Dental microscopy / by A. Hopewell Smith.

Dental Microscopy

A. Hopewell Smith

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DENTAL MICROSCOPY

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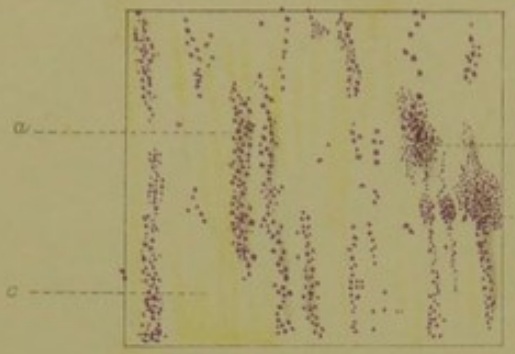
FRONTISPIECE.

Fig. 2.



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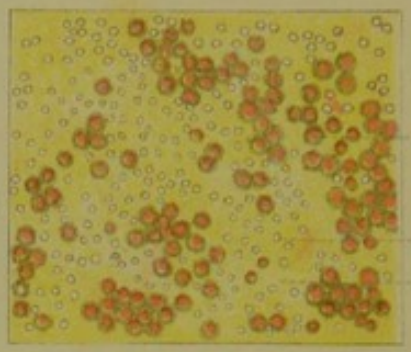

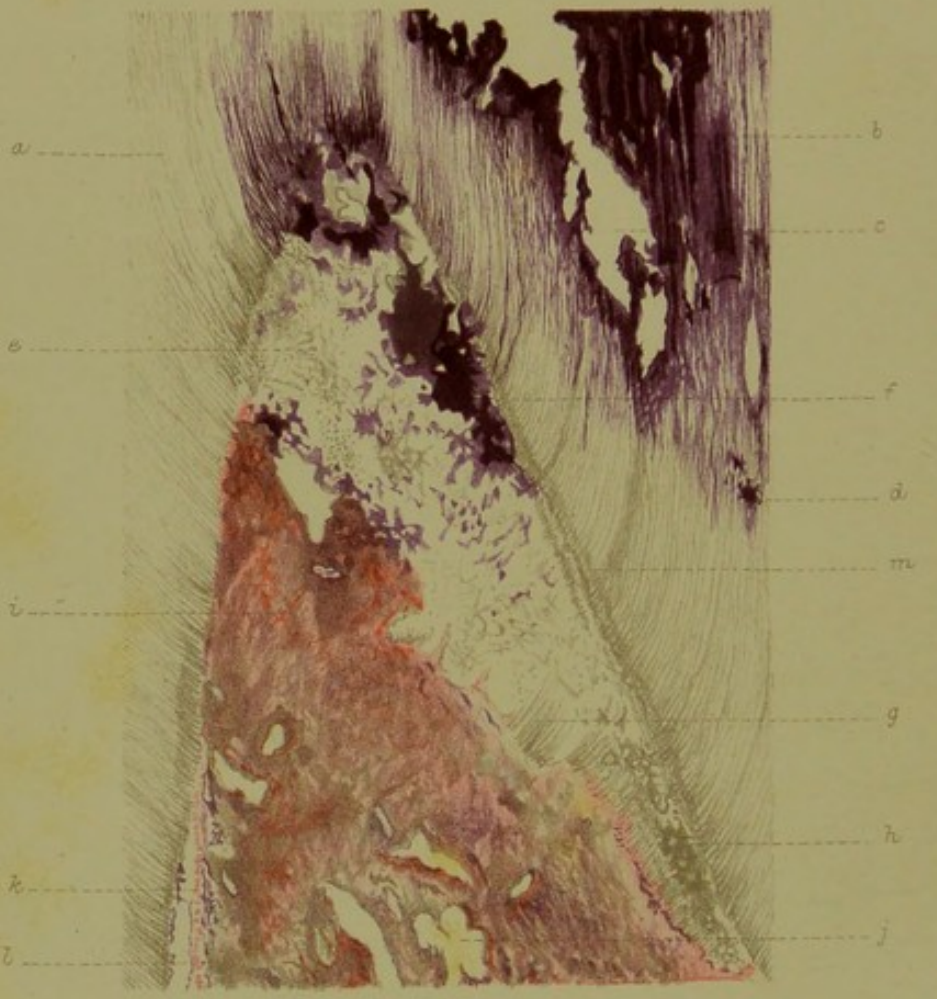

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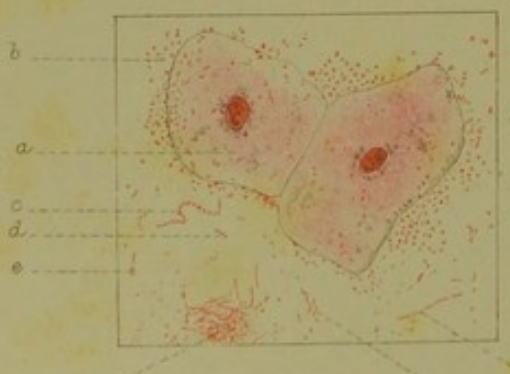

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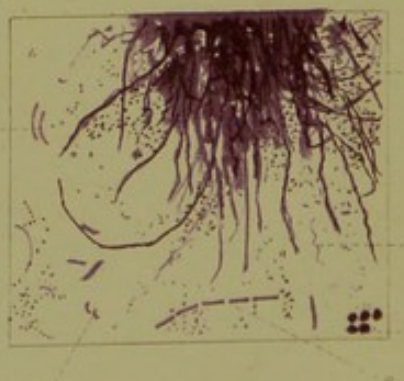

Fig. 5.

A Hopewell Smith fec.

West Newman chr

DENTAL MICROSCOPY

BY

A. HOPEWELL SMITH

L.R.C.P. Lond., M.R.C.S. Eng., L.D.S. Eng.

DEMONSTRATOR OF DENTAL HISTOLOGY AT THE DENTAL HOSPITAL OF LONDON

LATE LECTURER ON DENTAL SURGERY AND PATHOLOGY AND ALSO ON PRACTICAL DENTAL HISTOLOGY AT THE NATIONAL DENTAL HOSPITAL AND COLLEGE LONDON; LATE ASSISTANT DEMONSTRATOR OF HISTOLOGY AT CHARING CROSS HOSPITAL MEDICAL SCHOOL

WITH COLOURED FRONTISPIECE, EIGHT LITHOGRAPH PLATES, AND NUMEROUS ILLUSTRATIONS FROM THE AUTHOR'S ORIGINAL DRAWINGS

SECOND EDITION, REVISED AND ENLARGED

LONDON, ENGLAND
THE DENTAL MANUFACTURING COMPANY, LIMITED
6 TO 10 LEXINGTON STREET

PHILADELPHIA, U.S.A.
THE S. S. WHITE DENTAL MANUFACTURING COMPANY
CHESTNUT STREET

1899

IN APPRECIATION

OF

CHARLES S. TOMES, Esq., M.A., F.R.S.

THE AUTHOR

DEDICATES THIS WORK TO HIM

PREFACE

TO

THE SECOND EDITION

A KNOWLEDGE of the science and art of practical Dental Microscopy serves several ends. First, it gives the student a clear insight into the preparation of the various dental tissues, for the purposes of microscopical examination, and thus forms the basis for a complete study of the histology and patho-histology of the organs with which he is so closely associated. Secondly, it imparts to the earnest worker a degree of digital dexterity not wholly obtained otherwise, thereby enabling him to appreciate and acquire, in a unique way, the niceties of touch and manipulation so desirable in his daily work. And, thirdly, it fulfils the regulations of the Royal College of Surgeons of England, by helping to prepare the student for his final test at that College.

The introduction of subjects connected with certain pathological and bacteriological conditions of the teeth and adjacent parts has seemed to be desirable: therefore the present volume contains several more chapters than its predecessor.

Additional drawings have been supplied to illustrate

as completely as possible the subjects under discussion ; and, to prevent any possibility of the student's mind being reduced to a chaotic state by attempting to assimilate these subjects through the help of photo-micrographs, plain line drawings, carefully copied from original photographs, have been reproduced.

The Author desires to convey his thanks to Messrs. Morton Smale and Colyer for the use of the woodcuts of his own drawings which appear in their work on 'The Diseases and Injuries of the Teeth,' to Mr. Leon Williams for his figures illustrating caries of enamel, and to many gentlemen in England and on the Continent who have generously offered advice or lent preparations or micro-scopical specimens to aid the task of producing the present volume.

BERKELEY SQUARE, W.
March, 1899.

PREFACE

TO

THE FIRST EDITION

THIS hand-book has been written with a two-fold purpose. It is offered to the student of Dental Histology and others interested in this particular branch of Science, with a view of presenting in a concise manner the chief methods of making microscopical preparations, and of enabling the student, through the medium of the illustrations, to recognise the most important features of the dental tissues. To the advanced worker the notes may appear somewhat amplified; but to those who are desirous of acquiring the art of Dental Microscopy, it is hoped that they will prove to be a guide not exhaustive, but of some value, stimulating original research, and facilitating the means by which this end may be attained.

The Author's best thanks are due to Mr. W. H. Dolamore for his revision of the proof-sheets, and for his superintendence of the work during its passage through the press; and also to those gentlemen who have kindly supplied him with their special methods of procedure.

January, 1895.

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Fig. 2.—Longitudinal section of carious dentine ; decalcified and cut on a microtome ; stained by Gram's method ; $\frac{1}{12}$-inch objective and C ocular ; shows (*a*) micrococci in tubules ; (*b*) liquefaction foci ; (*c*) matrix of dentine.

Fig. 3.—Transverse section of carious dentine ; 'pipe-stem' appearance ; decalcified and cut on a microtome ; stained picrocarmine ; $\frac{1}{12}$-inch objective and C ocular ; shows (*a*) carious tubules ; (*b*) empty normal tubules ; (*c*) matrix of dentine.

Fig. 4.—Some varieties of the micro-organisms of the mouth ; film preparation ; stained carbol-fuchsine ; $\frac{1}{12}$-inch objective and C ocular ; shows (*a*) stratified squamous epithelial cells detached from the mucous membrane of the mouth, with their nuclei ; (*b*) masses of micrococci ranged about the cells, which in many cases become destroyed by them ; (*c*) *Streptococcus longus* [its individual members ought to have been drawn the same size and shape] ; (*d*) *Streptococcus brevis* ; (*e*) *Diplococci* ; (*f*) *Spirochæte dentium* ; (*g*) *Bacillus buccalis maximus* ; (*h*) mass of bacteria containing round, thread, and rod forms.

Fig. 5.—Various types of micro-organisms found beneath a mass of salivary calculus in a well-cared-for mouth ; film preparation ; stained by Gram's method ; $\frac{1}{12}$-inch objective and D ocular ; shows (*a*) mass of bacteria containing cocci and thread forms ; (*b*) *Spirillum sputigenum* ; (*c*) large curved rods, which are particularly numerous in the mouths of some aged people ; (*d*) *Spirochæte dentium* ; (*e*) *Bacillus buccalis maximus* ; (*f*) *Sarcina lutea.*

PLATE I.

Fig. 1.

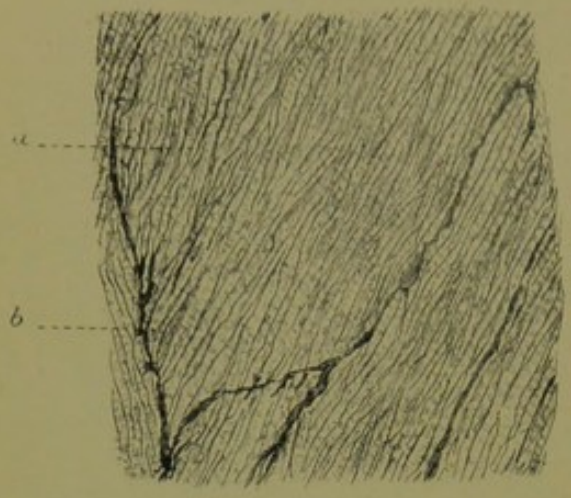


Fig. 2.

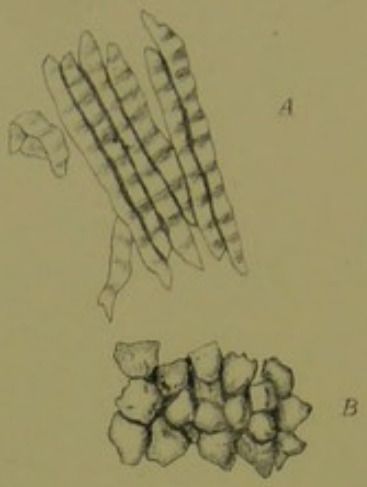


Fig. 3.

Fig. 4.

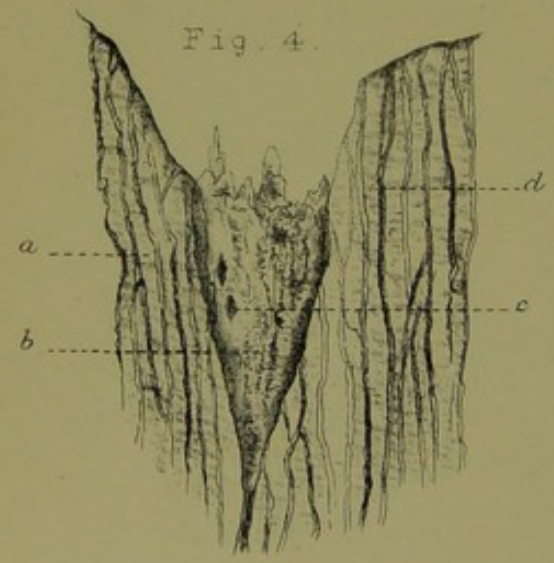


Fig. 5.

