, when the sweat-glands are easily distinguished upon making a section (Giraldès, quoted by Kölliker).

Warts, *Corns*, and other growths, which consist of thickened epidermis, may be subjected to examination, and sections obtained in a similar manner.

The subcutaneous areolar tissue is sometimes found thickened over a considerable extent, or over small circumscribed spaces; in which latter case the sensation of small subcutaneous tumours is produced if the affected portions of skin be pinched up between the finger and thumb. The condition termed elephantiasis appears to consist of a thickening and hypertrophy of the subcutaneous cellular tissue, and the pouring out of lymph into the areolæ or cellular interspaces, which subsequently becomes a part of the tissue. Sections of skin in this state may be made after being hardened in alcohol or in a solution of chromic acid.

181. Examination of Mucous Membrane.—The epithelium of mucous membranes is very readily subjected to examination, and its character is found to vary much according to the locality from which it is taken. The chief varieties of epithelium, and the method of examining them have already been referred to (§ 171–176). In order to obtain a specimen of epithelium from a mucous membrane all that is required is to scrape the surface gently with a knife, and place what has been removed upon a glass slide, and, after moistening it with a little water, syrup, or a mixture of glycerine and water, which does not cause the cells to become so turgid from endosmosis, the specimen may be placed in the microscope. The thin glass cover should not be allowed to press too hard upon the specimen,

which may be prevented by inserting one or two pieces of hair or thin hog's bristles.

The basement membrane is sometimes demonstrated with difficulty, but from various accidental circumstances its presence may now and then be seen in the examination of pieces of mucous membrane. Frequently, however, the epithelium cannot be sufficiently removed in order to see this structure distinctly; perhaps the most favorable situation to look for it is the kidney (§ 141). The submucous areolar tissue may be very readily demonstrated by removing a small piece from the under surface of the mucous membrane with scissors, and tearing it up with needles.

A thin layer of pale muscular fibres has been described by Brücke situated immediately beneath the basement membrane of the small intestine. The contractile fibre cells of which it is composed are arranged in two layers, one of which takes a circular and the other a longitudinal direction. This is termed the muscular layer of the mucous coat, to distinguish it from the muscular coat of the intestine which lies external to the submucous tisssue.

182. **Villi. Muscular Fibres.**—The villi are best shown by making a perpendicular section of the mucous membrane of the small intestines with a very sharp knife, taking care, if possible, to make them take one direction by allowing a stream of water to flow over them, as referred to in describing the method of examining the papillæ of the skin (§ 179).

The muscular fibres of the villi, demonstrated first by Brücke, are to be shown by washing off the epithelium, and treating them with a solution of acetic and nitric acid, composed of about four parts of water to one of acid. For a description of the arrangement of these fibres I must refer to Kölliker's work.

The elementary structure of the muscular coat may be demonstrated by soaking small shreds in nitric acid diluted with four parts of water (§ 164).

183. **Hypertrophy of Submucous Tissue. Cancer of Stomach.**—In certain morbid conditions, the submucous tissue in this region is found as a hard, dense, somewhat transparent-looking

layer, varying in different cases from the eighth or tenth of an inch to an inch or even more in thickness, and almost of a cartilaginous consistence. Thin sections may be very readily examined; but nothing more than the original elements of the tissue with granular matter, and a few badly-defined cells, can usually be made out.

In certain cases, however, the meshes or areolæ of the tissue are much enlarged, and filled with cells having the general aspect of cancer cells, while the fibres composing the walls of these spaces are found to be more numerous and of increased thickness. After a time these morbid changes involve the mucous membrane itself, and an irregular ulcerating surface is formed. Such is a general description of a common form of the disease known under the name of cancer of the stomach. In examining an ulcer of this description, a little of the secretion on the surface, the surface itself, and the hardened tissue beneath, should be separately subjected to examination.

In the majority of cases of the so-called "cancer of the pylorus," nothing more than the thickening of the submucous areolar tissue above referred to can be observed, and upon microscopical examination none of the cells characteristic of malignant growths can be detected. A similar condition is not unfrequently found affecting the submucous tissue of the colon, cæcum, and other situations. It is important to distinguish this affection from cancer, as in its general appearance to the naked eye it is found so closely to resemble scirrhus, although it is essentially different from this disease in a pathological point of view.

In the examination of these structures, thin sections entirely through the thickened mass should be obtained with the aid of a Valentin's knife. The section, after being slightly washed, may be subjected to examination with the usual precautions.

184. Ulcers of the Intestines.—The surface of ulcers of the intestine may be examined by scraping, or by cutting off small pieces with curved scissors. Sometimes the villi situated immediately around the ulcer will be found to be very much increased in length. This change had taken place to a great extent around some ulcers of the small intestine of a patient,

who died of fever some time ago in King's College Hospital. The villi round the margin of the ulcers were many of them three or four times as long as those in other parts of the intestine. Whenever ulcers of the intestinal canal are examined, we must always endeavour to ascertain if the ulcer has eaten into the muscular coat of the intestine, a point which can easily be decided by the presence or absence of unstriped fibre in the tissue which forms the base of the ulcer.

185. Serous and Synovial Membranes.— Serous membranes may be examined according to the general directions previously given. It will sometimes be found difficult to demonstrate the delicate cells upon their surface, and fresh specimens only should be examined. The epithelium of serous membranes is generally of the pavement or tessellated variety, and appears to form one layer on the surface of the delicate basement membrane.

A small portion of the peritoneum of a mouse or other small animal will be found to display well the fibres of the sub-basement tissue, and often vessels and nerves may be seen beautifully distinct in this situation. The greater part of the thickness of serous membranes is made up of condensed areolar tissue, in which the yellow fibrous element is very abundant. This areolar tissue becomes less dense at a greater distance from the surface, and often contains fat cells like the subcutaneous cellular tissue. In disease the epithelium often increases very much in quantity; and in old cases of ascites, or pleurisy, it is not uncommon to find the serous membrane completely altered in structure, its surface being covered by a tolerably thick cellular layer. Frequently the fluid contained in the cavity is rendered turbid by the presence of a great number of cells of a similar character.

In order to examine the distribution of the vessels in synovial membranes, an injected specimen is necessary. The fringe-like processes which project into many of the joints are highly vascular, and a well-injected specimen forms a beautiful object. The surface in the recent state is covered with large cells of a more or less globular form.

The vessels which run between synovial membrane and

cartilage are very tortuous, and exhibit considerable dilatations and varicosities.

186. Adipose Tissue.—Adipose tissue may be examined by cutting off a thin section, and placing it with a little water between two pieces of glass, care being taken not to allow the thin glass cover to press upon the section. The surface of one of the small collections of fat cells which may be taken from the subcutaneous areolar tissue may be subjected to examination as an opaque object (§ 79).

In order to examine the arrangement of the vessels, the specimen must be previously injected.

Frequently the more solid portion of the contents, consisting of margaric acid and margarine, will crystallize on the surface of the more oily part in small acicular crystals, which radiate from a centre forming a star-like mass.

Adipose tissue should be examined by low as well as by high powers (a quarter-of-an-inch object-glass).

Fat cells are sometimes found degenerated, the fat having much diminished in quantity, and the greater part of the cell being occupied with a serous fluid, which, however, exists in very small quantity in a state of health; and under these circumstances the nucleus is often very distinct. The cell may also be in a shrivelled condition, and of a more irregular and frequently angular form. The fluid in its interior has been found to contain granular matter with many small oil-globules.

Adipose tissue obtained from emaciated subjects often shows the stellate crystals of margarine or margaric acid; and not unfrequently also the nuclei of the cells may be seen here and there, after treatment with a little acetic acid.

187. Of making Sections of the Cornea and Retina.—Thin sections of the cornea and retina are made upon the same principle as those of the skin (§ 180). The sclerotic is first cleaned by cutting away all the muscles adherent to it with sharp scissors, and the eye is then cut in two parts with a sharp knife, without removing the vitreous humour. It may be divided either transversely or in a longitudinal direction. When the cornea is to be examined, the anterior part may be well washed with water, and the ciliary processes, &c., removed;

after making little notches around it with scissors, in order that it may dry as flat as possible, it is to be pinned out upon a small piece of board with numerous pins. It is allowed to dry spontaneously, and then thin sections may be made with a sharp scalpel and moistened with water, when they swell out to their former size. The section may be treated with a drop of acetic acid, when the structures of which it is composed will become clearly visible.

In order to obtain a section of the retina, the posterior part of the eye with the vitreous humor adhering is carefully notched, and pinned out as in the former case. With care the greater part of the vitreous may be cut away with scissors, but a thin layer should be allowed to dry upon the surface of the retina. Thin sections may be made and treated as in the case of the cornea. Dilute acetic acid or dilute caustic soda may be applied to the section after it has been examined in pure water.

188. **Examination of the Crystalline Lens.**—The crystalline lens may be examined in the recent state by moistening a portion with a drop of water. It may be boiled, and some of the fibres carefully torn off, and afterwards moistened. Or it may be soaked for some time in a solution of chromic acid, and then subjected to examination; or lastly, it may be dried, soaked in oil for a considerable time, and a thick perfectly transparent section made, which may be ground to any required degree of tenuity; the surfaces may be afterwards polished. Sections prepared in this manner may be mounted in Canada balsam.

In examining cataracts we should carefully observe the microscopical characters of the soft external pulpy part, as well as of the hard internal nucleus. In many of these cases numerous oil-globules will be observed, which, from my own observations, appear to consist chiefly of cholesterine held in solution in an oily fat; and other larger globules, consisting of some very transparent substances presenting nearly the same refracting powers as this portion of the lens, but evidently of very different composition, as they are not miscible with it. Occasionally, also, small plates of cholesterine have been noticed. There is always much granular matter.

In examining the character of the fibres of the lens, it is better to boil it previously, and tear off a few fibres with forceps: these may be afterwards carefully separated from each other with needles. The arrangement of the fibres should be examined in different animals, especially in the human subject, the ox, the horse, and in fishes.*

If the disposition of the fibres of the surface of the lens is to be shown, it requires hardening in a solution of chromic acid. The lens may then be examined in a deep glass cell (§ 73), in some of the chromic acid solution with a low power.

* Todd and Bowman's "Physiology," chap. xvii.

Upon the subjects treated of in the last chapter, amongst many others, the following works should be consulted:—

Various articles in the "Cyclopædia of Anatomy and Physiology;" "Kölliker's Handbook," translated by Busk and Huxley for the Sydenham Society; Todd and Bowman's "Physiology;" "Chemistry of Vegetable and Animal Physiology," Mulder; "Lectures on Clinical Medicine," Dr. Bennett, 1853. "Chemie und Mikroskop am Krankenbette," Dr. Hœfle.—Erlangen, 1850.

CHAPTER XI.

MORBID GROWTHS—ENTOZOA—VEGETABLE PARASITIC STRUCTURES.

189. General Characters of Tumors.—Morbid growths and tumors are met with in various parts of the body, sometimes appearing quite superficially, being united to the adjacent tissue, by the intervention of a long narrow pedicle containing the necessary vessels and nerves for the supply of the tumor; while in other instances we find tumors deeply imbedded in the substance of solid organs, such as the liver or brain, and deriving their nutriment from every point of the surrounding texture.

Sometimes a tumor is produced by the rapid growth of a tissue at a particular point, and it therefore consists simply of the elements of this tissue. Fatty tumors, certain tumors of a fibrous structure, exostoses from bones, and many others, are produced in this way, and, as might be expected, but little difference can be made out between their minute structure and that of the tissue of which they are, as it were, the off-growth. In other instances, however, and these extremely numerous, the morbid growth is found to possess a structure of a much more complicated character; and although it may contain the elements of one or more of the tissues in a healthy state, it cannot be compared with any texture of the body in a normal condition; neither can any individual characters be laid down, by which many of these growths could be included in well-defined classes.

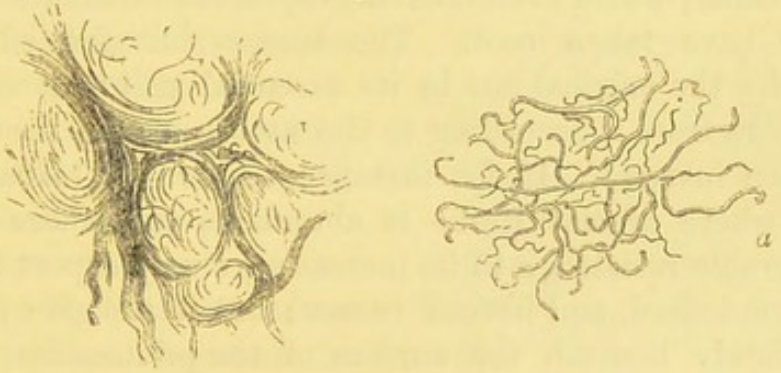
Although possessing certain points in which many resemble

one another, there are other peculiarities which often render it difficult to apply to them any specific name. Not only is there a difficulty in defining the species of different tumors by their microscopical characters, but the so-called benign tumors pass by almost imperceptible shades into those of a malignant and dangerous nature.

It is hardly possible to indicate the most important points in the structure of tumors without referring more or less to their distinctive anatomical peculiarities. I must, therefore, very briefly enumerate those which appear to me the most important, referring for more complete information on the microscopical structure to the different works on the subject, especially to Professor Bennett's work, and those referred to in the note in page 165.

In taking a general survey of the more common morbid growths which are brought under our notice, and examining carefully into the tissues involved, or inquiring from what particular texture the morbid structure has originally sprung, we cannot fail to remark the peculiarly localized condition of many of these tumors: often an enormous mass appears to have been formed by the rapid and circumscribed growth of one or more elements of a tissue; as, for instance, by a redundant growth of epithelium on some part of the cutaneous

Fig. 128.



surface, large warts are produced. By simple hypertrophy of the subcutaneous areolar tissue of the leg and foot, or of that of the scrotum, most formidable diseases are caused. Subcutaneous fibrous tumors depend upon a morbid development of

the same tissue, only circumscribed. Fig. 128 shows the general appearance of hypertrophied cellular tissue. The specimen from which this drawing was made, was taken from the scrotum of a patient operated upon by Mr. Fergusson. Upon the addition of acetic acid to the preparation, the fibres of the yellow element, *a*, became very distinct. By a rapid and irregular development of epithelium in various parts either of the cutaneous or mucous surface, which extends inwards, and gradually invades deeper structures, a class of tumors and ulcers are produced which have been deservedly termed "malignant," in many senses in which that word has been used. To these tumors, which more or less closely resemble those of a truly cancerous nature, the term "cancroid" has lately been applied.

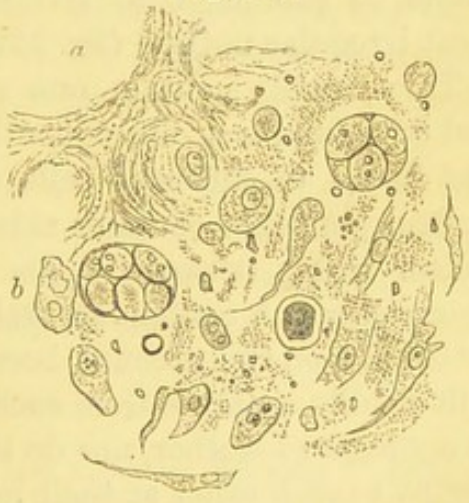
The truly *cancerous* growths frequently commence deep in the substance of a tissue, and gradually make their way towards the surface: they arise from a peculiar condition of the system generally, which we know, in many instances, to be hereditary, and they often exist in different and very distant parts of the body at the same time. The cells, of which these tumors are in great part composed, possess an inherent power of multiplication, so that if one of them, or even, perhaps, a portion of its contents, obtains entrance into the blood, it may be carried to distant parts of the body, and there become the germ of another tumor, which shall encroach upon the structure in which it may have taken root. The tumor thus formed usually resembles the original one in its essential points of structure, but differs from it according to the nature of the tissue which has been invaded. If, for instance, the growth takes place in a part where areolar tissue is abundant, and where there is considerable resistance to its increase, we may expect to find a hard, condensed, and fibrous tumor; but if the growth begins immediately beneath the surface of the peritoneum, or in a like situation, where it will encounter little resistance, a tumor of a very different character will be produced In this latter case, a soft spongy tumor will probably be formed, in which the cells bear a much larger proportion to the fibrous element than in the former instance.

190. **Cancer.**—A cancerous growth may be described as consisting of a fibrous matrix (fig. 129), more or less abundant, and arranged so as to form areolæ, or interspaces, upon the walls of which the vessels ramify. These interspaces contain cells in considerable number, suspended in a more or less viscid fluid, with much granular matter.

The great difficulty of deciding as to the cancerous or non-cancerous nature of a tumor, arises principally from the fact, that no single element, of which the structure is composed, can be looked upon as characteristic of true cancer. Neither the character of the cells, nor the nature of the matrix, nor the arrangement of the elementary constituents can separately determine the point, and it is only by carefully noting the collective appearances observed upon a microscopical examination, that we shall be enabled to decide. In the great majority of cases, however, it is possible to speak with tolerable certainty; but at the same time it must be borne in mind that instances come under notice from time to time, in which the most careful and experienced observers would be unable, from a microscopical examination, to determine the nature of the tumor.

The distinction between cancerous growths and osseous tumors, fatty tumors, and many fibrous tumors, is too obvious to need explanation; and it will be necessary here to refer only to a few of those instances, in which the non-malignant structure approaches very closely, in its microscopical characters, to the cancerous growth.

Fig. 129.



A cancerous growth, in its microscopical characters, does not resemble, and cannot be confounded with any healthy texture; while many of the non-malignant tumors, in their essential characters, bear great similarity to certain healthy tissues, or are actually identical with them in structure. A section

of a fatty tumor cannot often be distinguished from a section of ordinary adipose tissue: many fibrous tumors resemble ordinary fibrous tissue. Epithelial growths frequently appear to be made up entirely of cells, which cannot be distinguished from healthy epithelium. Again, there are certain large tumors of the mamma, which, in intimate structure, are found to be composed entirely of the ordinary gland tissue.

From the freshly-cut surface of a cancerous tumor, a more or less turbid juice exudes, which, upon examination in the microscope, is found to contain cells varying much in size and form, as well as in the character of their contents: a few fragments of fibrous tissue; a number of free oil-globules, and, perhaps, a few cells containing oil-globules; and much free granular matter (fig. 129).

Upon examining a thin section made with a Valentin's knife, the relation of these structural elements to each other may be observed. The fibres will be seen to form meshes or interspaces, in which the cells and fluid are contained (*a*). In some instances the fibres resemble those of ordinary areolar tissue; sometimes they consist chiefly of fibres resembling those of yellow elastic tissue; and not unfrequently the fibres become perfectly transparent, upon being treated with acetic acid, showing the absence of the yellow element.

The cells vary much in size and form: they may be perfectly round, or prolonged at either end into delicate fibres, or of most irregular outline (fig. 129).

They usually contain one nucleus, but very often two are met with, and not unfrequently many more may be observed. The nuclei of different cells often differ much in size. The nucleus generally contains several granules, and much granular matter exists between it and the cell-wall. Cells are often observed which contain several smaller ones in their interior; these have, on this account, been termed "mother-cells" (*b*). The cells readily separate from each other, and exhibit no tendency to aggregate together, nor do they appear ever to have been adherent to each other at their margins.

A tumor from which a milky juice is poured out from the cut surface, and which, upon microscopical examination,

is found to consist principally of cells exhibiting the above general characters, and arranged in the meshes of a fibrous stroma, may be pronounced to be of a cancerous nature.

Cancerous tumors have been divided into three principal varieties by Dr. Walshe, determined by the relative quantities of the viscous juice, fibrous, or cellular elements present. "If the fibrous element be in excess, it constitutes scirrhus, or hard cancer; if the cells be numerous, encephaloma, or soft cancer; and if the fluid abound, and be collected into loculi, or little cysts, it is called colloid cancer." (*Bennett.*)

191. Cancroid Growths.—*Tubercle corpuscles and pus globules* are distinguished from cancer-cells by their more general uniformity in size, by the nature of their contents, and by other characters.—See '*Tubercle*' and '*Pus.*'

Enchondroma is distinguished from cancer by its general cartilaginous aspect, by the absence of milky juice, by the character and arrangement of the cells, and by the clear transparent intercellular substance. Cartilaginous tumors may, however, become softened and broken down into a pulpy mass, which closely resembles, in its microscopical characters, true cancer. (*Bennett.*)

Fibre-cells.—Certain forms of fibrous tumors contain long spindle-shaped cells, somewhat resembling some cancer-cells: so also in inflammatory exudations cells are met with, which in general characters, are very similar to true cancer-cells; but, as observed before, the character of the cells alone must not be looked upon as pathognomonic of the nature of the growth from which they were taken.

Epithelial Growths.—Epithelial Cancer.—Epithelioma.—The tumors included under these heads resemble the cancerous growths more closely than any other. The distinctive characters of these have been carefully investigated by Professor Paget.*

---

* See also Bennett, "On Cancerous and Cancroid Growths." Lebert, "Traité pratique des maladies Cancéreuses et des affections curable confondues avec le Cancer." Paget, "Lectures on Tumours," 1853. Walshe, "The Nature and Treatment of Cancer."

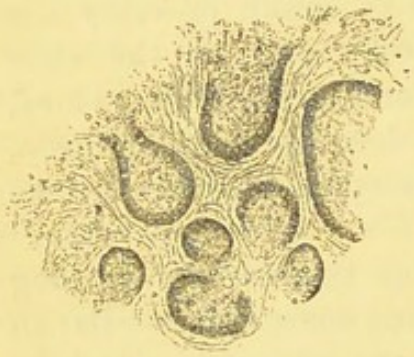
Under this head are included the following forms of disease: cancer of the lip, noli me tangere, cauliflower excrescence of the uterus, chimney-sweeps' cancer, &c.

Common warts consist merely of a superabundant secretion of the epithelial cells of the cuticle; and the tubercles, which occur on the external generative organs, present a similar structure.

In cancer of the lip, tongue, &c., fissures are formed, in which an abundant growth of epithelium takes place, accompanied with an ichorous discharge. The edges become indurated, and as the disease gradually advances, it proceeds from the surface, and invades deeper structures.

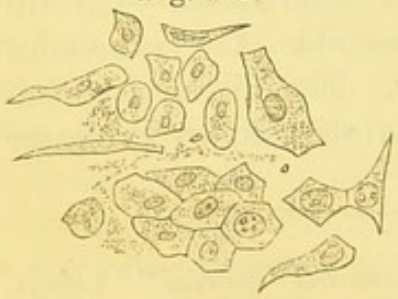
If a thin section of one of these growths be examined, interspaces will be observed (fig. 130), from the walls of which the cells appear to grow. The cells often seem to be arranged in laminæ; they do not vary so much in size and form as the cells of true cancer; the nuclei do not differ much in size; they rarely contain many nucleoli, and usually adhere to each other by their margins; frequently three or four, or more, will be found united together. In fact, these cells very nearly resemble in their general characters the ordinary epithelial cells of the surface, upon which the growth is developed.

Fig. 130.



In fig. 130 is shown the appearance of a section of a specimen of epithelial cancer magnified about 40 diameters. Fig. 131 represents the cells from the same tumor under a quarter-of-an-inch object-glass.

Fig. 131.



The most important distinctive characters between the cells of cancerous tumors, and those of non-cancerous (cancroid) growths, which are most liable to be mistaken for them, are represented in a tabular form as follows :—

Cancerous.	*Cancroid.*
Cells not connected with the matrix in a regular manner, or forming laminæ.	Cells connected with the matrix, often forming distinct laminæ.
Cells differing much from each other in size and form.	Cells resembling each other in size and general outline.
Cells readily separable from each other.	Cells often cohering by their edges, which generally form straight lines; three or four cells being frequently found united together.
Cells not connected together at their margins; their edges seldom forming straight lines.	
Cells containing several smaller cells in their interior often met with.	Cells usually containing one nucleus.
Nuclei varying much in size and number in different cells.	Nuclei not varying much in size in different cells.
Juice scraped from the cut surface containing many cells floating freely in the fluid, and not connected with each other.	Juice scraped from the cut surface containing small collections of cells, which are often connected with each other.

192. **Examination of Morbid Growths.**—The secretion, if such exist, on the free surface of the tumor should be first separately examined: secondly, the microscopical characters of the juice, which exudes from the freshly-cut surface should be ascertained: and, lastly, a thin section ought to be made, in order to determine the relation of the constituents of the tumor to each other, and especially the proportions in which the different elements are present. Its connexion with surrounding structures may be seen by examining a thin section, which should include a portion of the adjacent texture; and these observations should be made first with low powers, and afterwards with a power of about 200 diameters.

The disposition, arrangement, and general direction of the fibres in the fibrous portion of the tumor should be carefully noted, and the form, size, shape, and contents of the cells (especially with reference to the presence or absence of granular matter, nuclei, &c.), should be especially dwelt upon. Every opportunity should be taken of carefully delineating the appearances observed, in order that the structure of one tumor may be compared with that of others which may subsequently

fall under notice, and if the growth presents anything unusual a section ought to be put up in some preservative fluid. Accurate notes should be made of every examination, and entered in a note-book kept for the purpose.

Sections of most tumors, provided they do not contain osseous deposit, are advantageously made with a Valentin's knife.

In examining the cells it is better to wet them with a little serum or gum-water, for sometimes if water alone be employed, they become distended and burst.

Lastly, the influence of certain chemical reagents upon the sections and portions scraped from the cut surface must be ascertained. The most important reagents in the examination of morbid growths are acetic acid, solution of soda, and ether; but the stronger acids and other tests will occasionally be required. The two former are of advantage in rendering the tissues more transparent, and displaying the nuclei. Ether is sometimes required to ascertain if certain globules which resemble fatty matter, are really of this nature.

193. Preservation of Morbid Growths. — The fluid, I believe, which will be found most efficacious for this purpose, is the solution of creosote (97), but this is not in all cases satisfactory. The other preservative solutions which I have yet tried, so totally alter the characters of the cellular tumours, as to obliterate all appearance of their former structure. I have found it best to cut off small pieces of the tumor, and place them in a little bottle with creosote fluid, which must be changed two or three times. Each bottle should be carefully labelled. Sections must then be cut when it is intended to submit the tumor to examination. Thin sections put up in cells do not usually keep well.

ENTOZOA. EPIZOA.

194. Examination of Entozoa.—The microscopical examination of entozoa does not usually present any great difficulty. The smaller species may be examined entire in the usual way; but the larger ones require dissection, and as the structures are often very delicate, the dissection had better be performed

under water after the creature is quite dead, and muscular contractility has passed off.

Entozoa may be mounted in preservative fluids, or dried and placed in balsam.

The small thread-worms (*Ascaris vermicularis*) are very common in children, and occur chiefly in the rectum.

The Tricocephalus dispar is met with in the cæcum and colon.

The Ascaris lumbricoides is usually found in the small intestines.

195. Tape-Worm.—The common English tape-worm (*Tænia solium*) is often met with. A fresh joint may be placed under the microscope and examined with low powers. If dried upon a glass slide, and mounted in Canada balsam, it makes a very instructive preparation. The ovaries of many joints will be found to be quite distended with ova, some of which should be squeezed out and mounted separately.

The head is not easily procured. It may be examined in fluid with an inch object-glass as an opaque preparation, or it may be put up in balsam, but it must be dried with great care.*

The broad tape-worm (*Bothriocephalus latus*) is seldom met with in this country. It may be examined and preserved in the same manner as the common tape-worm.

---

* *Means of procuring the head of the Tape-worm.*—It may be advantageous just to refer to the most effectual manner of obtaining the head of the tape-worm. Of all the remedies I have seen tried, the ethereal oil of male fern is certainly the most efficacious. Out of about thirty cases which were treated by the physicians of King's College Hospital, the head was expelled in six or seven. Some of the patients had been treated with kousso, and others with the oil of male fern. All the successful cases had been treated with the latter; indeed, although I have seen many cases treated with kousso, I never was successful in finding the head; the greater part of the worm, however, was invariably expelled. The oil of male fern was administered as follows:—two drachms to half an ounce, according to age, &c., were suspended in eight ounces of water, with the aid of mucilage. After fasting for twenty-four hours, (only a little water, or, at most, milk being allowed,) the patient was made to take the draught early in the morning, and an hour or an hour and a half afterwards, a dose of castor-oil was given. The worm was usually expelled in the course of the day. The fasting appeared to be a very essential part of the treatment.

196. Hydatids, Echinococci.—Hydatids are not unfrequently met with in the post-mortem theatre. They are usually found in the form of large cysts, occupying a considerable portion of the liver. The parent cyst is often surrounded by a layer of purulent fluid. Upon opening this parent cyst numerous smaller round cysts (acephalo-cysts) with much fluid, escape. The walls of the cysts are usually quite white, not unlike the boiled white of egg; and they vary much in thickness. The external surface is smooth, but the internal appears more transparent and granular. The granular appearance arises from the presence of little elevations with which the surface is studded. By scraping these gently with a knife, not unfrequently many echinococci will be removed. These may also be obtained by allowing the fluid contents of the acephalo-cysts to flow into a conical glass. After a short time the echinococci sink, and may be removed with a pipette.

Fig. 132.

Fig. 132 represents the appearance of echinococci magnified with an inch object-glass, and in fig. 133 are shown two specimens magnified with a quarter (about 220 diameters). In one of these the hooks are seen to be extruded, a condition which has been considered to result from the occurrence of endosmosis and commencing decomposition.

Fig. 133.

A thin section of the walls of the cyst shows a laminated appearance, and often a considerable number of crystals of triple phosphate will be found, especially if the hydatid is not quite

fresh. The structure of the wall appears homogeneous, or at most slightly granular.

If a little of the fluid from the interior be evaporated upon a glass slide, numerous crystals of chloride of sodium will be formed (§ 313), their form depending upon the degree of rapidity with which the evaporation has been conducted.

The character of the claws (fig. 133 *a*) should be particularly noticed, as their presence is characteristic of echinococci.

Hydatids are occasionally expectorated; usually in consequence of the cyst in the liver opening into the base of one lung. The appearance of the cysts in the sputum will generally direct attention to the origin of the pulmonary mischief, but the observation should be always confirmed, if possible, by the microscopical examination of the claws or hooks.

The echinococci may be preserved in the creosote solution (§ 97), or in preservative gelatine (§ 99).

The hooks may be readily preserved moist in fluid, or dry in Canada balsam.

197. Other Entozoa.—The Trichina spiralis is a rare species of entozoon contained in small cysts, which are sometimes found in the voluntary muscles.

The Cysticercus cellulosæ, like the Trichina, also infests the muscles, but it has been met with in a few instances within the chamber of the eye.

The common fluke (*Distoma hepaticum*) forms a very interesting object for examination. One species may generally be met with in the bile-ducts of the ox and sheep.

The only other entozoon which need be alluded to here is the Strongylus gigas, the largest of the entozoa. This is very rarely met with in man, but is not unfrequently seen amongst animals. It is usually found in the kidney. Some years ago I met with three of these creatures, two males and a female, coiled up in what had been the kidney of a dog, but which was now reduced to a thin membranous cyst. The ureter was quite pervious, and the mucus on the surface of its mucous membrane, with that of the bladder, contained very numerous ova. For microscopical examination of the tissues of this creature, it must be dissected under water. The intestine is

square and contains altered blood. The ova form beautiful objects.

198. **Epizoa.**—The only epizoa which need be referred to here, are the itch insect (*Acarus scabiei*) and the entozoon from the sebaceous follicles (*entozoon*, or *demodex folliculorum*). The first is rather difficult to procure. They must be extracted from the itch pustules or vesicles by passing a fine needle into the burrow, the opening of which is always at the side, and may be known by the presence of a little dark point; but this Acarus will only be found in a few instances. To catch the insects requires practice and dexterity. The male is much smaller than the female. They may be dried carefully at a gentle heat, and preserved in Canada balsam.