A. Hopewell Smith del.

West Newman lith.

DESCRIPTION OF PLATE I

Fig. 1.—Longitudinal section of enamel, human; ground down; unstained; $\frac{1}{6}$-inch objective and A ocular; shows (*a*) prisms; (*b*) fissures produced by grinding.

Fig. 2.—Human enamel, decalcified; stained picro-carmine; $\frac{1}{12}$-inch oil immersion and C ocular; shows (*a*) longitudinal section, striæ, and interprismatic substance; (*b*) transverse section of same, shows the outline of the prisms.

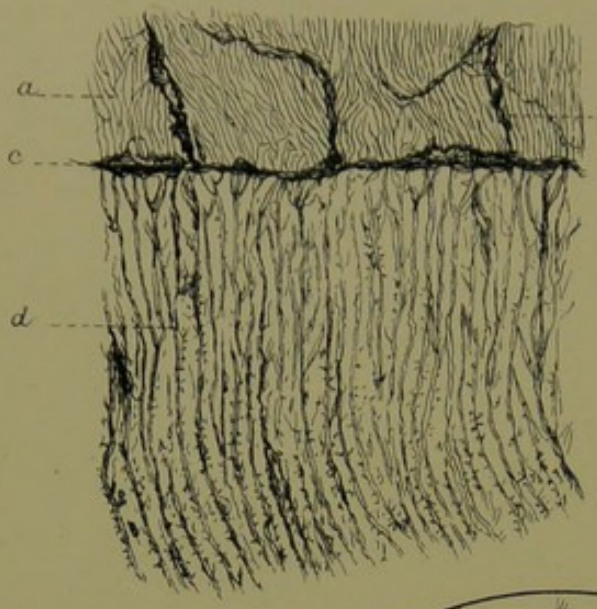
Fig. 3.—Striæ of Retzius (human); ground down; stained orange rubine; 1-inch objective and A ocular; shows (*a*) enamel; (*b*) free edge of enamel; (*c*) striæ of Retzius; (*d*) dentine.

Fig. 4.—Pit in enamel, with Nasmyth's membrane *in situ*; ground down; unstained; $\frac{1}{12}$-inch objective and C ocular; shows (*a*) enamel; (*b*) Nasmyth's membrane; (*c*) lacunæ; (*d*) interprismatic substance.

Fig. 5.—Lacunal cells from Nasmyth's membrane; decalcified in hydrochloric acid; unstained; $\frac{1}{6}$-inch objective and C ocular; shows (*a*) cells; (*b*) homogeneous matrix.

PLATE II.

Fig. 1.



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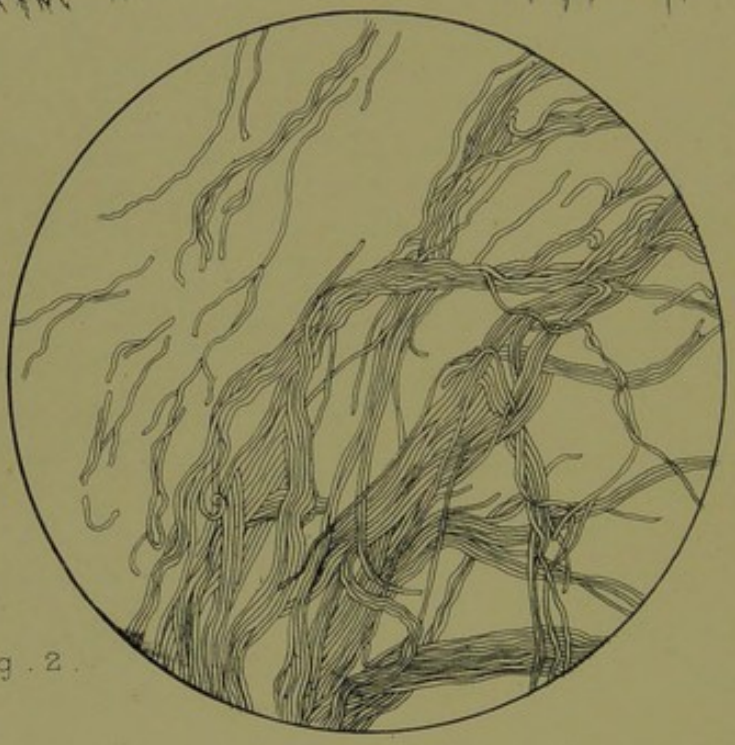

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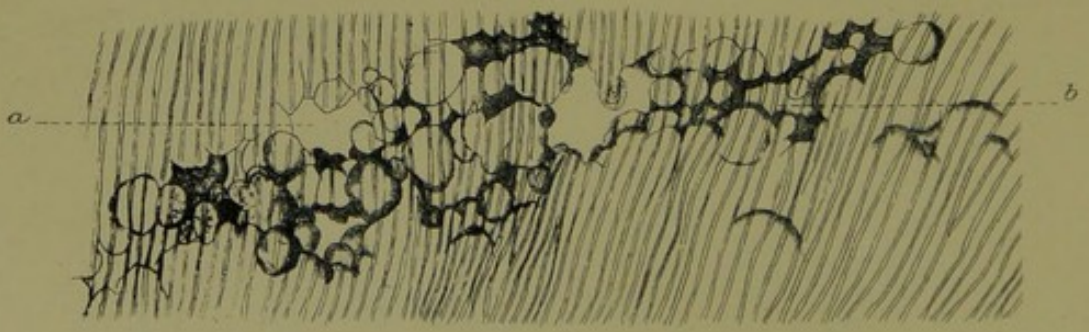

Fig. 4.

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West, Newman lith.

DESCRIPTION OF PLATE II

Fig. 1.—Amelo-dentinal junction, longitudinal section ; ground down ; unstained ; $\frac{1}{6}$-inch objective and A ocular ; shows (*a*) enamel ; (*b*) fissures in enamel ; (*c*) amelo-dentinal junction ; (*d*) dentinal tubules, their branches and terminations.

Fig. 2.—Sheaths of Neumann ; decalcified and teased out ; carmine ; $\frac{1}{6}$-inch and A ocular.

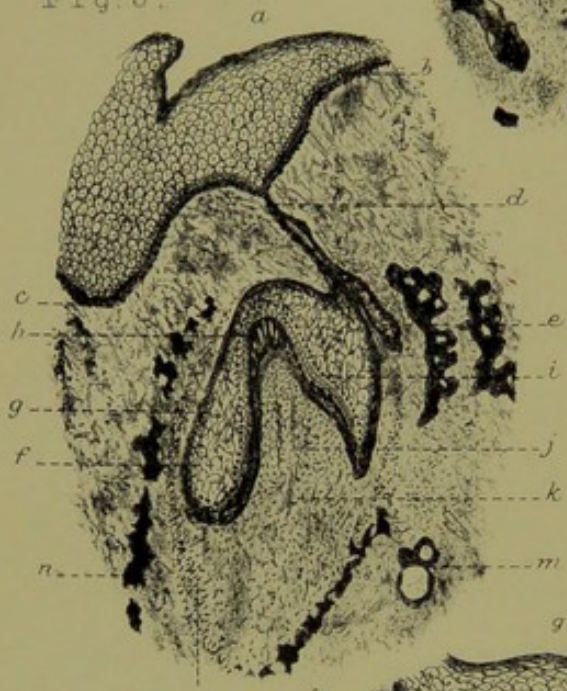
Fig. 3.—Dentine, transverse section ; ground down ; stained methylene blue ; $\frac{1}{6}$-inch and A ocular ; shows (*a*) lacunæ of cementum ; (*b*) tubules terminating here and there in lacunæ.

Fig. 4.—Dentine, interglobular spaces, longitudinal section ; ground down ; unstained ; $\frac{1}{6}$-inch and A ocular ; shows (*a*) interglobular spaces ; (*b*) dentinal tubules crossing over the interglobular spaces.

PLATE III

Fig. 1.

Fig. 3.



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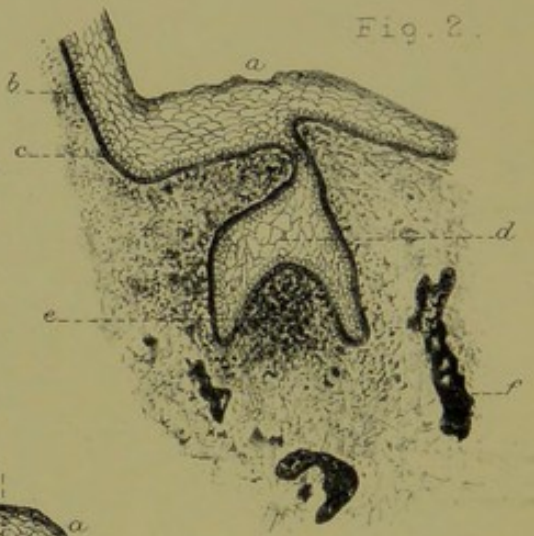

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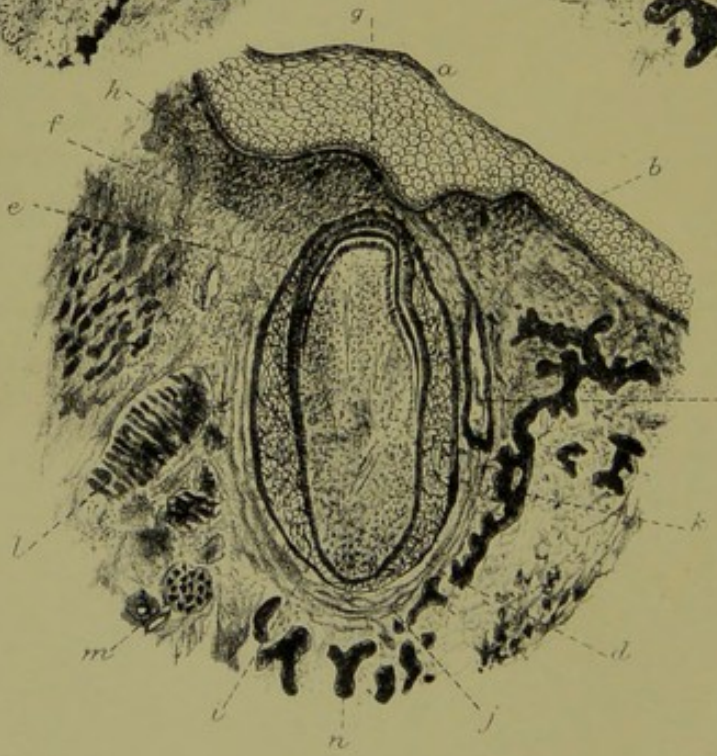

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DESCRIPTION OF PLATE III

Fig. 1.—This and the accompanying figures are intended to show some of the stages of development of teeth in mammalia. From the embryos of pigs of varying lengths.

First Stage.—Longitudinal section; hardened in perchloride of mercury, or Müller's fluid and alcohol; cut on a microtome; stained hæmatoxylene; 1-inch objective and C ocular; shows (*a*) oral epithelium; (*b*) deeper layer of epithelium; (*c*) Rete Malpighi; (*d*) primary inflection of enamel germ; (*e*) commencement of formation of dental sac; (*f*) bone of jaw.

Fig. 2—*Second Stage.*—Prepared and cut as above; stained carmine; 1-inch and C ocular; shows (*a*) oral epithelium; (*b*) deeper layer of epithelium; (*c*) Rete Malpighi; (*d*) stellate reticulum of enamel organ; (*e*) dentine papilla; (*f*) bone of jaw.

Fig. 3—*Third Stage.*—Prepared and cut as above; stained hæmatoxylene; 1-inch and C ocular; shows (*a*) oral epithelium; (*b*) deep layer of epithelium; (*c*) Rete Malpighi; (*d*) neck of enamel organ; (*e*) permanent enamel germ; (*f*) stellate reticulum of enamel organ; (*g*) external epithelium; (*h*) internal epithelium; (*i*) cells of stratum intermedium; (*j*) dentine papilla; (*k*) rudimentary blood supply of pulp; (*l*) formation of dental sac; (*m*) artery, vein, and nerve of jaw; (*n*) bone of jaw.

Fig. 4—*Fourth Stage.*—Prepared and cut as above; stained hæmatoxylene; 2-inch and A ocular; shows (*a*) oral epithelium; (*b*) neck of enamel organ; (*c*) permanent enamel germ; (*d*) dental pulp; (*e*) layer of odontoblasts; (*f*) dentine; (*g*) enamel, (*h*) ameloblasts; (*i*) dental sac, inner portion; (*j*) dental sac, outer portion; (*k*) stellate reticulum; (*l*) muscle fasciculi; (*m*) artery, vein, and nerve; (*n*) bone of jaw.

PLATE IV.

Fig.1.