The entozoon folliculorum is generally present in the follicles of the skin of the scalp, chin, and other parts of the face. It may usually be procured very readily from the nose, by squeezing out the contents of the sebaceous follicles by pressing the skin firmly between the finger and thumb, or between two of the finger-nails. The white cheesy matter thus expressed must be torn with needles, and then placed on a slide in a drop of oil, and covered with thin glass. One or two of the entozoa will usually be found. There are two varieties, and these are constantly met with in the same individual. One is much longer, and the body more thin and taper than the other.*

I have found them in considerable number in the wax which collects in the ear. If the wax is tolerably moist the addition of oil is unnecessary.

EXAMINATION OF VEGETABLE PARASITIC STRUCTURES.

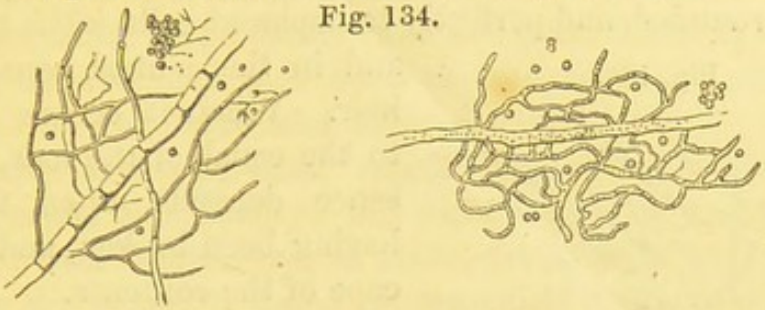
There are certain vegetable structures of a very low organization, which not unfrequently fall under the notice of the practitioner. Some of these are found growing upon the surface of the skin or mucous membrane in certain forms of disease, while others are met with in the recent fluid secretions, or become developed at different periods of time after the secretions have left the body. A few of the most important will be briefly referred to in this place.

---

* Todd and Bowman's "Physiology," vol. i. p. 425.

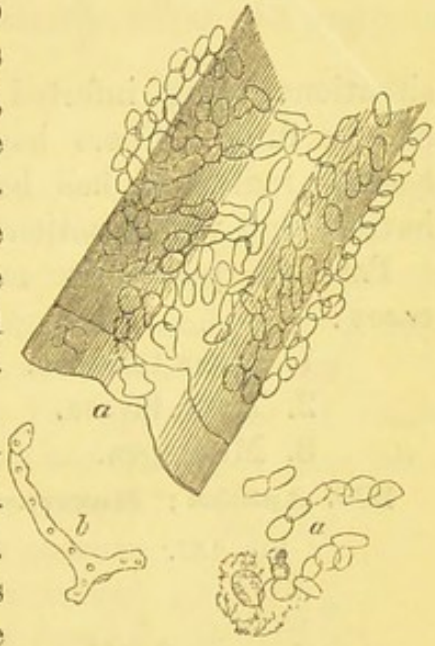
Fig. 134 shows the general characters of a fungus which is often developed in acid urine (*Penicilium glaucum*).

Fig. 134.



199. The Achorion Schœnleinii usually appears as elongated vesicles, of a more or less oval form (fig. 135 *a*), many of them being rather irregular and varying much in size, but often joined end to end so as to form branches. This fungus grows in the hair follicle, and is also found in abundance amongst the epithelium in the neighbourhood. It may frequently be seen within the hair in considerable quantity (fig. 135), and may be found in abundance in the little honeycomb-like masses, termed favus crusts.

Fig. 135.

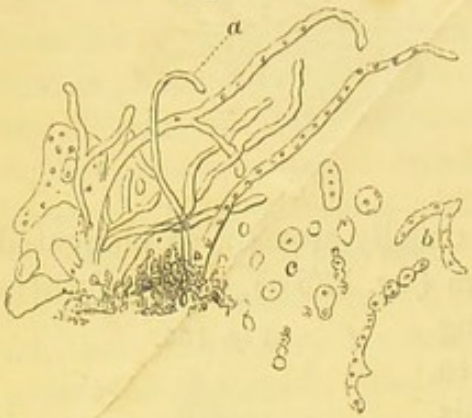


The *favus* consists of a little cavity filled with spores of the fungus, granules, and epithelial cells (fig. 136). One or two hairs usually pass through the centre of the favus. The fungus is composed of the *mycelium* (*a*), or the proper substance of the plant; of a *receptacle* (*b*), or *sporangium*, which contains the reproductive organs; and the reproductive organs themselves, or the *spores*.

This fungus occurs in *Tinea favosa*, *Porrigo favosa*, *scutulata*, &c. The favus may be placed upon a glass slide, moistened with water, and subjected to microscopical examination. When the hair is to be examined the same course is pursued, but it will often be found advantageous to treat it with a drop of solution of potash, which renders the hair more transparent, and the fungus more distinct.

There are several other species of fungi infesting the hair. The *Tricophyton tonsurans* is found in the form of very minute oval or rounded, and perfectly transparent cells, *within* the bulb, and in the central canal of the hair. When it occurs external to the canal of the hair, its presence depends upon the hair having been broken, and the escape of the contents.

Fig. 136.



Other species are found in the epithelium of the skin. That condition of the skin termed *Pityriasis versicolor* depends upon the epithelial cells in the coloured situations being infested with spores of another of these minute fungi. Cases have occurred in which a previously healthy individual has been infected with the disease, after having slept with a patient suffering from this affection.

Parasitic plants are met with in the following skin diseases :—

1. Tinea tondens.
2. Tinea favosa.
3. Mentagra.
4. Pityriasis versicolor.
5. Porrigo decalvans.
6. Plica polonica.

200. **Aphthæ ; Muguet.**—The aphthæ which occur upon the mucous membrane of the mouth and pharynx of ill-nourished infants, and the whitish matter resembling false membrane, which is sometimes formed in the same situations in adults, who have long suffered from exhausting diseases, and to which the term *muguet* has been applied, are composed of a vegetable fungus, which was first described in 1842 by Gruby, and has been spoken of by him under the names of aphtaphyte and cryptogames du muguet. It is placed under the genus Oidium, and termed *Oidium*

Fig. 137.

albicans by Robin. The appearance of this fungus is shown in fig. 137, which is taken from M. Robin's work.*

Low forms of cryptogamia have also been found in the lung by Prof. Bennett, and have been noticed in the stools by him as well as by Dr. Farre, and other observers.

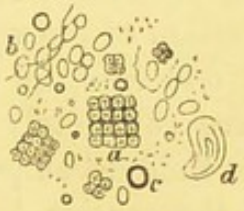
The examination of these vegetable growths in the microscope presents no difficulty; but without care they may readily be passed over unobserved, as their structure is very delicate, and they are generally found accompanied with epithelial cells and much debris. A very small piece only should be submitted to examination, and should be moistened with a little water or dilute syrup. They may be seen with a power of 200; but to bring out their characters clearly, requires a power of 500 to 600.

ALGÆ.

201. **Sarcina Ventriculi.**†—This body is a species of alga which was originally discovered by Goodsir, in 1842, among the matters vomited by a patient. Since this period it has been found by a great many observers, and, indeed, may now be looked upon as by no means uncommon (fig. 138).

The vomited matters have much the appearance of yeast, and fermentation proceeds for some time after they have been ejected. In vomit presenting these characters, the sarcinæ are, I believe, never absent; but they have been found in other cases and in other situations: by Lebert, in a case of cancer, accompanied with black vomiting; and by myself in a case in which there was a very abundant ejection of coffee-ground vomit for a few days before death. In this specimen the

Fig. 138.



---

* "Histoire naturelle des végétaux parasites qui croissent sur l'homme, et sur les animaux vivants." Paris, 1853.—See also a review of this work, by Dr. Parkes, in the "British and Foreign Medico-Chirurgical Review,' October, 1853.

† M. Robin has arranged it under the genus Merismopœdia (Meyen), and he calls it Merismopœdia ventriculi.

sarcinæ were very abundant, but there was no tendency to fermentation.*

The sarcina has been found in the urine, three times by Heller, once by Dr. Mackay of Edinburgh, twice by Dr. Johnson, and twice by myself.† In the fæces it has been met with frequently by Bennett and Hasse; it was observed by Virchow in an abscess of the lung, and once by Dr. Jenner in the fluid of the ventricles of the brain.

In all the cases which have come under my own observation, the matter in which the sarcina was present was acid, although in several instances, in consequence of the ejection of much clear fluid (pyrosis), the vomit generally had an alkaline reaction. But in these cases, the brown flocculi which contained the sarcinæ were intensely acid. The sarcina is generally, but not invariably, accompanied with a great number of oval torulæ, which vary considerably in size and form in different cases (fig. 138 *b*). These torulæ were not present in the specimens of urine which contained the sarcinæ. In the accompanying wood-cut (p. 175) a partially digested starch granule is shown at *d*, and an oil globule at *c*.

By the action of acids and alkalies the sarcina becomes paler, but is not destroyed by these reagents even if warm. The cells, however, exhibit a tendency to separate from each other in a quadruplicate manner. Iodine communicates a slightly brown colour to it. It is not destroyed by the decomposition of the vomited matters in which it was developed; but in one case, in which it was present in the urine, the cells were completely broken down, and all traces of them lost, as the fluid decomposed and became alkaline. The development of the sarcina has been investigated by Frerichs in a dog with a fistula in his stomach.

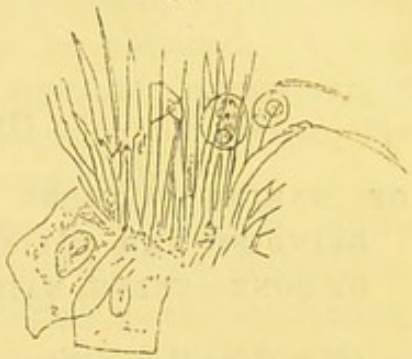
---

* With reference to the symptoms occurring in these cases, see Dr. Todd's "Clinical Lectures," "Medical Times and Gazette," May 2nd, 1851.

† For the opportunity of observing the sarcina in one of these cases, I have to thank my friend Mr. Brown, of Lichfield.

202. **Other forms of algæ** are found in different situations; for instance, in the cavity of the mouth, especially towards the back; mixed with, and adhering to, or growing from the cells of epithelium, will be seen, with a power of 200 or higher, a vast number of little hair-like bodies, which consist of filaments of a very minute alga (*Leptothrix buccalis*). The filaments grow upon any small particles of food which may remain entangled in the epithelium of the mouth. The papillæ at the back of the tongue are thickly covered with very long filaments, consisting almost entirely of this alga (fig. 139); it is very abundant between the teeth, and the so-called tartar is partly composed of it.

Fig. 139.



Similar vegetable growths have been found in the stomach, intestines, and fæces. One species occurs in the mucus of the uterus.

The examination of these substances presents no difficulty. Sarcinæ may be removed with a pipette from fluids in which they subside as a deposit, or, in cases where the mass is very viscid, with the handle of a knife. If necessary, a little water may be added, and the whole covered with thin glass, which often requires to be pressed down firmly, in order to obtain a very thin stratum for examination.

To examine the algæ from the mouth, it is only necessary to scrape the upper surface of the tongue, and examine the epithelium and debris removed in the usual way, and moistened with a little water under the microscope.

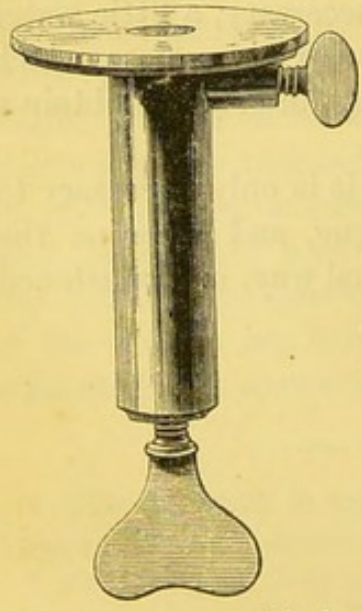
CHAPTER XII.

OF MAKING SECTIONS OF HARD TISSUES—INSTRUMENTS—GRINDING AND POLISHING THIN SECTIONS—EXAMINATION OF BONE—TEETH, NAILS, HAIR, &C.

Of making thin sections of hard tissues.—The methods of examination which have been already described, are such as are applicable to the investigation of the microscopical characters of soft tissues only, or textures which could be readily cut with an ordinary knife; but the different methods of operating when it is necessary to obtain very thin sections of hard, unyielding tissues, such as horn, bone, ivory, teeth, &c., will now be considered.

203. Cutting thin sections with the Knife.—Many hard substances, such as nail, horn, and dried animal textures, may be cut with a strong, sharp knife, or with a razor; an operation which is easily performed by placing the substance upon a piece of soft deal board, and, after cutting a smooth edge, removing a thin shaving, which may be examined dry, or in fluid, or may be placed in Canada balsam, as occasion may require.

Fig. 140.



Another plan of cutting thin sections of tissues of this description, is to employ a little instrument similar to that which is used for cutting thin sections of wood. It consists essentially of a small table, in which there is a hole in the centre, provided with a fine threaded screw at the bottom, by turning which any substance placed in the hole may be forced

any required height above the surface of the table. A sharp knife is fixed to one end of the upper surface, with a moveable hinge. This knife can be moved backwards and forwards over the hole, and as the substance can be gradually forced up by the screw, sections of great tenuity may thus be obtained. A very simple modification of this piece of apparatus is represented in the woodcut (fig. 140). This is made entirely of brass. The sections are cut off with a razor or strong knife.

204. Cutting thin sections with the Saw.—In order to obtain sections of bone and ivory, and tissues of this description, sufficiently thin and transparent for microscopical examination, it becomes necessary first to cut a thin section with the aid of a sharp saw, and then to grind this down to the required tenuity upon a hone or other smooth stone. The scratches, which are in this process produced upon the surface, are obliterated by subsequent polishing.

For cutting the sections a fine-toothed saw is required; those made of watch-spring, set in a bow, answer very well, but almost any saw with fine teeth, and made of tolerably hard steel, will be found efficient. The simplest and most convenient

Fig. 141.

form which I have seen is represented in fig. 141. This saw may be purchased for a shilling or eighteenpence according to the size.

205. Grinding thin sections of hard Tissues.—The section, after having been cut off with the saw, requires to be ground thin before it can be subjected to examination. It may perhaps be as much as the tenth of an inch in thickness when the grinding is commenced, but by rubbing it for a short time upon a smooth stone it may be reduced to the proper degree of tenuity. Stones which are well adapted for this purpose are the 'Charley Forest' stones, the Turkey stones, or the Water of Ayr stones, about an inch or more in width, and six inches in length. Each of the four sides should be perfectly smooth. Other stones, or even a piece of slate, answer also very well,

and may be procured at much less cost.* The stone is wetted with a little water, and the section rubbed up and down with the finger, or with a piece of cork or leather.

A very good plan also is to imbed the section slightly in a piece of warm gutta percha, which should extend only a very short distance beyond the edges. This is to be rubbed up and down on the wet hone, water being added as required, till the surface is perfectly smooth, when the section is to be taken off, turned round, and ground down on the opposite side until it is sufficiently thin. The section may also be ground down expeditiously by rubbing it between two hones. If very thick, it will be better to reduce it somewhat with the aid of a flat file before commencing the grinding. After being ground to what is considered the proper thinness, the section may be placed in the microscope, when numerous dark lines will be found all over the surfaces; these must be removed by polishing. The deepest of the scratches may be obliterated by rubbing the specimen upon a very smooth part of the hone quite dry.

206. **Polishing thin sections of hard Tissues.**—The polishing must, however, be completed upon a piece of smooth plate glass, or upon a sort of leather strap prepared for the purpose. A piece of board about two inches broad, ten or twelve long, and half an inch in depth, is covered on one surface with three or four layers of cloth, soft leather, or flannel. The whole being laid perfectly smooth is to be surmounted with a piece of smooth leather, sufficiently large to be tacked to the sides of the board. In this way a firm and even pad is obtained. On the surface a little putty powder (peroxide of tin) is sprinkled and moistened with water. The section is then to be carefully rubbed up and down, and afterwards the polishing is to be completed by rubbing it upon a part of the strap which is not wet. Whitening may be used instead of putty powder. If, upon microscopical examination, the surface is found to be still covered with scratches, the process of polishing must be repeated.

In order to polish a thin section of a hard tissue upon glass,

* Common stones adapted for grinding cost about threepence or fourpence each. The Arkansas oil-stone is the best, but very costly.

it is only necessary to rub it rapidly backwards and forwards with the fingers, upon a piece of plate glass, until the surface becomes quite smooth.

207. **Examination of Bone.**—In order to obtain a thin section of bone we proceed as above described; grinding the section which has been cut off with the saw, upon a hone, or between two pieces of hone, until sufficiently thin; or the piece of bone may be attached to a slip of glass with a little Canada balsam, or other cement, and then rubbed down upon one side. Afterwards the slide may be warmed, and the section turned round, in order to grind the opposite side. The section is to be polished upon the strap, or by rubbing it upon a slip of plate glass.

If the bone contains much fat, it may be removed from the sections by placing them for a few minutes in a little ether.

For examining the lacunæ, a dry section should be taken and submitted to a power of 200, when they will appear as a number of small black corpuscles of irregular form. If now the specimen be wetted with a small drop of turpentine, upon microscopical examination it will be found that this penetrating substance enters the canaliculi, and may be seen to fill the lacunæ, displacing the air contained in them, to the presence of which the dark, almost black appearance was due. When the specimen has remained for a short time in turpentine, the lacunæ become so transparent as to be scarcely visible. By careful management the turpentine may be seen to enter the lacunæ, while the section is under the microscope.

In order to examine the cartilage or organic basis of bone, pieces of bone must be soaked in dilute hydrochloric acid (consisting of about one part of acid to twenty of water, or rather stronger), until the mineral matter has been entirely dissolved out, which may be known by ammonia not giving a precipitate in the solution which has been repeatedly changed, or by the absence of solid matter, if a drop of the solution be evaporated to dryness upon a glass slide. This operation of soaking the bone in acid may take two or three days. Upon making a thin section of this animal substance and examining it, the Haversian canals will be remarked, and their arrangement may

be readily demonstrated by cutting sections in different directions. The lamellæ are also visible. The section should be afterwards treated with dilute solution of potash. If a section be macerated for some time in water the lamellæ separate.

Thin sections of bone may be carefully burnt in a platinum capsule, and the white ash, which remains after the mass has been for a short time exposed to a red-heat, examined in the microscope. The organic matter may also in great measure be removed by boiling the sections in potash.

In examining the development of bone, the long bones of any young animal may be employed. Thin sections of the ossifying cartilage may be cut off with a sharp knife; and a portion of the bone already ossified should also, if possible, be removed with the cartilage. The ossifying portions of the skull and other bones may also be examined with advantage, and the appearances presented under different magnifying powers should be carefully observed as in other cases.

208. Mounting Specimens of Bone.—Sections of bone may be preserved as dry objects in fluid, or in balsam (Chapter VII.). The first is the method usually employed, but specimens should also be mounted in Canada balsam. Tolerably thick sections show well in this manner; but they must not be too thin, for in this case the balsam enters the lacunæ, and renders them almost invisible, precisely analogous to what occurs when a section of bone is moistened with turpentine. Sections of ossifying cartilage keep very well in weak spirit, and also in creosote solution, if not too strong.

209. Examination of Teeth.—The microscopical examination of teeth is conducted upon the same principle as that of bone; but in consequence of the greater hardness and very brittle nature of the enamel, sections are not so easily prepared.

In order to cut a thin section of a tooth we require a very fine saw, and great care must be taken not to chip the enamel. The section is rubbed down in the manner just described, and afterwards polished by rubbing it first upon the dry hone, and subsequently upon the leather, or a piece of plate glass.

Teeth may be ground down upon a lapidary's wheel, or upon a dentist's emery wheel. Sections can also be readily

cut with a diamond saw (an iron wheel, the edge of which is covered with diamond dust).

The thin section is now to be soaked for a short time in ether to remove the fatty matter, and then allowed to dry.

It is to be subjected to examination in the dry way, moistened with water, turpentine, or Canada balsam, and the different appearances in each case should be carefully observed.

The cartilaginous basis is to be examined also in thin sections, which may be cut either before macerating in acid, or subsequently. A whole tooth placed in moderately strong acid will become soft in four or five days, when thin sections of different parts may readily be cut with a sharp knife.

The dentinal tubes may be isolated from each other by longer maceration in acid, and afterwards by soaking for a few hours in dilute caustic soda or potash. It is better in this investigation to cut the thin section before maceration in acid, or to macerate the tooth until moderately soft, and then remove a thin section, which is to be further exposed to the action of the strong acid. A mixture of sulphuric and hydrochloric acids has also been recommended.

In investigating the development of teeth it will be necessary to obtain embryos of different ages from two months upwards; but this subject may also be studied with advantage in the lower animals.

Sections of teeth may be preserved in the dry way and in Canada balsam.

The cartilaginous basis will keep in the creosote solution pretty well, and also in other dilute preservative solutions.

210. Examination of Nails.—Sections of nails may be very easily obtained with a sharp knife. They may be examined dry, in aqueous fluids, and in balsam. The action of nitric and acetic acids, and also that of caustic soda upon the cells should be observed. The nail may be readily removed from the skin by maceration in water. The different characters of the cells in various parts of the nail should be carefully noticed. If a portion of nail be boiled in a solution of caustic soda, its component cells are rendered distinct, and in each a nucleus is developed.

In order to see the connection between the nail and the cutis, the skin with the nail attached must be dissected off and allowed to dry, when sections are to be obtained in a similar manner to that described under the examination of the skin (§ 180). The cells of nail and horn behave very similarly to those of the epidermis when acted upon by the stronger acids. Nitric acid causes them to assume a deep-yellow colour, and often renders the outlines of the individual cells very distinct.

211. **Examination of Hair.**—Hair may be examined in the dry way, or mounted in fluids, or in Canada balsam. The examination of hair in different media (§ 82) is very instructive. The hair of different animals, especially that of the dog, cat, horse, rat, mouse, bat, &c., should be examined; but for displaying the minute structure, a white hair is to be preferred.

The hair-bulb may be seen in a carefully prepared section of skin in any situation, in which they are abundant, such as the scalp. In order to obtain a good section, the skin should be first dried. The section must be made in the same direction as that which the hair-bulb takes through it. Fatty matter may be removed by soaking it for a short time in ether, and after the ether has evaporated it may be moistened with water in the usual manner. It will also be instructive to note the effect produced on treating it with a little dilute caustic soda.

The reagents most useful in demonstrating the minute structure of hair are solutions of soda or potash, strong sulphuric acid, and acetic acid. For displaying clearly the cortical part it is better to treat the hair with strong sulphuric acid (Kölliker). The cuticle of the hair (surrounding the cortex) should be treated with caustic soda, which will cause several of the cells to separate from one another.

212. **Longitudinal sections of Hair.**—In order to obtain a longitudinal section of hair, a small bundle should be made by sticking several hairs together with gum water, and as soon as this is quite dry thin shavings may be removed with a sharp knife or razor, the sections becoming separated from each other as soon as they are moistened with water.

213. **Transverse sections of Hair.**—Transverse sections may

be obtained by cutting thin shavings off the end of the gummed bundle, or several hairs may be placed between two pieces of card, or between two pieces of cork, which may be pressed together in a vice, or tied tightly with strong string, when thin sections of hair may be readily removed from the surface with a sharp knife, along with a shaving of the cork, from which the former can very easily be separated. The knife should be slightly moistened on the upper surface, in order to prevent the sections from being lost as they are cut off.

Hair is usually mounted in Canada balsam, or preserved in the dry way between glasses.

214. Examination of Osseous Tumors. — Sections of bony tumors and of osseous plates, such as those frequently deposited in arteries, may be obtained in the same way as sections of bone. Good sections of tumors which have partly an osseous, and partly a fibrous or fibro-cartilaginous consistence, are very difficult to make; but a thin section may generally be removed with care with a strong knife, in such a manner as to cut through both the fibrous and ossified portions, if the latter is not very hard and abundant. The facility of this operation is sometimes increased by drying the tumor, and after removing a section, remoistening it with water.

In examining growths of this description, we may dissolve out the ossific matter with hydrochloric acid, as described under the examination of bone and teeth.

On the examination of hard tissues reference may be made to the following works:—Kölliker, op. cit.; Paragraphs at the end of each article on the preparation of specimens; Quekett, op. cit.; Todd and Bowman, op. cit.; Strausdurkheim, op. cit.; and articles in the "Cyclopædia of Anatomy and Physiology."

CHAPTER XIII.

EXAMINATION OF SUBSTANCES WHICH FORM DEPOSITS FROM FLUIDS, AND OF THEIR PRESERVATION. — APPARATUS EMPLOYED.—EXAMINATION OF URINE, AND THE METHODS OF COLLECTING URINARY DEPOSITS.

THE most important pieces of apparatus required in the examination of deposits which subside from fluids after they have been allowed to stand still for some time in a glass vessel, are the following:—Test tubes, pipettes of different sizes, conical glasses, wash-bottle, and watch-glasses, which have been already referred to (§ 48), funnels for filtering, and cells in which the deposit is to be subjected to microscopical examination. The application of chemical reagents will frequently be required; but for information on this subject the reader is referred to Chapter XVI.

Fig. 142.

215. Test Tubes.—Test tubes (fig. 142) will often be required in microscopical examination. The observer should be provided with several, varying in length from five or six inches to an inch and a half, or even less. The smaller tubes are very convenient for preserving small quantities of deposits in a preservative solution for examination on a subsequent occasion. In boiling a specimen of urine in a test tube over a lamp, it may be held by twisting a piece of paper three or four times folded round the neck, so as to serve for a sort of handle; or a little support made of wire, and mounted in a wooden handle may be used; or the tube may be placed through the smallest ring of the stand (fig. 147), and in this manner suspended over the lamp.

216. **Pipettes.**—The pipettes required in microscopical examination should be of various sizes, according to the depth of the vessel which contains the deposit, and according to the diameter of the orifice through which the pipette is to pass. When it is required to remove some of the deposit from the bottom of a bottle with a narrow neck, for instance, we shall want a pipette of very small calibre. On the other hand, if the deposit be very thick and viscid, the pipette must be wide, or it will not enter it.

Fig.143.

Fig.144.

Pipettes are made of common glass tube of various sizes; the opening at the bottom being drawn out slightly in the blowpipe, in order to make it a little narrower than the tube itself.*

It is convenient to have a sort of collar to the pipette, about two inches from the top, which will prevent the finger and thumb from slipping when the instrument is used (figs. 143, 145). Occasionally a pipette, the end of which is slightly bent round (fig. 144), will be found useful; and sometimes when we wish to decant a considerable quantity of fluid from a watch-glass, &c., a pipette, upon the stem of which a bulb has been blown, will be found of great advantage. The top of the pipette should be slightly bent over in the form of a lip, and perfectly smooth, so that it may be completely covered with the fore-finger, while the middle finger and thumb are placed on either side of the tube immediately below the ring (fig. 145).

Various other forms of pipettes have been employed, but the above will be found most useful to the microscopical observer. A small pipette with a narrow opening is convenient for removing any superfluous liquid which may escape outside the thin glass cover when preparations are being mounted in fluids in cells.

---

* Glass tubing adapted for making pipettes may be purchased at the operative chemists.

217. Conical Glasses.—Glasses of the shape figured in 145 are the most convenient vessels in which to place fluids holding certain substances in suspension, which we wish to examine. After the fluid has been allowed to stand for some time, the deposit collects in the narrow portion of the glass, and however small in quantity, may be very easily removed with the pipette. These glasses can be obtained of various sizes. In choosing them, it is better to select those in which the narrow part terminates in a round or flat extremity, for many will be found to have a little prominence around which the deposit collects, and in this case it is with difficulty removed by the pipette.

Fig. 145.

218. Wash-bottle.—This simple piece of apparatus is of great use to the microscopical observer, as well as to the chemist. He will find it most convenient for washing away the parenchymatous part of tissues in order to leave the more fibrous portions, removing epithelium from the surface of membranes, &c. It is employed by the chemist chiefly for washing precipitates on filters, &c.

Fig. 146.

The wash-bottle is made with an ordinary bottle or glass flask, having a moderately wide mouth. Two tubes bent, as shown in the figure, are accurately fitted into a cork adapted to the neck of the bottle (fig. 146). Upon nearly filling the bottle with water, and blowing through the shorter tube, the water will be projected from the capillary orifice of the longer

one in the form of a fine jet, which may be directed upon any desired point.

219. Funnels; Filtering.—The funnels required in microscopical examination are very small. Those of about two or three inches in diameter will be found large enough for most purposes. Glass funnels are the cheapest and the best. The funnel is supported in the small retort stand (fig. 147) or upon the tripod. The filtering paper may be obtained already cut in packets of circular pieces of any size required. One of these is folded in the manner shown in fig. 148, when used. Before filtering, the filter should always be moistened with a few drops of water, or with a fluid of the same nature as that which is to be filtered.

Fig. 147.

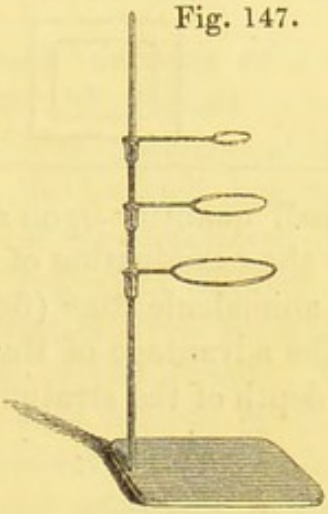
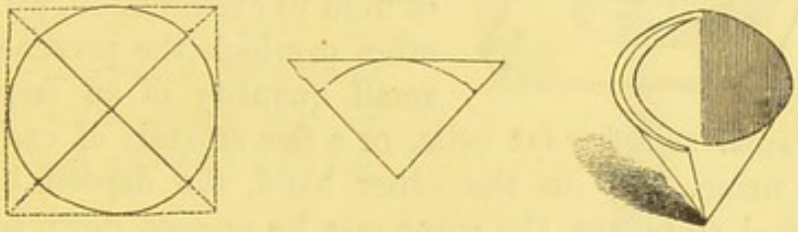


Fig. 148.

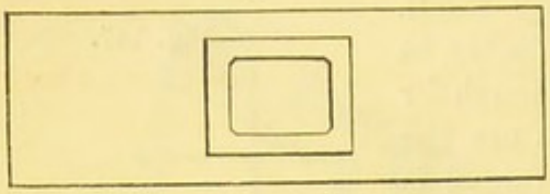


220. Straining through muslin is sometimes a convenient method of separating fine and coarse particles from each other, or for separating a crystalline deposit from viscid mucus in which the crystals may be entangled. By projecting a stream of water from the wash-bottle, the crystals may be washed through the muslin into a vessel placed beneath to catch them, while the mucus remains behind. In the separation of starch particles from gluten, a similar plan may be pursued.

The muslin may be tied over a glass or funnel with a piece of thread, or it may be conveniently fixed in its place by one of the vulcanized India-rubber rings commonly sold at stationers' shops.

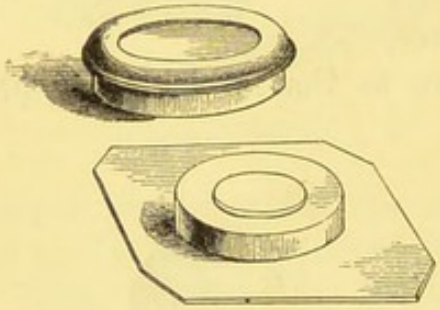
221. Cells for the examination of Deposits.—The thin glass cell (fig. 149) will be found a very convenient form of cell for the examination of deposits from fluids, especially when they exist only in small quantity. If the deposit is very abundant, it will only be necessary to place a small quantity upon a glass slip, and cover it with thin glass. For the examination of urine, I have been in the habit of using the animalcule cage (fig. 150).

Fig. 149.



The advantage of this cell consists in the facility with which the depth of the stratum of fluid to be examined may be altered, according to the quantity of the deposit which it contains. This is a point of great practical importance when the amount of sediment is very small, for by submitting only a very thin stratum of fluid to examination, we might often overlook the presence of a small quantity of an important deposit, such as a few fat cells, or a few crystals of oxalate of lime in urine. If, on the other hand, the deposit be very opaque and abundant, the cover may be pressed down so as to come very nearly into contact with the glass upon which it is placed, and a very thin stratum only may in this manner be examined (§ 23).