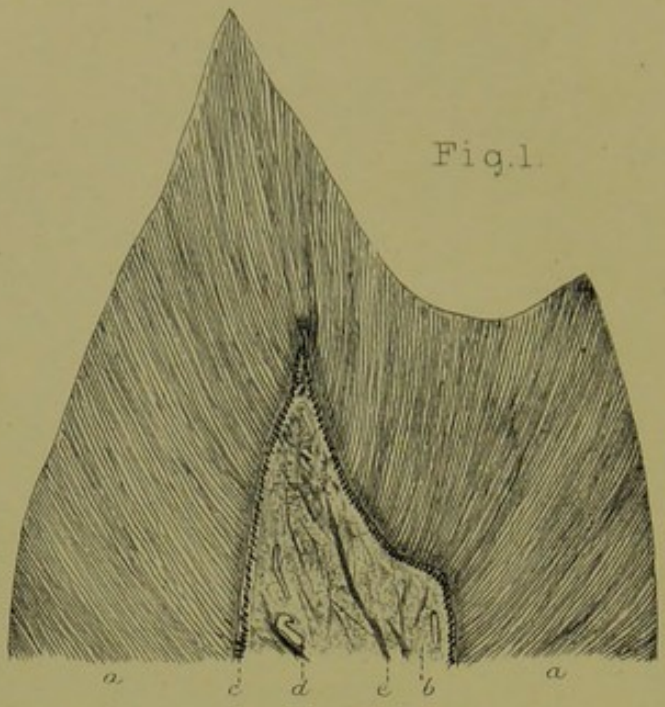
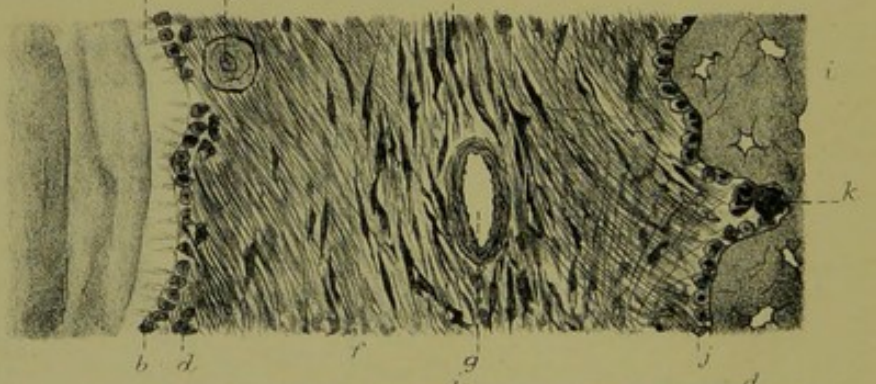


Fig.2.

Fig.3.



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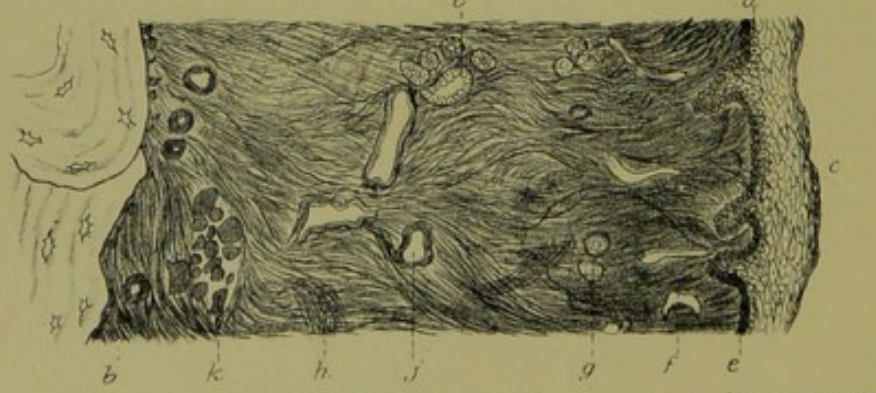

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West, Newman lith.

DESCRIPTION OF PLATE IV

Fig. 1.—Longitudinal section of crown of bicuspid to show the pulp *in situ* ; decalcified (Author's process) ; stained gold chloride ; 2-inch objective and A ocular ; shows (*a*) dentine ; (*b*) pulp *in situ* ; (*c*) membrana eboris ; (*d*) capillary ; (*e*) nerve bundles.

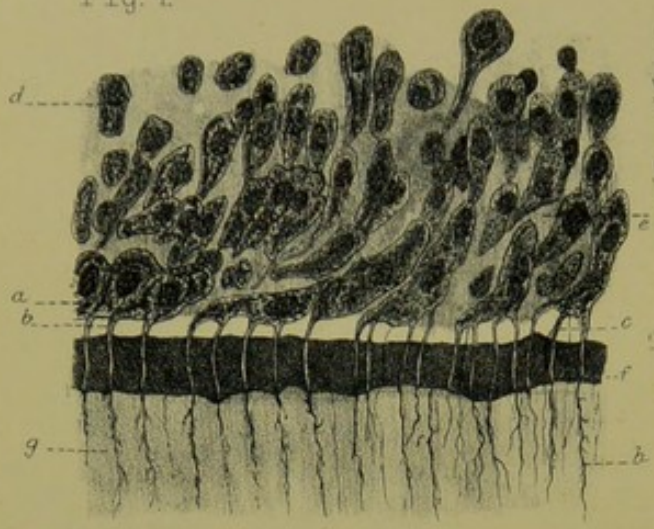
Fig. 2.—Transverse section of pulp *in situ* ; young canine tooth below cervical region ; decalcified ; stained rubine ; $\frac{1}{8}$-inch and A ocular ; shows (*a*) dentine ; (*b*) odontoblasts (diagrammatic) ; (*c*) basal layer of Weil ; (*d*) pulp tissue ; (*e*) arteriole ; (*f*) venule ; (*g*) nerve bundle.

Fig. 3.—Longitudinal section of periodontal membrane *in situ* ; decalcified ; stained hæmatoxylene ; $\frac{1}{8}$-inch and A ocular ; shows (*a*) cementum with lamellæ ; (*b*) cementoblasts ; (*c*) Sharpey's fibres ; (*d*) lymphatics ; (*e*) calcospherite spherule ; (*f*) connective tissue fibres ; (*g*) blood-vessel cut obliquely ; (*h*) 'principal' fibres of membrane ; (*i*) bone of alveolus of jaw ; (*j*) osteoblasts ; (*k*) two osteoclasts.

Fig. 4.—Transverse section of gum *in situ* ; decalcified ; stained rubine ; 2-inch and C ocular ; shows (*a*) bone of alveolus ; (*b*) its periosteum ; (*c*) oral epithelium (stratified) ; (*d*) deeper layer of epithelium ; (*e*) Rete Malpighi ; (*f*) submucous tissue ; (*g*) 'glands' of Serres ; (*h*) fasciculi of fibrous tissue ; (*i*) mucous glands ; (*j*) blood-vessels ; (*k*) muscle fibres.

PLATE V.

Fig. 1.



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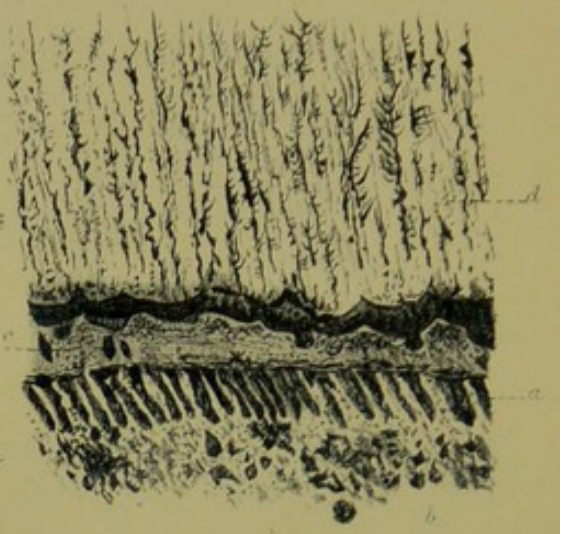

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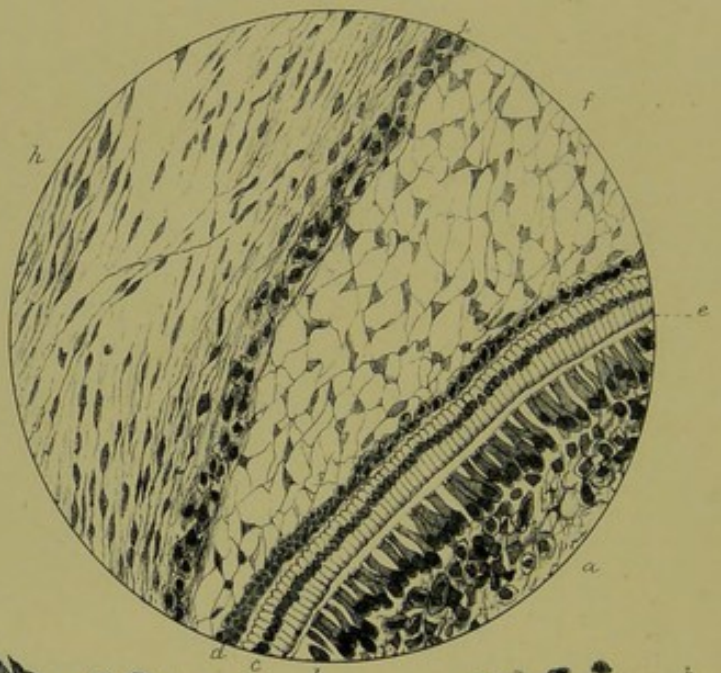

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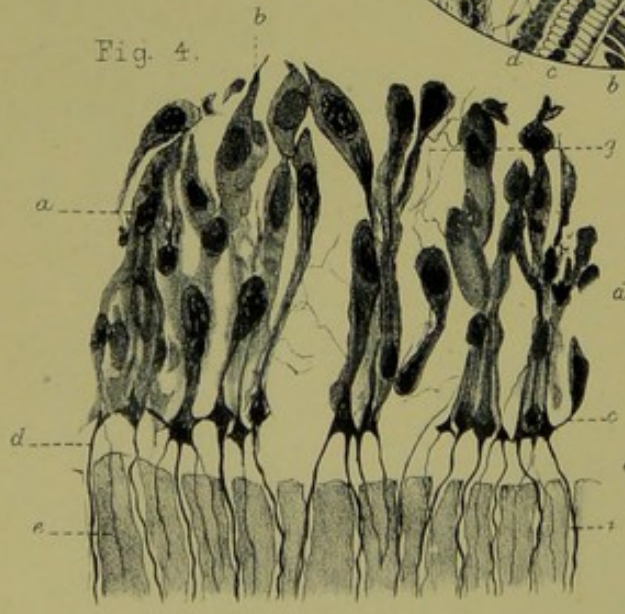

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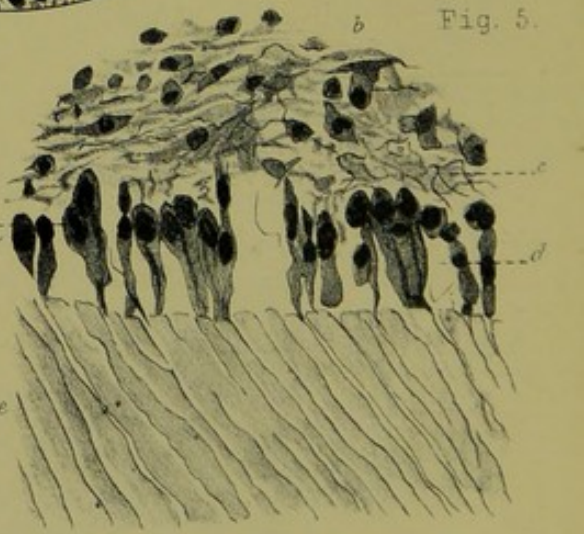


A.Hopewell Smith del.

West, Newman lith.

DESCRIPTION OF PLATE V

Fig. 1.—Longitudinal section through the dentine organ of a pup at birth, hardened in Müller's fluid and alcohol ; cut on a microtome ; stained hæmatoxylene ; ¼-inch objective and C ocular ; shows (*a*) layer of odontoblast cells ; (*b*) thick processes of odontoblasts at apex of dental papilla ; (*c*) thin processes producing thinner fibrils ; (*d*) undeveloped odontoblasts and dentogenetic cells of pulp ; (*e*) homogeneous matrix in which cells are imbedded ; (*f*) formed but uncalcified dentine ; (*g*) calcified dentine matrix ; (*h*) dentinal tubules.

Fig. 2.—Transverse section through the uncompleted apex of root of a tooth, from a photograph by Mr. Howard Mummery (see 'Trans. Odonto. Soc.,' Vol. XXII., No. 7, Plate II., fig. 2), × 170 ; prepared by Weil's process ; shows (*a*) odontoblasts with square extremities ; (*b*) dental pulp ; (*c*) formed but uncalcified dentine, showing manner of its deposition ; (*d*) dentine.

Fig. 3.—Longitudinal section through the enamel and dentine organs in the incisor region of the mandible of a fœtal pup ; prepared as in Fig. 1 ; stained hæmatoxylene ; ⅛-inch and A ocular ; shows (*a*) pulp ; (*b*) odontoblasts ; (*c*) ameloblasts ; (*d*) cells of stratum intermedium ; (*e*) commencement of formation of dentine and enamel ; (*f*) stellate reticulum ; (*g*) cells forming dental sac, its inner portion ; (*h*) its outer portion.

Fig. 4.—Transverse section of the pulp of an adult canine (human), at the broadest part of the cervical region, prepared by Author's process ; stained rubine ; $\frac{1}{12}$-inch and C ocular ; shows (*a*) odontoblasts ; (*b*) their basal poles ; (*c*) their median poles ; (*d*) their distal processes ; (*e*) dentine matrix ; (*f*) dentine tubules ; (*g*) fine network formed by the 'supporting fibres' of the pulp.