Fig. 150.

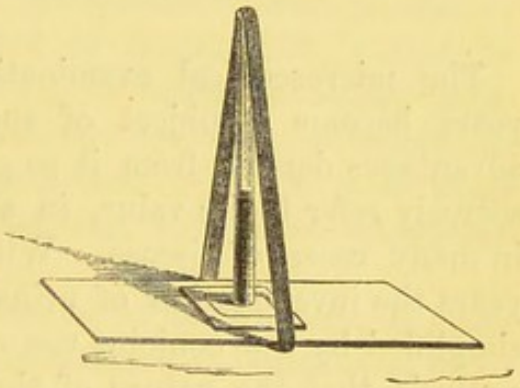


222. Removal of the Deposit from the Vessel containing it.—This is effected as follows:—The upper end of the pipette being firmly closed with the forefinger, and the tube held by the thumb and middle-finger, the lower end is carried down to the bottom of the vessel containing the deposit (fig. 145). If the fore-finger be now raised very slightly, but not removed, a few drops of the fluid with the deposit will enter the tube. When a sufficient quantity for examination has entered, the forefinger must again be firmly pressed upon the upper opening, and the pipette carefully removed. A

certain quantity of the deposit is then allowed to flow from the pipette on to the glass slide or cell, by gently raising the fore-finger from the top; over the deposit is then placed the thin glass cover, and it is subjected to examination in the usual way.

223. Method of collecting a very small quantity of a Deposit from a Fluid.—When the quantity of deposit is very small, the following plan will be found of practical utility. After allowing the lower part of the fluid which has been standing to flow into the pipette as above described, and removing it in the usual manner, the finger is applied to the orifice, in order to prevent the escape of fluid when the upper orifice is opened by the removal of the finger. The upper opening is then carefully closed with a piece of cork. Upon now removing the finger from the lower orifice, the fluid will not run out. A glass slide is placed under the pipette, which is allowed to rest upon it for a short time. It may be suspended with a piece of string, or supported by the little retort stand (fig. 147). Any traces of deposit will subside to the lower part of the fluid, and must of necessity be collected in a small drop upon the glass slide, which may be removed and examined in the usual way.

Fig. 151.



Another plan is to place the fluid with the deposit removed by the pipette in a narrow tube, closed at one end, the bore of which is rather less than a quarter of an inch in diameter. This may be inverted on a glass slide, and kept in this position with a broad elastic India-rubber band. The deposit, with a drop or two of fluid, will fall upon the slide, but the escape of a further quantity is prevented by the nature of the arrangement (fig. 151).

224. Separation of the Deposit from the Fluid in which it was suspended.—After allowing time for the complete subsidence of the deposit, the supernatant fluid is poured off, and

the glass filled up with water, or some fluid which approaches in density more nearly to that which was removed, as weak syrup, glycerine, saline solutions, &c., in cases in which the endosmosis of water into cells is to be feared. After again allowing time for the subsidence of the deposit the operation of pouring off the fluid is repeated, and more water, or the preservative solution added, and again poured off, until the deposit is considered to be free from the original fluid. Two or three washings generally suffice. In this way a deposit may be thoroughly saturated with any fluid in which it is to be preserved.

After being thoroughly washed, the deposit may be removed with a pipette in the usual way, placed upon a slide or in a small test tube, which may then be corked up and labelled. The latter plan is the most satisfactory with which I am acquainted for preserving small quantities of deposits, and if the tube be nearly filled with the preservative fluid, the deposit will keep for a length of time.

ON THE EXAMINATION OF URINE, AND THE METHOD OF DETECTING URINARY DEPOSITS.

The microscopical examination of the urine has of late years become a subject of such great importance, and the advantages derived from it so generally admitted, that I need scarcely refer to its value, in assisting us to form a diagnosis in many cases of disease. Within the last fifteen or twenty years the investigation of urinary deposits has been so much simplified by the conjoint use of the microscope and chemical analysis, that the nature of the greater number has been correctly ascertained. The investigations of Dr. Prout, followed by those of Drs. Golding Bird, Bence Jones, Christison, Owen Rees, Johnson, and many others, have shown the importance of the examination of the urine in disease, and the advantages derived from such an examination with reference to diagnosis and treatment. By frequent examination of different specimens of urine, the student will soon become familiar with most of the deposits he is likely to meet. At first,

however, he will encounter some serious difficulties, some of which will be referred to in this and the following chapter.

In many specimens he is surprised to discover nothing, whilst, in examining others, the whole field of the microscope is found covered with substances of various shapes and colours, the nature of which he is unable to ascertain by reference to works on the subject. Many of the substances, the presence of which leads to this difficulty, have obtained entrance into the urine accidentally. Portions of hair have been mistaken for casts of the renal tubes, starch granules for cells; and other substances of extraneous origin, such as small portions of woody fibre, pieces of feathers, wool, cotton, &c., often take the form of some of the urinary deposits, and so resemble some of the drawings of them in their general appearance, as to mislead the student in his inferences, and retard his progress in microscopical investigation. It is hardly the province of the present work to enter into an accurate description of the various urinary deposits; but at the same time it is desirable briefly to refer to some of the most important, and the means by which they may be distinguished from one another.

225. **Collection of Urine for Microscopical Examination.**— Urine, which is to be submitted to examination, should be collected in considerable quantity, in order to obtain sufficient of the deposit for examination. In many instances the amount of sediment even from a pint of urine is so small that, without great care in collecting, it may be altogether passed over.

Bottles used for carrying specimens of urine should be made of white glass, with tolerably wide mouths, and capable of holding at least four ounces; but, if the sediment only of the urine is required, the clear supernatant fluid may be poured off, after the urine has been allowed to stand for several hours, and the remaining deposit may then be poured into small bottles of an ounce capacity, or even less. The only objection to this latter mode of collecting urine is, that no idea of the amount of sediment deposited by a given quantity of urine can be formed. The bottles may be arranged in a case capable of containing two, four, or six.

226. Importance of examining the Urine soon after it has been passed, and also at a later period.—In all cases the urine should, if possible, be examined within a few hours after its secretion, and, in many instances, it is important to institute a second examination after it has been allowed to stand for twenty-four hours. Some specimens of urine pass into decomposition within a very short time after they have escaped from the bladder; or the urine may even be drawn from the bladder actually decomposed. Under these circumstances we should expect to find the secretion highly alkaline, having a strongly ammoniacal odour, and containing crystals of triple phosphate, with granules of earthy phosphate; and upon carefully focussing, numerous vibriones may generally be observed.

In other instances, the urine does not appear to undergo decomposition for a considerable period, and may be found clear, and without any deposit a day or two, or even longer, after it has been passed.

In those cases in which lithic acid or oxalate of lime are present, we shall find that the deposit increases in quantity after the urine has stood some time. The latter salt is frequently not discoverable in urine immediately after it is passed, but makes its appearance in the course of a few hours; depending upon a kind of acid fermentation, which has been the subject of some beautiful investigations of Scherer's.

In order to obtain sufficient of the deposit from a specimen of urine for microscopical examination, we must place a certain quantity of the fluid in a conical glass, in which it must be permitted to remain for a sufficient time to allow the deposit to subside into the lower part (§ 217).

227. Magnifying Powers required in the Examination of the Urine.—Urinary deposits often require to be examined with different magnifying powers, those which are most frequently used being the inch and the quarter of an inch. Large crystals of lithic acid are often readily distinguished by the former, but crystals of this substance are sometimes so minute that it is absolutely necessary to use high powers. Octohedra of oxalate of lime are frequently found so small that they cannot be seen with any power lower than a quarter; and, in order to bring

out the form of the crystals, higher magnifying powers than this are sometimes necessary. Spermatozoa may be seen with a quarter, but they then appear very minute. In these cases, an eighth of an inch object-glass will be of advantage. The casts of the tubes, epithelium, and the great majority of urinary deposits can, however, be very satisfactorily demonstrated with a quarter of an inch object-glass.

In some cases, it will be well to subject the deposit to examination in various fluids, such as water, spirit, mucilage, turpentine, Canada balsam, &c. (§ 82).

228. Importance of chemical Examination of Urinary Deposits.—In the investigation of those deposits which are prone to assume very various and widely-different forms, such as lithic acid, it will sometimes be found necessary to apply some simple chemical tests, before the nature of the substance under examination can be positively ascertained.

Suppose, for instance, a deposit which is found, upon microscopical examination, not to possess any characteristic form, be suspected to consist of lithic acid, or of an alkaline lithate, we have only to add a drop of solution of potash, which would dissolve it, and then excess of acetic acid, when the crystals of lithic acid will be deposited after some time in their well-known rhomboidal form; or other chemical tests may be applied.

When it is necessary to resort to chemical reagents, a drop of the test solution is to be added to the deposit which is placed in the cell, or upon the glass slide. If necessary, heat may be applied to the slip of glass by a spirit-lamp, and, with a little practice, the student will soon be able to perform a qualitative analysis of a few drops of urine, or of a very small portion of a deposit.

229. Examination of the Deposit in the Microscope.—The drop of urine, with the deposit removed by the pipette, being now enclosed in one or other form of cell, before described, various parts of the specimen are to be brought into the field of the microscope. It is better to examine the object as regularly as possible, commencing on one side, and moving it up and down, until the whole has been traversed. After one specimen has been examined, and the nature of its contents noted, another

may be treated in a similar manner. Specimens should be taken from the deposit at different levels, for while some deposits soon sink to the bottom, others are kept buoyed up, as it were, either by the small quantity of mucus which the urine contains, as is the case with small crystals of oxalate of lime, or from the flocculent nature of the deposit itself.

As each part of the deposit is brought under the field of the microscope, the student should endeavour to recognize every object as it passes under view.

This, however, will for some time be found a matter of considerable difficulty, arising partly from the great number of deposits which commonly occur, and partly from the very various forms which many of these substances are liable to assume; but chiefly, I believe, from the great number of substances of accidental presence which are found in almost every specimen of urine subjected to examination; and this is more especially true of urine obtained in the wards of a hospital, upon which the first microscopical observations of the student are usually made.

230. Matters of extraneous origin frequently met with in Urine.—The substances named in the following list are among those which are constantly met with amongst urinary deposits, and their general characters are represented in fig. 152.

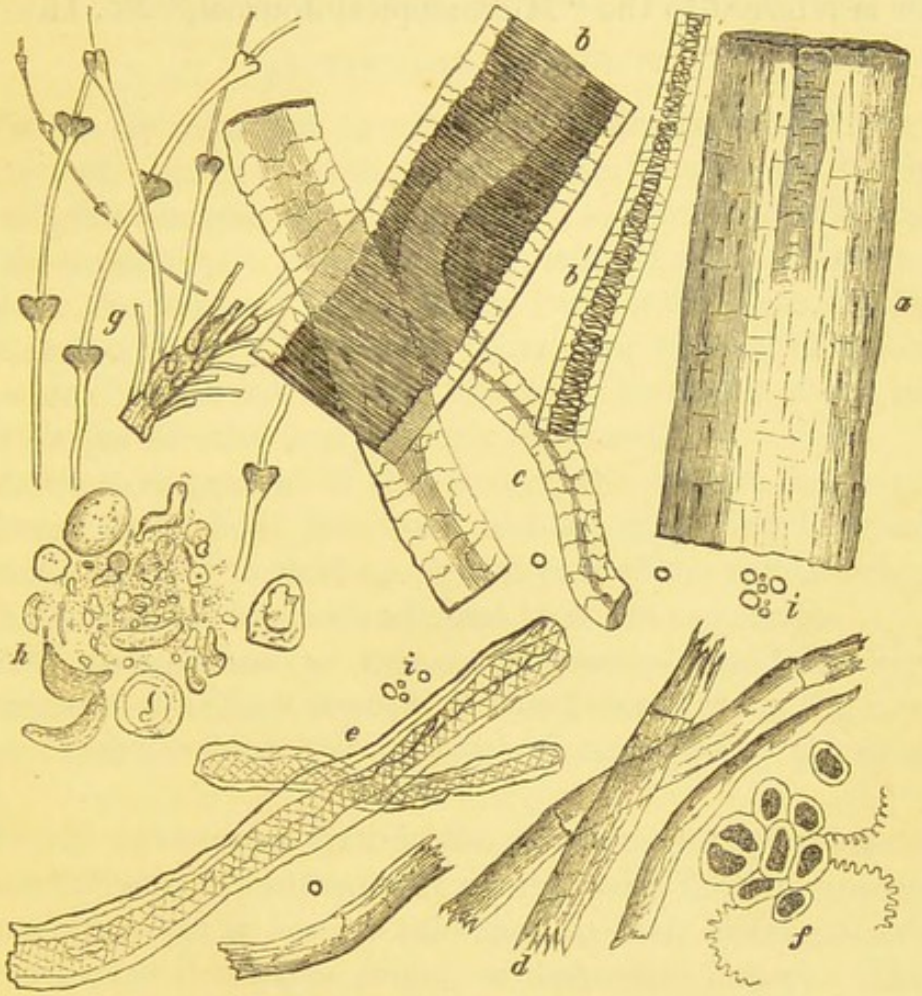
Extraneous Substances.

Fragments of human hair, *a*.
Cat's hair, *b*.
Hair from blankets, *c*.
Portions of feathers, *g*.
Fibres of worsted of various colours.
Fibres of cotton of various colours, *e*.
Fibres of flax, *d*.
Potato starch (fig. 179).
Rice starch.
Wheat starch, bread-crumbs, *h*.
Fragments of tea-leaves, or separated spiral vessels and cellular tissue, *f*.
Fibres of coniferous or other wood swept off the floor (fig. 173).
Particles of sand.
Oily matter — in distinct globules arising from the use of an oiled catheter, or from the accidental presence of milk or butter, *i*.

Besides the above, there are many other substances, met

with less frequently, as, for instance, fragments of silk, mustard flour, cheese, small fragments of the skin of potato, or of different kinds of fruit, and many others which will occur to the mind of every one. With the microscopical characters of these bodies, the student should be perfectly familiar as soon as possible; and, as they may be obtained without the slightest difficulty, this is easily effected. Without this precaution, he will

Fig. 152.*



find himself in constant difficulty, and his ignorance will cause him to make the most ludicrous mistakes.

The origin of most of these substances is obvious;—some of

* These figures are all magnified with a power of 220 diameters.

them become slightly altered by being allowed to stand in the urine.

It is impossible, as a general rule, to prevent the chance of matters falling accidentally into the urine. In wards of hospitals, where the floors are constantly swept, the disadvantage is greatly increased, and much inconvenience arising from the presence of extraneous matters would be prevented if each vessel were provided with a light simple cover. For further remarks on the subject of extraneous matters in the urine, the reader is referred to the "Microscopical Journal," No. II.

CHAPTER XIV.

URINARY DEPOSITS, AND THE METHOD OF PRESERVING THEM AS PERMANENT OBJECTS.

It is not my intention to enter into a description of the characters of urinary deposits, further than is necessary to render intelligible remarks upon the practical examination of the urine in the microscope; and for further detail, I must refer the student to the works of Drs. Golding Bird, Owen Rees, and Johnson, and to Mr. J. E. Bowman's "Manual of Medical Chemistry," in which last he will find the chemical characters of the urine, and its quantitative analysis fully discussed.

In the arrangement of this part of the subject, the principle followed throughout this work, namely, supposing the student actually engaged in working at that part of the subject which is being treated of, has been adhered to as far as possible.

231. Arrangement of Urinary Deposits.—The following arrangement of urinary deposits is based simply upon the appearance which the deposit assumes when examined by the unaided eye.

It will serve to associate in the mind the general appearances which different deposits usually assume, with their microscopical characters; but it has no reference to their chemical nature, microscopical characters, origin, or diagnostic import. That it has some practical advantage will, I think, be admitted; but it must not be considered in the light of a scientific classification.

Upon taking a superficial glance at the more common forms of urinary deposits, it will be noticed that while some are transparent, light, and flocculent, others present the converse of these characters, while again there are several granular or crys-

talline substances which form a small dense sediment which sinks to the bottom of the vessel, leaving a perfectly clear supernatant fluid. Deposits will, therefore, be divided into three classes, according to the general characters which they exhibit.

1. *Light and flocculent deposits, usually transparent, and occupying considerable volume.*—Mucus, with epithelium of different characters, spermatozoa, vibriones, certain forms of fungi, various forms of casts of the uriniferous tubes, and certain matters of extraneous origin.

2. *Dense and opaque deposits, occupying considerable bulk.*—Lithate of ammonia, pus, phosphates, and certain extraneous matters.

3. *Granular or crystalline deposits, occupying a small bulk, sinking to the bottom, or deposited upon the sides of the vessel.*—Lithic acid, oxalate of lime. Small quantities of triple phosphate, cystine, carbonate of lime, blood corpuscles, &c., with matters of extraneous origin.

FIRST CLASS OF URINARY DEPOSITS.

232. Mucus.—If healthy urine be allowed to stand for a few hours after it has been passed, a bulky, flocculent, and very transparent cloud will be deposited towards the lower part. Upon examining this in the microscope, a few delicately-granular cells, rather larger than a blood corpuscle, will be observed sparingly scattered through a clear and perfectly transparent substance, in which only a few minute granular points can be detected. This is all that is observed in examining healthy mucus in urine. In some diseases, however, this mucus increases in quantity, and forms a viscid transparent deposit, containing numerous cells similar to those above referred to, with much epithelium, the character of which depends upon the particular part of the urinary mucous membrane from which the mucus has been poured out. Fig. 153 represents the general appearance of mucus found in urine. In the upper part of the figure is represented a cell of bladder epithelium.

Fig. 153.

A very thick, glairy, gelatinous deposit, which is frequently

found in the urine in cases of disease of the bladder, must not be mistaken for mucus. This consists of pus altered by the action of carbonate of ammonia which has been set free in consequence of the decomposition of the urea by the mucus or some other animal matter acting upon it as a ferment, after it has left the bladder. In some cases this change even commences in the bladder itself, when the urine will be found to exhibit a highly-alkaline reaction, to evolve an ammoniacal odour, and frequently to contain a considerable deposit of crystals of the triple or ammoniaco-magnesian phosphate with granules of phosphate of lime.

The mucus which is deposited from many specimens of urine often contains a great number of octohedral crystals of oxalate of lime, frequently so very minute as to appear under a power of 200, like a number of dark square-shaped spots (fig. 199). Their crystalline form may be demonstrated by the use of a higher power, but they may be recognized with certainty with a power of 200 with a little practice, as their square shape presents a characteristic appearance which soon becomes familiar to the eye. They will be found to be insoluble in a solution of potash, and also in strong acetic acid. These crystals are commonly not deposited until after the urine has left the bladder, and if it be allowed to stand for a longer period, they frequently undergo a great increase in size. Upon examination, fragments of hair, small portions of cotton fibre, and other substances of accidental presence, will not unfrequently be found encrusted with these minute crystals.

233. Vibriones.—After mucus has been allowed to stand for some time in urine, numerous vibriones are developed in it. These are seen as minute lines under the microscope, which undergo very active movements; the longer ones twisting about in a serpentine manner. They are sometimes developed in urine before it has left the bladder, and always occur in decomposing urine. Fig. 154, *b*, represents the appearance of some of the commonest vibriones met with in urine, under a power of 200 diameters.

Fig. 154.

234. Torulæ.—Certain forms of vegetable fungi or torulæ are developed in urine after it has been standing some time. The period which elapses before the appearance of the fungi, and the particular species which is developed vary much in different specimens of urine, and in different cases of disease. In diabetes, torulæ are often developed in quantity very soon (within a few hours) after the urine has been passed; and their growth at this early period leads the observer to suspect the presence of sugar, which must be confirmed by the application of chemical tests.*

Fig. 155.

Fig. 156.

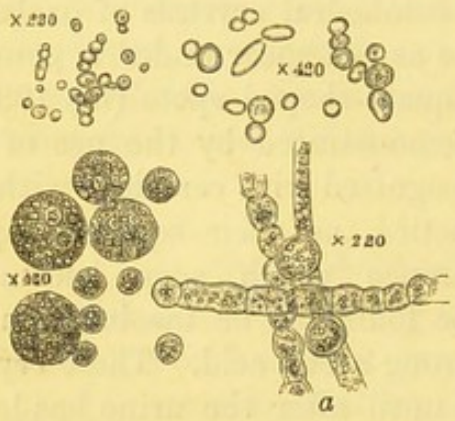


Fig. 154, *a*, represents sporules of three different characters.

Fig. 155 shows the appearance of fungi often developed in urine. All these were found in acid urine, and lithic acid was present in the specimen which contained the fungi represented in the two lower drawings.

235. Penicilium Glaucum. Sugar Fungus.—Dr. Hassall has communicated a most interesting paper upon the development of torulæ in the urine, to the Medico-Chirurgical Society, which will be found in the volume of Transactions for 1853. Dr. Hassall arrives at the conclusion that there is a species of fungus which is developed in specimens of urine, containing even very minute traces of sugar, and which may be looked upon as characteristic of the presence of this substance, as it occurs in no other condition of the urine. This is the sugar fungus, which is represented in different stages of growth in fig. 156.

---

* Vide Bowman's "Medical Chemistry;" Golding Bird on "Urinary Deposits," &c.

The sugar fungus in diabetic urine is identical with the yeast plant. The sporule state is represented in the figure, and at *a* is shown the thallus of the sugar fungus. These drawings have been copied from Dr. Hassall's paper.

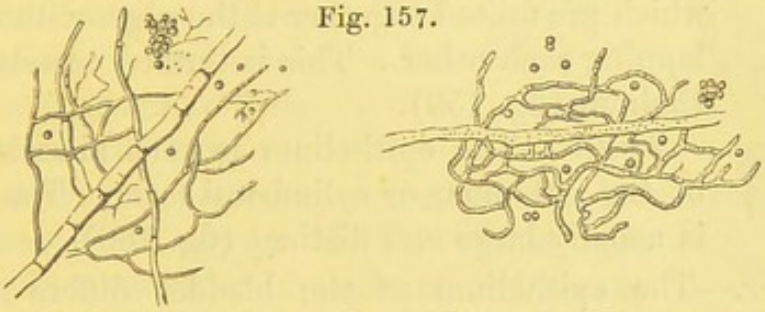
Besides the sugar fungus, however, there is another species which is very commonly met with in acid urine containing albumen, if exposed to the air. This is the penicilium glaucum, the same fungus which is developed in the lactic-acid fermentation.

This species is represented in different stages of growth in figs. 154 *a*, 155, 157.

The microscopical characters of the fungi in different specimens of urine vary considerably; but, according to the observations of Dr. Hassall, these differences depend not upon the existence of several distinct species of plants, but rather upon the stage of development which the fungus has reached.

Thus, in some specimens of urine, the growth of the fungus is arrested at the sporule stage (figs. 154, *a*, 155), in another not until a thallus (fig. 157) is formed, and in a third it goes on

Fig. 157.

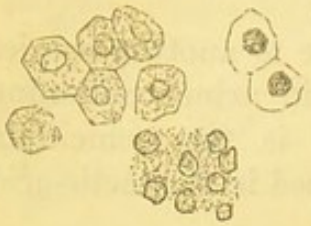


until aerial fructification takes place, and new spores are developed. The degree of acidity of the urine, and the length of time during which it has been exposed to the air, appear to determine, in great measure, the stage of development which the fungus attains.

The penicilium glaucum, as well as the sugar fungus, may be met with in saccharine urine, because all the necessary conditions for its development may be present, namely, exposure to air, an acid liquid, and a certain quantity of nitrogenous matter; but the sugar fungus is found only in those specimens of urine in which to these three conditions is added a fourth, viz., the presence of sugar.

236. Epithelium of the Genito-urinary Passages.—The forms of epithelium which may occur in urine are very numerous, as the characters of the cells differ very much in different parts of the genito-urinary mucous membrane.

Fig. 158.



Figures 158, 159, 160, 161, *a*, 162, were taken from specimens of epithelium carefully removed from the mucous membrane of the urinary passages of a male subject. Figure 161 *b*, *c*, from specimens found in urine.

Kidney.—Convoluted portion of the tubes.—The epithelium is of the variety termed glandular, or secreting epithelium, and forms a thick layer upon the basement membrane (fig. 158).

Fig. 159.

Straight portion. — The epithelium is flatter, and approaches more nearly to the scaly variety. It forms a thin layer on the surface of the basement membrane.

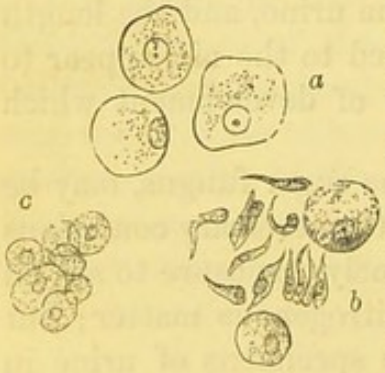
Pelvis of the Kidney.—The epithelium consists of flat thin scales, which are united together at the edges without overlapping each other. This is termed tesselated epithelium (fig. 159).

Fig. 160.

Ureter.—The epithelium is very abundant, and of the columnar or cylindrical form. The nucleus is usually large and distinct (fig. 160).

Bladder.—The epithelium of the bladder differs much in different parts. In the fundus there is much columnar epithelium mixed with the large oval cells figured in 161;—whereas, in that part termed the trigone, the large flattened cells, with a very distinct nucleus and nucleolus are most abundant. The columnar epithelium appears to line the mucous follicles, while the scaly lies on the surface of the mucous membrane between them. In fig. 161 the columnar variety is shown at *b*.

Fig. 161.



Urethra.—The epithelial cells of the urethra (fig. 162) are, for

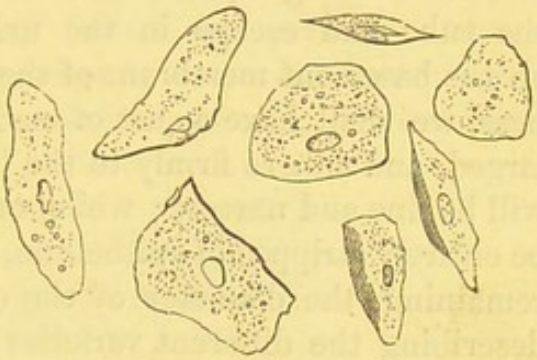
the most part, of the columnar form, but, mixed with this there is also a good deal of scaly epithelium. Towards the orifice, the epithelium is almost entirely of the scaly variety.

Fig. 162.

Vagina.—The large cells of scaly epithelium, so commonly met with in the urine of females, and derived from the vagina, are represented in figure 163. They vary, however, much in size and form, and are sometimes very irregular in shape, with uneven ragged edges.

237. Spermatozoa.—The urine should be examined for spermatozoa as soon as possible after it has been passed, as they very rapidly become destroyed. They may be readily detected with a power of 200 diameters (fig. 164), if the eye is familiar with their appearance; but to demonstrate them to persons who have not seen them before, it is better to employ a power of from 300 to 400 diameters.

Fig. 163.



The occasional presence of spermatozoa in the urine is not inconsistent with perfect health. It is only when their appearance is constant and accompanied with other more important symptoms that the practitioner is justified in interfering. Hence the occasional presence of spermatozoa in urine must not be looked upon, in itself, as evidence of the existence of that condition, to which the name of *spermatorrhœa* has been applied.

Fig. 164.

238. Casts of the Uriniferous Tubes.—This forms to the practitioner a most important and interesting class of urinary deposits, and one to which, until lately, very little attention has been paid. The microscopical characters of casts, in different forms of kidney disease, particularly with reference to the diagnosis of pathological changes taking place in the organ, have

been investigated chiefly by Dr. Johnson, to whom we are indebted for much that we know upon this subject.

The microscopical characters of the forms of casts most commonly met with in urine will alone be referred to; and for a full description of the characters of the urine in which they occur, and their pathological interpretation, I must refer to Dr. Johnson's work.

A cast consists of a mould of a uriniferous tube, and is formed of some transparent material allied to fibrine (but perhaps not identical with it) which is poured out into the canal, and there coagulates, entangling in its meshes whatever may be in the tube at the time of its effusion. It varies in diameter with that of the central canal; but probably, after its formation, it contracts slightly, and is thus enabled readily to pass from the tube and escape in the urine. If the epithelial layer, on the basement membrane of the tube, be of its ordinary thickness, we shall have a cast of medium size. If the cells be enlarged, and adhere firmly to the basement membrane, the cast will be fine and narrow; while, on the other hand, if the tubes be entirely stripped of epithelium, the basement membrane alone remaining, the diameter of the cast will be considerable. In describing the different varieties of casts, it will be convenient to divide them into three classes, according to their diameter.

239. Casts of medium diameter. — 1. *Casts of medium diameter, about the* 1-700*th of an inch.*

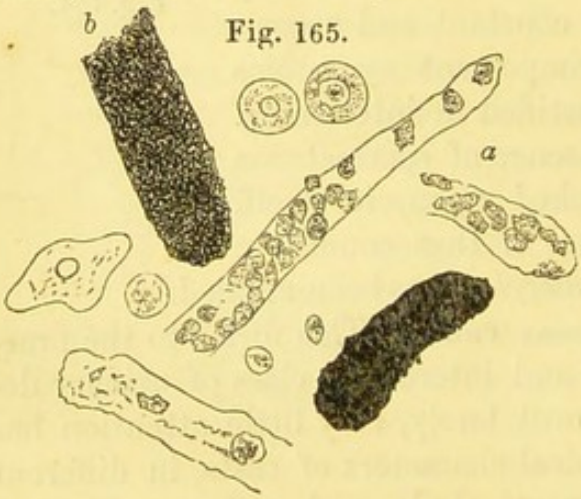
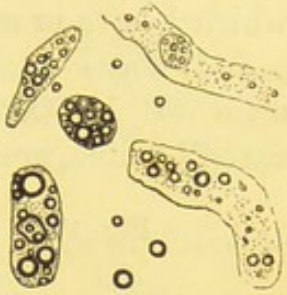


Fig. 165.

"Epithelial casts" consist of moulds of the tubes in which cells of epithelium are entangled. Some of the cells may be entire, while others are disintegrated (fig. 165). Some casts contain only granular matter (figs. 165, *b*, 167) with epithelial débris; more rarely casts will be found to contain blood or pus globules. In some instances,

entangled in the cast, are numerous oil-globules, readily distinguished by their highly-refracting nature, with or without cells of epithelium, larger than natural and gorged with oil (fig. 166).

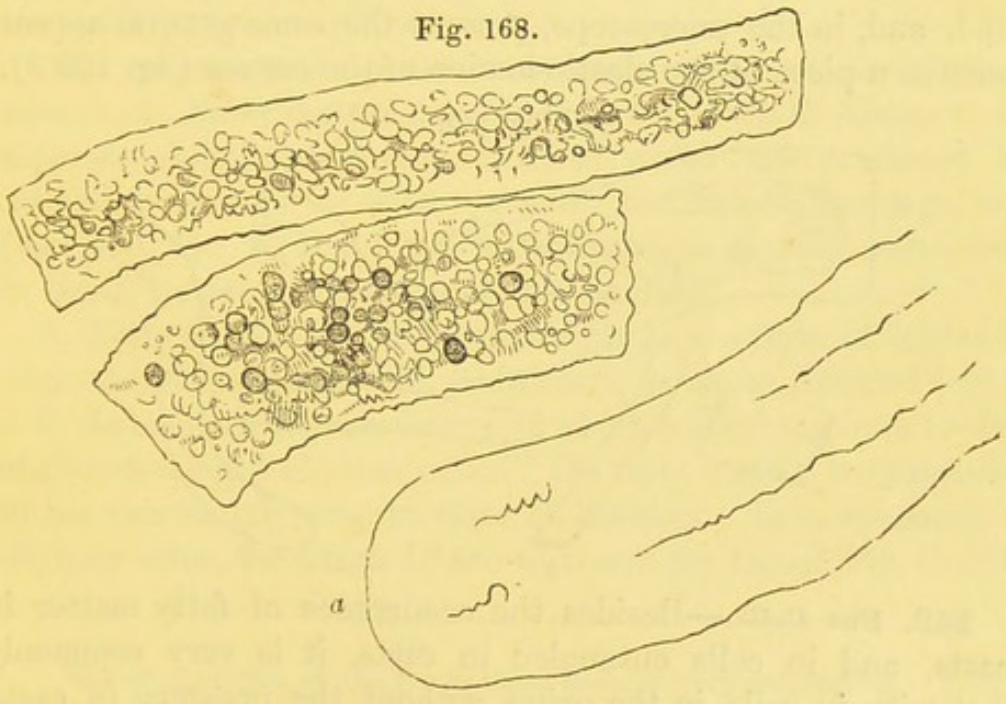
Fig. 166.



Once I have met with casts of medium diameter, containing well-formed dumb-bell crystals of oxalate of lime. These casts were found in the urine of a patient suffering from cholera. In the same specimen, also, several octohedra of oxalate of lime were present, but these latter were not entangled in the casts.

240. Casts of considerable diameter. — 2. *Casts of considerable diameter, about the* 1-500*th of an inch.* Dr. Johnson speaks of "large waxy casts," which are perfectly transparent, and have a glistening aspect, somewhat resembling in appearance the surface of wax as it cools after having been

Fig. 168.



melted. Casts of considerable diameter also occur, of a granular character, and one portion of a cast is often granular while the other is transparent, and containing perhaps a few epithelial cells. Large waxy casts are represented in fig. 168; at *a* is

represented a large cast perfectly transparent. Two of the casts in the figure, and the one represented at *a*, fig. 169, are seen to be composed of a material in the interior, differing from that which forms the circumference of the cast—an appearance which I have in several instances observed.

241. Casts of small diameter.—3. *Casts of small diameter, about the* 1-1000*th of an inch.* These are the "small waxy casts," which, according to Dr. Johnson, are formed in cases in which the epithelium manifests no tendency to desquamate (*non-desquamative nephritis*). The diameter of the cast is, therefore, that of the central canal only (fig. 167); and, not unfrequently, we meet with casts of less than 1000th of an inch in diameter, having a perfectly smooth and glistening surface, and without the slightest trace of granular matter. These appear perfectly hyaloid, and, in the microscope, present the same general appearance as a piece of the elastic lamina of the cornea (fig. 169 *b*).

Fig. 167.

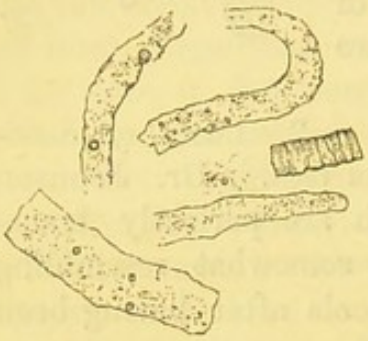
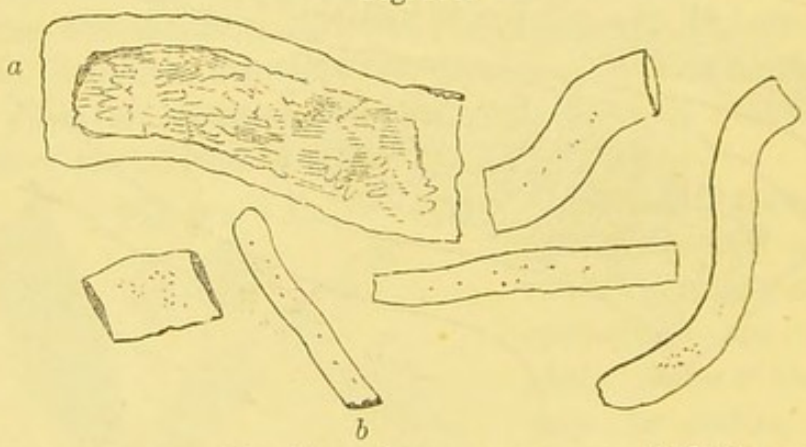


Fig. 169.



242. Fat Cells.—Besides the occurrence of fatty matter in casts, and in cells entangled in casts, it is very commonly met with in cells, in the urine, without the presence of casts. These cells consist usually of epithelial cells of the kidney, enlarged and gorged with oil (figs. 166, 170, *a*). Sometimes they contain a few oil-globules, which are well defined, and are seen to be distinct from each other; while, in other instances, the

globules are very minute, and so crowded together, that the cell appears perfectly opaque and dark, resembling the so-called inflammatory globules. Occasionally cells containing oil-globules may be derived from some other part of the mucous surface of the urinary passages. Fig. 171 represents the appearance of some epithelial cells, and collections of oil globules taken from the membranous portion of the urethra. These could hardly be mistaken for the cells and casts met with in the urine in cases of fatty degeneration of the kidney; but at the same time it is important to bear in mind that cells containing oil-globules are occasionally met with under other circumstances. Of late, much attention has been paid to the presence of fatty matter in the urine, and it may be of advantage here to refer to the various states in which it may be met with in this secretion.

Fig. 170.

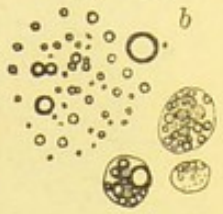
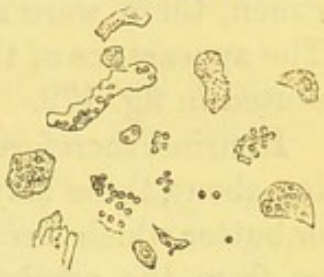


Fig. 171.



243. Conditions in which Fatty Matter may be met with in Urine.—1. Fatty matter may occur in the urine as distinct and separate globules, resembling those which are produced by intimately mixing oil with water by the aid of mucilage, &c. (fig. 170, *b*). When fatty matter occurs in this state only in urine, its presence is usually accidental.

2. Fatty matter occurs in the urine in the form of globules, enclosed within a cell-wall, or in casts, as above referred to.*

3. In some of the rare instances which occur from time to time of the so-called "chylous urine," the fatty matter is suspended in an exceedingly-minute state of division. In a specimen of chylous urine, for which I have to thank my friend Mr. Cubitt,

---

* The composition of the fat in these cases is very interesting. I have found it to contain much cholesterine dissolved in a more fluid fat, from which it may be readily separated in a crystalline form. From the fatty matter contained in cells obtained from morbid structures in other parts of the body, I have also been able to extract much cholesterine; and also from some organs in a state of fatty degeneration.

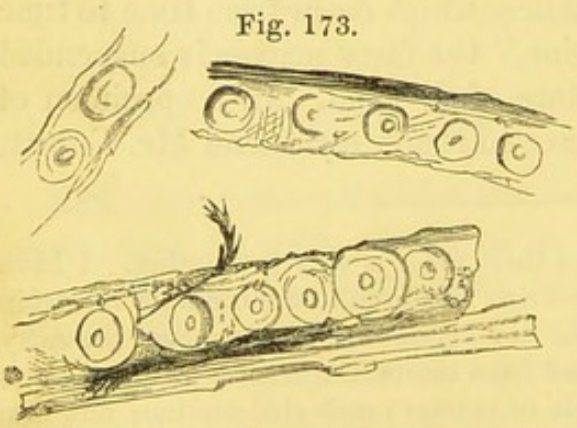
there existed nearly thirteen grains of fatty matter in a thousand of urine. I could not detect any oil-globules. The whole of this large quantity of fatty matter was in an extremely minute state of division, to which the term "molecular" has been applied, and in which state the fatty matter is found in chyle. Upon microscopical examination, the field was seen to be covered with very minute molecules, like small dots, revolving, with a slight wavering motion, about each other. In this specimen, there were also a few delicately-granular cells present. The appearance of this urine, examined with a quarter, is represented in fig. 172.

Fig. 172.

In urine, therefore, in which we find distinct oil-globules floating about, these may be derived from the presence of a little oil or butter which has accidentally fallen into the urine (fig. 152 *i*), or from the admixture of milk. When the oil-globules are enclosed within a cell-wall, or entangled in casts, the condition may be looked upon as indicative of "fatty degeneration" of the kidney, or of the epithelium in some other situation; and where the fatty matter is in a molecular state, the case is one of "chylous urine."

244. Extraneous Matters.—These have been already referred to. Cotton fibres, blanket hair, &c., will often be found among flocculent urinary deposits. The student must be careful not to mistake short portions of hair or other substances for casts. The only matter of extraneous origin which need be particularly referred to here, is one which may very easily be mistaken, and, indeed, frequently has been, for tube casts. The substance to which I refer consists of the delicate fibres of coniferous wood which are swept off the deal floor, and thus get into the urine (fig. 173).

Fig. 173.



The fibres become soft and swollen by soaking, and may easily be mistaken for casts. The round pores which they contain much resemble epithelial cells. These bodies, of course, will only be met with when the floor is of deal and often swept. I have found them in very many specimens of urine obtained from King's College Hospital.

SECOND CLASS OF URINARY DEPOSITS.

The three deposits included in this class often resemble each other very closely when examined only by the unaided eye; but they differ widely in their microscopical characters, in their behaviour with chemical reagents, and also in their pathological importance.

245. Pus.—The microscopical characters of pus will be more fully described in Chapter XV. The form of the globules becomes somewhat altered if they have been soaking in urine for a long time, and ultimately they undergo complete disintegration. Fig. 174 shows the appearance of pus-globules: at *a* some cells are shown, acted upon by acetic acid. If decomposition of the urea, accompanied with the development of carbonate of ammonia, occurs, the globules become converted into a glairy viscid mass (see Mucus, § 232). A deposit of pus is very frequently accompanied with crystals of triple phosphate, but this is by no means invariably the case. I have noticed that when the pus is derived from the bladder, the crystals are very frequently present; but in several cases in which large quantities of pus were passed in the urine, but derived from the kidney, the crystals were altogether absent.*

Fig. 174.

246. Earthy Phosphates. — The earthy phosphates which occur in large quantity in urine are triple phosphate, generally accompanied with amorphous granules, or small rounded globules of phosphate of lime. This deposit often occurs in considerable quantity. Upon microscopical examination, numerous

---

* "Clinical Lecture," by Dr. Todd. "Medical Times and Gazette," No. 185.

prisms of triple phosphate (HO,NH_4O,MgO,PO_5), in their well-known triangular form, with obliquely-truncated extremities will be observed (fig. 175).

Some of the crystals are of a more quadrilateral form, while others appear almost like an octohedron, in consequence of the central part of the crystal not being developed. A crystal of this form is represented. In consequence of the two ends being closely approximated, the appearance of a square crystal, the opposite angles of which are connected with straight lines, is produced. Various modifications of the above forms will also be very frequently met with. The faces of the crystals become roughened by standing long in the urine, or, indeed, in pure water, unless a small quantity of some ammoniacal salt be dissolved in it, in which case the crystals will keep unimpaired for a length of time.

Fig. 175.

The more uncommon modifications of the crystals of triple phosphate will be referred to in the next class, as they most frequently occur only in small quantity. The deposit of triple phosphate is always accompanied with phosphate of lime ($2CaO,HO,PO_5$) if the urine be alkaline. This phosphate occurs in the form of small spherical crystals, or amorphous granules. Often two small globules are joined together so as to resemble a small dumb-bell crystal.

Fig. 176.

247. Lithates.—This deposit is often very abundant. It may vary in colour from a pale buff to a tolerably deep red; often, however, it is almost colourless. It is this deposit to which the terms "nut-brown sediment," "lateritious deposit," &c., have been applied, according to the proportion of colouring matter it may contain. It consists principally of lithate of soda, with small and variable proportions of lithates of ammonia and lime, with, perhaps, also a trace of lithate of magnesia.*

Fig. 177.

* "Lehrbach der Zoochemie," Heintz. Berlin, 1853.

Upon microscopical examination it is found to consist entirely of minute granules which are unequally aggregated together in different parts of the field (fig. 176). More rarely the deposit contains spherical masses of the lithate, or small rounded globules.

The appearance of lithate of ammonia artificially prepared is shown in fig. 177.

Fig. 178.

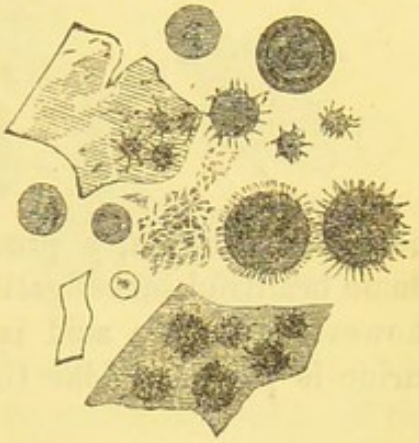


Fig. 178 shows the appearance of the spherical masses of lithate of soda, which form part of the scum of urine while it is evaporating. The smooth flat portions consist of phosphates which form a very thin film to which the lithates adhere. For the chemical characters of these deposits, see Lehmann's "Physiological Chemistry," vol. i., Bowman's "Medical Chemistry," &c.

Lithate of soda is not unfrequently met with in urinary deposits in the form of small spherical masses, from the surface of which spicules of lithic acid project in various directions (fig. 182, *c*).

Fig. 179.

Occasionally, the very dark granular appearance of certain casts is due to a deposition of lithates upon its surface after the cast has been passed. In these cases the granular appearance is removed upon applying a gentle heat to the slide upon which the deposit is placed.

248. The most common extraneous matters which simulate deposits of this class are starch (fig. 179) and sand, which are occasionally added for purposes of deception. In one instance which came under the notice of my friend Dr. Stewart, a large quantity of peroxide of iron had been added to the urine, with the view of deceiving the practitioner.*

---

* "Microscopical Journal," No. II., p. 93.

THIRD CLASS OF URINARY DEPOSITS.

249. Uric or Lithic Acid.—This is one of the most common urinary deposits. Uric acid is frequently deposited after the urine has left the bladder, in consequence of the occurrence of

Fig. 180.

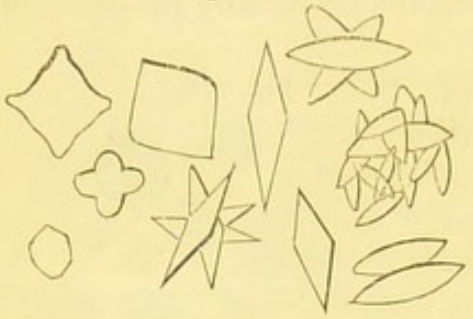
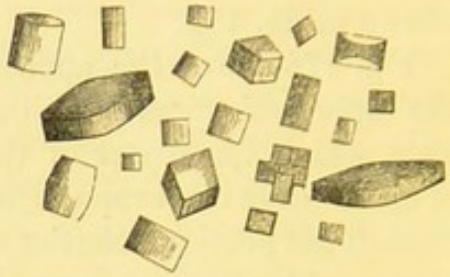
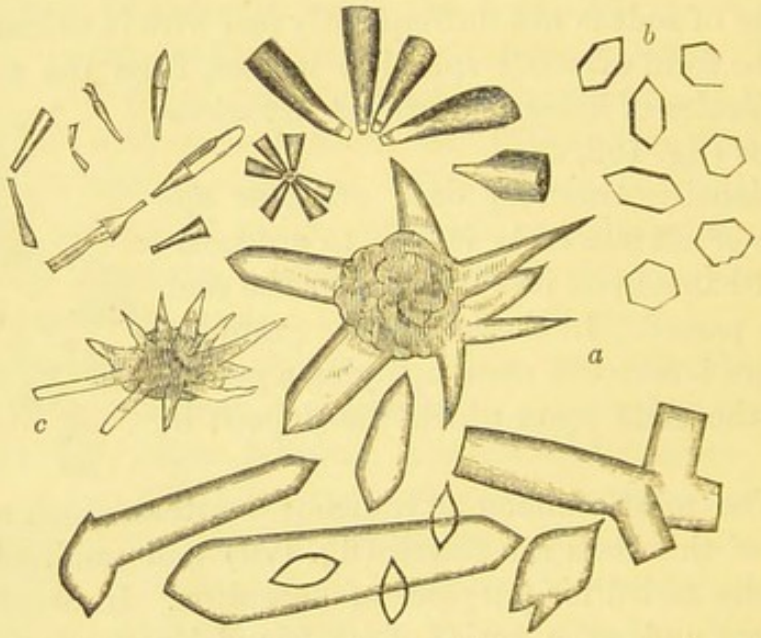


Fig. 181.



acid fermentation, a process which has been the subject of some beautiful investigations by Scherer.* In some instances, however, the uric acid is undoubtedly deposited before the urine is passed. Like the lithates, deposits of uric acid vary

Fig. 182.

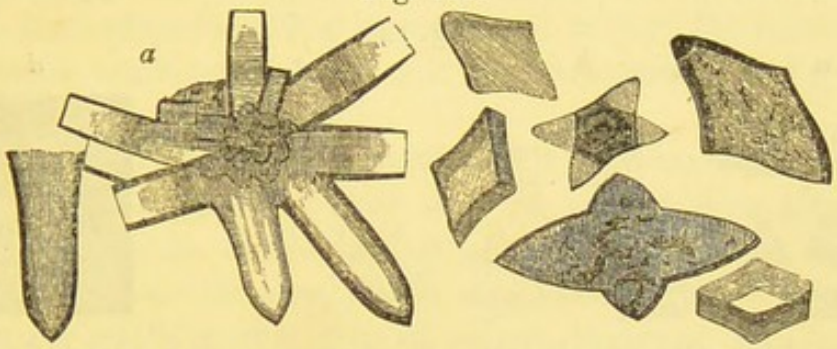


very much in colour. Sometimes they are nearly colourless, while, in other instances, the crystals are arranged in the form of large grains of a deep red colour, "cayenne pepper grains."

* "Untersuchungen," 1843. Lehmann's "Chemistry;" translated by Day, vol. ii., p. 408.

In figs. 182, *a*, 183, *a*, the appearance of two of these crystalline masses, examined by powers of 200 and 100 respectively, is depicted. The crystals also vary much in size, so that the deposit may appear to the unaided eye as a granular layer, or as a distinctly crystalline sediment. Deposits of lithic acid usually occupy an inconsiderable bulk, compared with that of the urine from which they have been precipitated.

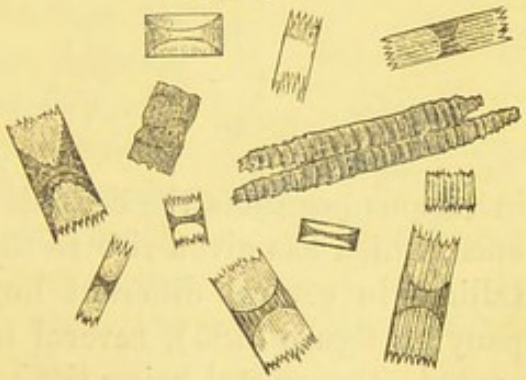
Fig. 183.



The forms which the crystals assume are very various. The most characteristic, and that most frequently met with, is the rhomb, and it is in this form that this substance is deposited when any of its salts are decomposed by the addition of a stronger acid. Some of the most common forms are represented in figs. 180, 181. The latter figure represents the form which lithic acid usually assumes in the urine of cases of "acute dropsy," and of "dropsy after scarlatina."

Figs. 182 and 183 represent some curious modifications of crystals of lithic acid. The six-sided crystals (fig. 182, *b*) must not be mistaken for cystine. They may readily be distinguished by two of their sides being longer than the others, and also by their chemical characters.

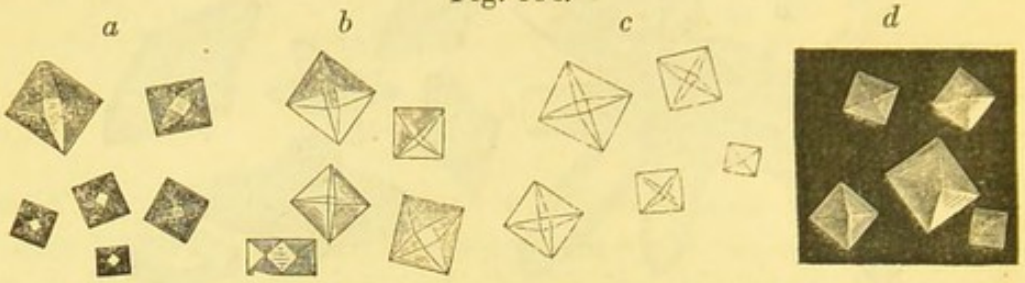
Fig. 183.*



In Fig. 183* are represented some crystals of lithic acid, which are occasionally met with. They may often be produced by the rapid crystallization of lithic acid in urine, to which nitric or hydrochloric acid has been added.

250. Oxalate of Lime.—Oxalate of lime was first shown to be a common urinary deposit by Dr. Golding Bird. It occurs as a scanty sediment, in which the crystals, if they are large, are seen as minute glistening points to the unaided eye. Large crystals of oxalate of lime present a beautiful appearance when examined by reflected light (fig. 184, *d*). If they are subjected to examination in the dry way, they appear like dark cubes, with a clear bright centre (fig. 184, *a*). Their appearance in

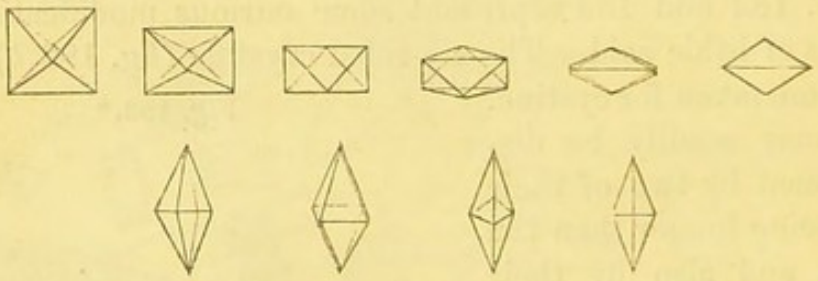
Fig. 184.



fluid and in Canada balsam is shown in the same figure at *b* and *c*. More commonly, however, the crystals do not all sink to the bottom of the liquid, but are, as it were, buoyed up by the small quantity of mucus present. They vary very much in size.

Oxalate of lime crystallizes in well-defined octohedra, one axis of which is much shorter than the other two. Viewed

Fig. 185.



in various positions the crystals present a very different appearance, which has given rise to the idea that this substance crystallizes in several different forms in urine. In the accompanying figure (185), several of these appearances are represented; the crystal being the same in each case, but viewed in a different position.

In the four lower figures a crystal is shown with one of its

lateral angles towards the observer, and rotated upon its long axis.

I have been able to observe all these different forms by causing the crystals to rotate in the field of the microscope. With the aid of a little glass model it is very easy to demonstrate the different appearances to any one.

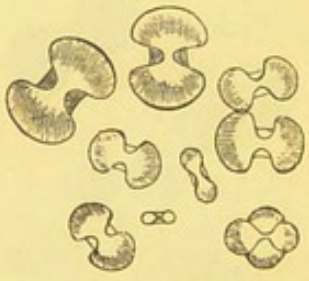
Octohedra of oxalate of lime are frequently deposited after the urine has left the bladder, and continue to increase in size for some time after their first appearance; so that the urine should always be examined soon after it has been passed, and also after the lapse of several hours.

Not unfrequently the crystals are very minute, and without care in the examination they may be passed over altogether. Minute crystals of oxalate of lime often occur amongst deposits of pale lithates, which may obscure them from view. Upon the addition of a drop of potash, however, the lithate is dissolved, while the crystals of oxalate of lime are not affected, and can be distinguished readily upon microscopical examination.

251. **Dumb-bell Crystals.**—*Dumb-bell crystals of Oxalate (Oxalurate?) of Lime.* These crystals were also first described by Dr. Golding Bird, as consisting of oxalate of lime; but in consequence of their power of polarizing light, he considers it probable that they may be composed of oxalurate of lime, for substances which crystallize in the octohedral form do not possess this property.

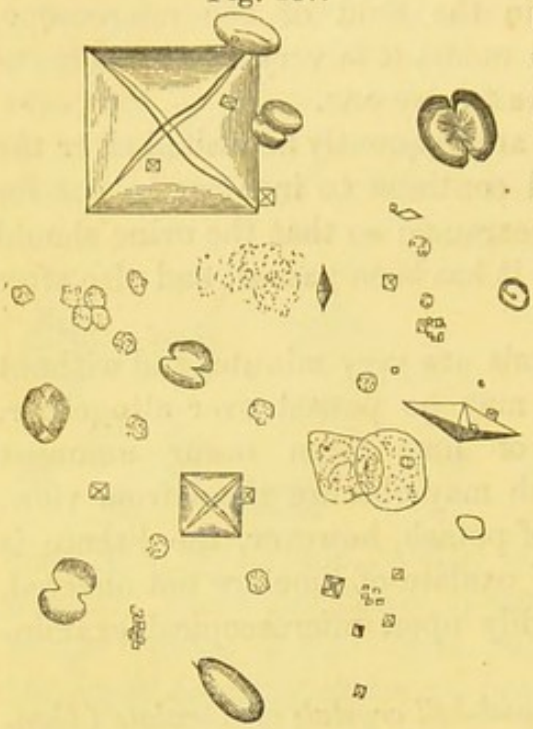
A very perfect form of these dumb-bell crystals is represented in fig. 186: they were obtained from the urine of a child, two years of age, suffering from jaundice. Besides the dumb-bell crystals, other allied forms are very often present, such as oval and perfectly circular crystals (fig. 187); and not unfrequently crystals of an irregular form occur, one side being even and regular, while the opposite presents different characters. Dumb-bells will generally only be met with in urine for a few consecutive days, and they are almost always accompanied with

Fig. 186.



octohedral crystals (fig. 187). I have observed on several occasions that the appearance of the more perfectly-formed dumb-bell crystals is preceded and succeeded by the presence of the circular, oval, and less regular forms of crystals.

Fig. 187.



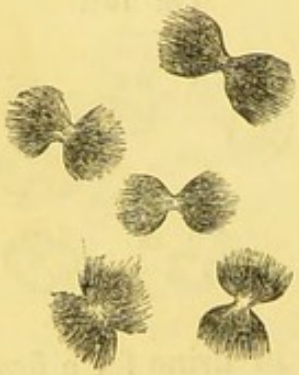
These crystals are certainly formed in the kidney; for I have seen them in the tubes after death on several occasions, and once I met with them in the fibrinous casts of the uriniferous tubes which had escaped in the urine.

By the prolonged action of acetic acid, I have found that the crystalline matter was dissolved, leaving a small quantity of organic matter, taking the precise form of the original crystal, and appearing like a cell-wall (fig. 188).* A similar change takes place in the case of the spherical and dumb-bell-shaped crystals of carbonate of lime, so common in the urine of the horse and other herbivora.

Fig. 188.

The dumb-bell crystals appear to be formed by the aggregation of minute acicular crystals; an arrangement which is well seen in the crystallization of other substances, which, under certain circumstances, assume this form. In fig. 189, some crystals of lithate of potash (prepared artificially) are represented in this form, but these do not possess any investing membrane. Figures 186–190 are all magnified with a power of about 200 diameters.

Fig. 189.



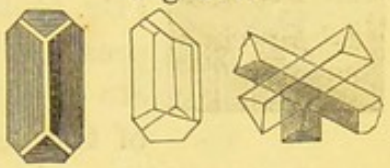
* "Medical Times," 1851, p. 374.

Phosphate of lime also appears to assume the dumb-bell form occasionally.—The crystals delineated in fig. 190 were obtained from the decomposing mucus of the gall-bladder of an ox.

Fig. 190.

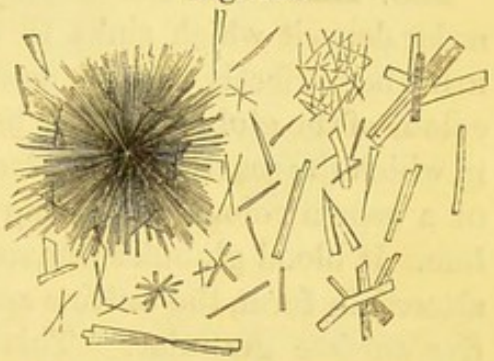
Lithic acid also assumes the dumb-bell form; but these crystals are readily distinguished from those of the oxalurate of lime by their solubility in solution of potash, and by the difference of their refracting power. Dr. Bacon has added some interesting observations upon the composition of these crystals, which will be found in Dr. Golding Bird's work on Urinary Deposits, to which the reader is referred for further information upon this subject.

Fig. 191.



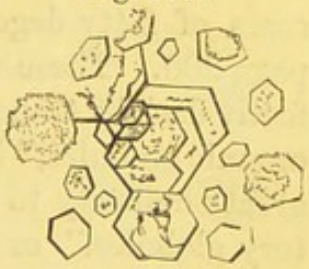
252. Triple Phosphate.—Besides the ordinary form of crystals of triple phosphate (figs. 175, 191), there are others which occur more rarely in small quantities. Fig. 192 represents some crystals of triple phosphate, of a beautiful form, not very frequently met with. The specimen from which this drawing was taken contained also many octohedral crystals of oxalate of lime. If a little ammonia be added to healthy urine, the ammoniaco-magnesian phosphate is precipitated in the form of beautiful stellate feathery crystals.

Fig. 192.



253. Cystine.—Cystine forms a deposit much resembling that of the pale urates; from which, however, it is readily distinguished by not being dissolved upon the application of heat. Cystine is readily soluble in ammonia; and as the ammoniacal solution evaporates, the characteristic six-sided plates are formed. For the deposit from

Fig. 193.



which the accompanying drawing (fig. 193) was taken, I am indebted to my friend Dr. Sankey, of the London Fever Hospital.

254. **Carbonate of Lime** is very rarely met with in a crystalline form in human urine. Not unfrequently it occurs, mixed with a deposit of triple phosphate and phosphate of lime, as an amorphous powder, or forming very small round masses. Occasionally, however, it has been found forming dense spherical stellar masses composed of aggregations of minute acicular crystals (Dr. Golding Bird). Fig. 194 represents the appearance of carbonate of lime as it occurs in the urine of the horse, under the influence of reflected light; and in fig. 195 some crystals, viewed in Canada balsam, with transmitted light, are shown.

Fig. 194.

Fig. 195.

255. **Blood Globules** usually form a red or brownish-red granular deposit which sinks to the bottom of the vessel. If the urine be perfectly neutral, or slightly alkaline in its reaction, the colour of the globules will be bright red; while, in those instances in which the reaction is decidedy acid, the globules will be found of a brown colour, imparting to the supernatant fluid a smoky hue. If blood globules remain long in urine they become much altered in form, the outline appearing irregular and ragged, and the surface granular. This change no doubt is chiefly dependent upon physical causes.

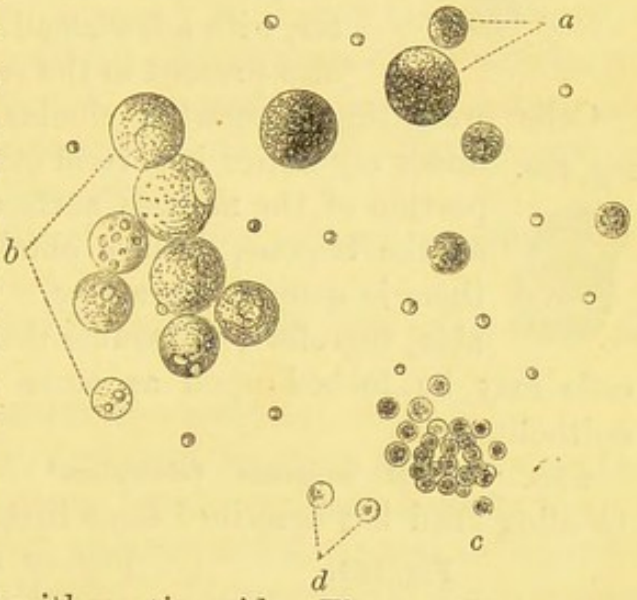
On the subject of extraneous matters sufficient has already been said.