Fig. 5.—Transverse section of same at the narrowest part of the pulp cavity ; preparation and stain as above ; ⅛-inch and C ocular ; shows (*a*) odontoblasts ; (*b*) pulp ; (*c*) network of fibres apparently connected with the odontoblasts ; (*d*) wide intercellular spaces ; (*e*) dentine.

PLATE VI.

Fig. 2.

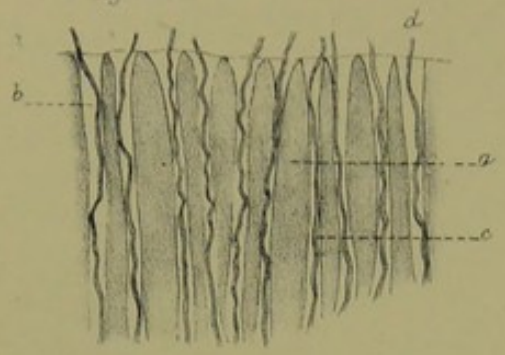
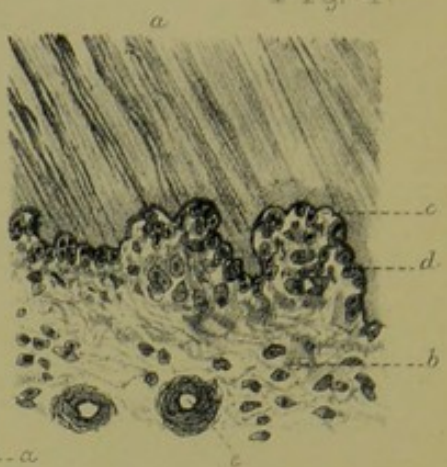


Fig. 4.



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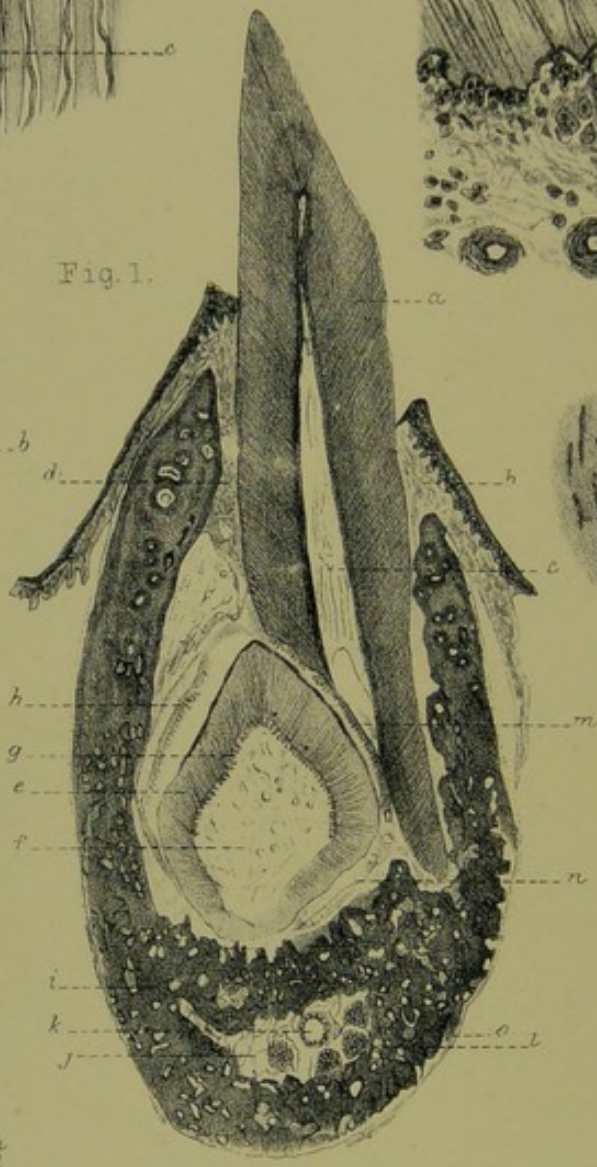

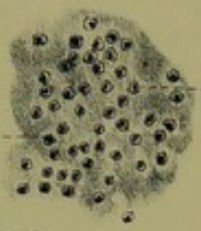

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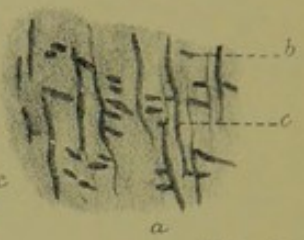
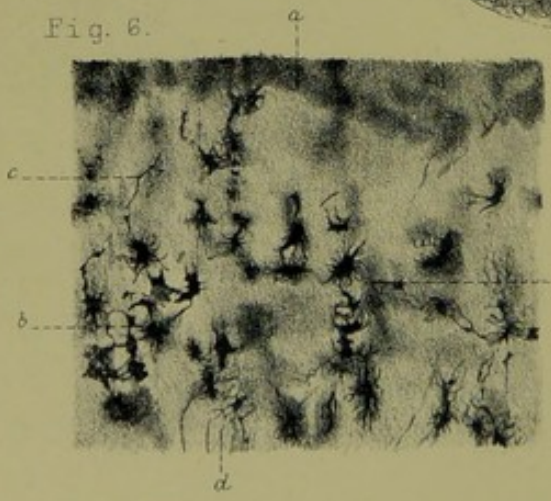


Fig. 5.

Fig. 6.



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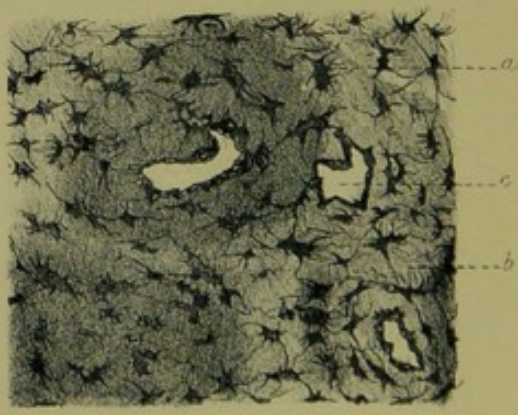


A. Hopewell Smith del.

West, Newman lith.

DESCRIPTION OF PLATE VI

Fig. 1.—Vertical section of mandible of a kitten, with temporary and permanent teeth *in situ*, decalcified in chromic acid ; stained carmine and methyl green ; 2-inch objective and A ocular ; shows (*a*) dentine of temporary tooth ; (*b*) flange of gum ; (*c*) pulp of temporary tooth ; (*d*) periodontal membrane ; (*e*) dentine of permanent tooth ; (*f*) its pulp ; (*g*) odontoblast layer ; (*h*) ameloblasts torn away ; (*i*) bone of jaw ; (*j*) inferior dental canal ; (*k*) inferior dental artery ; (*l*) nerve ; (*m*) commencement of formation of absorbent organ ; (*n*) dental sac ; (*o*) alveolar periosteum.

Fig. 2.—Longitudinal section of dentine near the pulp cavity, with dentinal fibrils *in situ* ; decalcified (Author's process) ; stained rubine ; $\frac{1}{12}$-inch and C ocular ; shows (*a*) dentine matrix ; (*b*) tubule ; (*c*) fibril ; (*d*) fibril passing from pulp into tubule.

Fig. 3.—Transverse section of same ; stained gold chloride ; $\frac{1}{12}$-inch and C ocular ; shows (*a*) tubule with fibril ; (*b*) matrix.

Fig. 4.—Longitudinal section of absorbent organ *in situ* ; decalcified ; stained carmine ; $\frac{1}{6}$-inch and A ocular ; shows (*a*) dentine of temporary tooth ; (*b*) absorbent organ ; (*c*) Howship's lacunæ ; (*d*) multi-nucleated cells ; (*e*) blood-vessels.

Fig. 5.—Concentric lamellæ in dentine, longitudinal section of molar (radicular portion) ; ground down ; unstained ; $\frac{1}{6}$-inch and A ocular ; shows (*a*) dentine matrix ; (*b*) tubule obliquely cut ; (*c*) lamellæ running parallel to pulp cavity.

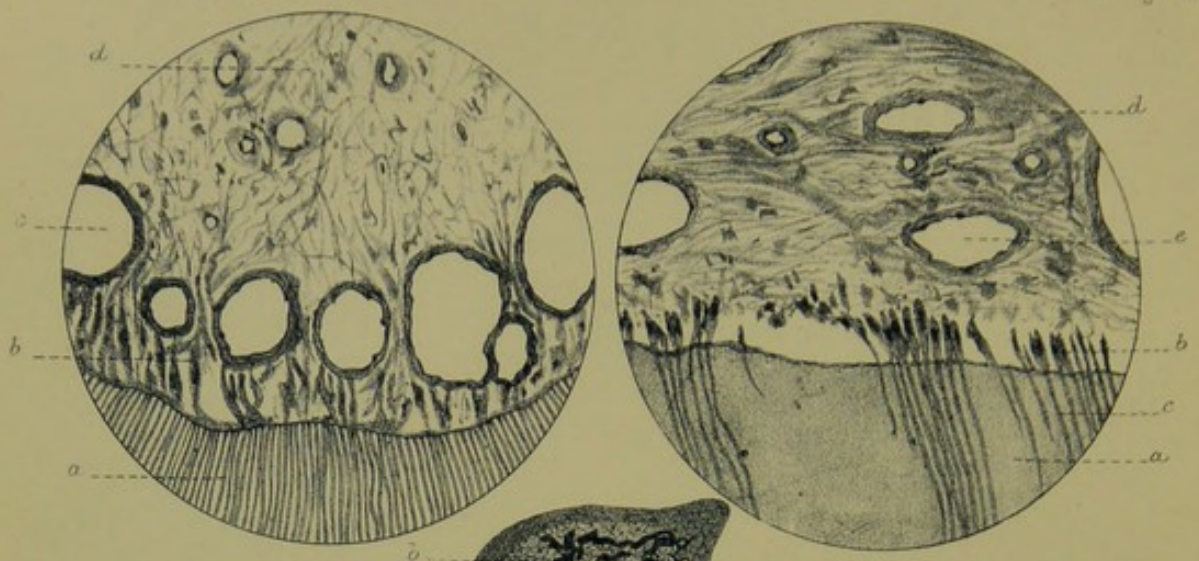
Fig. 6.—Transverse section of cementum, somewhat hyperplasic ; ground down ; stained methylene blue ; $\frac{1}{6}$-inch and A ocular ; shows (*a*) structureless outer portion ; (*b*) lacunæ ; (*c*) canaliculi ; (*d*) dentinal portion with termination of tubules ; (*e*) lamellæ of cementum.

Fig. 7.—Section of alveolus of jaw, dense cancellous bone ; soft parts hardened and section ground down (Caush's method) ; stained carmine ; shows (*a*) lacunæ containing cells ; (*b*) canaliculi ; (*c*) spaces in cancellous bone.

Fig. 1.

PLATE VII.

Fig. 2.



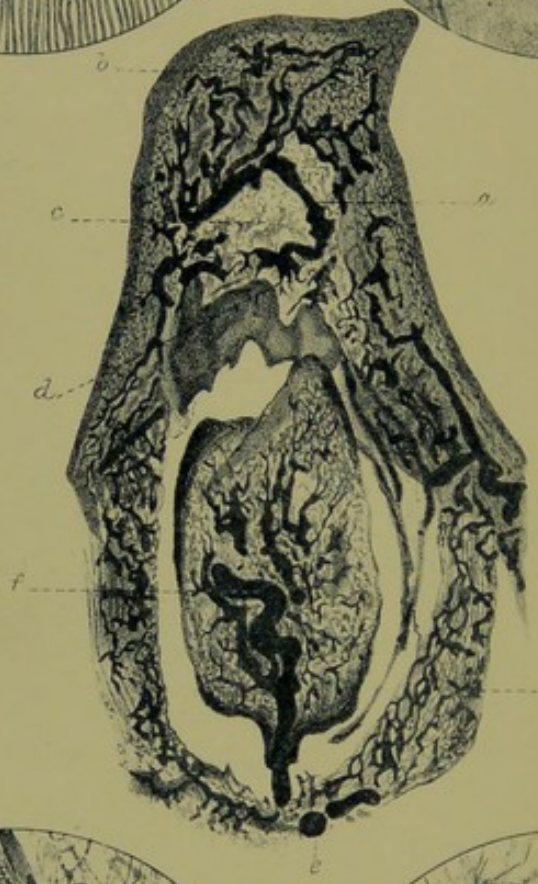
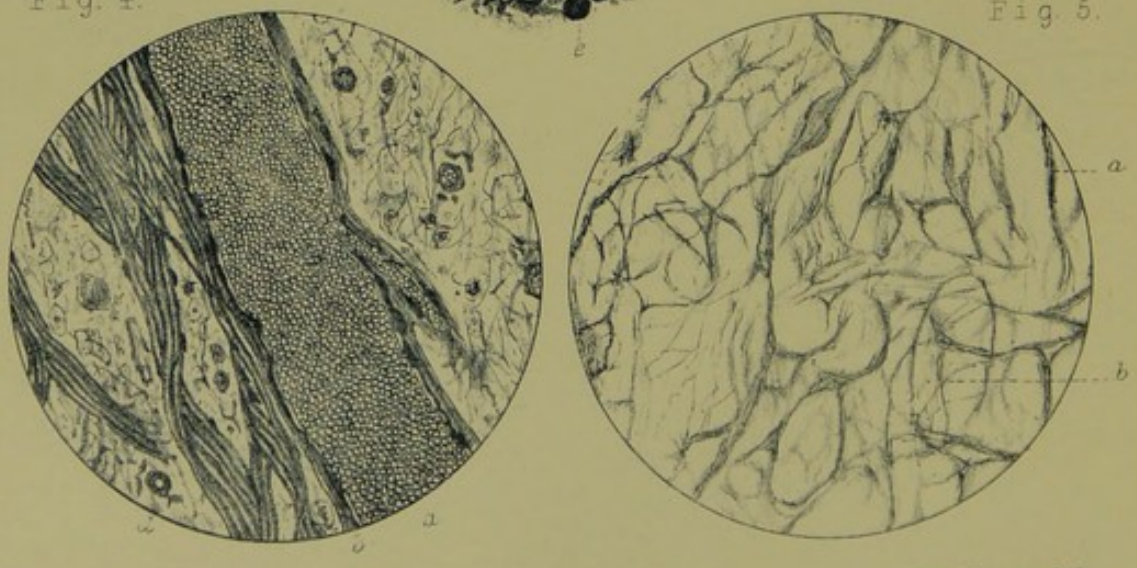


Fig. 3.