256. **Large organic Globules, Exudation Cells, &c.**—Large cells filled with oil globules, which are met with in the urine in cases of fatty degeneration, have already been referred to in page 209. These when completely filled with oil, appear perfectly dark by transmitted light. They have been termed "large organic globules," by Dr. Golding Bird, and in structure present great similarity to the so-called "exudation cells," "inflammatory globules," or "compound granular cells." They consist essentially of a cell-wall, enclosing within it a greater or less

number of oil or fat *globules*,* distinguished by their dark outline and clear transparent centre. By reflected light these cells appear opaque and perfectly white. They must be distinguished from the cells, in which no oil globules can be detected, in which even with very high powers the dark parts appear to be composed only of minute granules or molecules, which appear as very fine dots.

257. Spherical Cells containing Nuclei and Granular Matter.—Cells presenting these characters are not unfrequently met with in specimens of urine, but I have not been able to determine with accuracy the portion of the mucous tract from which they are derived, or their pathological importance.

Fig. 196.



The cells represented in fig. 196 were found in the urine of a patient suffering from rheumatic fever, at *a*, as they occurred; *b*, the same treated with acetic acid; *c*, cells much resembling pus; *d*, the same treated with acetic acid. The smaller round bodies are altered blood corpuscles.

The large cells above referred to contained several transparent bodies within them, which became very distinct upon the addition of acetic acid (nuclei?). The central bodies did

---

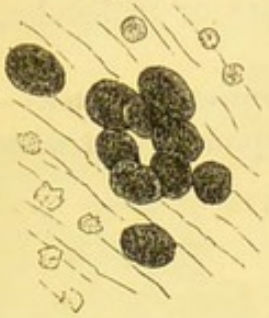
* The term "globule" is here restricted to a spherical body, with a dark and well-defined outline, and a clear bright centre. Cells containing oil globules are represented in the figure.

"Granules" and "molecules," under the microscope, appear as minute points or dots, in which no distinct structure can be made out. They exhibit the peculiar molecular movements (see Chylous urine). In the figure, three cells containing granular matter are represented. Granular cells are also shown in fig. 196; granular casts in figs. 165, *b*, 167.

not refract light as oil globules, nor did they present the circular dark and well-defined outline so characteristic of the latter.

In fig. 197 are represented specimens of large cells filled with dark granular matter, but not containing any oil particles, from the urine of a case of chronic bronchitis. There were also a few pus globules present in this specimen. Fig. 198 represents a curious form of cell found in the urine of a case of renal dropsy of seven weeks' duration. Casts of medium diameter, with a few small cells containing oil, were also present in the same specimen of urine.

Fig. 197.



Cells presenting somewhat similar characters have come under my notice in several other cases; and from that portion of the mucous surface of the bladder known as the trigone, I have obtained cells agreeing with them in general characters. It appears not unreasonable, therefore, to assume that many of these peculiar cells may be looked upon as some modification of bladder epithelium.

Fig. 198.

258. **"Small organic Globules."** — Under this name Dr. Golding Bird has described some little bodies smaller than the pus or mucous corpuscles, with a perfectly smooth exterior, and unaffected by acetic acid. Dr. Bird suggests that they may be nuclei which have been set free from a cell by the bursting of the investing membrane.*

Fig. 199.

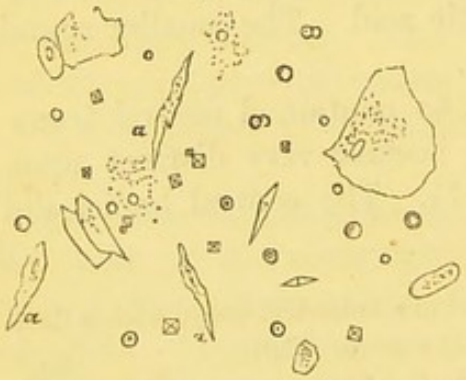


Fig. 199 represents the appearance of the deposit from the urine of a patient suffering from calculus. The small round bodies represented in different parts of the figure were insoluble in strong acetic acid, and were unaltered on the addition of ether or potash. Many of them contained a central dark spot. They were accompa-

* "Urinary Deposits," fourth edition, p. 356.

nied with numerous small octohedral crystals of oxalate of lime. From their highly refractive properties and chemical characters just referred to, it is probable that they were composed of oxalate or oxalurate of lime.

ON THE PRESERVATION OF URINARY DEPOSITS.

It is very desirable to be able to preserve many urinary deposits permanently, particularly when their nature is doubtful, in order that they may be compared with other specimens; and as this point presents some difficulties to the student, although it has been referred to in Chapter VII., I shall nevertheless discuss it here somewhat in detail. While some of the substances met with are preserved with comparative facility, others are only prevented from decomposing with great difficulty, and by the use of good preservative solutions.

There are three methods of mounting urinary deposits:—

1st. As dry preparations.

2nd. In Canada balsam, turpentine, oil, and other fluids of similar characters.

3rd. In an aqueous preservative solution.

The first method is only applicable in a very few cases, as the greater number of substances forming urinary deposits are so altered by the processes of washing and drying as to be afterwards recognized with difficulty. Large crystals of lithic acid, crystals of oxalate of lime, and certain forms of phosphates and lithates may, however, be mounted as dry objects, but they of course exhibit different characters when examined in fluid (§ 82).

259. Preservation of Urinary Deposits in the Dry way.—Specimens which are to be mounted in the dry way must undergo the same preliminary washing and drying as those which are to be put up in Canada balsam. The same description will, therefore, serve for both. Suppose we require to dry some crystals of lithic acid:—after the crystals have been allowed to collect at the bottom of a conical glass vessel, the clear supernatant fluid is to be poured off, and the crystals are to be washed with a little dilute alcohol, or with a very weak solution of acetic acid. When the process of washing has been repeated

two or three times (§ 224), a small quantity of the deposit is to be transferred by means of a pipette to a glass slide, and the greater part of the fluid soaked up with a small piece of blotting paper. The crystals are next to be spread a little over the glass, with the aid of a fine needle, in order to separate the individual crystals from each other, and the slide is to be placed in a warm place, or in the sun, until quite dry; but care must be taken that the drying is not carried on too rapidly, and that too great a degree of heat is not employed. A narrow rim of paper or cardboard is next to be gummed on the slide so as to include the crystals in a sort of shallow cell; and, lastly, the glass cover is to be put on, and kept in its place either by anointing the edges with a little gum-water, or by pasting it down with narrow strips of paper, which may be variously arranged and ornamented according to taste.

260. Preservation of Urinary Deposits in Canada Balsam.— If the crystals of lithic acid are to be mounted in Canada balsam, they should be carefully dried first, as above directed, and afterwards over sulphuric acid (§ 282), and then moistened with a small drop of spirits of turpentine. The slide is now to be slightly warmed, in order to volatilize the greater part of the turpentine, and a drop of Canada balsam is to be dropped upon the preparation from the end of a wire, which may be readily effected by holding the wire with the balsam over the lamp or hot brass plate for a minute or two in order to soften it. The slide is next to be held over a lamp, in order to keep the balsam fluid until any air-bubbles which may be present have collected into one spot on the surface of the liquid balsam, an operation which is expedited by gently moving the slide from side to side. The air-bubbles may now be removed by touching them with a fine-pointed wire, as directed in § 105. Lastly, the glass cover is to be taken up with a pair of forceps, slightly warmed over a lamp, and one edge is allowed to touch the balsam. The surface is permitted to fall gradually upon the balsam, so that it is wetted by it regularly, and only by very slow degrees, for otherwise, air-bubbles would yet be included in the preparation. The glass slide with the preparation may now be set aside to cool.

261. Preservation of Urinary Deposits in Aqueous Solutions.—For the preservation of urinary deposits, the most important method is that of putting them up in some preservative fluid, for in this manner alone can the characteristic appearances of many specimens be retained. Mounted in the dry way, and in Canada balsam, it need scarcely be said that the object presents different characters to those observed when it was examined in the urine; and although the two former methods are of great advantage in examining the structure of some crystals, they are ill adapted for preserving the great majority of urinary deposits, and are wholly inapplicable for the preservation of epithelium, casts of the renal tubes, &c.

When preservative solutions are employed, the objects must always be placed in shallow cells; and the most convenient form of cell for this purpose, according to my experience, is that which is made by painting upon the glass slide, with a fine brush, a narrow border of Brunswick black (§ 69), enclosing either a square or circular space, as may be most convenient. In cases where a deeper cell is required, those composed of thin glass (§ 71) or tinfoil (§ 70) are the most useful. The forms of cells just referred to, I can recommend from experience, for I have many preparations put up in them which have been well preserved for some years.

Next, with regard to the preservative fluids best adapted for mounting urinary deposits—weak spirit answers pretty well for some sediments, but as a general rule is not suitable for substances occurring in urine.

Glycerine may be employed in some cases diluted with a little water. The preservative gelatine (§ 99) I have found answer exceedingly well for the preservation of dumb-bell crystals of oxalate of lime and some other crystalline deposits: with care, epithelium may also be preserved in it. I have used the creosote and naphtha solution (§ 97) most successfully for the preservation of casts and various kinds of epithelium, &c.

Whatever preservative fluid is used, care should be taken that the deposit to be put up is thoroughly saturated with it, for unless this object be attained, there is danger of the preparation being destroyed after a time.

262. Method of separating the Deposit from the Urine, and placing it in the preservative Fluid.—The most simple manner of mounting deposits by the use of these fluids is by allowing the sediment to subside to the bottom of a conical glass, pouring off the supernatant urine, and adding a small quantity of the preservative solution. The deposit is again allowed to subside, and the solution poured off, and replaced by a fresh quantity. After the subsidence of the deposit, a small portion may be removed with a pipette, placed in one of the forms of cells above referred to, and the glass cover placed on the surface of the liquid, care being taken that the whole surface of the glass be wetted with the solution, in order that no air-bubbles may be included in the preparation (§ 91). Any excess of fluid is now to be soaked up with a clean cloth, or with blotting-paper, and the cover cemented to the cell by applying a little Brunswick black or other varnish with a camel's-hair brush. The name of the deposit, with any other particulars, is to be appended to the slide, and the preparation laid flat in the cabinet.

In the manner just detailed, the following may be readily preserved: various kinds of epithelium, casts, fat cells, torulæ, confervæ, pus, mucus, lithic acid, oxalate of lime, lithates of soda and ammonia, and other substances, whose characteristic appearance is not altered by aqueous fluids. If lithic acid, oxalates, phosphates, or other crystals, are to be put up as objects for examination with polarized light, they should be mounted in balsam or turpentine.

263. Preservation of Crystals of Triple Phosphate. Cystine.—Crystals of the triple phosphate may be preserved in water to which a little ammonia and muriate of ammonia have been added. In this solution the surfaces of the crystals preserve their beautiful smooth character, while in pure water or in creosote fluid the surface becomes roughened rapidly. Dumb-bells, as I before noticed, may be preserved in the preservative gelatine, and they are not liable to shift their position in consequence of being well supported by the jelly. Crystals of cystine cannot be preserved in the creosote solution, because they are slowly dissolved by it; but as they are insoluble in

vegetable acids, a dilute solution of acetic acid will be found to keep them unchanged.

The figures represented in Chapter XIV. are magnified with one of Powell's quarters (about 220 diameters) unless stated to the contrary.

On the subjects treated of in the last two Chapters, the following works may be consulted:—"Urinary Deposits," by Dr. Golding Bird; "Diseases of the Kidney," by Dr. Johnson; "Brightsche Nierenkrankheit," Braunschweig, 1851, by Dr. Frerichs; "Medical Chemistry," by J. E. Bowman; "Physiological Chemistry," Lehmann, translated by the Cavendish Society; "Traité de Chimie Anatomique et Physiologique," Robin and Verdeil; "Lehrbuch der Zoochemie," Heintz, Berlin, 1853; Schmidt, op. cit. Chapter III.

CHAPTER XV.

BLOOD — MILK — SEROUS FLUIDS — SPUTUM — VOMIT — SUBSTANCES PASSED BY THE BOWEL—DISCHARGES FROM THE UTERUS AND VAGINA—PUS.

264. Examination of Blood.—In order to examine the blood, a small drop is placed upon a glass slide, and covered with thin glass, which is to be pressed down until a very thin, transparent, and almost colourless stratum only remains. If in this manner the individual globules cannot be seen distinctly, a little syrup or serum must be added; but it is better to avoid the addition of any fluid, if possible. Upon carefully focussing, the red globules will appear to present a dark centre and light circumference, or the reverse, according as the focus is altered (fig. 200), and here and there a white corpuscle may be observed.

Fig. 200.

If a little strong syrup be added to a drop of blood, the corpuscles will be found to have become flatter from exosmosis of a part of their contents; while, on the other hand, if placed in water, they become spherical from endosmosis, and ultimately burst. It is not difficult to make a solution of similar density to that in the interior of the corpuscle; and in this manner, as Dr. Rees expresses it, we may take the specific gravity of a blood corpuscle, if we ascertain the specific gravity of the solution which has been added to the blood.

Acetic acid causes the membrane of the corpuscle to become more transparent and clear, and to swell up from endosmosis. After the application of this reagent, the blood corpuscle may be scarcely visible, but the membrane is not dissolved by it. Strong hydrochloric and nitric acid do not dissolve

the globules; with the latter reagent the outline is often rendered darker and thicker, while the entire globule becomes smaller. The corpuscles are entirely soluble in ammonia and alkalies. They are rendered darker, and the walls corrugated, by the acid of the gastric juice; and, after remaining in acid urine for some time, a similar change occurs; hence the black colour of blood, which has been effused into the stomach, and the dark smoky hue of acid urine containing blood.

Blood crystals, and the method of obtaining them, will be spoken of in Chapter XVII.

265. **Blood in Disease.**—In looking at a drop of healthy blood, besides the red corpuscles, here and there a larger white or colourless corpuscle is seen. The relative number of these should be carefully noted, as in disease they are liable to increase enormously. In health there is one white corpuscle to about fifty red ones. The condition in which they are much increased in number is frequently associated with enlargement of the spleen, and has been termed 'Leukhemia;' or 'white-cell blood disease' (Leucocythemia), by Professor Bennett.

In extreme cases, white or colourless corpuscles are almost as numerous as the red, and they appear much more so, because the red-blood corpuscles collect together in little piles, while the white remain separate and distinct, and occupy the intervals or spaces thus formed. The surrounding fluid contains much granular matter. Upon being treated with acetic acid, the cells swell up a little, become more transparent, and usually display one, two, or even more roundish bodies in the centre, much resembling those developed in the pus globule, by the action of the same reagent.*

In some cases of cholera, several cells, much larger than the white corpuscle, have been found in the blood. In a case which I had an opportunity of examining, a short time ago, many of these large cells contained oil globules, collected together in one part, leaving the remainder of the cell perfectly clear and transparent. The outline of the cell was distinct and well defined.

---

* Bennett on "Leucocythemia," 1852.

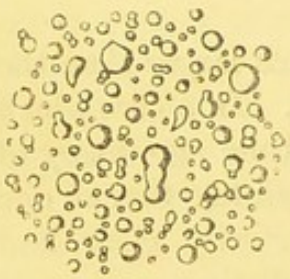
266. Blood of lower Animals.—The blood of the lower animals, particularly of the rat or mouse, bird, frog, newt, and fish, should be examined when an opportunity occurs. The size of the colourless corpuscles in these different animals may be compared, and it is interesting to observe the relation which they bear in size and number to the red globules in them as well as in man.

In some of the blood corpuscles of the spleen of the dog, and of certain fish, as the perch, and other animals, two or three little yellowish crystals have been observed in the interior (Funke, Kölliker). Sometimes, in examining a clot of blood, which has been effused in the brain, or in other situations, and which has remained there for some time, red crystals of hæmatine may be found in it. Splenic blood has also been found to contain free crystals. This subject will be again referred to in Chapter XVII.

The phenomena of the circulation of the blood are better studied in the foot of the frog, or in the branchiæ of the young newt. The former is to be placed in a moist bag and carefully tied down to the frog plate. Among mammalia, in the wing of the bat.

267. Examination of Milk.—The examination of milk presents no difficulties. All that is necessary is to place a drop upon a glass slide and cover it with a piece of thin glass. The general characters of the oil globules, and the fact of their not running together, and forming larger globules when pressed, should be noticed (fig. 201). This is prevented by the albuminous investment which surrounds each globule, and which may be demonstrated as follows. If the drop of milk be treated with a little acetic acid, the form of the globules is much altered, and if the acid be strong, the membrane will be dissolved, and several will run together, forming a larger globule. Again it will be found that ether will not dissolve the oil globules of the milk unless a little carbonate of soda, or some other alkaline salt, capable of dissolving this membrane, be previously added, when the

Fig. 201.



ether immediately effects the solution of the oily matter. This very instructive experiment may be performed in a test tube, or upon a glass slide, under the microscope ; the reagents being most conveniently applied by using the little bulbs (§ 300). In the figure some globules thus treated are seen running together.

The colostrum, or milk secreted first after delivery, will be found to contain many large cells, consisting of an investing membrane filled with oil globules, resembling those which are floating free in the surrounding fluid.

By microscopical examination, the most common adulterations of milk can be readily detected,—such, for instance, as chalk and flour (starch globules). It has been said that milk has been adulterated by the addition of sheep's or other brains. Such cases are, no doubt, very rare. Fragments of vessels, nerve-tubes, and cells, would be readily detected upon microscopical examination.

268. Examination of Serous Fluids.—Serous fluids may be poured into a conical glass-vessel, and allowed to stand until all the deposit has collected. A small quantity may then be removed by a pipette, in the usual way, and examined in the microscope. The microscopical characters of the fluid should be contrasted with those of ascitic fluid, the fluid of hydrocele, and serum from ovarian and other cysts.

269. Fluid from Serous Cavities.—The clear serous fluid which collects sometimes to a great extent in the peritoneal cavity (ascites), will be found, if recently effused, to contain but traces of cells, or cell debris ; but after the disease has been of long standing, the surface of the peritoneum becomes altered, and covered with a vast number of granular and almost spherical cells, varying very much in size, and not usually containing a distinct nucleus. A moderately-abundant deposit often takes place after the fluid has stood for some time. In other cases, which are of a more acute character, the fluid is found to be of a greenish or dirty-yellow colour,—opaque, with numerous flocculi and shreds of false membrane suspended in it, or attached to the surface of the peritonæum. In such a specimen, pus globules, with many of the cells above referred to, would probably be found, with others, which are darker in consequence of

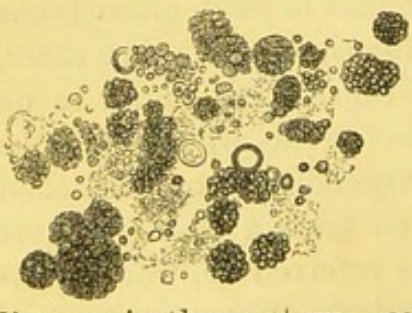
being filled with minute oil globules. The flocculi present usually a delicately fibrous appearance, with numerous cells entangled in the meshes formed by the interlacement of the fibres. Plates of cholesterine are sometimes found in ascitic fluid.

The fluid which accumulates in hydrocele is usually perfectly clear, containing a few delicate cells, and, perhaps, a few free oil globules, with not unfrequently spermatozoa, and sometimes many plates of cholesterine are also present.

270. Fluids from Cysts.—Upon contrasting serous fluids of this description with those which are found within the cavities of cysts, a marked difference is always observed, both in their chemical as well as in their microscopical characters. As an example of a fluid of this description, ovarian serum may be instanced; but the fluid found in cysts in different parts of the body, as in the antrum, or in the eyeball, thyroid gland, &c., will be found to present very similar characters.

The deposit of ovarian fluid will consist usually of cells, free granular matter, oil globules, and perhaps blood globules. Not unfrequently, many crystalline plates of cholesterine will be observed in it. The cells are composed of at least two distinct forms:—1. Small, delicate, transparent, and faintly granular cells, without the slightest appearance of a nucleus, some being somewhat larger, and others smaller than a pus corpuscle. 2. Large cells, often as much as the thousandth of an inch in diameter, but varying in size, of a dark colour by transmitted, and white by reflected light. These cells are crammed with minute oil globules, which, however, cannot easily be demonstrated without carefully focussing. They are almost constantly present in the fluids which are now under consideration, and have a structure apparently identical with that of cells presenting similar characters, and found frequently in softening of the brain (fig. 202),—sometimes in the coats of vessels undergoing fatty degeneration, in the urine in Bright's disease, in the sputum—especially in pneumonia in an advanced

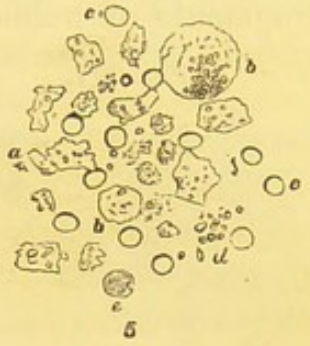
Fig. 202.



stage, in cystic tumors of the breast, in malignant growths, &c. In many instances, the fatty matter abounds in cholesterine, which crystallizes out from an alcoholic solution. The attention of the student is particularly directed to the occurrence of cells of this description in various morbid products.

Fig. 203 represents the appearance of the deposit from a specimen of serum obtained from a case of ovarian dropsy. The clear well-defined bodies, *e*, are blood corpuscles rendered spherical by endosmosis. Some small granular cells, and one of the large cells, containing a few oil globules, *b*, are also represented. The large dark cells are not shown in this figure, but the general characters of these may be seen by referring to the last figure.

Fig. 203.



271. **Examination of Sputum.**—In order to examine sputum, it is only necessary to place a small piece upon the glass slide, and cover it with thin glass. If any dark spots be observed in the mass, or any appearance of fibrous tissue, or other peculiarities affecting certain parts only of the sputum be noticed, these portions should be examined separately. They may be removed easily by the aid of forceps with broad points, and a pair of sharp scissors.

Sputum in different diseases presents very different characters, and in some cases by subjecting it to examination we are enabled to confirm an opinion with reference to the changes going on within the chest, which we have formed from physical signs; and hence the microscopical examination of the sputum is well worthy of attention, although it is a subject involved in great difficulty. See Dr. Black's papers, "Association Journal," 1853.

Epithelium from the cavity of the mouth and air-passages, with portions of any fungous vegetations which are so commonly found in the mouth, especially about the back of the tongue, and in the matter secreted by the tonsils, will commonly be met with in sputum, as well as portions of food from the same situations, such as muscular fibre, starch, oil globules,

&c. It must not, therefore, be inferred that everything present in the sputum comes from the air-tubes.

Sputum sometimes contains small fragments of pulmonary tissue, which are frequently distinct and well defined, but unless they are very abundant, may be readily passed over unnoticed. The conditions in which they are met with are phthisis and gangrene of the lung. It has been stated that fragments of pulmonary tissue may be found in the sputum before evidence of the existence of cavities can be obtained by physical examination. If this could be established, it would certainly form a very important aid in the diagnosis of phthisis in the early stage.

Fig. 204.

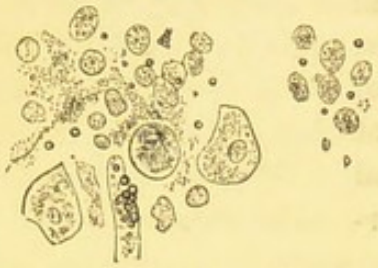


Fig. 204 represents the microscopical characters of a specimen of transparent, frothy, viscid, and almost colourless bronchial sputum. Some of the cells which have been treated with acetic acid, are shown to the right of the figure.

In phthisis portions of soft tubercle are often expectorated. These are frequently observed in the sputum as small roundish, somewhat transparent spots, which are found upon examination to be composed chiefly of small cells, more or less oval in form, and having a granular appearance (fig. 205) (tubercle corpuscles), with much free granular matter and oil globules.

Fig. 205.

The small, hard, white masses of very irregular form, which are occasionally expectorated by patients, consist chiefly of amorphous masses of phosphate of lime, with a little carbonate. For the examination of these see § 167.

Cholesterine has been found in the sputa, but it is not very frequently present in a crystalline form. In solution, in oily matter, forming minute oil globules and granules, it is often met with.

272. Pus, &c.; Claws of Echinococci, or portions of Hydatids in Sputum.—Pus is very frequently present in sputum, and so also are blood corpuscles. In the sputum of pneumonia, particularly in the more advanced stages, numerous large cells

containing oil globules will be found, with a vast number of finely-granular cells much resembling pus globules, but which do not exhibit the presence of central bodies when treated with acetic acid.

In a few rare cases, portions of the skins of hydatids, with adhering claws of echinococci, have been expectorated in the sputa. These may be separated by stirring the sputum well in water, removing the tenacious mass, and allowing time for the deposit to subside. Two such cases have fallen under my own observation. For the characters of the claws see fig. 133 *a*.

A knowledge of the microscopical characters of the different kinds of food, and of extraneous matters likely to be present in sputum, is as important in the microscopical examination of this substance as in the case of urine (§ 230), for we are of course liable to meet with fragments of anything that passes through the mouth.

273. Examination of Vomit.—As vomit usually contains a vast number of substances often separated from each other, it becomes necessary to examine several specimens taken from different parts, in order to ascertain the general microscopical characters. Portions may be removed upon the point of a knife; by the pipette if the vomit be very fluid; and with the aid of scissors and forceps, if it be very viscid, as in the case of sputa.

Vomit always contains fragments of vegetable and animal tissues, which have been taken as food, more or less altered by the processes of digestion. Starch globules are usually met with in great numbers; but if sufficient time has been allowed for the change to take place, the membranous coverings of the starch granules will alone remain.

Considerable attention has been given to the appearance presented by the uredo of wheat, as it occurs in vomit, and also in stools. In the time of the cholera, the undigested uredo found in the stools was looked upon as a fungus connected with the cause of this affection, but its true nature was pointed out by Mr. Busk.

Torulæ are very frequently present in considerable numbers in vomited matters; several other forms of vegetable fungi are

not unfrequently met with, and vibriones are often very abundant. The sarcina (fig. 138) has been already described.

The colour of the so-called coffee-ground vomit appears to be due to the presence of a dark-brown pigment in considerable quantity, forming small aggregations or minute granules, and probably consisting of the altered colouring matter of the blood; often with a considerable number of blood globules, somewhat changed in form. In some specimens of cholera vomit, numerous flocculi, consisting partly of large cells of scaly epithelium, and partly of cylindrical epithelium from the intestines, have been found.

The clear fluid which is brought up in certain cases (Pyrosis or Waterbrash) contains only a little epithelium, and a few small oil globules.

The green vomit, depending upon the presence of bile, contains cylindrical epithelium (gall-bladder ?), scaly epithelium, flakes and small masses of biliary colouring matter, often of a very bright colour, and fat globules.

In cases in which cancer of the stomach is suspected, the vomit should always be examined for cancer cells, although usually these will be found so much broken down as not to be recognizable.

274. Examination of Matters passed by the Bowels.—The microscopical examination of the fæces is in certain cases of considerable importance. In dysentery, shreds of fibrinous matter, blood corpuscles, pus globules, and cylindrical as well as squamous epithelium, are sometimes present. Crystals of triple phosphate are also often met with.

In typhus stools, crystals of triple phosphate are frequently present in great numbers; altered blood, and vast numbers of vibriones, with different kinds of vegetable fungi, are not uncommonly found.

The stools of cholera patients are remarkable for the large quantity of cylindrical epithelium they frequently contain. In many instances the white flocculi are almost entirely composed of it. Sheaths of the villi are often found in great numbers quite entire,—perhaps forced off by the violent contraction of the villus (§ 182). Undigested muscular fibre, exhi-

biting the transverse striæ very beautifully: large crystals of triple phosphate, and fragments of substances taken as food, are also generally met with.

Masses of vegetable confervoid growths have occasionally been passed by the bowels, but such cases are not common; one is mentioned by Dr. Farre, and another by Professor Bennett.

Professor Quekett has met with some elastic fibres in the fæces, exhibiting the transverse striæ, which are normal in the fibres of the ligamentum nuchæ of the giraffe. This transverse division depended probably upon incipient decomposition. The division is sometimes so distinct and complete as to have led to these fibres being mistaken for confervoid growths.*

275. Discharges from the Uterus and Vagina.—The character of these discharges varies very much. In subjecting them to microscopical examination, it is better to avoid the addition of water or other fluid if possible.

In uterine and vaginal discharges, the following substances are not unfrequently met with. Epithelium of the vagina, pus globules, blood corpuscles, small transparent oval or circular granular cells, usually occurring in abundance in the mucus about the os and cervix uteri, and small oil globules.†

In cases of cancer of the uterus, we should expect to meet with cancer cells in the discharge, but these are often so broken down as not to be distinguishable; still, when this condition is suspected, the discharge and also the urine should be subjected to very careful and repeated microscopical examination. In this investigation, the resemblance of the cells of columnar epithelium from the ureter to spindle-shaped cancer cells must be borne in mind, and the student must be careful not to mistake the former for the latter.

276. Examination of Pus.—The microscopical examination of pus is easily performed; but in many instances the inferences to

---

* "Principles of Human Physiology," fourth edition, Dr. Carpenter, p. 438, note.

† Upon the microscopical characters of Leucorrhæal discharges, the Memoir of Dr. Tyler Smith, in vol. xxxv. of the "Medico-Chirurgical Transactions," should be consulted.

be drawn from the examination must only be arrived at with great caution.

Pus corpuscles become smaller upon being placed in saline solutions of greater specific gravity than the serum in which they float, by exosmose of part of their contents. The corpuscles of pus are destroyed by the action of caustic alkalies, and converted into a thick glairy mass, which cannot be poured from the vessel containing it in drops. Upon examining this glairy mass in the microscope, only a few granules can be observed.

Water containing a trace of iodine in solution causes the pus globule to swell, and displays the central mass.

The most characteristic reaction, however, is produced by the addition of acetic acid. This reagent, if not very strong, causes the corpuscle to swell, so that it may become nearly twice its previous diameter; the outline of the cell being very thin, but clear and distinct, and from one to four little bodies become developed in the centre (fig. 205 *a*). These have a dark outline, are of rather an irregular form, highly refractive, but do not appear to be soluble in ether.

Fig. 205.

Occasionally I have found pus cells in which the central contents were very dark, and slightly separated from the cell-wall (fig. 206). Upon treating these with acetic acid, the same reaction ensued (*a*). It appeared as if the acid caused the central mass to contract, probably after dissolving part of its constituents. The central bodies have been termed nuclei, but they cannot be looked upon in the same light as the nuclei of cells generally.

Fig. 206.

277. Microscopical Characters of the Pus Globule.—I must here offer a few very brief observations upon detecting the pus cell, and the inferences to be drawn from its presence.

When a small quantity of pus is placed between glasses, and examined with a power of about two hundred diameters, numerous granular cells larger than a blood globule, but with a circular outline and finely-tuberculated surface are noticed.

The serum in which these cells float usually contains a few free fat globules. The cells above referred to have been considered as characteristic of pus, and much trouble was taken in the earlier days of microscopical research to assign definite characters to them, by which they might be distinguished from the so-called mucous corpuscle, and other cells which they much resemble. Such a distinction, however, cannot be made, for, in the first place, cells may be obtained which present various stages, apparently intermediate between an ordinary epithelial cell and a pus globule; secondly, cells agreeing in their microscopical characters with the pus globule, are not unfrequently formed on the surface of a mucous membrane, without its functions being seriously impaired, and certainly without the occurrence of those preliminary changes which usually precede the formation of pus; and, thirdly, cells are found in the lymph, in the blood, in the lymphatic glands, in the serous fluid in the interior of certain cysts, and in many other situations, which in their size, form, and general appearance, so much resemble the globules found in true pus, that it is quite impossible to assign characters by which they may be distinguished. The figures of these cells, as they appeared before and after treatment by acetic acid, often could not be distinguished from the figures of pus cells, treated in a similar manner, given by the same authors.

Cases occur in which it appears almost useless to attempt to decide as to the presence or absence of pus, if only a few globules are to be found (nor do I think that if such were possible, it would be of any advantage), because no characters by which the globules can be distinguished individually have been laid down.

At the same time it must not be supposed that the diagnosis of pus is a matter of secondary importance; and all that is intended in introducing these observations is to impress upon the student the importance of not stating that pus has been found in any particular locality, or in any particular fluid, merely because a few cells having all the characters of a pus globule have been observed. To say that "pus had been found in the blood," or that "the casts of the uriniferous tubes con-

tained pus," would lead to a very different inference from that derived from the statement that "cells having all the characters of pus globules had been found in the blood," or that the "casts of the tubes contained cells resembling those of pus." The former will be true in extremely few cases; the latter in a vast number that fall under the observation of every practitioner. If, however, we find a considerable number of globules under the field of the microscope, of nearly uniform size, agreeing in general characters with the pus globule, and upon the addition of acetic acid exhibiting the characteristic reaction, we shall seldom be wrong in inferring that they are really pus cells.

In examining the blood in cases in which the white corpuscles are enormously increased in number, there can be no difficulty in deciding, since we have every reason to believe that pus globules could not possibly exist in the blood under the same circumstances.

CHAPTER XVI.

THE APPLICATION OF CHEMICAL ANALYSIS TO MICROSCOPICAL INVESTIGATION.—METHOD OF APPLYING TESTS.—EFFECTS OF REAGENTS UPON ANIMAL STRUCTURES.

278. Importance of Chemical Analysis in Microscopical Investigation.—By microscopical examination alone we are enabled very readily to distinguish with certainty the presence of exceedingly minute quantities of substances presenting definite crystalline forms, or exhibiting special anatomical characters. If the composition of a crystalline body has been once made out, by resorting simply to the microscope we are enabled by observing the form, even of a single crystal, to indicate its nature and properties. By the aid of the microscopical examinations of some of the secretions, we have seen that the course of pathological changes may be investigated, and morbid alterations of structure detected, which cannot be rendered appreciable to the senses by any other known methods of investigation.

By an acquaintance with the behaviour of certain substances with particular chemical reagents, and the application of this knowledge to microscopical investigation, we are often enabled to distinguish peculiarities of structure, to ascertain the chemical composition of minute quantities of matter, and to demonstrate clearly the existence of compounds in the animal frame with the greatest certainty, which would entirely escape our observation if we subjected them separately to the most careful chemical analysis, or to the most searching microscopical examination.

The application of chemical analysis to microscopical investigation, and the examination of crystalline forms in the microscope, is fast throwing a new light upon the physiological

changes which are constantly taking place in organized bodies in health, and the modifications which these changes undergo when influenced by circumstances interfering with or counteracting healthy actions; the consideration of which must especially interest practitioners, in the various forms of disease which are constantly being brought under notice.

The laboratory has already become a most necessary adjunct to the dissecting-room, the museum, the post-mortem room, and the clinical wards of our hospitals; and he who would wish to apply all the means at present at our disposal to unravel the mysteries of disease to aid him in forming a correct diagnosis, or to assist him in the investigation of those changes which occur in different organs, and are familiar to him in the post-mortem theatre, will do well to make medical and pathological chemistry, with microscopical examination, essential parts of his studies.

The works of Vogel, Schmidt, Scherer, Hœfle, and others, which have been published within the last ten or twelve years, have done much to advance this branch of investigation; while those of Golding Bird, Schwann, Robin and Verdeil, Lehmann, and Gorup-Besanez, and the excellent Atlas of plates by Dr. Funke, show the vast importance which the combined methods of chemical and microscopical investigation are very fast assuming.*

It is not within the compass of the present work to do more than refer to the general principle upon which such examinations are conducted, and to give examples of those processes

---

* "Anleitung zum Gebr. des Mikroskopes zur Zooch. Anal. u. zur Microscop. Chemisch. Untersuch," Dr. Julius Vogel, 1841.—"Chemische und Mikroskopische Untersuchungen zur Pathologie," Dr. J. J. Scherer, Heidelberg, 1843.—"Entwurf: einer Allg. Untersuchungsmethode der Säfte u. Excrete des Thierischen Organismus," Dr. Carl. Schmidt, 1846.—"Chemie und Mikroskop am Krankenbette," Dr. Hœfle, 1850.—"Physiological Chemistry," Dr. Lehmann, translated by Dr. Day, Cavendish Society, 1851.—"Atlas of Physiological Chemistry," Dr. Otto Funke, Cavendish Society, 1852.—"Traité de Chemie Anatomique et Physiologique," Robin et Verdeil.—"Urinary Deposits," Dr. Golding Bird, 1853.—"Manual of Zoo-chemical Analysis," Gorup-Besanez, translated by J. W. Slater.

which appear to be of the greatest importance to the student in medicine, and which he will be frequently called upon to perform.

As an instance of the great advantage of the application of a few simple tests to microscopical investigation, I may refer to the effects of ether upon fat globules, which are so commonly found in different tissues, and crystalline bodies composed of phosphate or carbonate of lime, which sometimes resemble them so nearly in refractive properties, in form, and in general appearance, as to have led to mistakes with reference to their nature. The application of a drop of ether has no effect whatever upon the latter, but instantly dissolves the former, so that by this very simple plan we are enabled at once to decide a very important question, and one which has led to much discussion, in consequence of the solubility of the globules in ether not having been ascertained in certain cases, which have been since considered as of a doubtful nature.

The detection of the presence of mere traces of urea, lithic acid, and other substances, by the application of reagents, and subsequent microscopical examination, will be referred to in Chapter XVII.

ON THE CHEMICAL AND MICROSCOPICAL EXAMINATION OF ANIMAL SOLIDS AND FLUIDS.

Preliminary Operations.—After having noted carefully the general characters which the substance exhibits, with reference to form, colour, size, weight, hardness, &c.; and fluidity, transparency, tenacity, &c., in the case of liquids; and after portions of solid textures, and the deposit from fluids have been subjected to microscopical examination, we may proceed to ascertain the reaction.

279. **Reaction.**—The reaction of any moist substance is ascertained by testing it with a piece of blue and reddened litmus or turmeric paper. If the substance be dry, or the reaction of a vapour is to be tested, the paper must be first moistened with a drop of distilled water. The blue paper is reddened by acids, while the red is rendered blue, and the

turmeric brown, by alkalies. As the change of turmeric is only visible when the alkaline reaction is very decided, it is not much employed in animal chemistry.

If the acid reaction is due to the presence of carbonic acid, the blue colour will be restored upon gently warming the paper over a lamp, upon a glass slide, or upon a warm plate.

An alkaline reaction may depend upon the presence of *volatile* or *fixed alkali*. The red colour is restored upon warming the paper which has been rendered blue by the presence of volatile alkali (ammonia or carbonate of ammonia), while it is not restored if the change is produced by the presence of a fixed alkali (potash, soda, or their carbonates, or an alkaline phosphate, &c.)

280. **Specific gravity—Solids.**—The specific gravity of animal solids may be taken in two ways.

First. By weighing in air, and afterwards in water, which is the process usually followed, and that which affords the most accurate results. The precautions necessary to be observed in carrying out this process will be found in "Gorup-Besanez' Zoo-chemical Analysis," "Bowman's Practical Chemistry," and other analytical works on chemistry.

Secondly. The specific gravity of solids may be obtained by placing small portions in certain solutions, the specific gravity of which has been previously ascertained by experiment: this latter method has been employed lately for ascertaining the specific gravity of the brain in different cases.*

The solutions are prepared in considerable quantities at a time, and kept in large bottles numbered according to the specific gravity.

Several glasses are nearly filled with the solutions from different bottles, and arranged in regular order. The piece of tissue is thrown into one, and, if it sinks, it must be placed in the fluid of the next higher specific gravity, and so on, until it neither sinks towards the bottom nor rises to the surface, when

---

* Dr. Bucknill "On the Specific Gravity of Cerebral Substance," Lancet, 1852.—Dr. Sankey in the "British and Foreign Medico-chirurgical Review," Jan. 1853, p. 40.

the specific gravity marked upon the bottle will correspond to that of the substance itself, since a solid will only displace an equal bulk of a solution which is of the same density as itself.

The soluble substances employed for making the solutions may be syrup, various salts, glycerine, and other compounds, which do not exert any chemical action upon the tissue, the specific gravity of which we wish to determine.

281. Specific Gravity—Liquids.

First. By the converse of the last operation, namely, by placing little glass bulbs, the specific gravity of which is marked upon them, into the solution, the density of which we wish to know, until one is found which neither sinks nor swims. This will indicate the specific gravity of the fluid. This method is not so correct, nor so easily applicable to general purposes as the two following.

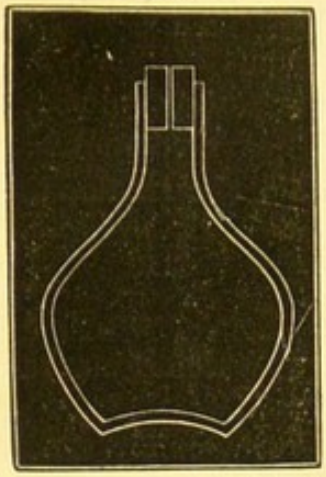
Fig. 207.

Secondly, by the hydrometer or urinometer. The number which is on a line with the surface of the fluid, when the instrument comes to rest, indicates its specific gravity. This method is tolerably correct, if the observer is careful to obtain the best instruments; but many which I have examined, indicated a specific gravity eight or ten degrees from the truth. The hydrometer or urinometer should always be tested by the specific gravity bottle. It may be remarked that the degrees marked upon the stem should gradually diminish in length, from above, downwards. If they are equal, as in the figure, the instrument may at once be pronounced as incorrect, without resorting to an experiment. Fig. 207 is an exact copy of one of the *imperfect* instruments commonly sold.

Thirdly, by the specific gravity bottle, which consists of a small glass flask. When quite dry it is accurately counterpoised in a delicate balance, and filled up to a certain point with distilled water, and weighed. The distilled water is then poured

out, and it is filled up to the same point exactly with the liquid to be tested, and again weighed. The specific gravity is then readily calculated from these data.

Fig. 208.



Some bottles are made to hold exactly one thousand or five hundred grains of distilled water, and are provided with a perforated stopper, through which the excess of fluid escapes, after the bottle has been filled, care being taken not to include air-bubbles (fig. 208). The outside of the bottle is wiped dry, and the whole weighed. The weight shows the specific gravity at once, upon deducting the weight of the thousand-grain bottle; or, when a five hundred-grain bottle is employed, the amount only requires to be doubled.

282. Evaporation and Drying.—The evaporation of animal fluids, and the desiccation of animal solids, must always be conducted over a water-bath, otherwise there is great danger of decomposition occurring. For operations upon small quantities, the water-bath, described in § 47, will suffice, or the cans of the injecting apparatus (fig. 104) may be removed, and basins placed over the holes.

In endeavouring to obtain crystals of organic substances, it is always advantageous, to evaporate the solution over the surface of sulphuric acid under a bell-jar, or, what is better still, in vacuo. In some instances, the evaporation may be conducted by simply exposing the liquid placed in a basin or watch-glass, and covered lightly with paper, to the air; or, where very slow evaporation is necessary, the watch-glass may be covered over with a bell-glass.

When quantitative analysis is to be performed, much greater care must be observed in the process of drying. For a description of the apparatus required and the cautions to be observed, see "Gorup-Besanez' Zoo-chemical Analysis," &c.

283. Incineration.—By incinerating a small portion of any organic substance, upon a piece of platinum foil, we are enabled to ascertain whether it contains inorganic salts, or con-

sists entirely of organic matter, in which case the substance leaves only a black residue, which burns off entirely after a short time.

The destruction of organic matter, by exposing the substance to a red heat, upon platinum foil, or in a platinum capsule, has been already adverted to. In order to obtain the inorganic constituents perfectly free from carbon, it is sometimes necessary to keep the mass, for a considerable time, at a dull red heat. If the temperature be too high, the process is often much retarded, in consequence of the fusion of some of the salts, as the phosphates and chlorides, and the inclusion of small masses of carbon, which are thus protected from the action of the atmosphere.

The platinum basin or foil may be supported over the lamp upon a piece of wire, bent in the form of a triangle, or upon one of the small rings attached to the spirit-lamp (fig. 147). It may be removed from the lamp with the aid of an old pair of forceps.

284. Apparatus required.—The chemical apparatus which is necessary in the course of microscopical investigation is very simple, and the greater number of instruments have already been referred to. The following are among the most important pieces of apparatus :—

A few conical glasses of different sizes (§ 217). Test-tubes of various sizes (§ 215) arranged on a stand. Spirit-lamps, with various supports (§§ 43–45), or, where gas is laid on, the gas-lamp (fig. 81). Small porcelain basins, watch-glasses ; a simple water-bath (§ 47), or the injecting-can (§ 116), may be used, if several evaporations are to be conducted at once. A small platinum capsule, a strip of platinum foil, a blowpipe, pipettes (§ 216), and glass stirring-rods.

REAGENTS.

The reagents necessary are not very numerous ;—they should be perfectly pure. Of the greater number only very little is required; but of alcohol, ether, and one or two others, it is necessary to have a moderate quantity.

The reagents should be kept in stoppered bottles, of about the capacity of two ounces.

285. **Alcohol.**—Alcohol of different strengths will be required for the purpose of dissolving certain animal substances, and for separating them from other constituents, which are insoluble in this reagent.

Alcohol should always be diluted with distilled water, and it is better to prepare a considerable quantity at a time. It is convenient to have two or three bottles which will hold about two quarts each. The strength of each specimen should be written upon a label and attached to the bottle.

The importance of alcohol, as a preservative solution, has been already referred to (§ 94).

286. **Ether.** — An ounce or two of ether will be quite sufficient for microscopical purposes. It should be kept in a stoppered bottle, provided with a glass cap, to prevent loss by evaporation. A little should also be kept in one of the small glass bottles with capillary orifices (§ 300), for the convenience of applying to cells containing highly refracting globules, resembling oil, &c., under the microscope.

287. **Nitric Acid** should be kept of two different degrees of concentration: one the strongest that can be procured, and another containing about 20 per cent. of the strong acid. This last is the acid most used by the microscopist, especially in separating muscular fibre cells. It is prepared by mixing one part of the strong commercial acid with five parts of water.

288. **Sulphuric Acid** is sometimes required undiluted, but a small bottle of diluted acid (one of acid to five of water) should also be at hand. The pure colourless acid should always be procured;—it is to be purchased for about 1*s.* 6*d.* a pound, but only very small quantities are required.

289. **Hydrochloric Acid** may be obtained perfectly colourless. It may be kept in the pure state and diluted as required.

290. **Acetic Acid.**—Two specimens of acetic acid will be found convenient. One, a solution of the strongest acid which can be procured; the other, containing about 20 per cent. This is prepared by dissolving one part of the strongest liquid acid, or of the pure glacial acetic acid, in five of water.

291. **Chromic Acid** is usually required very dilute. For the purpose of hardening tissues, a watery solution of a straw-colour will be found strong enough. It is easily prepared by dissolving a little of the crystallized chromic acid in distilled water. For the method of preparing the crystallized acid, see § 98.

292. **Solution of Potash** should be kept of two or three different strengths. One, the strongest which can be procured; another, made by mixing one part of the strong with three or four of water; and a solution consisting of one part of liquor potassæ to eight or ten of water will be found of a useful strength for the examination of many preparations.

293. **Solution of Soda** is generally required very dilute. It may be made by mixing one part of the strong solution of the shops with five or six of water; but this, for many purposes, will require to be still further diluted. Or, about twenty-five grains of the fused soda may be dissolved in an ounce of distilled water.

294. **Ammonia.**—Solution of ammonia, made by mixing one part of the strongest liquor ammoniæ with three of water will be found sufficiently strong for all the purposes for which this reagent will be required.

295. **Nitrate of Barytes.**—A cold saturated solution of the salt forms a test solution of convenient strength. It should be filtered before use.

A solution of nitrate of barytes is employed as a test for sulphuric and phosphoric acids. The precipitated sulphate of baryta being insoluble both in acids and alkalies; while the phosphate of baryta is readily soluble in acids, but insoluble in ammonia.

296. **Nitrate of Silver.**—A solution of nitrate of silver is prepared by dissolving one hundred and twenty grains of the crystallized nitrate in two ounces of distilled water, and filtering if necessary.

Nitrate of silver is employed as a test for chlorides and phosphates. The *white* precipitate of chloride of silver is soluble in ammonia, but insoluble in nitric acid. The *yellow* precipitate of tribasic phosphate of silver is soluble in excess of ammonia, as well as in excess of nitric acid.

297. **Oxalate of Ammonia.**—Some crystals may be dissolved in distilled water, and, after allowing time for the solution to become saturated, it may be filtered.

Oxalate of ammonia is used as a test for salts of lime. Oxalate of lime is insoluble in alkalies and in acetic acid, but soluble in the strong mineral acids. In testing an insoluble deposit for lime, it may be dissolved in nitric acid and excess of ammonia added; the flocculent precipitate is readily dissolved by excess of acetic acid, and to this solution the oxalate of ammonia may be added. The precipitation of oxalate of lime is favoured by the application of heat. Many deposits of phosphate are with great difficulty soluble in acetic acid, hence the necessity of first adding nitric acid, as above directed.

298. **Iodine Solutions.**—An aqueous solution of iodine is easily prepared, by dissolving a few grains of iodine in some distilled water, until it acquires a brownish-yellow colour. A solution of iodine is sometimes useful for colouring certain substances which are so transparent as to be scarcely distinguishable upon microscopical examination.

A darker solution may be obtained by employing a solution of iodide of potassium to dissolve the iodine (one grain of iodine and three grains of iodide of potassium, to one ounce of distilled water). For testing bodies suspected to consist of starch, the following solution is recommended by Professor Schultz. Zinc is dissolved in hydrochloric acid;—the solution is permitted to evaporate in contact with metallic zinc until it attains the thickness of a syrup; and the syrup is then saturated with iodide of potassium. The iodine is next added, and the solution, if necessary, is diluted with water.*

It will be found convenient to keep small quantities of those solutions, in most frequent use, in the small capillary tubes or bulbs (§ 300). A small box containing twelve bulbs will be quite sufficient for all ordinary purposes. For the examination of the urine, not more than six or seven will be necessary.

---

* "The Microscope, in its special Application to Vegetable Anatomy and Physiology," Schacht, translated by Currey.

METHOD OF APPLYING TESTS TO SUBSTANCES INTENDED FOR MICROSCOPICAL EXAMINATION.

299. Tests kept in Glass Bottles.—The matter to be tested may be placed upon a glass slide, and, if necessary, a drop of water added, to moisten or dissolve it, as the case may be.

In these operations we usually require only a small drop of a solution, and it will be found most convenient, in applying it to the object, to take a drop from the bottle by dipping a stirring-rod into it, and withdrawing it immediately. Enough will be found adhering to the stirring-rod for the purpose required. The rod should not be dipped in a second time, without being first well washed in water,—for if this be not scrupulously attended to, there is great danger of conveying some of the substance intended for examination into the test bottle, in which case the whole contents are spoiled.

Without great care in all our manipulations, we shall be in danger of removing a portion of one substance from a glass slide and carrying it to a deposit which is subsequently examined;—a result which might lead to great inconvenience. Claws of echinococci, and other minute bodies, in themselves highly characteristic, may thus be transported, and find their way into deposits in which we should not expect their presence; and from such an accident we might be led to infer, very erroneously, the existence of hydatids when the presence of the claws of the echinococci really resulted from accident. Without the greatest attention to cleanliness, the microscopical observer will be constantly led into error, and thereby bring discredit upon himself and upon the science.

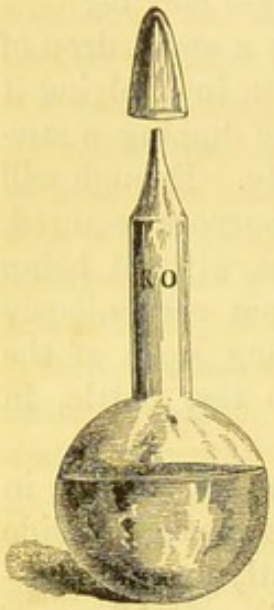
Nothing is more common than to find a specimen which we are examining in the microscope covered with a vast number of starch granules, which have been introduced from without. Usually they are derived from the squares of thin glass which are commonly kept in a little starch powder to prevent fracture.

Accidents of this kind can always be avoided, by not allowing the drop of the reagent to touch the deposit until the rod has been removed. This can be effected by placing the drop near the substance intended for examination, and then allowing it to come in contact with it, either by inclining the

glass slide, or by leading it with a glass rod or other small instrument to the matter to be tested.

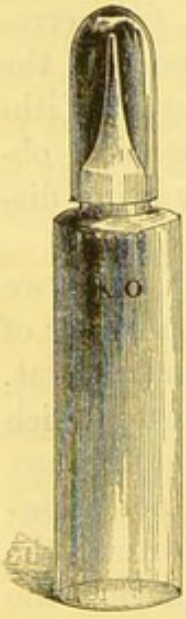
300. **Tests kept in Glass Bulbs with Capillary orifices.**—By far the most convenient method with which I am acquainted, of applying chemical reagents to minute quantities of matter, consists in allowing a drop to issue from a small glass vessel, having a capillary orifice, by which means a quantity even much less than a single drop can be readily obtained, and there is no chance of any portion of the preparation being introduced into the test solution.

Fig. 209.



With this view a small bulb, about an inch in diameter, was blown at one end of a piece of glass tube, the other was drawn out to a moderately fine capillary point, and a small cap, made either of glass or gutta percha, was adapted to the end (fig. 209). These bulbs were easily filled, by expanding the air within them, by the heat of a spirit-lamp, and then inverting them in a small vessel containing the solution which was to be introduced. As the bulb cooled, the liquid rushed into it, to supply the place of the previously expanded air. A small bubble of air should, however, be retained in the bulb, by the expansion of which some of the fluid can afterwards be expelled. The bulbs containing the strong acids and alkalies should be furnished with glass caps, but gutta percha will be sufficient for the other tests. When it is required to expel a drop of the solution, the bulb is taken in the hand, and the air in the interior being expanded by the warmth, a small quantity of the solution is forced out.

Fig. 210.



Mr. Highley, of Fleet-street, has had some small bottles made of the form shown in the accompanying figure (fig. 210). These are all capped with glass, and, as the bottom is flat, they stand very readily. They are fitted up in small cases, and will be found

exceedingly convenient to the microscopical observer. It is better to have the cap made of a conical shape, corresponding to that of the end of the bottle, otherwise, a little of the fluid is liable to collect between the cap and the neck, which runs down the sides when the cap is removed.

301. **Application of the Reagent to minute quantities of matter.**—With the aid of the bulbs just referred to, the most minute traces of different substances may be readily detected. The solution of the substance, consisting perhaps of only one drop, is placed upon a glass slide. This drop may be very readily divided into four or five smaller drops, if necessary, to each of which a separate test may be applied. For instance, suppose we have a minute quantity of the ash of an animal tissue, or of the solid residue of an animal fluid, to examine, and we wish to ascertain if it contains carbonates, sulphates, chlorides, and phosphates, and whether phosphate of lime and magnesia are present, we may proceed as follows :—the portion of ash, which may, perhaps, be half the size of a pin's head, or even less, is removed from the platinum foil, upon which it has been ignited in order to remove organic matter, and placed upon a glass slide. It is moistened with the smallest quantity of water, and then treated with a minute drop of nitric acid. If effervescence takes place, a carbonate is present. The acid solution is then divided into three portions, with the aid of a small stirring-rod, and the solutions tested as follows :—

1st portion.—If a drop of a solution of nitrate of silver gives a cloudy precipitate, chlorides are present.

2nd portion.—If nitrate of barytes produces a white precipitate, sulphates are present. Upon the addition of excess of ammonia, the precipitate produced by nitrate of barytes will be increased if phosphates exist in the solution. The precipitate of phosphate of baryta is flocculent, and readily distinguished from that of sulphate of baryta (which is dense and granular), by its solubility in acids.

3rd portion.—If lime or magnesia are present, in the form of phosphate, a precipitate will be produced upon adding excess of ammonia to the nitric acid solution. The mixture may be stirred a little, with a fine piece of glass or platinum wire, and

then allowed to stand for some time. A piece of thin glass is now applied, and the precipitate subjected to microscopical examination. Phosphate of lime occurs as a granular amorphous sediment, while the ammoniaco-magnesian, or triple phosphate, is usually found crystallized in a beautiful stellar form, or as minute prismatic crystals.

302. Testing for Carbonates. — As carbonates are often present in very minute quantity in the ash of organic substances, a slight modification of the plan above given may be pursued, and, in this way, the smallest traces may be detected. If only a few bubbles of carbonic acid are given off upon the application of the acid to the substance; or if, in consequence of the solubility of the carbonate present, they are evolved very rapidly, they frequently elude observation.

In testing for minute traces of carbonates we may proceed as follows:—The portion of ash, deposit, or tissue (as the case may be), is placed upon a glass slide, and lightly covered with a piece of thin glass. A minute drop of nitric or acetic acid, not too strong, is then allowed to escape from one of the bulbs. This is drawn by capillary attraction between the glasses, and soon comes in contact with the substance to be tested. Any bubbles which may be given off are thus confined, and they may generally be seen clearly enough. In some instances, however, advantage is derived from subjecting the specimen to microscopical examination, when the formation of the gas can be seen; and the bubbles set free cannot possibly be mistaken for air-bubbles, which had been included in the interstices of the tissue previously, and afterwards expelled upon the addition of the fluid, because they may be seen gradually to increase in size and number as the action of the acid continues. In testing for carbonates, the possibility of this occurrence, however, must always be borne in mind, and the fallacy carefully guarded against.

EFFECTS OF REAGENTS UPON ANIMAL STRUCTURES.

303. Effects of Acids.—The effects of the application of cold strong acids to animal textures is very variable; in some instances the tissue is completely destroyed, while in others

scarcely any effect seems to be produced. The mineral acids generally coagulate albuminous tissues, and render their microscopical characters confused and indistinct. Tribasic phosphoric acid, however, is an exception to this rule. Acetic acid dissolves many of the substances allied to albumen.

The appearance of some structures is scarcely altered by the application of a strong acid; for instance, the blood corpuscles exhibit their usual form and general character for some time after the addition of strong nitric acid, and the cells of the epidermis and nail, although turned of a yellow colour, are not destroyed; the latter are separated somewhat from each other, but their outline may often be seen beautifully distinct upon microscopical examination. On most of the mineral constituents of the body, insoluble in water, the acids act as direct solvents.

304. Acetic Acid.—Acetic acid is one of the most useful reagents to the microscopical observer. It has the property of rendering the cell-wall very clear, while the nucleus is often rendered darker and more distinct. In many instances the action of the acid upon the cell-wall probably arises from endosmosis; the cell becomes much larger, and the wall more pulpy and thicker, and approaches more nearly in density and refracting power to that of the solution in which it is immersed. In numerous instances, by adding a saline solution to cells which have been previously rendered transparent by acetic acid, they again contract, and the outline becomes distinct. In some cases, however, the cell-wall is actually dissolved by the acid, and its contents set free. Acetic acid will be required of various strengths, the most useful proportion being one part of the strong acid to three or five of water. Acetic acid is very frequently used to make epithelial structures transparent, in order that the arrangement of the minute vessels and nerves in papillæ, &c., may be demonstrated, as in the case of the tongue, skin, &c. Sections of preparations which have been hardened by maceration in alcohol, often require boiling slightly in acetic acid before they can be rendered transparent. The action of acetic acid on white fibrous tissue is very characteristic, as it converts it into a transparent jelly-like mass, in which a few

nuclei are visible. Upon the yellow element, on the other hand, this reagent exerts no action whatever.

The action of acetic acid upon pus-globules has been already adverted to (§ 276).

Acetic acid may also be employed for testing crystalline bodies as phosphates and carbonates. It distinguishes phosphate or carbonate of lime from oxalate of lime (all of which are insoluble in water), by dissolving the two former, while it does not affect the latter even if boiled with it.

The action of acetic acid upon any particular tissue, upon any form of cells, fibres, &c., that are subjected to examination, should always be specially noted.

305. **Dilute Nitric Acid** is much employed in microscopical research.—An acid composed of one part of acid to two or three of water forms a good solution for hardening some structures, previous to cutting thin sections. The thin sections may sometimes be rendered very transparent by being treated afterwards with dilute caustic soda. For demonstrating fibre-cells in organic muscle, nitric acid is a valuable reagent. For this purpose the solution should contain about twenty per cent. of strong acid, and the muscular fibre should be allowed to macerate in it for some time, when small pieces may be removed with scissors, and after being carefully torn up with fine needles, subjected to examination.

By boiling animal tissues in strong nitric acid, they become destroyed, while any siliceous constituents remain behind. In this manner, the siliceous skeletons of the *Diatomaceæ* may be separated from any organic matter with which they may be combined. This is one of the processes employed for obtaining these beautiful objects from guano.

306. **Sulphuric Acid.**—**Hydrochloric Acid.**—The pure concentrated acids only should be used for microscopical investigation. They may be obtained at most of the operative chemists.

Concentrated sulphuric acid causes epidermic structures to swell up very much, and the cells to separate from each other so as to be readily isolated. Boiling acid completely dissolves them. In the examination of hair, strong sulphuric acid will be found to render the outline of the cells very distinct.

Hydrochloric acid is usually employed for dissolving out the mineral constituents of certain tissues, such as bone or teeth. As a rule, it is better to use dilute acid (one of acid to three or four of water), in which case, however, a longer time must of course be allowed, than when the acid is concentrated.

307. **Effects of Alkalies.**—The action of alkalies, even when cold in a very dilute state, is to dissolve most animal textures. Cell-membranes are frequently almost instantly dissolved, while the nucleus appears to be acted upon with greater difficulty.

Alkalies are also employed for dissolving certain crystalline substances which are occasionally found in animal tissues, such, for instance, as deposits of alkaline lithates, which are not unfrequently met with in the form of considerable deposits in the tissues of gouty persons.

308. **Potash and Soda.**—The action of potash and soda upon animal structures is very similar. Both very readily dissolve substances of an albuminous nature, but the effect of soda is more gradual, and it has been found that for most purposes in microscopical research, this reagent possesses advantages over potash.

These reagents are usually employed to dissolve the layer of epithelium covering mucous membranes, in order to examine the arrangement of the structures beneath the basement membrane; and in investigating the termination of the nerves and vessels in papillæ and other structures, they, especially the latter, are very valuable.

Dilute solution of caustic soda is also a valuable reagent in pursuing investigations on the nerves and nervous centres.

For the purposes above mentioned, the alkalies should be diluted with water. One part of solution of caustic soda to eight or ten parts of water, will be found a convenient strength. The changes are expedited by the application of heat, which, however, must not be too great, for fear of complete solution taking place. Where the structures are hard and dry, the action is much facilitated by warming the substance with the reagent in an ordinary test tube,—a plan which is much recommended by Kölliker.

Carbonates of Potash and Soda.—Some animal textures become

hardened by prolonged maceration in carbonate of potash, but this plan does not appear to be so generally useful as others previously indicated. Epidermic structures are not much altered by these salts. Gurlt recommends skin to be hardened in solution of carbonate of potash for the examination of the sweat ducts.

309. Effects of Alcohol and other Substances in hardening Animal Structures.—There are several reagents which render soft tissues quite hard and firm, so that very thin sections may be cut off easily with a sharp knife; of these alcohol is most commonly employed. Alcohol permeates animal tissues readily, and coagulates the albuminous structures very firmly: it may be used of different strengths according to circumstances.

In hardening structures with alcohol, it is better to add weak spirit first, and then to replace this by stronger, increasing the strength each time the spirit is changed. By proceeding in this way, the tissue contracts very gradually, and its form and general characters do not become so much changed as when it is plunged at once into strong alcohol.

A weak solution of chromic acid hardens parts of the nervous system and other tissues very effectually, so that thin sections may be readily removed from structures hardened in this manner.

Solutions of soluble salts are also used for hardening animal textures, especially Goadby's solution, and solutions of common salt, or nitrate of potash. Animal textures may also be hardened in wood naphtha.

CHAPTER XVII.

OF OBTAINING CRYSTALLINE SUBSTANCES FROM THE FLUIDS AND TEXTURES OF THE ANIMAL BODY, AND OF THEIR MICROSCOPICAL EXAMINATION.