Fig. 4.

Fig. 5.



A. Hopewell Smith. del.

West, Newman. lith.

DESCRIPTION OF PLATE VII

Fig. 1.—Transverse section of the pulp of an adult bicuspid (human) at the broadest part of the extreme radicular region of the tooth; prepared by the Author's process; stained hæmatoxylene; $\frac{1}{6}$-inch objective and A ocular; shows (*a*) dentine; (*b*) the insignificant nature of the odontoblasts; (*c*) large capillaries at the periphery of the pulp; (*d*) pulp tissue.

Fig. 2.—Longitudinal section of the pulp of an adult canine (human) at the apex of the radicular region; Author's process; stained rubine; $\frac{1}{6}$-inch and A ocular; shows (*a*) dentine; (*b*) insignificant odontoblasts; (*c*) tubules corresponding in direction with the long axes of the odontoblasts and coincidence of absence of both; (*d*) pulp tissue; (*e*) capillary, obliquely cut.

Fig. 3.—Vertical section of the mandible of a fœtal kitten, injected to show the vascular supply, hardened in Müller's fluid and alcohol; general tissues stained carmine, vessels injected with Prussian blue; 2-inch and A ocular; shows (*a*) external set of capillaries, supplying: (*b*) gum, (*c*) enamel organ, and (*d*) ameloblasts; (*e*) internal set, supplying: (*f*) pulp, and (*g*) various periodontal tissues.

Fig. 4.—Longitudinal section of the dental pulp, hardened in Müller's fluid; stained chloride of gold; $\frac{1}{6}$-inch and A ocular; shows (*a*) capillary filled with blood corpuscles; (*b*) capillary wall; (*c*) bundles of nerve fibres; (*d*) pulp tissue.

Fig. 5.—Fibrous stroma of pulp from temporary tooth of a monkey; hardened in perchloride of mercury and alcohol; stained carmine; $\frac{1}{6}$-inch and A ocular; shows (*a*) large fibres; (*b*) delicate reticulum of fine 'supporting fibres.'

PLATE VIII.

Fig. 1.

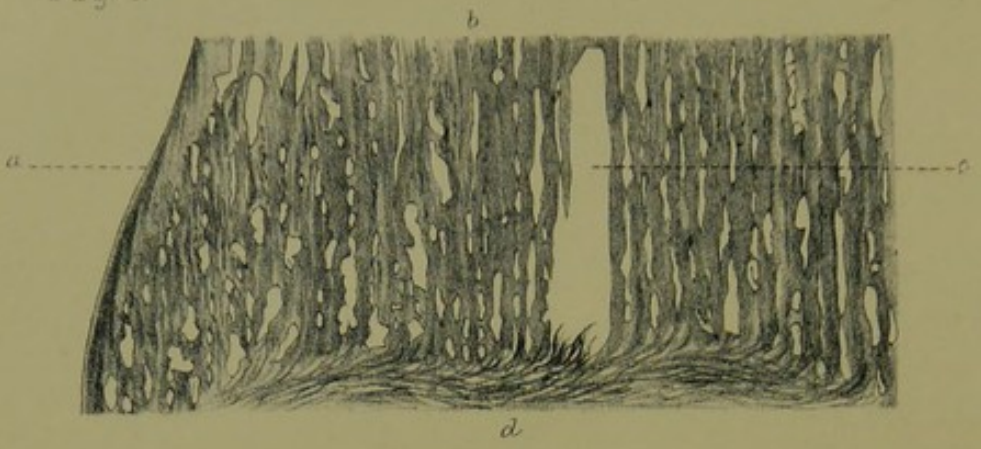
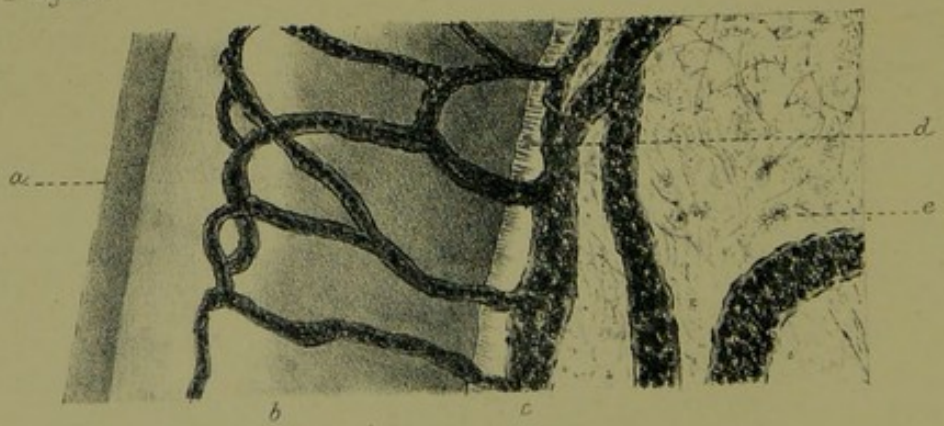


Fig. 2.

Fig. 3.



A. Hopewell Smith del.

West, Newman lith.

DESCRIPTION OF PLATE VIII

Fig. 1.—This and the accompanying figures are intended to show three of the chief varieties of dentine. *Osteo-dentine.*—Longitudinal section of an anchylosed tooth of the pike (*Esox lucius*); decalcified; stained carmine; 1-inch objective and A ocular; shows (*a*) free surface of dentine; (*b*) osteo-dentine; (*c*) inter-trabecular spaces; (*d*) bone of attachment.

Fig. 2.—*Plici-dentine.*—Transverse section of a tooth of the basking shark (*Selache maxima*); decalcified; stained carmine; 2-inch and A ocular; shows (*a*) folded free surface of tooth; (*b*) large tubes; (*c*) pulp chamber, soft tissues not retained; (*d*) plici-dentine.

Fig. 3.—*Vaso-dentine.*—Longitudinal section of a tooth of the hake (*Gadus merlucius*); prepared by the Author's process; stained picro-carmine; ¼-inch and A ocular; shows (*a*) free surface of tooth; (*b*) vaso-dentine; (*c*) naturally injected capillaries traversing canals in dentine (Tomes's thorn-like processes of canals not shown); (*d*) layer of connective tissue fibre bundles; (*e*) pulp tissue.

DENTAL MICROSCOPY

CHAPTER I

INTRODUCTORY

Introduction

THE non-existence of a treatise on Dental Microscopy may be safely advanced as an excuse for the Author's presentation of the following pages. He has been induced to give publicity to his work with the microscope in this special branch of science, practical instruction and hints in manipulation being his main object.

It is interesting to observe how rapidly this scientific dental work has of late years advanced, and how many of the younger members of the profession are taking it up. By the establishment of special classes for Dental Histology, at the various schools; by a much needed examination, in this subject, at the termination of the student's curriculum; and, by the wide fields of research still open to the original worker, it will be readily seen that we have to deal with a question of no mean importance. This fact is now recognised by the members of the British Dental Association, at whose Annual Meetings, papers, demonstrations, and discussions form an interesting and valuable feature.

Reasons for Publication

The Manuals of General Histology, which are

NOTE.—The drawings throughout this work are made, for the most part, from original photographs.

present day text-books, refer but very briefly to this special subject: mere outlines of practical work are, as a rule, only given, the demonstrators, at the schools, adopting in class their own methods of carrying out the principles there laid down. With a view, therefore, of helping dental students in their work, it is the writer's desire to place on record the details of those methods chiefly in vogue at the present time, which he has found of greatest use, thereby hoping to stimulate individual work at home, as an addition to that of the hospital practical class. The notes will be didactic, helpful, and instructive. No attempts will be made to teach Dental Histology; students must rely, for this purpose, on their own lecturers and text-books.

The Illustrations

These pages, however, are accompanied by original illustrations, drawn by the author from his own preparations, and instructions are given as to the methods recommended for making such specimens. The plates are thus intended to be a useful feature, and a help to the student. A short description will be found accompanying each figure, in order that he, who has prepared and mounted a section, may be able, under the microscope, to recognise its various structures, and seeing, learn and interpret its meaning. He will thus become familiar with his own sections, and, by comparison with those of others, quickly understand the broad facts of Dental Histology and Patho-histology.

General remarks

As with everything that requires for its proper performance the application of technical manipulative skill in addition to complete foreknowledge of the subject, so it is with Dental Microscopy. It is essentially made up of minutiæ; it is a matter of much detail; and the student must begin at the very beginning if he wishes to become a microscopist and histologist. Many difficulties will necessarily arise at first, but experience and practice will, in time, lead

the diligent inquirer to successful and gratifying achievements.

The simpler the apparatus he employs, the better; the less complicated the method he adopts, the truer the results.

The Microscope.—A simple form of compound microscope should be used at the commencement of histological studies, this being more suitable, firstly, because Instruments

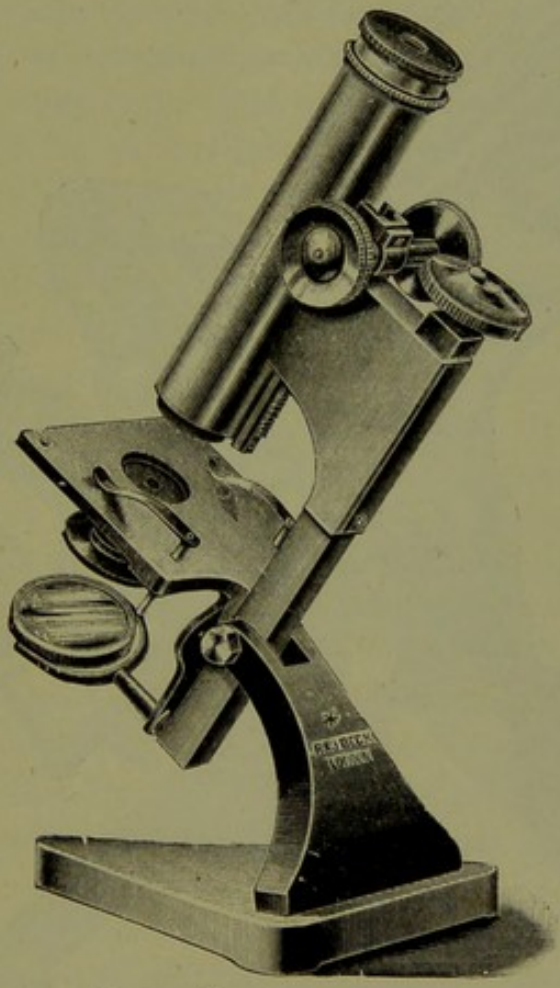


FIG. 1.—The 'Star' Microscope.

of the ease of management, and secondly, because it involves no great initial outlay. The shape or size of stand is immaterial; it should be heavy and quite firm: the objectives must, however, be good. These should be, for ordinary purposes, 1 inch and $\frac{1}{6}$ inch, which, with The Objectives

two eye-pieces—'A' and 'C' or 'D,' as they are usually called—give a magnification ranging variously from 35