310. Formation of Crystals in Animal Fluids.—Some crystalline bodies are deposited from their solution in animal fluids by simple evaporation; others less soluble may be deposited by allowing the fluid to stand still for a short time, when certain changes occur in some of its constituents, which lead to the precipitation of bodies in a crystalline form, such, for instance, as lithic acid, or crystals of triple phosphate. In other cases it becomes necessary to add some reagent before the crystals are thrown down, while not unfrequently a long and often complicated chemical analysis is required, in order to isolate some of the substances which were previously held in solution, and obtain them in a crystalline form. The addition of water in some cases causes the most rapid crystallization, especially when the crystallizable material is contained in a cell, as when water is added to blood, in order to obtain blood crystals. Instead of water, in other instances, it becomes necessary to add alcohol, in which fluid the crystals may be much less soluble than in water.

311. Influence of other constituents upon the Crystallization.—In many instances it is exceedingly difficult to separate some crystalline bodies from other constituents of the animal substance, the presence of which much increases their solubility and prevents their crystallization. The extractive matters exert this influence in a marked degree, and it is only of late years that several new bodies of definite chemical composition have been isolated. Creatin and creatinine may be instanced amongst the number, for these were not very long ago included

under the indefinite term 'extractives,' and it is very probable that as the analytical methods at our disposal become improved, many new crystalline bodies will be isolated from the extractive matters. A very small quantity of extractive matter entirely prevents the crystallization of urea, while the presence of chloride of sodium favours the separation of the urea, by forming with it a compound which readily crystallizes in large octohedral crystals even in the presence of extractive matter. The existence of carbonic acid in excess may cause carbonate of lime, triple phosphate, and other salts, to be held in solution. Excess of alkali prevents the precipitation of lithic acid, and excess of acid that of phosphate of lime. Fatty matters dissolve cholesterine.

Some crystalline substances, which are soluble at the temperature of the body, crystallize when the solutions containing them are cooled 30 or 40 degrees. The effect of dilution upon retaining crystals in solution need scarcely be alluded to.

Hence, before the presence of many substances can be detected by microscopic examination, certain chemical operations are required in order to separate them from their combinations in the animal body, or for the removal of other substances which interfere with their crystallization.

The method of detecting some of the most important of these crystalline substances will be given, but as it is not possible to do more than introduce a few of the processes in this place, the reader who wishes to pursue these beautiful investigations is referred to the works in the note.*

312. Separation of Crystals from Animal Substances.—From what was stated in the last paragraph, it follows that in many instances this is a matter of some difficulty. Not unfrequently, if the crystals are not soon separated from the fluid in which they were formed, they again undergo solution or become decomposed. If the crystals are not very soluble, the super-

---

* "Lehmann's Physiological Chemistry," translated for the Cavendish Society; Gorup-Besanez' "Zoo-chemical Analysis," translated by Slater; Bowman's "Medical Chemistry." Also to the excellent "Lehrbuch der Zoochemic," by Heintz, which however is only published in German.

natant fluid may be poured off, and the crystalline deposit washed with ice-cold water, and subsequently dried on filtering paper over sulphuric acid without the application of heat.

If the crystals will not bear the application of water, as much of the fluid as possible must be poured off, and the remainder absorbed with bibulous paper, or by placing them upon a porous tile, and exposure over sulphuric acid in vacuo. In many instances we are enabled to wash the crystals with water, holding a little acid or alkali, or some alkaline salt, in solution, or with alcohol, ether, or some other fluid in which we know them to be quite insoluble.

In cases in which crystals insoluble in water are deposited in animal solids, they may be separated by agitation in this fluid, when, being heavier, they subside to the bottom, and the lighter animal matter may be partly removed by forceps, and partly by being poured off with the supernatant fluid; or it may be separated by straining while the crystals are washed through muslin in the manner described in § 220.

313. Examination of Crystals under the Microscope.—Some crystals which have been entirely separated from the fluid in which they were originally deposited may be examined in the dry way, in water, or other fluid in which they are known to be insoluble, or in Canada balsam; but, as a general rule, it is necessary to examine the crystals as they lie in some of the fluid in which they have been formed. When they have been obtained by allowing a concentrated solution to cool, some of the thick solution must be removed with the crystals, placed upon a glass slide, or in a thin glass cell, covered with a piece of thin glass, and examined in the usual way,—first using a low power (an inch), and afterwards a higher power (a quarter), because, although some of the crystals are of a large size, others amongst them, the form of which is very perfect, are often exceedingly minute. The crystals and mother-liquor should not be exposed to the air previous to examination, because, in many instances, water is absorbed, and partial solution takes place.

In order to accustom himself to the necessary manipulation required in the process, the student may evaporate a

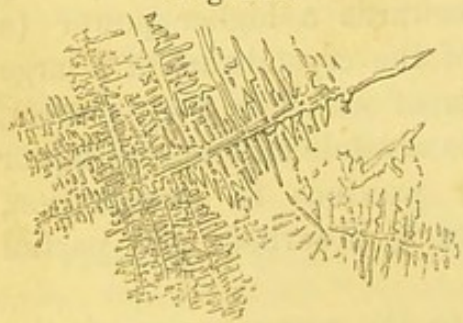
solution of common salt upon a glass slide, and when it has become sufficiently concentrated, he may cover it with a small piece of thin glass, and allow it to cool. When cold it may be subjected to microscopical examination, and beautiful cubes of chloride of sodium will be observed (fig. 211). Crystals of several salts may be made in the same simple manner, and form very instructive specimens. Phosphate of soda, phosphates of soda and ammonia, sulphates of potash and soda, muriate of ammonia, and a variety of other salts, can be readily obtained in microscopic crystals in this manner.

Fig. 211.

Different faces of the crystal, as it lies in the liquid, may be brought into view by slightly moving the thin glass cover with a fine-pointed instrument, such as a needle, while the preparation is in the field of the microscope. With a little practice, crystals may in this manner be made to rotate in the mother-liquor. Crystals which are precipitated by the addition of some reagent, such as nitrate of urea by nitric acid, must be examined in a little of the solution. The addition of water would, in many instances, destroy them immediately.

The influence of the crystals upon polarized light (§§ 29, 30) should be examined, and in cases in which the nature of the crystal has not been ascertained, its angles should be carefully measured (§ 38), and accurate drawings made. Their behaviour with chemical reagents is next to be ascertained, and their solubility in water, alcohol, and other fluids must be noted. For these experiments different portions must be taken and separately tested in the manner referred to in §§ 300, 301.

Fig. 212.



A drop of the solution should also be evaporated rapidly nearly to dryness, and allowed to crystallize upon the slide without being covered over, when the substance will often be found to assume a variety of beautiful forms, such as croslets, dendritic expansions, &c., which vary

according to the rapidity with which the evaporation has been conducted, and other circumstances (fig. 212).

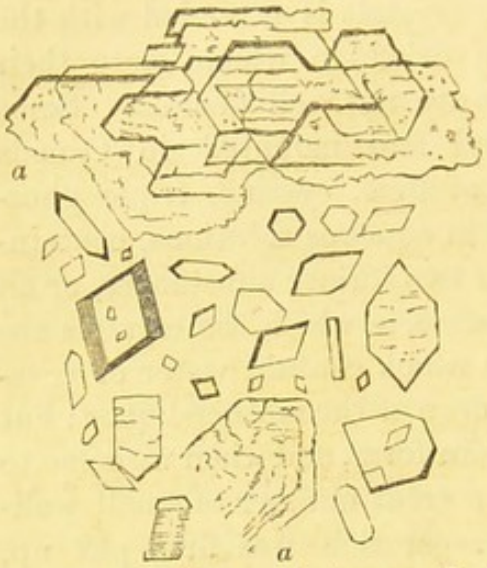
314. Preservation of Crystals as permanent objects.—The preservation of the more soluble crystals is attended with the greatest difficulty, except when dried, in which state their characters under the microscope are not well defined. Some crystals may, however, be dried and mounted in Canada balsam; others, such as oxalate of lime, cystine, triple phosphate, &c., can be well preserved in aqueous solutions, containing a little acid in the case of the two former substances, or an ammoniacal salt, in the latter instance, in which the crystals are known to be insoluble. Crystals which contain water of crystallization must be preserved in a drop of the mother-liquor; but in many instances they alter much in form, and when we come to examine them, instead of finding a great number of small well-formed crystals, as when the preparation was first put up, nothing remains but one or two large ill-shaped ones. The concentrated mother-liquor often acts upon the cement with which the glass cover is fixed on the cell, and very soon air enters, and the preparation is destroyed.

A preparation of nitrate of urea in my possession has kept well for a considerable time, in a very thin cell, containing only just sufficient of the solution to preserve the form of the crystals. This cell is made of Brunswick black. Crystals of chloride of sodium appear to keep pretty well in their mother-liquor, and the same will be found to be the case with a great number of substances. The more soluble crystals of an organic nature can seldom be preserved unless they are perfectly pure.

315. Urea.—Traces of urea may always be detected by observing the crystalline characters of the nitrate of urea in the microscope. Upon adding a drop of nitric acid to a drop of cold concentrated urine, placed upon a glass slide, a crystalline precipitate of nitrate of urea will immediately take place. Upon covering this with a piece of thin glass, and subjecting it to microscopical examination, the characteristic rhomboidal plates will be observed. Fig. 213 represents the appearance of nitrate of urea examined with a quarter of an inch object-glass. At *a*

are shown some crystals of the impure nitrate, as obtained from urine; the other crystals in the figure were formed by adding some nitric acid to a solution of pure urea.

Fig. 213.



Another drop of the concentrated urine may be treated with a strong solution of oxalic acid, when we shall obtain crystals of oxalate of urea, the form of which is represented in fig. 214, under a power of 200 diameters.

When mere traces are supposed to exist in animal fluids or solids, we must proceed to separate the urea from albuminous or other substances, before the addition of the nitric acid.

If the urea exist in an albuminous solution (serum of blood, or in a dropsical fluid), we must remove the albumen by boiling with a few drops of acetic acid, and subsequent filtration. The filtered solution is to be evaporated to dryness over a water-bath, and the dry residue treated with cold alcohol. Much chloride of sodium separates from the alcoholic solution, as it is evaporated. If to a drop of the cold mother-liquor a drop of nitric acid be added, as above described, crystals of nitrate of urea will be formed, if urea was present in the original solution. In all cases, the fluid suspected to contain urea must be operated upon when quite fresh, as this substance readily becomes decomposed into carbonate of ammonia.

Fig. 214.

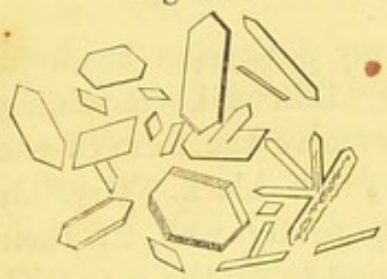
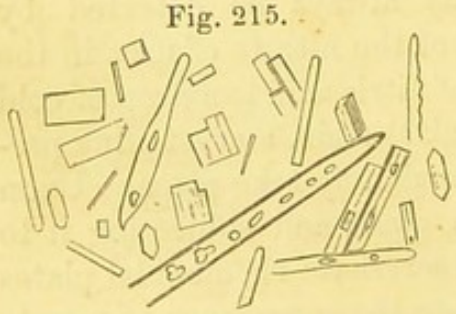


Fig. 215.



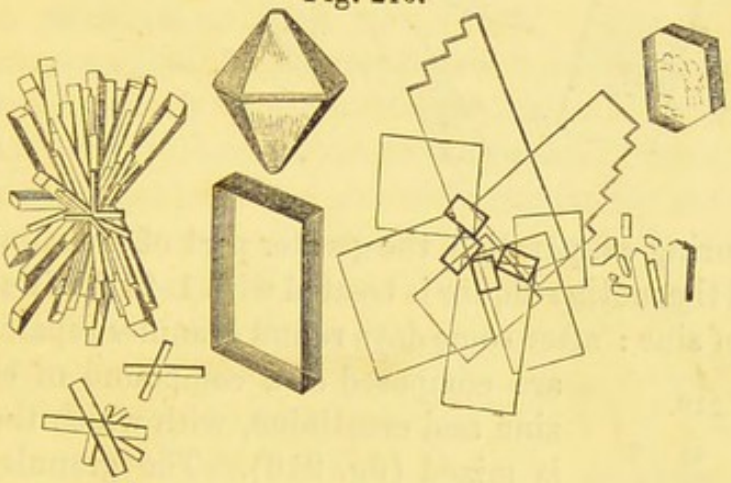
In examining solid tissues for the presence of urea, the fresh tissue may be broken up,

extracted with several portions of hot water, the liquid boiled with a few drops of acetic acid, and the clear solution filtered from coagulated matters and evaporated, when the residue may be treated with alcohol, as just now described.

Crystals of pure urea, obtained by decomposing a solution of the oxalate of urea with chalk, and carefully evaporating the filtered liquid, are shown in fig. 215. The cavities represented in many of the crystals are filled with air.

316. Creatine—Creatinine.—Creatine exists only in very small quantity in muscular fibre. According to Dr. Gregory, it is most readily prepared from the flesh of the cod-fish; from 25lbs. of which, in one experiment, he obtained 164 grains of creatine. The flesh is to be chopped in small pieces, and well kneaded with water. After all the fluid has been expressed by

Fig. 216.

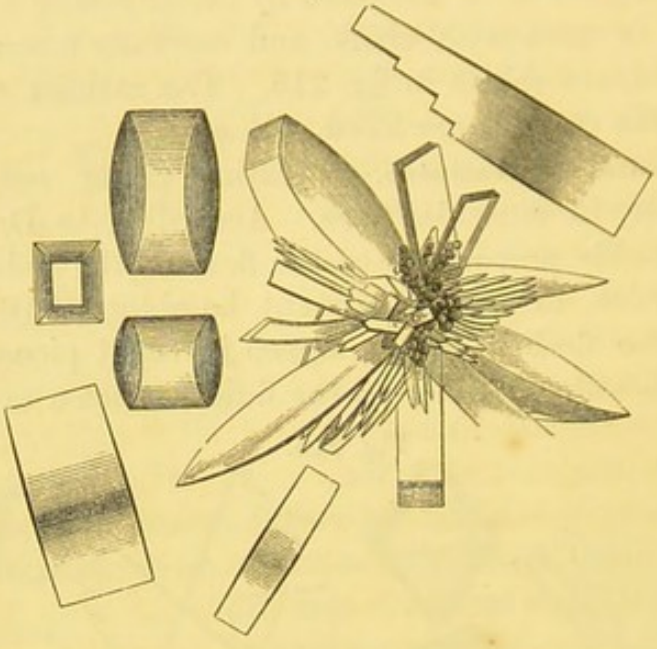


powerful pressure, it is very carefully raised to the boiling-point, and the coagulated matter removed by filtration. The phosphatic salts are precipitated by caustic baryta. The solution must be again filtered, and evaporated at a gentle heat (130°–140°) to about one-twentieth of its volume, or to the consistence of syrup; any scum which forms being, from time to time, removed from the surface. This concentrated solution may then be set aside. On cooling it forms a thin jelly, and, after standing for some time, crystals of creatine are deposited.

Crystals of creatine are represented in fig. 216, and those of creatinine in fig. 217, which have been copied from M. Robin's Atlas (see 272).

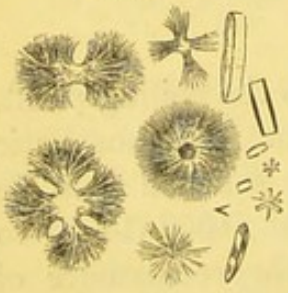
Liebig obtains creatine and creatinine from urine, by evaporating the fluid, after the precipitate produced by the addition of lime-water and chloride of calcium has been separated by filtra-

Fig. 217.



tion. During evaporation, the greater part of the salts are deposited, and the mother-liquor is treated with 1–24th of its weight of chloride of zinc : after some days round granules separate ; these are composed of a compound of chloride of zinc and creatinine, with which the creatine is mixed (fig. 218). The granules are dissolved in boiling water, and treated with hydrated oxide of lead, until the reaction becomes alkaline. The fluid is next filtered, decolorized with animal charcoal, and evaporated to dryness. The residue consists of creatine and creatinine, the latter of which may be removed by boiling alcohol, in which creatine is almost insoluble.

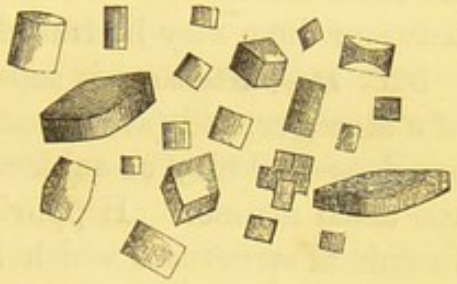
Fig. 218.



317. Uric or Lithic Acid.—The presence of lithic acid, in a crystalline form, can be readily detected in animal fluids and solids, by microscopical examination of the crystals, even although the most minute traces may be present.

In order to ascertain if an amorphous or other deposit contain lithic acid, or a lithate, we must treat it with a few drops of potash, which will dissolve any of the acid that may be present. This alkaline solution is to be decomposed with excess of acetic acid; and, after it has been allowed to stand for some hours, to allow time for the separation of the crystals of uric acid, any deposit that may have formed is to be subjected to microscopical examination. The microscopic crystals obtained, in this manner, are usually in the form of rhombic tablets (figs. 219, 220), and not unfrequently assume that of six-sided plates.

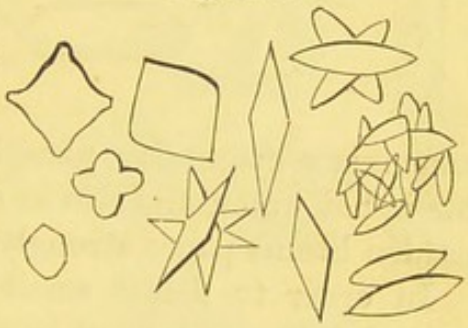
Fig. 219.



Uric acid is soluble in alkaline fluids, and is usually present in serum, in combination with an alkali; hence we shall be able to detect it in aqueous extracts, if it existed originally in the fluids. All that is necessary is to concentrate the solution, and then add excess of acetic acid.

Dr. Garrod * has proposed an excellent plan for detecting the presence of uric acid in the blood of gouty patients, which is very simple and easy of execution. A little of the serum is poured into a watch-glass, and a few drops of acetic acid added to it. Two or three very fine filaments of silk, or tow, are then placed in the mixture, and the whole allowed to stand in a still place, under a glass shade, for twenty-four hours or longer. Upon submitting the filaments of tow to microscopical examination, they will often be found studded with minute crystals of lithic acid, frequently exhibiting some of the forms shown in the above figures.

Fig. 220.



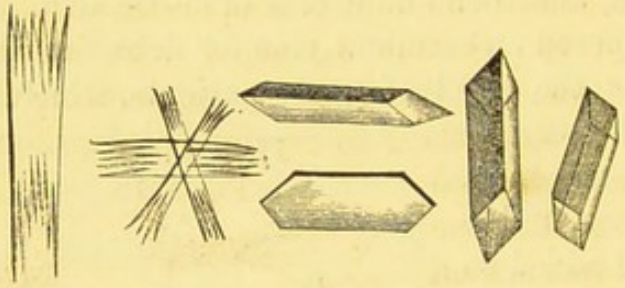
The student will gain much practical information as to the characters and various forms which this substance assumes, by

* "Medico-Chir. Trans." Vol. XXXI.

dissolving some of the crystals obtained from urine in alkaline solutions (potash, soda, alkaline carbonates, phosphates, &c.), and then causing the crystals of lithic acid to be precipitated by the addition of excess of acid. To some specimens he may add hydrochloric, to others acetic or nitric acids, &c. Upon examining the crystals obtained by these various processes, in the microscope, he will notice a great variety of forms, but, upon careful examination, it will be found that most of them are mere modifications of the same form, and that a connection between them may be traced in many instances.

318. **Hippuric Acid** is separated from its salts by the addition of a stronger acid (as hydrochloric acid), and the crystals which are deposited may be subjected to microscopical examination in the usual manner. Hippuric acid should always be sought for in animal structures which are quite fresh, as it undergoes decomposition very rapidly, and then becomes entirely converted into benzoic acid. The microscopical characters of these two acids are very distinct. Benzoic acid crystallizes in scales, while the crystals of hippuric acid occur in the form of beautiful prisms (fig. 221), not unlike those of the ammoniaco-magnesian phosphate. Hippuric acid is very soluble in hot water, and also in alcohol. Solutions of hippuric acid redden litmus paper strongly.

Fig. 221.



In order to detect small quantities of hippuric acid, the animal fluid, which must be perfectly fresh, is evaporated nearly to dryness, and then treated with alcohol sp. gr. ·830. After the addition of a crystal of oxalic acid, the spirituous solution is evaporated to the consistence of syrup. The residue is next to be extracted with ether, which contains about one-sixth of its volume of alcohol. The solution is again evaporated, and the remaining extract treated with water, which dissolves the hippuric acid, while any fatty matter which is present is left behind in an insoluble state. The solution may be filtered into a

watch-glass, and allowed to evaporate slowly that crystals may form.

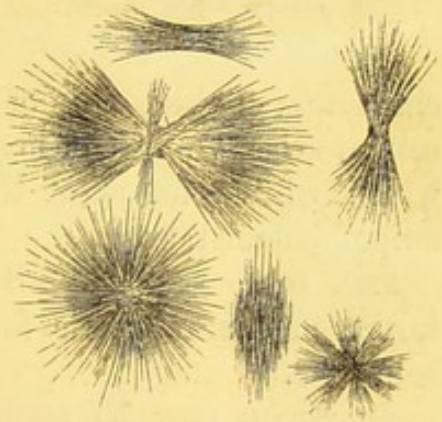
Hippuric acid may always be obtained from the fresh urine of horses or oxen. After the administration of benzoic acid, it is found in human urine; and Lehmann has remarked the presence of hippuric acid in diabetic urine, in every instance in which he has sought for it. Hippuric acid has been found in the blood of oxen by Verdeil and Dolfuss.*

319. Lactic Acid—Lactates.—The presence of this acid is often detected with difficulty in animal substances, in consequence of its characteristic reactions being interfered with by the presence of many organic bodies. Its separation from other substances is attended with much difficulty, especially when it is present only in very minute proportions.

Its presence is most readily determined by the microscopical characters of certain of its crystalline salts. Of these the lactates of zinc, copper, and lime, are the most characteristic.

In order to detect the presence of lactic acid in animal fluids, Lehmann proceeds as follows: the fluid is evaporated carefully over a water-bath, and the residue extracted with alcohol. After the separation of some of the salts, by evaporating this alcoholic solution, and allowing them to crystallize out, the remaining mother-liquor is treated with sulphuric or oxalic acid. The sulphate or oxalate of potash is then precipitated by means of alcohol, and the impure lactic acid remains in solution. To this solution baryta water is next added, and the excess of baryta removed by carbonic acid. The solution filtered from the precipitate is evaporated to a syrupy consistence, treated with alcohol, filtered, again evaporated, and then allowed to stand for some time, in order that any baryta salts may crys-

Fig. 222.



* Lehmann's "Physiological Chemistry," Cavendish Society, Vol. II. p. 212.

tallize out. The syrup is next removed, and decomposed with sulphate of lime. The solution filtered from the sulphate of baryta is evaporated to a small bulk, when crystals of lactate of lime, in the form of double brushes (fig. 222), with crystals of sulphate of lime, may be observed upon microscopical examination. The crystals of lactate of lime may be dissolved in alcohol and sulphate of copper added. After the removal of the excess of sulphates of lime and copper by evaporation and crystallization, the remaining solution is to be concentrated, and the crystals of lactate of copper examined in the microscope (fig. 223). If distinct and measurable crystals are not obtained in this manner, Lehmann dissolves the residue in a little water to separate any butyric acid that may be present, and after being strongly boiled, the solution is filtered, and a zinc bar placed in it, which, in the course of a short time, becomes covered with crystals of lactate of zinc, the angles of which may be measured with the goniometer (fig. 224). The microscopical characters of these salts of lactic acid are well shown in the excellent atlas of plates of my friend Dr. Funke, of Leipzig, a work which will be found of the greatest value to the student in animal chemistry.*

Fig. 223.

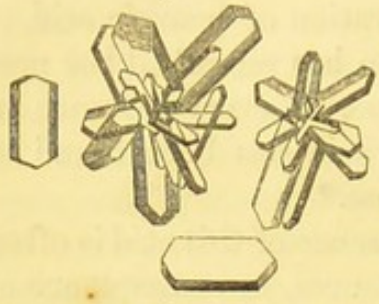
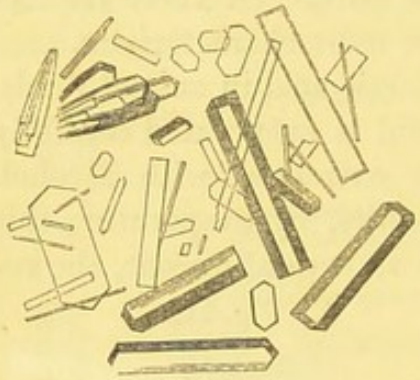


Fig. 224.



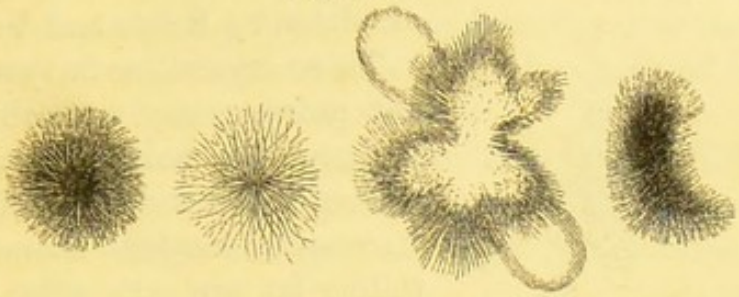
320. Fatty Matters. — Some substances of this nature crystallize in characteristic forms, from their ethereal or alcoholic solutions.

Margarine may be readily obtained from human fat: it is deposited from its alcoholic solution in round spherical masses, which appear almost black by transmitted light, in consequence of being composed of dense aggregations of minute crystals

* Cavendish Society, 1853.

(fig. 225). Almost the whole of the oily fat remains in solution in the alcohol.

Fig. 225.



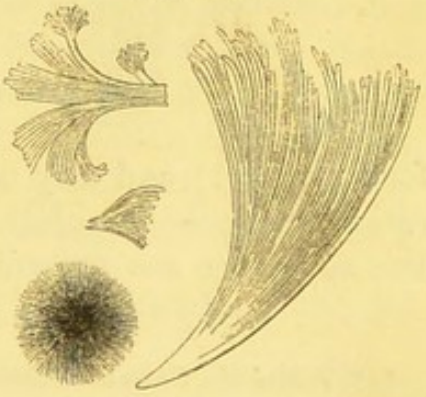
Minute stellæ of this substance may be obtained from a concentrated alcoholic solution of human fat, and not unfrequently separate spontaneously from the oily fat in which they have been previously dissolved. This crystallization may sometimes be seen in the contents of the fat vesicle of adipose tissue, particularly if putrefaction has commenced, and also in many mixed fatty matters which have been extracted from animal substances, if they be subjected to microscopical examination.

Margarine crystallizes from its solutions in tufts composed of somewhat wavy, minute, acicular crystals, or in separate free, short crystals, which are usually somewhat curved.

Margaric acid also crystallizes in minute tufts composed of very small and much-curved crystals (fig. 227).

Stearine may be obtained in large quantity from mutton fat: it is only slightly soluble in hot alcohol; from which solution it readily crystallizes in a form much resembling that of margarine, but the needle-like crystals are for the most part thinner, and their direction is straight. Stearine also very commonly crystallizes in quadrangular tablets.

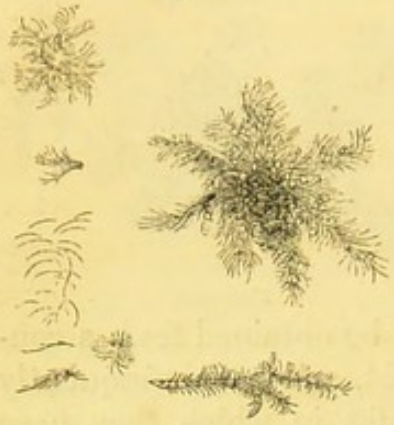
Fig. 226.



In examining the crystals of these fatty matters, deposited from ethereal or alcoholic solutions, obtained by digesting the dried animal substances in alcohol or ether, a large number of oil-globules will also be observed in the majority of instances. The characters

of stearic acid under the microscope are shown in fig. 226, and those of margaric acid in fig. 227. The accompanying figures were taken from the excellent atlas of plates by Robin and Verdeil.*

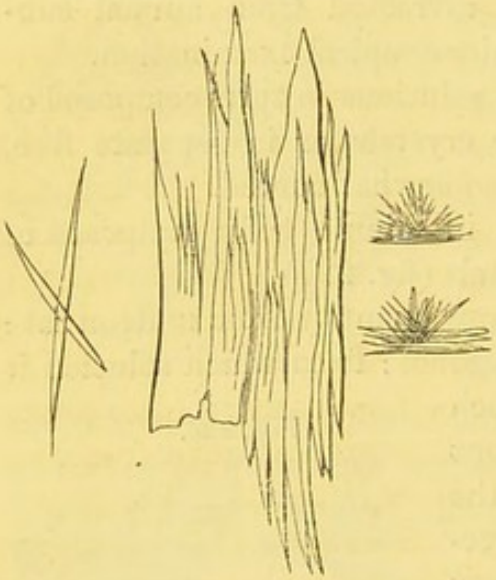
Fig. 227.



These crystalline fatty matters are not unfrequently met with in morbid growths, and very commonly in various fluids and solids of the body. In vomited matters, masses of crystalline fat are very often observed; and in vomit containing sarcinæ, the stellar crystalline masses are very frequently present.

Cholesterine is a non-saponifiable fat, and occurs in many situations in the human body. There is always a small quantity of cholesterine in bile, and the colourless gall-stones consist almost entirely of this substance. It may be extracted from many of the tissues in a state of health; I have even obtained it from the healthy crystalline lens of the eye. In disease it often occurs in serous fluids, especially in the serum of ovarian and other cysts, and in the fluid of hydrocele.

Fig. 228.



Cholesterine may always be recognized by its crystalline form (fig. 121), and may usually be obtained by the slow evaporation of alcoholic solutions; but where only mere traces of this substance are present, it is necessary to remove the other

* "Traité de Chimie Anatomique et Physiologique;" a work that may be consulted with great advantage by all interested in the microscopical characters of the various crystalline substances met with in, or obtained from, the animal body.

fatty matters before the cholesterine can be obtained in the form of crystals. By boiling the fats with water and oxide of lead, the saponifiable fats form a plaster with the latter substance, and the cholesterine is dissolved by treating the latter with dilute alcohol, from which solution it may be obtained in a crystalline form by subsequent evaporation.

Seroline.—This is another non-saponifiable fat, but differs from cholesterine in not forming distinct and well-defined crystals; it separates in large transparent flakes from alcoholic solutions (fig. 228).

321. Crystallizable Substance from the Blood.—This beautiful compound is held in solution in the red blood-corpuscles of man and animals. It was first examined by Funke, and afterwards by Kunde.* The subject has lately been very carefully investigated by Lehmann.† In the "Medical Times and Gazette" for 1852, will be found a very interesting paper on the subject by Dr. Parkes.‡ The various forms of the crystals are well delineated by Dr. Funke.§ See also frontispiece.

These crystals are very readily obtained by diluting blood with water. A drop of blood may be placed upon a glass slide, and after the addition of a drop of water, alcohol, or ether, the whole should be lightly covered with thin glass. A hair, or a small piece of thin paper or wood, may be placed between the glasses, in order that a stratum of fluid of sufficient thickness may be retained. Whenever it is possible, it is preferable to use defibrinated blood. Often the corpuscles and a little serum may be removed from the clot by firm pressure, and from this very perfect crystals may frequently be obtained. The blood-corpuscles become ruptured by endosmosis, their contents escape, and crystallize as the solution gradually becomes con-

---

* Dissert. inaug. Lips. 1851. (O. Funke.) Zeitschrift. f. rat. Med. N. F. Bd. I. II.