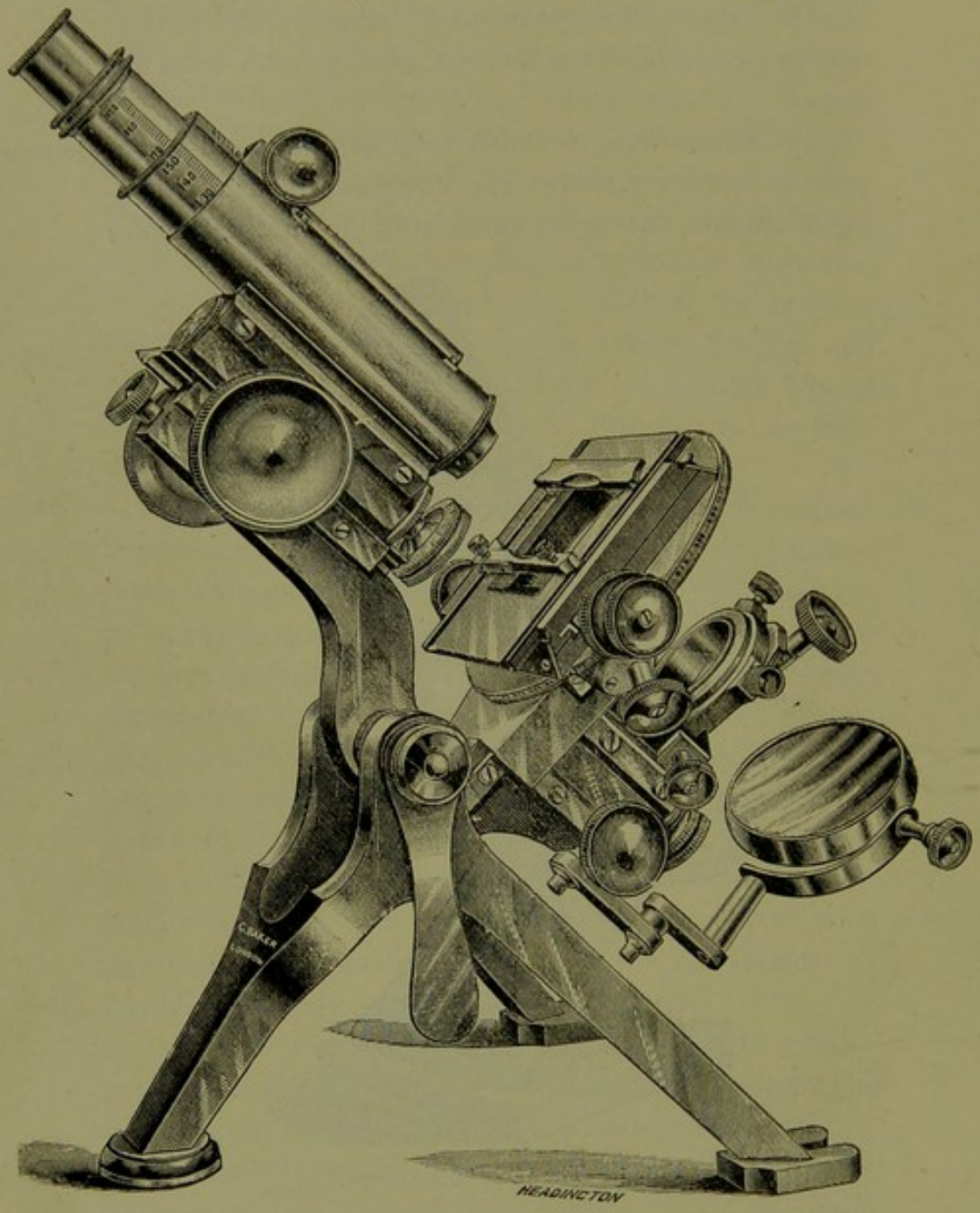


FIG. 2.—Shows a most useful form of Compound Microscope, with mechanical stage and sub-stage fittings. Such an instrument is capable of the highest critical bacteriological research work.

to 750 diameters. Later on, a $\frac{1}{12}$-inch water or oil immersion will be found indispensable for the examina-

tion of bacteria and for fine work generally. The microscope should be provided with rack and pinion, or 'coarse,' and screw, or 'fine' adjustments, and a reversible mirror. It is advisable to have a double nose-piece attached to the draw tube, much time and trouble being thus saved. This accessory is not, however, quite essential at first, and a mechanical stage need not be purchased until later. A sub-stage with focussing and swinging adjustments, an Abbé condenser, iris diaphragm, and bull's-eye condenser, on stand, make up the necessary primary equipments. The student should obtain catalogues of the various instrument makers and dealers, among whom may be mentioned Messrs. Baker of Holborn, Beck of Cornhill, Powell and Lealand of Euston Road, Steward of Strand, Swift of Tottenham Court Road, Watson of Holborn, the Dental Manufacturing Company of Lexington Street, London, Frazer of Edinburgh, and Parke of Birmingham. It is invidious to make distinctions, but the writer can specially recommend Beck's 'Star' microscope as being eminently suited, at first, for all the requirements of the student. It is a cheap form of instrument, but the objectives are certainly very good.

Makers of Microscopes

The various parts of a microscope can be more readily understood by reference to the accompanying drawing than by a long verbal description.

Use of Parts of Microscopes

The uses and actions of these parts are briefly as follow :—

The *Foot* supports the microscope, and keeps it rigid and fixed in one position. It should be heavy and firm, and the form shown in the drawing, known as the Jackson, is the best, for it allows the instrument to be placed in a horizontal position without losing its firmness and support.

Foot

The *Limb* is very solid. It sometimes carries, at the end furthest from the foot, the fine adjustment.

Limb

Body

The *Body* is the optical tube through which the objects are seen. It is provided at one end with the objective, and at the other with the *Draw Tube*, which accurately fits its interior. It is attached to the limb by means of a rack and pinion adjustment. Some cheap forms of microscope have no rack and pinion, but for convenience and usefulness this is really necessary.

Objectives and Eye-Pieces

The *Objectives* and *Eye-Pieces* are the magnifiers, and the magnification of the object depends on these

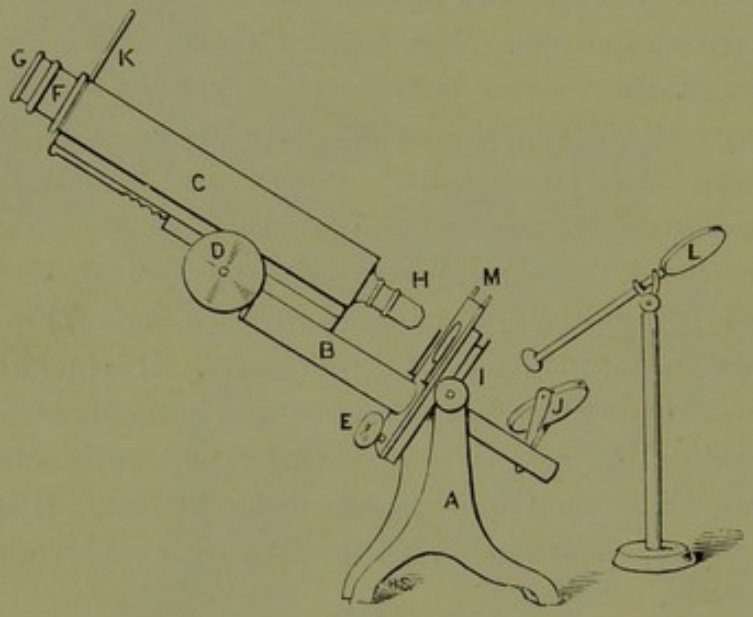


FIG. 3.—The Parts of a Microscope.

A. Foot or stand of microscope.
B. Limb.
C. Body.
D. Coarse adjustment.
E. Fine adjustment.
F. Draw tube.
G. Eye-piece or ocular.
H. Objective lens.
I. Sub-stage with Abbé condenser.
J. Reversible mirror carried by tail-piece.
K. Screen mentioned in text (moved aside).
L. Bull's-eye condenser.
M. The stage.

Their Magnifying Powers

only, and not on the size of the stand of the microscope, as is sometimes supposed. The powers of the former are expressed as 1 inch, $\frac{1}{6}$ inch, etc. These terms do not indicate the distance at which they focus on the object, but mean the actual magnifying power of the

objective. Thus, a 1-inch objective should have a power of 10 diameters, $\frac{1}{6}$-inch 60, and so on. The ocular again amplifies the image formed by the objective, 3, 5, or more times.

The Adjustments

The *Coarse Adjustment* raises and lowers the body, and is required for focussing with the lower powers. The *Fine Adjustment* is to be used with higher powers for very delicately focussing. The *Sub-Stage* carries the illuminating apparatus, Abbé condenser, and diaphragms for concentrating the light on to the object. The function of the diaphragms is to cut off circumfluent rays of light, and make the image well defined and bright.

Sub-Stage and Diaphragms

Mirrors

The *Plane Mirror* is used with the condenser. The *Concave* is employed when a maximum amount of light is required, and for high and low powers by lamplight as well as daylight.

Stage

The *Stage* holds the glass slide in position by means of clips.

Condenser

The *Bull's-Eye Condenser* is necessary for condensing light on opaque objects, and for photo-micrography.

Screen

A useful adjunct to the microscope is a home-made *Screen* for protecting the eye. It should be a dull black piece of cardboard, 2 inches by $4\frac{3}{4}$, in which a circle has been made towards one end, to slip over the draw tube. When in position (see fig. 3), the unoccupied eye can remain open, and much comfort, with no fatigue of vision, will thus be assured. The dark background renders the image, through the microscope lens, free from all extraneous objects lying on the table top. Both eyes remain open, one of course being used for viewing the object.

Apparatus required

The room for work should be well lighted, and should contain a firm table and cupboard.

Guy's Hospital List

Appended is a list of instruments suggested by the Histology Demonstrator (Mr. A. E. Baker) at Guy's

Useful Instruments

Hospital Dental School, and used by the students in that Institution :—

A COMPOUND MICROSCOPE.—NOTE.—The instrument should have a firm stand, and be fitted with rack and pinion, coarse and fine adjustments, and an 'Indicator.'

The OBJECTIVES should be well 'corrected,' and have good penetrating power, with flatness of field.

A double NOSE-PIECE (fig. 4).

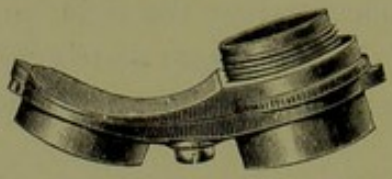


FIG. 4.—Double nose-piece for quickly changing objectives of varying powers. A triple nose-piece is often employed.

PINE-WOOD BOX to hold six dozen slides, lying flat.

Six dozen or more GLASS SLIPS, 3 × 1 inch, ground edges.

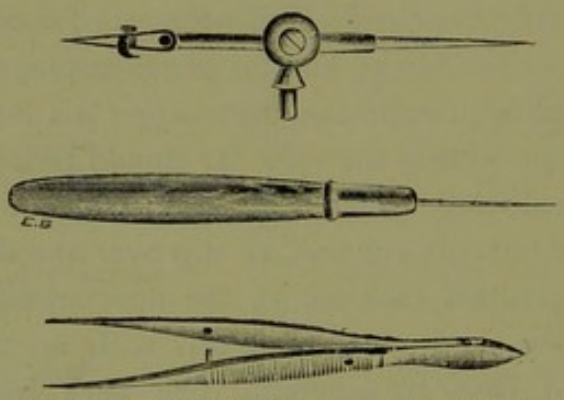


FIG. 5.—Mounted needle and forceps. The upper figure represents an instrument which can be attached to the microscope stage, and consists of a pointer at one end and a pair of stage forceps at the other.

Half-ounce thin COVER-GLASSES, $\frac{3}{4}$-inch diameter, circular.

One-eighth ounce thin COVER-GLASSES, $1\frac{1}{4} \times \frac{3}{4}$ inch, oval or oblong.

FORCEPS and needles, set in handles (fig. 5).

Useful Instruments

Camels'-hair BRUSHES, two large and two small.
Small pair of SCISSORS, two section LIFTERS.
Three sizes glass CAPSULES, with glass lids.
Small glass FUNNEL and filter papers.
Half-dozen deep WATCH GLASSES.
HOT TABLE, 6 in. high, the top 4 in. square.
Small SPIRIT LAMP, to go under same.
Half-dozen CLIPS (fig. 6).

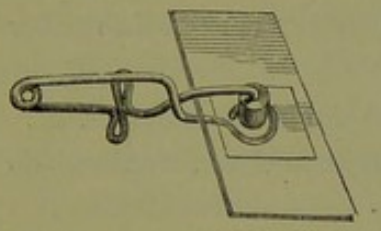


FIG. 6.—Brass clip for keeping cover-glass flat after mounting.

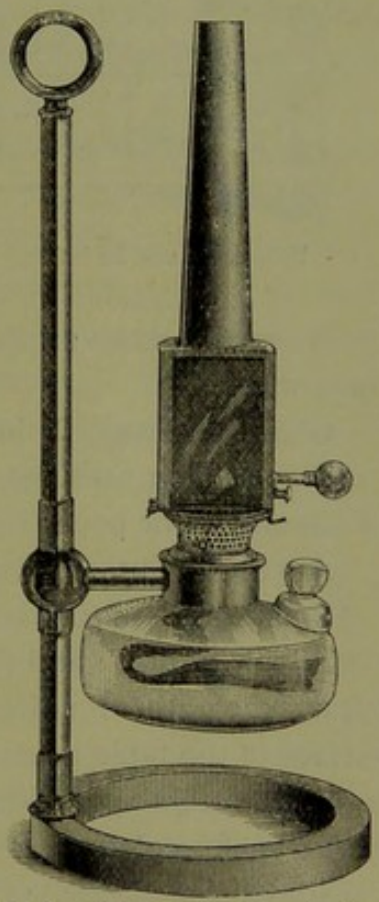


FIG. 7.—Oil lamp for illuminating the object.

An Arkansas and an oil STONE.
One box of neat square LABELS.
A silk HANDKERCHIEF.

Additions to List

The above are those instruments generally used in class work. In addition, may be mentioned for private use :—

A good microscope LAMP (fig. 7).

An ether-freezing MICROTOME (fig. 15).

Two or three large STONE JARS, corked.

Stoppered glass BOTTLES of 2 oz., 4 oz., and 8 oz. capacities.

A RACK containing half a dozen Wolrab gold cylinder bottles (fig. 8).

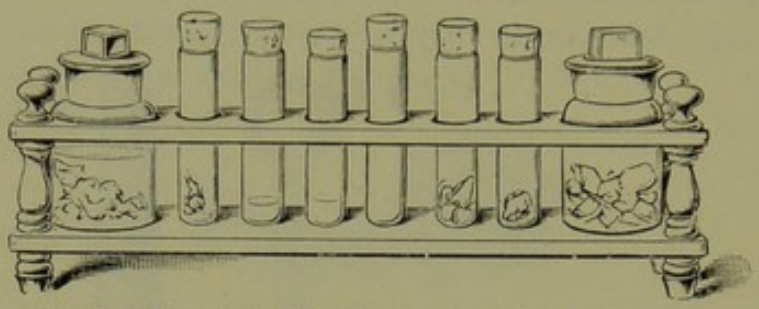


FIG. 8.—Rack for holding bottles containing specimens, &c.

A small glass PIPETTE, fitted with india-rubber suction tube.

Glass MEASURE, divided into cubic centimetres.

Two section LIFTERS, one large and one small, made of aluminium or bone (for using with acid solutions).

A TURN-TABLE for 'ringing' slides (fig. 24).

Small dissecting FORCEPS and SCALPEL.

A pair of black and white GLASS SLABS or porcelain TILES, separate or united, loose or let into the upper surface of the table near its edge; the former for facilitating the staining and mounting of unstained sections, the latter for coloured preparations.

Finally, a NOTE BOOK, unruled, and a supply of pencils and coloured chalks or water-colours, for making drawings of sections.

About Slides

The slides should be as thin and flat as possible, have rounded edges, and be free from scratches.

About Cover-Glasses

The cover-glasses should be the thinnest (·004) procurable, and measure in diameter not less than 21 mm. Circles are more useful than squares, except in a few cases, because they are easily 'rung' after

the section has been mounted. They should be kept in cotton wool in a small box, and wiped carefully with a silk handkerchief, flat on the table, or between the fingers. Some workers prefer to keep them in a jar of water acidulated with nitric acid.

Cover-glasses may be *chemically* cleaned by a method given in Chapter VIII.

The objectives and eye-pieces should always be cleaned by means of a small piece of soft wash-leather, both before and after using, and the microscope kept, when not wanted, under a glass shade.

The nose-piece is a useful addition to the microscope. It is better for the student to purchase one made of aluminium rather than brass. For, after some years, it will be found that the fine adjustment gets a little out of order, in consequence of the extra weight on the body and draw-tube of the instrument.

Method of using the Microscope

To those students who are quite beginners and have never handled a microscope, the following remarks may be of service :—

The Question of Light

The instrument having been placed on a firm table, near a window facing N. or W. in the morning, S. or E. in the afternoon, out of the rays of direct sunlight, should be tilted to a convenient angle, the student being seated. The 1-inch objective, and 'A' or No. 1 ocular in place, and the specimen fixed firmly on the stage by means of the clips, the concave mirror is now to be moved in such a manner that a flood of light passes through the aperture of the stage, and illuminates equally, in all parts, the section to be examined. It is advisable to make sure of this by looking at the stage and section, not through the microscope tube at all, but at the side of the instrument. No diaphragm is to be used for the low powers. The coarse adjustment must now be carefully screwed up or down, as the case may be, until the specimen is seen to be in focus. If this has been done

as just described, the field of vision will be equally bright and clear, and the image sharp, but reversed.

Examination of Specimen

Every part of the section should be examined, by moving the slide about, using for this purpose the fingers of both hands to steady it. With Beck's 1-inch objective and 'A' ocular, thus, the magnification—an important point in histological work—will be about 35, and if 'C' ocular is substituted for the lower power, the magnification equals 90 diameters.

To get a higher Magnification

If it is the wish of the observer to examine more minutely any particular part of the section, he should place that in the centre of the field. The $\frac{1}{6}$-inch objective is then moved or screwed into position, after having first racked up the coarse adjustment as far as possible, to prevent all risk of breaking the cover-glass or damaging the lens. If 'A' ocular is used, and the concave changed for the plane mirror (care being again taken to equally illuminate the field of vision by means of the condenser and a small diaphragm), the coarse adjustment should bring the objective close to, but not touching the cover-glass, the fine adjustment being finally used for focussing. The magnification is now nearly 200. When 'A' ocular is removed and 'C' takes its place, the magnification exceeds 510. To get a still higher magnification, the draw-tube of the microscope may be extended one, two, or three inches. If it is thus lengthened, the section will be magnified 750 times.

Choice of Powers

The student, however, should be satisfied with using only, for many weeks, the low-power objective and eye-piece. Later on he can combine them with the higher powers, and produce the various magnifications obtainable.

Low Powers

That the employment of low powers for ordinary work is the best, may be well conceived, when it is remembered that a clear steady illumination is produced by simple means, that the precision of the focal adjust-

ment is not of the greatest consequence, and that the tendency towards errors of refraction of light is considerably lessened. A large field of vision is presented, and the position that cells and other structures occupy, with regard to the surrounding tissues, their relative size and number, and general characteristics, are all best observed under a low amplification.

High Powers

With high powers, the minute anatomy of the tissues —the markings of cells, structure of their walls, etc.—is brought out; but, *per contra*, the object is less defined, the illumination considerably reduced, and there is greater fear of spherical and chromatic aberration, leading to distortion and a false appearance of the image.

It will be some time before the beginner can manipulate the sub-stage condenser in a satisfactory manner. It is unnecessary to add a long account of it—experience in its use, as throughout microscopy, is by far the best instructor.

The above is the plan for viewing transparent objects by daylight or lamplight, opaque specimens being seldom, if ever, used in dental work.

Preliminary Exercise

The student having by this time become well acquainted with the various parts of the microscope, their functions and movements, should proceed to follow the good advice given by Schäfer in his 'Essentials of Histology.' He should make temporary slides of the common objects of his room, *e.g.* dust, fibres of cotton, silk, etc., and also mount a few air-bubbles. By so doing he will be spared much trouble, annoyance, and loss of time. But the golden rule in Practical Microscopy is to thoroughly learn to use the low powers before work with $\frac{1}{6}$ or $\frac{1}{12}$ inch objectives is attempted.

Practical Examples

As before mentioned, each chapter will contain the methods of preparing sections of histological specimens. If the instructions, there given, are carefully followed,

the student will, by using the objectives and oculars indicated, and by comparing the sections he has made, be enabled to see, on the stage of his own microscope, images almost identical with those figured on the plates.

Comparison of Sections with Figures

Thus, a human tooth, having been prepared according to the plans detailed in the following chapter, is mounted and examined with $\frac{1}{6}$-inch objective and 'A' eye-piece. The student will observe, through his own instrument, appearances similar to those found in plate I., fig. 1. He should compare the drawing with the actual preparation, and, although the former will generally be somewhat diagrammatic—for the sake of clearness—he will soon be able to readily recognise the most prominent features of his own slide. In connection with each figure, the methods of preparation, the stain, and the magnifying powers used are mentioned, and the salient histological structure of the section brought out.

CHAPTER II

ON THE PREPARATION OF SECTIONS OF THE HARD TISSUES

Aim of the Student

AT the outset of practical Dental Microscopy, it must be clearly understood by the student that, having mastered all the details of the management of a microscope, it is of the highest importance, in the preparation of tissues for histological purposes, to treat his specimens, both chemically and mechanically, in such a manner that their structures will be altered as little as possible. It is a very great question whether or not one ever sees through the microscope tissues which are not somewhat changed in appearance, either by swelling or shrinkage. The student's aim, therefore, is to prepare sections free from these defects, and the employment of fresh materials, reagents, and reliable processes cannot be too strongly insisted upon.

GENERAL PRINCIPLES

There are two chief principles of treatment to be adopted when preparing the hard dental tissues for microscopical examination and research, and each, in its own way, answers the purpose admirably. These are:—(*a*) RUBBING or GRINDING DOWN the tissue in its hard calcified state; (*b*) CUTTING BY MICROTOME after decalcification.

A

Grinding down Hard Tissues:

The former is the older and more general method. For many years it has been a favourite with histo-

Advantages and Disadvantages

logists, but it possesses several serious drawbacks. Broken-down detritus, dust, and air get into and are retained in the interspaces, and though the sections show degrees of contrast well, they are very much spoilt if extraneous matters are also present. It is difficult, too, to get a portion of tooth thin enough for examination with the higher powers. Very often pieces of the part to be preserved break in a most disappointing fashion at the last moment, and the work of hours is thereby rendered useless. Ground-down sections take stains badly, as a rule—that is to say, the colouring matter penetrates the parts somewhat imperfectly. Only two or three specimens can be obtained from one tooth, and sometimes it is necessary, when investigating matters, to make sections from a single specimen, in transverse, horizontal, and in vertical directions. This can easily be done if the tissue be decalcified, but only with very great difficulty if the simple grinding-down process is adopted. It must also be remembered that such mechanical manipulations are at times tedious, irksome, and lengthy. Still, the method of grinding-down must not be considered by any means obsolete. The structure of the hard parts is well retained and exhibited.

Various Methods

The following are the chief methods employed:—

(1) *Grinding-down Sections of Hard Tissues on a Wheel*

Charters White's Method modified

The tissue is held by means of the finger tips on the right-hand side of a vertical, rapidly revolving, sharp, dry corundum or carborundum wheel, until a perfectly flat surface results. Great care must be taken, for, if the wheel revolves too rapidly, the tooth will probably be cracked or broken. Much heat and a smell of burning dentine are evolved, which make it rather unpleasant. The flat surface is now rubbed down on a dry Arkansas stone, and finally, to remove

The Dry Method

all scratches, polished on a razor strop, also used dry. The finished side is then fastened on to a piece of thick glass—a microscope slide will do—with hard Canada balsam, which has been previously warmed and softened over a spirit lamp. After the balsam has cooled a little, the polished tooth surface is pressed into it, flat on to the glass, and allowed to remain there. When the balsam is set hard, which, in practice, may take some days, the exposed surface of tooth may be ground and polished in the same way. The tooth is then ready for mounting.[1] Soluble glass (silicate of soda) with hydrate of sodium may be used, and is even preferable to Canada balsam for fastening the specimen on the slide. A drop is placed on a slide, and the polished surface of the section pressed on to it. In a few hours the slice of tooth is quite firmly fixed, and grinding and polishing can be proceeded with at once. Thus much saving of time is effected. Care must be taken to avoid wetting the specimen, as water dissolves soluble glass. Resin and wax (3 to 1) may be also substituted for the above media. It will be noticed that everything here is done in a dry condition, and a mere skeleton of the tooth remains.

Mr. Charters White, who originally introduced this plan in 1885,[2] recommended the use of a wet buff leather, with putty powder sprinkled on it, but the above-mentioned method is perhaps better.

Alpmêr stone—a very fine brownish stone—is excellent for rubbing-down and finally polishing hard sections.

(2) *Rubbing-down between Plates of Glass*

The Wet Method

Slices of the hard portions of a tooth are cut under dripping water with a fine fret-saw. If it is sharp it easily cuts dentine. The enamel, being extremely hard,

[1] The methods of mounting will be found on page 103

[2] Transactions of British Dental Association, Annual Meeting, 1885.

will require notching previously with a very thin corundum wheel.

If it is not desired to make more than one section from the same specimen (*e.g.* the lower jaw of a rat with teeth *in situ*), it saves time to do the preliminary grinding under water, on a corundum wheel. In this case the tissue need not be fixed by balsam on to glass, but should be ground down on both sides, the fingers holding it on the wheel.

Place a slice of the specimen between two plates of ground glass, their dull sides meeting. It is convenient to have the lower plate the larger of the two, 18–20 inches square. The upper should be 10–15 inches square. On the top of the tooth pour a little water, and add a small amount of the finest pumice powder. With a rotary movement of the upper glass, rub down the section till it is thin. Towards the end of the process, carefully watch the section through the upper glass, using no pumice, but plenty of water. Old and worn ground-glass plates are useful for polishing already thinned sections, and should be kept for this purpose. When the section is quite transparent, remove it from the glass carefully with the fingers, or a pair of fine forceps, wash in water, and then place it in a bottle of alcohol, till the time has come to mount it.[1]

The following modifications have recently been introduced :—

Dunkerley's Method

Mr. Dunkerley ('Journal Royal Microscopical Society,' part 6, 1892) proceeds as follows :—

'Sections are cut off the tooth, by means of a thin copper disc, fitted to a dental lathe, and revolving in a trough containing water and fine corundum powder. The thin disc is now replaced by a thick one, with the same trough and contents. The sides of this disc are

[1] In connection with this, the student is referred to Mr. Charters White's work on 'The Microscope, and How to Use it,' 1893, pp. 38-42.

used as a lapidary's stone to grind these sections thinner, one side of which is next polished on Water of Ayr stone, under running water, this surface being afterwards secured to a glass slip by thick Canada balsam. The grinding of the section on the thick copper disc is now proceeded with, until the section is thin enough to see the structure; then proceed to polish this surface on the Water of Ayr stone, until all details are visible under the microscope, when, after careful washing, the section is mounted.'

The plan seems well adapted for cutting sections of specially large teeth, *e.g.* molars of horse, elephant, etc.

Andrew's Method

Mr. J. J. Andrew's Plan.—The tooth to be ground is cut into as fine a slice as possible, by the aid of small saws. It is then reduced in thickness by grinding on a corundum wheel on the lathe, a copious supply of water being allowed meanwhile to flow over the section. The finger presses it carefully against the wheel, until it is very nearly thin enough for microscopic examination. It is finally ground between a couple of fine soft hones, made of Hindostan or other stone of similar fineness of grit, finishing to remove scratches between two Arkansas hones. The section is then well washed, the water dried off between the folds of fine blotting paper, and mounted in Canada balsam.