† "Lehrbuch d. Physiolog. Chemie," Vol. I. second edition, 1853. Bei. d. k. Sächs. Gesel. d. Wiss. 1852-1853.

‡ See also a paper on "Albuminous Crystallization," by Dr. Sieveking, in the "British and Foreign Medico-Chirurgical Review," for October 1853, in which some excellent woodcuts of blood-crystals are given.

§ Cavendish Society, 1853.

centrated. The time which elapses before crystallization takes place varies from an hour to several hours, or days, in different specimens of blood. Crystals may also be obtained in a similar manner from the coagulum of blood.

The form of the crystal often varies slightly in the same specimen, but the blood of different animals yields crystals of very different forms. The prismatic form is that most commonly obtained from the blood of man, the carnivora, and fishes. Tetrahedral crystals appear most common in some of the rodentia, as the guinea-pig, while six-sided tables are formed in the blood of the squirrel, mouse, and some others. By the kindness of Professor Lehmann, I have had an opportunity of seeing some beautiful rhomboidal crystals, which he had obtained from the blood of the hamster (another of the rodentia). Frogs' blood cannot be made to crystallize, in consequence of the density of the cell membrane; but Professor Lehmann tells me he has obtained crystals readily from the blood of the Italian lizard.*

The crystals form more readily in daylight than in the dark, but most rapidly when the slide is exposed in the light of the sun. For the method of preparing these blood-crystals in considerable quantity, by passing first a stream of oxygen, and subsequently one of carbonic acid through the defibrinated blood, I must refer to Lehmann's "Physiologischen Chemie," band. II. s. 162, 2nd edition, where the observer will also find much important information on this highly interesting-subject.

Guinea-pig's blood crystallizes in the course of half-an-hour, or even sooner, if it be diluted with a little water or alcohol. I have seen crystals form in guinea-pig's blood without the addition of any fluid, and without any evaporation whatever. Dog's blood also crystallizes in the course of a short time upon the addition of a little alcohol. Human blood crystallizes after the addition of water, slowly if only just removed from the

---

* Since the above was in type, Teichmann has succeeded in obtaining crystals from frog's blood by the addition of a very large quantity of water at a very low temperature. Zeitschrift. für. rat. Med. N. F. Band III. Heft. 3.—*Brit. and Foreign Med. Chir. Review, April* 1854.

body, but more rapidly if the blood be not quite fresh. The crystals shown in the frontispiece (fig. 1) were obtained by diluting a drop of fresh blood from the finger, with a drop of distilled water; and after covering the mixture with thin glass, the slide was placed in a light place. Crystallization commenced about forty hours after the addition of water to this specimen of blood.

It is excessively difficult to preserve specimens of these blood crystals as permanent objects. I have succeeded, however, in keeping some human-blood crystals mounted in the dry way; some from dog's blood have been mounted in Canada balsam, while the beautiful octohedral crystals from guinea-pig's blood have kept pretty well in the fluid to which spirit had been added, although they soon exhibited a tendency to change colour.

322. Crystallization of Bile.—The glycocholates of potash and soda were first obtained in a crystalline form by Platner. The crystallizable substance of the bile may readily be obtained as follows:—Perfectly fresh ox-bile is rapidly evaporated to dryness over the water-bath, and the dry residue powdered and extracted with absolute alcohol; the dark-green alcoholic solution is quickly filtered into a small flask or bottle, and then ether is gradually added until the white precipitate, at first formed, ceases to be redissolved upon agitation. Care should be taken to add the ether very gradually, for otherwise a bulky, amorphous precipitate occurs which does not become crystalline. The bottle is to be lightly corked, and allowed to stand in a still place. After a few days, stellar masses of beautiful and almost colourless crystals appear; these increase until tufts of a considerable size are produced. The crystals may be subjected to microscopical examination, immersed in a drop of the solution in which they were produced, and are beautiful objects; or they may be carefully washed with alcohol, to which a tenth of its bulk of ether has been added, and rapidly dried in vacuo.

When quite dry, the crystals may be mounted in a cell in the dry way, but if exposed to the air while moist, they rapidly deliquesce. I have preserved some of these crystals in the solution in which they were formed in a thin glass cell for some months.

APPENDIX.

323. Illumination of Objects.—Mr. Rainey has devised a beautiful arrangement for producing a perfectly white artificial light for examining microscopical objects. The bright dazzling effect of the sun and of artificial lights is due chiefly to the influence of the calorific rays; and in order to obtain a light favourable to examination, these calorific rays must be stopped in their course by transmitting the light through some transparent medium which will absorb them.

The combination by which this desirable object is effected in the case of artificial light is the following: "One piece of dark-blue glass, free from any tint of red, one of a very pale blue with a slight shade of green, and two of thick white plate glass, all cemented together with Canada balsam." Mr. Rainey says that when this medium is used with Gillett's condenser, objects illuminated by the light of a camphine lamp appear as if they were seen by a bright day-light.*

324. Dark-ground Illumination.—By this mode of illumination, an object appears itself beautifully illuminated, while the entire field surrounding it is perfectly dark. It is effected by preventing any rays of light reflected from the mirror from passing through the object and object-glass, by placing a dark stop beneath the latter: and at the same time the arrangement is such that any oblique rays will impinge upon the object, and after having been refracted by it, they will pass through the object-glass. In consequence of the only rays that are transmitted through the instrument being those that are

* This apparatus may be obtained of Mr. Ross.

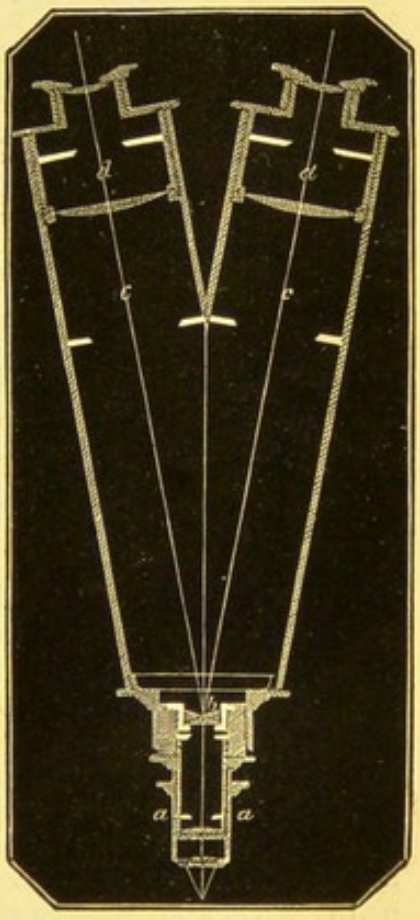
thus refracted by the object, the remainder of the field appears quite dark.

The same effect is produced by a beautiful arrangement of Mr. Wenham's, which consists of a truncated concave parabolic mirror with an opaque stop in the centre. This may be obtained under the name of Wenham's paraboloid reflector.

My friend Mr. Brooke has applied this instrument very successfully for the illumination of opaque objects under very high magnifying powers: Wenham's reflector is illuminated by a parallel pencil of rays, which are received, as they converge to the focus of the parabolic mirror, upon a small plane mirror attached to the object-glass, from whence they are reflected upon the object.

325. Gillett's Condenser.—This is a beautiful arrangement, by which objects can be examined either upon a dark or light ground, by oblique or by polarized light, without altering any of the adjustments of the microscope.

Fig. 229.



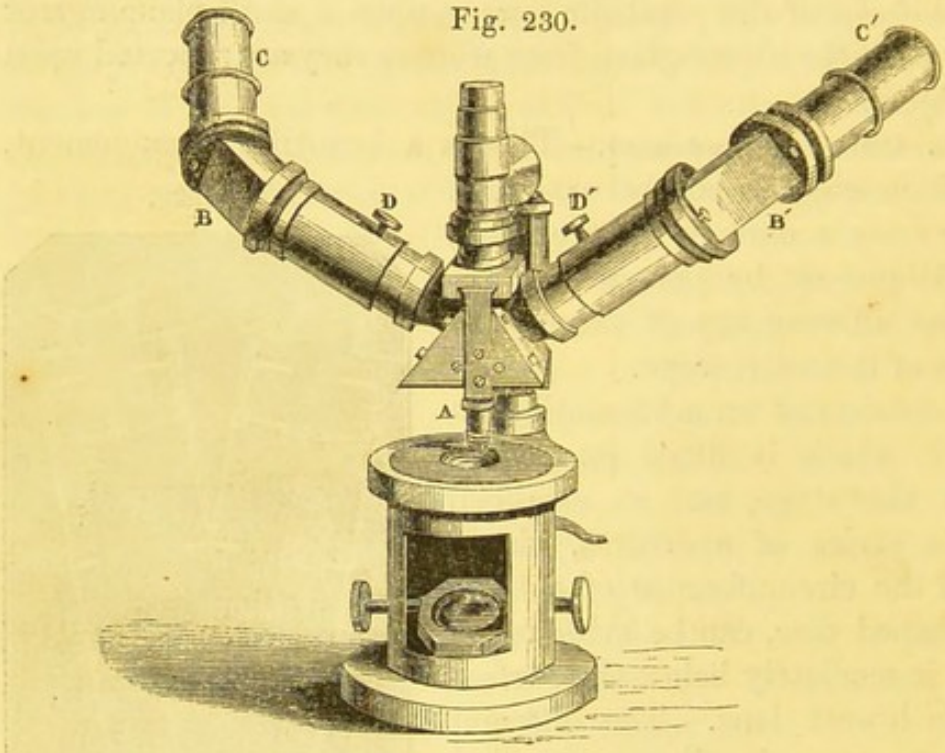
It consists of an achromatic condenser, which is fitted on underneath the stage, and so arranged that a series of apertures, placed round the circumference of a large cup-shaped disc, can be made to revolve immediately below the surface of the lowest lens. The different apertures vary in diameter. One is so arranged as to produce a dark ground; another is fitted with a tourmaline for examination of objects by polarized light, while a third will only admit an oblique pencil of light, &c.

In the examination of the more delicate test-objects, this instrument is invaluable, as points of structure by its aid can be readily

made out which are not visible by the ordinary methods of illumination; but for the general purposes of the medical practitioner it is not often required.

326. Binocular Microscope.—The attention of microscopical observers was directed many years ago to the advantage which would be derived from viewing an object with both eyes instead of only with one eye, by Professor Wheatstone, in his paper upon Binocular Vision. (Phil. Trans. 1838.) Of late years several attempts have been made to construct a microscope upon these principles, and very perfect instruments have been of late designed.*

Fig. 230.



The arrangement which Mr. Wenham has found to succeed best is shown in fig. 229, in which *b* represents a compound prism composed of two prisms of crown glass, and one large one of flint glass. By this arrangement two images are produced,

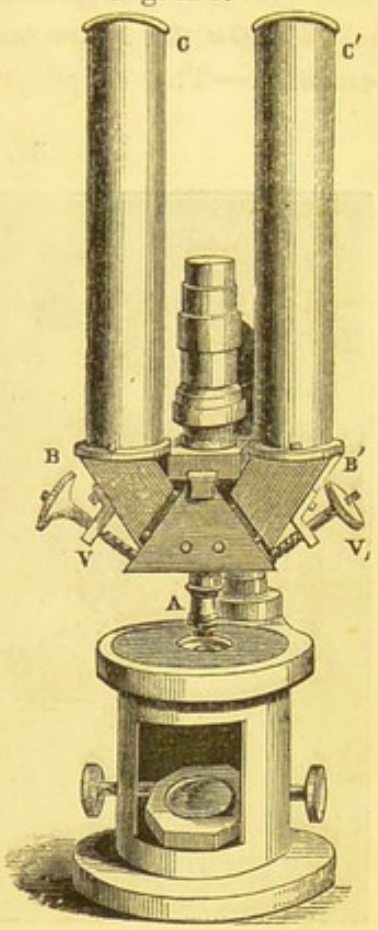
* "On the Application of Binocular Vision to the Microscope," by F. H. Wenham, Trans. of the Mic. Society of London, 1853, p. 1. "On the Binocular Microscope," Prof. Riddell, New Orleans, Mic. Journal, 1853, p. 18. "On a Microscope adapted for Anatomical Manipulations, and on a Binocular Microscope," by M. Nachet, Mic. Journal, 1854, p. 72.

one being transmitted through each of the tubes and eye-pieces *d*, *d*.

The very ingenious arrangement of M. Nachet is shown in fig. 230, by which two observers can examine an object placed in the field of the microscope at the same time. Immediately above the object-glass is placed a glass prism, the section of an equilateral triangle. At B B′ two other prisms are situated, by which an erect image of the object is produced in each tube.

By placing the two prisms B B′ nearer to the triangular prism, as shown in fig. 231, a binocular microscope of a most convenient and effective form is obtained. The course of the rays in the last arrangement is shown in fig. 232. For further information upon the subject of binocular microscopes, the reader is referred to the papers enumerated in the Note.

Fig. 231.

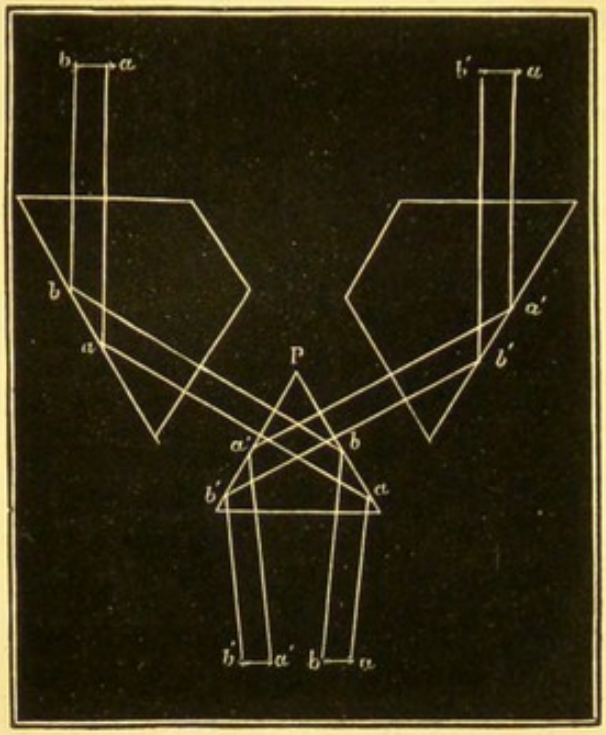


327. Nachet's Microscopes for Chemical Observations.—M. Nachet has contrived a beautiful arrangement for examining objects which are to be treated with chemical reagents, or to which heat is to be applied while under observation. In this instrument the object-glass is placed underneath the stage, and a prism beneath it, by which the rays of light transmitted by the object-glass are refracted through the tube of the microscope, inclined at an angle of about 45°, and conveniently arranged for observation. The light is thrown upon the object from above. The tube of the microscope, bearing the eye-piece with the object-glass, slides upon the base of the instrument, and can be withdrawn, while a spirit-lamp may be placed underneath the stage, and heat applied to the object when necessary. In this microscope the glasses cannot be injured by the fumes of acids, &c., which may be applied to objects. Mr. Highley has

proposed some additions to this instrument which render it very useful for investigations upon the optical properties of crystals.

328. **On the Manufacture of large Crystals of Sulphate of Iodoquinine.**—The employment of this substance for polarizing the light in microscopical examination has been already alluded to in page 30. Since this part of the work was in type, Dr. Herapath has succeeded in making crystals sufficiently large for the purposes desired.

Fig. 232.



Pure disulphate of quinine (that manufactured by Messrs. Howard and Kent is strongly recommended) must alone be employed. The quinine is dissolved in pyroligneous acid, sp. gr. 1·042, and the solution diluted with an equal quantity of proof spirit (made by mixing equal parts of spirit sp. gr. ·837 and distilled water).

A spirituous solution of iodine is made by dissolving 40 grains of iodine in one fluid ounce of rectified spirits-of-wine. The following is the formula:—

Disulphate of quinine . . .	50 grs.
Pyroligneous spirit	2 fluid ounces.
Proof spirit	2 fluid ounces.
Spirituous solution of iodine	50 drops.

The quinine is to be dissolved in the acid mixed with the spirit, and the solution heated to 130° F. The iodine solution is then to be added by drops, and the mixture agitated from time to time. As crystallization takes place, the largest plates rise to the surface of the fluid, and these increase in dimensions, being perfectly free from other crystals. The greatest care must be

taken to prevent the solution being agitated during the formation of the crystals, which may be effected by suspending the flask containing the mixture to a string which is made to stretch across the room.

The large crystals should appear in the course of six hours; but they should be allowed to remain in the solution for a period of from twelve to twenty-four hours. The largest plate is then removed by passing a small glass disk, attached to the end of a glass rod, beneath it. The glass with the crystal is then withdrawn, and the superfluous fluid imbibed with blotting-paper, the greatest care being taken not to touch the crystal, which is next dipped for an instant in cold distilled water in which iodine has been dissolved. The adhering fluid is again removed as before, and the crystal placed under a small glass shade, beneath which a watch-glass with a few drops of tincture of iodine is included. The iodizing of the plate takes about three hours, if the temperature be about 80°.

The crystal, being quite dry, is next mounted in thin Canada balsam (previously saturated with iodine) without the application of heat. For this purpose, however, Dr. Herapath recommends an etherial solution of Canada balsam which has been saturated with iodine. The most minute description of the whole process will be found in Dr. Herapath's paper, to which the reader is therefore referred. ("Microscopical Journal," No. VI. January, 1854.)

329. Corpora Amylacea.—Virchow has found that these little concretions which are met with in the brain, are composed of a substance allied to cellulose. In order to prove this, a watery solution of iodine is first applied, and this is followed by the addition of dilute sulphuric acid, when the characteristic colour is produced. The experiment succeeds better when the reagents are allowed to act very slowly (12 to 24 hours).

The little bodies which exhibit this reaction must be distinguished from the 'brain-sand,' consisting principally of mineral constituents, and found in the pineal body and choroid plexuses, the organic matrix of which consists of nitrogenized matter, and is coloured yellow by the iodine and sulphuric acid.

The cellulose bodies are found most abundantly in the

deeper layers of the membrane lining the ventricles of the brain, in immediate contiguity with the nerve fibres, and are more numerous when the lining membrane is very thick.

These observations of Virchow have been confirmed by Mr. Busk, who further states that the composition of these bodies is actually identical with that of starch, and that they exhibit the same characteristic reaction upon the application of Schultz's solution, composed of chloride of zinc and iodine (§ 298). For further information upon this very interesting subject, see Mr. Busk's Observations in No. VI. of the "Microscopical Journal," January, 1854.

330. Removing Stains from the Hands.—*Brunswick black* may be removed from the fingers by washing them with a little turpentine, or by rubbing them with lard or oil, which may afterwards be removed by soap and water.

Marine glue may generally be peeled off, or it can be dissolved with a little ether or naphtha.

Chromate of lead.—In using this substance for injection, the fingers often become stained with a very deep-yellow colour, which cannot be removed by ordinary washing. The application of a little hydrochloric acid at once dissolves the yellow precipitate. The hands should be plunged into water immediately after the stain has disappeared.

Sealing-wax varnish, and other varnishes soluble in spirits-of-wine, can always be removed by the application of a little spirit.

Lime and India-rubber cement, which adheres to the skin very firmly, can be removed by lard or oil, and subsequent washing.

Canada balsam may be removed by the application of a little turpentine or ether.

331. Table for the Mutual Conversion of British and Foreign Lineal Measurements.—This table is constructed upon the principle of those used for calculations in quantitative chemical analysis, and will enable the observer readily to compare the value of different measurements. It has been copied from a communication by Dr. Robertson, in the Edinburgh Monthly Journal of Science, for January, 1852.

To convert—	1	2	3	4	5	6	7	8	9	
1. British Inches into Millimetres .	25·39954	50·79908	76·19862	101·5982	126·9977	152·3972	177·7968	203·1963	228·5959	Millimetres.
2. Do. Old Paris Lines	11·25936	22·51872	33·77808	45·03744	56 29680	67·55616	78 81552	90·07488	101·33424	Paris Lines.
3. Do. { Rhineland or Prussian Lines }	11·65275	23·30550	34·95824	46·61099	58·26374	69·91649	81·56923	93·22198	104·87473	Prussian Lines.
4. Millimetres into British Inches . .	·03937079	·07874158	·11811237	·15748316	·19685395	·23622474	·27559553	·31496632	·35433711	British Inches.
5. Do. Old Paris Lines	·44329	·88658	1·32987	1·77316	2·21645	2·65974	3·10303	3·54632	3·98961	Paris Lines.
6. Do. { Rhineland or Prussian Lines }	·45878	91756	1·37633	1·83511	2·29389	2·75267	3·21145	3·67022	4·12900	Prussian Lines.
7. Old Paris Lines into British Inches .	·088815	·177630	·266445	·355260	·444075	·532890	·621705	·710520	·799335	British Inches.
8. Do. Millimetres .	2 25586	4 51172	6·76758	9·02344	11·27930	13·53516	15·79102	18·04688	20·30274	Millimetres.
9. Do. { Rhineland or Prussian Lines }	1·03494	2·06988	3·10482	4·13976	5·17469	6·20963	7·24457	8·27951	9·31445	Prussian Lines.
10. Rhineland or Prussian Lines into British Inches . .	·085817	·171633	·25745	·343267	·429083	·51490	·600717	·686532	·77235	British Inches.
11. Do. Millimetres .	2·179704	4·359408	6 539112	8·718816	10·89852	13·07822	15·25793	17·43763	19·61734	Millimetres.
12. Do. Old Paris Lines	·9662407	1·9324814	2·8987221	3 8649628	4·8312034	5·7974441	6·7636848	7·7299255	8·6961662	Paris Lines.

ILLUSTRATIONS OF USE OF THE ABOVE TABLE.

I.—EXAMPLE.

Given 245·9603 Paris Lines. Required the value in British Inches.

By line 7 of Table—

Old Paris Lines.		British Inches.
200	=	17·7630
+ 40	=	3·55260
+ 5	=	·444075
+ ·9	=	·0799335
+ ·0003	=	·0000266445
		21·8396351445 British In.

II.—EXAMPLE.

Given ·00215 Millimetres. Required the value in British Inches.

By line 4 of Table—

Millimetres.		British Inches.
·002	=	·00007874158
+ ·0001	=	·000003937079
+ ·00005	=	·0000019685395
		·0000846471985 British In.

III.—EXAMPLE.

Where extreme exactitude is not required, only one or two decimal places need be used. Thus—

Given 21·8396 British Inches. Required the value in Paris Lines.

By line 2 of Table—

British Inches.		Paris Lines.
20	=	225·19
+ 1	=	11·26
+ ·8	=	9·01
+ ·04	=	·45
		245·91 Paris Lines *very nearly*.

Data, from which the Table has been calculated, extracted from Mr. Woolhouse's Table, in the *Encyc. Metropolitana*,—British foot = 1. Metre = 3·2808992. Old Paris foot = 1·06578. Rhineland or Prussian foot = 1·0298.

INDEX.

ERRATA.

P. 47, line 2, for *is* read *are*.
P. 91, § 104, for *moving* read *removing*.

THE END.

LONDON :

PRINTED BY W. CLOWES AND SONS, STAMFORD STREET.

Highley's Scientific Library,

Fleet Street, London.

Mr. S. HIGHLEY, begs to inform Gentlemen interested in Scientific Literature, that on the Shelves of his Establishment will be found every Modern English and American Work on

CHEMISTRY—PHYSICS—PHOTOGRAPHY—MINERALOGY—GEOLOGY—PALÆONTOLOGY—BOTANY—ZOOLOGY—MICROSCOPY—ANATOMY—PHYSIOLOGY—SURGERY—MEDICINE—APPLIED SCIENCE.

Portraits of Scientific Men.

PHOTOGRAPHIC PLATES OF SCIENTIFIC SUBJECTS.

CHOICE SPECIMENS AND EDUCATIONAL COLLECTIONS
OF
MINERALS—ROCKS—FOSSILS—MINERALOGICAL APPARATUS AND MODELS.

STUDENT'S MICROSCOPES,
AND
SELECTED MICROSCOPICAL AND MICRO-PHOTOGRAPHIC APPARATUS.

CABINETS OF APPARATUS AND RE-AGENTS
FOR
EXAMINATIONS IN MEDICAL CHEMISTRY.

Osteological Specimens Human and Comparative.

A CATALOGUE OF
MR. S. HIGHLEY'S SCIENTIFIC PUBLICATIONS.

HIGHLEY'S GENERAL CATALOGUE
OF
Medical and Scientific Works, with their Dates and Prices.

TO WHICH IS ADDED AN INDEX OF SUBJECTS, WITH THE NAMES OF THE AUTHORS WHO HAVE WRITTEN UPON THEM.

Octavo, 2*s*. 6*d*. forwarded free by Post

HIGHLEY'S DESCRIPTIVE CATALOGUE
OF
Selected Educational Apparatus, Specimens Models, &c.,

USED IN MICROSCOPY, PHOTOGRAPHY, MEDICAL CHEMISTRY, MINERALOGY, OSTEOLOGY, ETC.

8vo. Illustrations. (*In Preparation.*)

Microscopical, Photographic, and Medical Apparatus.

HIGHLEY'S HOSPITAL MICROSCOPE on TRIPOD STAND, large Sliding Stage with Diaphragms, plain and concave Mirror, coarse and fine adjustment to body, Huyghenian Eye-piece, with Adapting-piece for Ross's or Smith and Beck's Object-glasses. 4*l.* 4*s.*

*** *This instrument combines economy with simplicity, elegance of form, and excellence of workmanship.*

ACHROMATIC OBJECT GLASSES. 1-inch, 1*l.* 1*s.*; ½-inch, 1*l.* 11*s.* 6*d.*; ¾-inch, 2*l.* 2*s.*

HIGHLEY'S ACHROMATIC GAS MICROSCOPE LAMP, with Reading Shade and Mounting Apparatus. Constructed to correct the yellow, glaring, and injurious light of the ordinary Gas-Lamp, as described in the *Quarterly Journal of Microscopical Science*, Part II., p. 142; and *Quekett on the Microscope*, p. 489. With great improvements, in Bronze, 2*l.* 10*s.*; or without Mounting Apparatus and Reflector, 2*l.* 2*s.*

HIGHLEY'S ARRANGEMENT for Object-Glass, Stage, and Mirror for Microscopical Photographs, as described in the *Quarterly Journal of Microscopical Science*, No. IV., and applicable to any Camera. 3*l.* 3*s.*

HIGHLEY'S MICROSCOPE CAMERA, consisting of the above Arrangement fitted to a double Telescope Chamber having a range from 12 to 24 inches, with Focussing Glass 6 inches square, Plate and Bath Frames, Glass Bath, Box fitting inside Camera, containing all the necessary Chemicals and Apparatus. The whole contained in a packing-case arranged for the adjustment of this instrument to any angle. Complete, 8*l.* 8*s.*

The Camera, Chemicals, Cases, &c., without Highley's Arrangement, but with Adapting-piece for any maker's Microscope, 5*l.* 5*s.*

DOUBLE COMBINATION CONDENSER for the above Arrangement, 1*l.* 1*s.*

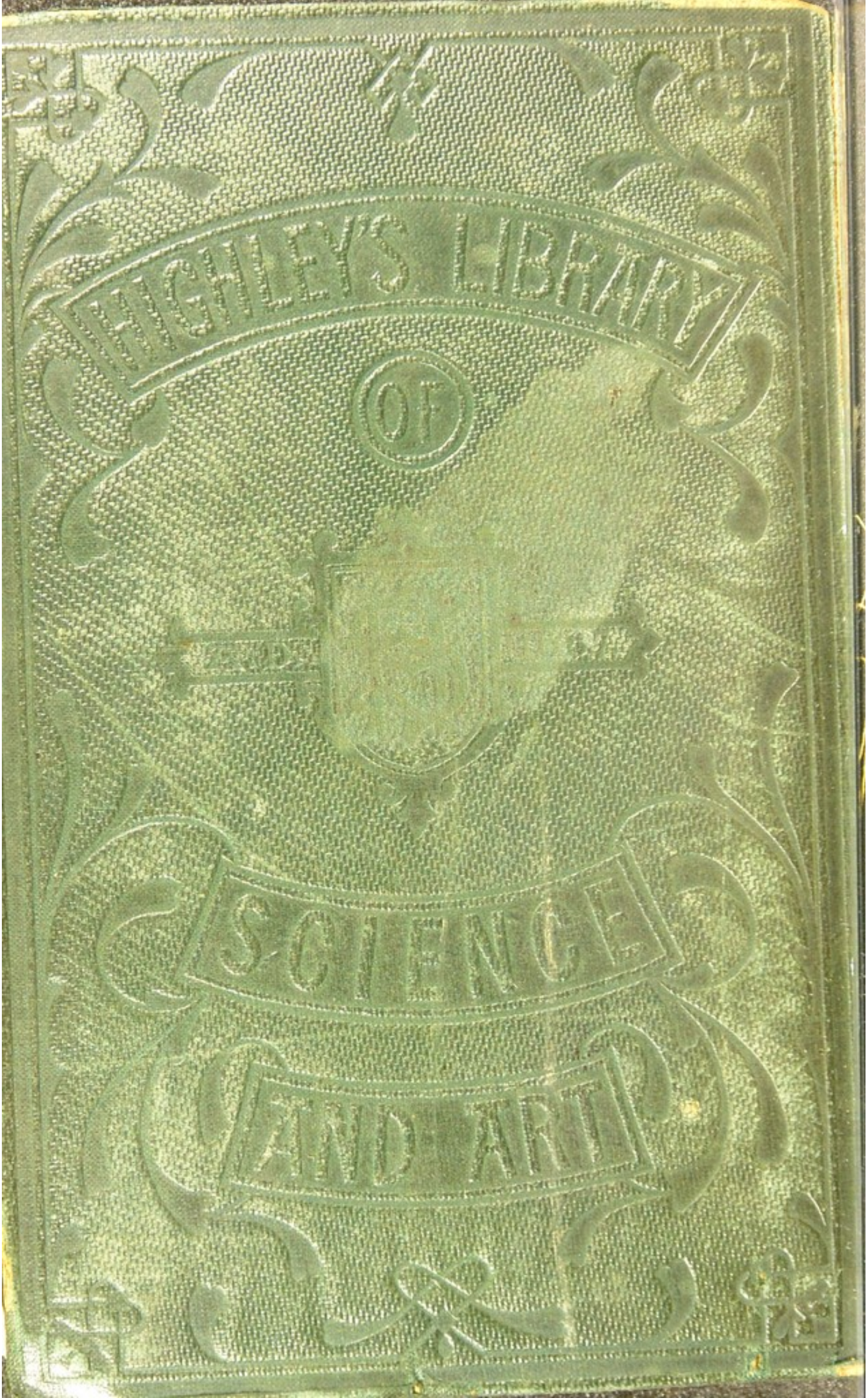
HIGHLEY'S CABINET OF APPARATUS & RE-AGENTS FOR EXAMINATIONS IN MEDICAL CHEMISTRY, as arranged by Dr. LIONEL BEALE.

CONTENTS.

APPARATUS. Urinometer in Case—Graduated 2-oz. Measure—Pipette—Stirring-rod—Microscopic Slides and Thin Glass—Watch-glasses—Test-Tubes—Tube-holder—Brass Forceps—Platinum Foil—Spirit Lamp with wire ring—Seven capped Dropping Bottles for the following Re-agents; Nitric Acid, Acetic Acid, Ammonia, Potash, Nitrate Barytes, Nitrate Silver, Oxalate Ammonia, Test-Papers. Price 1*l.* 11*s.* 6*d.*

HIGHLEY'S RE-AGENT CABINET FOR MICROSCOPICAL TESTING, containing 12 capped dropping bottles, 15*s.*

LONDON: SAMUEL HIGHLEY, 32, FLEET STREET.


HIGHLEY'S LIBRARY
OF
SCIENCE
AND ART