Whittles's Lathe

Mr. Dencer Whittles recently exhibited a lathe, which he has devised, for carrying stones for grinding-down purposes. It is placed on end and mounted on a table. The wheels revolve horizontally, in this case, the motor power being produced by the lathe band passing over pulleys which are placed at right angles to the treadle wheel. This arrangement will prove very useful for the final grinding and polishing of sections that have been prepared by Weil's process.

B

Decalcification of the Hard Tissues

Decalcification of Hard Tissues: Advantages and Disadvantages

It has been the fashion of late to decry the use of acids for decalcifying purposes, but rapid softening, which the writer strongly upholds, seems quite satisfactory. It is true that one cannot tell the precise chemical actions that occur when an acid is brought into contact with dentine or enamel: it is true, that it appears to be destructive to the tissues, and, it must be confessed, changes them somewhat, but, given the immense advantages accruing from its use, it will be found, that, as a rule, more instruction can be gained from decalcified sections than from those ground or rubbed down. To briefly mention these advantages, it may be said that the sections are much thinner than those of non-decalcified teeth. Any number can be obtained from one and the same tooth. They stain well, and can be rendered very transparent. It is also more easy to cut them, while the trouble of preparation is reduced to a minimum.

Decalcifying Agents

There are many decalcifying fluids and acids now used in histological work, the best known being hydrochloric, nitric, picric, chromic, acetic, and arsenic acids. The prolonged immersion of a tooth in commercial glycerine softens and removes the lime salts; but it is not used for decalcifying purposes, unless combined with hydrochloric acid in the proportion of glycerine 95 and acid 5 parts. The mixture acts slowly, too slowly almost, but preserves specimens while it softens them. It possesses the merit that it does not seem to blur or destroy the structure of dentine or cementum.

These decalcifying agents are here enumerated in the order of their importance and usefulness in Dental Microscopy. Their strengths vary considerably: thus,

hydrochloric acid is most useful when made up to a 10 per cent. solution, picric—a cold saturated aqueous solution—and nitric and chromic, in 1 per cent. solutions. The last named acid is also particularly serviceable in a crystalline form. Strong solutions are recommended in preference to weak ones. It is a rule, with but few exceptions, that the after-effects of the acid should be neutralised by immersion in an alkaline fluid. A saturated solution of bicarbonate of soda, one drachm to one pint of water, is perhaps the most convenient.

Acids

All acid solutions must be perfectly fresh and kept in glass-stoppered bottles, wooden instruments and aluminium or plated section lifters being used in manipulating the tissues.

It is very advisable to employ a fixed quantity of acid solution in decalcifying teeth and bone. The writer finds that 12 c.c., or 4 fluid drachms, is a good quantity to use. The bottles used by Wolrab for packing gold cylinders will easily contain the amount.[1] This rule applies chiefly to the hydrochloric acid solutions. By using this fixed quantity, the exact strength is known, and the probable length of time required for softening can be readily ascertained. The bottle should bear a small label, on which are noted the kind of specimen, the name of the solution in which it is immersed, and the date of immersion, to be followed by another small label, giving details of times of changing the solutions.

But, after all, the proper strength and periods of time for immersion can best be learnt by experience, because they depend, not only on the size of the tissue, but on its quality and structure. No hard and fast rules can be laid down.

[1] This is a form of corked sample bottle.

Other Methods of Decalcification

Decalcifying Methods

Dr. Black, in 'Periosteum and Peridental Membrane,' speaks of using a 3 per cent. solution of nitric acid. He remarks: 'It has been found that the element of time is . . . . more important than the strength of the acid solution employed.'

Bödecker[1] has been able to make specimens of teeth provided with *all* the hard tissues, by first grinding fresh teeth on a corundum wheel, and then decalcifying the thin section, in a large quantity of a ½ per cent. solution of chromic acid, for one or two days. Dilute glycerine is the solvent.

Ebner's decalcifying fluid consists of:—

Hydrochloric acid . .	1 gramme
Sodium chloride . .	10 grammes
Water to . . .	100 c.c.

This is a useful formula, but great quantities of the solution must be used, and it should be quite fresh.[2]

Hart, of New York,[3] divides a tooth into several pieces, and immerses them, after they have been ground thin, in a 6 per cent. solution of glacial acetic acid. Here they remain for ten hours, and are subsequently treated in the ordinary way.

Kleinenberg's formula is:—

Saturated aqueous solution of picric acid	100 parts
Strong sulphuric acid	2 „
Filter, and add distilled water . . .	300 „

Lepkowski[4] suggests the subjoined method for preparing sections of dentine, which possesses the advantages of simultaneously softening and staining it.

[1] Heitzmann's 'Microscopical Morphology of the Animal Body,' 1884, p. 613.

[2] Crookshank's 'Practical Bacteriology,' 1890, p. 25.

[3] 'Dental Cosmos,' September, 1891.

[4] 'Journal British Dental Association,' Vol. XIV., p. 248.

Pieces of teeth, which should be no thicker than ½ mm., are placed in a solution made of pure formic acid 3 parts, and 1 per cent. aqueous solution of gold chloride, 6 parts. They remain here for twenty-four hours, are then removed, washed with distilled water, and placed in a mixture of gum arabic and glycerine for twenty-four hours. On removal, they are again washed in distilled water, then alcohol, and finally imbedded in celloidin or paraffin.

The following are good methods for the preparation of specimens for the

DEMONSTRATION OF SPECIAL HARD TISSUES

To show Enamel Fibres

Enamel Fibres of man can be prepared by immersing a tooth in 12 c.c. of a 10 per cent. solution of hydrochloric acid. At the end of thirty hours, on removal from the acid, the enamel will be quite soft. A small portion should be taken up with a needle, or brush, and placed on a slide. The mass should then be teased out with needle points, and a drop of normal salt solution placed on the top, and a cover-glass applied. The staining is effected by allowing a solution of carmine or rubine to run beneath the cover-glass by capillary attraction. Excess of stain can be removed by blotting paper, and further washed away by irrigation with water acidulated with 1 per cent. acetic acid, and in this or in salt solution the enamel prisms are mounted (plate I., fig. 2). It is important to 'ring' the cover-glass as soon afterwards as practicable.

Under the microscope, in longitudinal preparations:

OBSERVE.—The wavy outlines of the individual prisms, their faint striation, and the interprismatic cement substance.

Pinna

The shell of *Pinna* affords an excellent opportunity of studying, by analogy, the structure of enamel fibres.

Pinna

The prisms are clearly demonstrated if a section is ground down and mounted unstained. If staining is desired, a fragment of shell is ground thin, then decalcified in a 10 per cent. solution of hydrochloric acid, till all bubbles have disappeared, then washed, stained with carbol-fuchsine or nigrosine ; washed again, dehydrated, cleared with xylol and mounted in balsam. The interprismatic substance is coloured a beautiful magenta with the former stain.

OBSERVE. — The honeycomb appearance of the tissue in transverse, and the marked striation in longitudinal sections.

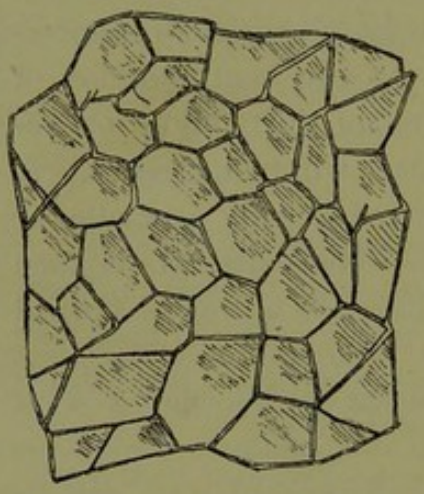


FIG. 9.—Transverse section of *Pinna* shell, ground down and unstained.

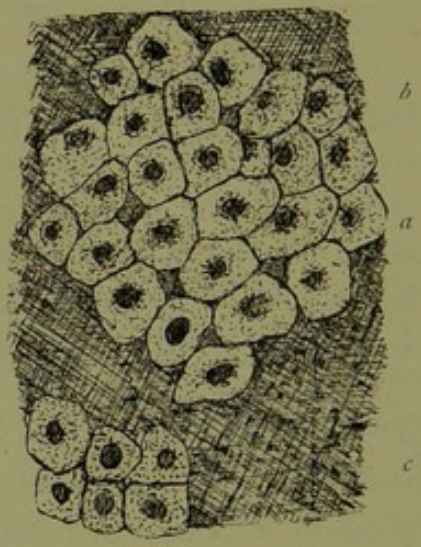


FIG. 10.—Nasmyth's membrane (Paul's method). *a*, Epithelial cell ; *b*, Matrix ; *c*, Impressions of Enamel Prisms.

Nasmyth's Membrane

Nasmyth's Membrane. — Immerse a permanent human tooth, by preference a newly erupted one (although the membrane remains over all, even old teeth), in a 10 per cent. solution of hydrochloric acid. In two and a half hours the membrane will begin to be separated from the surface of the enamel, and can soon be removed by means of a wooden needle-like point or brush. It should be washed in alkaline solu-

tion, then in water, and finally kept in rectified spirits of wine for further treatment (plate I., fig. 5). Sections of Nasmyth's membrane, *in situ*, may be obtained by first grinding down a tooth, as already described, placing it on a slide, and putting over it a cover-glass. If the hydrochloric acid solution be now run underneath the cover-glass, in a few hours the membrane will become detached from the free edge of enamel, and afterwards can be permanently mounted.

Another Method

Professor Paul's Method of Preparation. — This method of procedure is the best yet devised for bringing out the reticulated appearance of Nasmyth's membrane, and is to be recommended in addition to the methods previously described.

Paul's First Method

In a 2 per cent. solution of Bichromate of Ammonium are placed several fully developed unworn teeth of man, monkey, or sheep. They remain in this hardening fluid for a month, the fluid itself being constantly changed. After washing, they are transferred to two or three changes of alcohol, and then placed in a mixture of 5 per cent. nitric acid and weak spirit When the enamel is sufficiently decalcified, the tooth is immersed in water, and the membrane teased from off its surface with needle points. The membrane should be finally stained with eosine and mounted in Farrant's medium. In this manner its structure is rendered apparent, and the use of silver nitrate for staining purposes dispensed with.

Another, and still later method, devised by Professor Paul, is as follows :—

Paul's Second Method

A fresh unworn tooth is placed, immediately after extraction, for a few minutes in a mixture of

Phloroglucin	1 gramme.
Nitric acid (pure non-fuming) .	10 c.c.
to be added to	
Nitric acid (10 per cent. solution).	100 c.c.

When the membrane begins to separate from the enamel, the tooth is well washed, to get rid of the acid, stained in Ehrlich's acid hæmatoxylene, washed again, and placed in an aqueous solution of eosine. Lastly, the membrane is stripped from the tooth and mounted in Farrant's medium. The whole process occupies only a quarter of an hour.

OBSERVE.—Two layers, an outer, composed of large flat epithelial cells with large nuclei, and an inner thin translucent 'pellicle, usually marked with hexagonal impressions, derived from the ends of the enamel prisms.' Some of the cells have a cogged marginal outline.

Sheaths of Neumann

The Sheaths of Neumann (plate II., fig. 2).—Break a tooth into four or five pieces in a vice, and place the fragments in a 10 per cent. solution of hydrochloric acid. At the end of three days change the acid. At the end of five days, add 5 c.c. of pure nitric acid. At the end of seven days, remove the pieces from the solution. Transfer the soft mucoid deposit, which has collected at the bottom of the Wolrab bottle, to a glass slide, and tease it out with needle points, after a drop of distilled water has been placed over it. Press down a cover-glass, and stain with borax or lithium carmine, which should be allowed to run underneath, as first described.

Nigrosine or aniline blue-black may also be used as a stain. (Tomes.)

The sheaths are thus isolated, and very similar in appearance to yellow elastic connective tissue fibres.

Dentine and Cementum

To Show Dentine and Cementum.—(1) Take a newly extracted tooth (human) and place it in a solution of

Chromic acid (crystals) .	10 grains
Water	8 ounces.

It should remain here for two days, at the end of which time a fresh solution should be used.

(2) Then immerse it in a solution of

Chromic acid . . .	20 grains
Water	8 ounces

for four days.

(3) Finally, place the tooth in

Chromic acid	20 grains
Water	4 ounces
Hydrochloric acid ($2\frac{1}{2}$ per cent. sol.) .	4 ounces.

The latter should be added about ten minutes after the chromic acid solution is made.

(4) Remove the tissue to fresh solutions made up according to the last formula, every fourth day, until it is sufficiently soft (eleven or twelve days).

By using the chromic acid as mentioned, the advantages derived from the employment of fresh agents are assured.

Wash the tooth for twelve hours under running water, after it has been immersed for half an hour in an alkaline solution.

Another Method

Another and more usual method for efficiently showing the structure of enamel, dentine, and cementum, is to make a vertical section of an adult human tooth, by first grinding down on a wheel, and then between plates of glass, finally mounting the section, unstained, in Canada balsam.

If, however, such a section is stained after Golgi's method, its beauty and value are greatly enhanced. The dentinal tubules with their fibrils, the interglobular spaces and cemental lacunæ with their contents are coloured, and are thus well differentiated from the surrounding tissues. (See p. 95.)

In *Human Enamel*, which has been ground thin, and mounted unstained :

Human Enamel

OBSERVE.—Its great thickness over the cusps of the tooth; the cementum sometimes overlapping it at cervical region; the curvatures of the courses of the prisms, which are most marked over the masticating surfaces; the great numbers of supplemental prisms on the free surface; the faint striation of the prisms, very similar to that of striped muscle fibres; the brown striæ of Retzius running more or less parallel to the

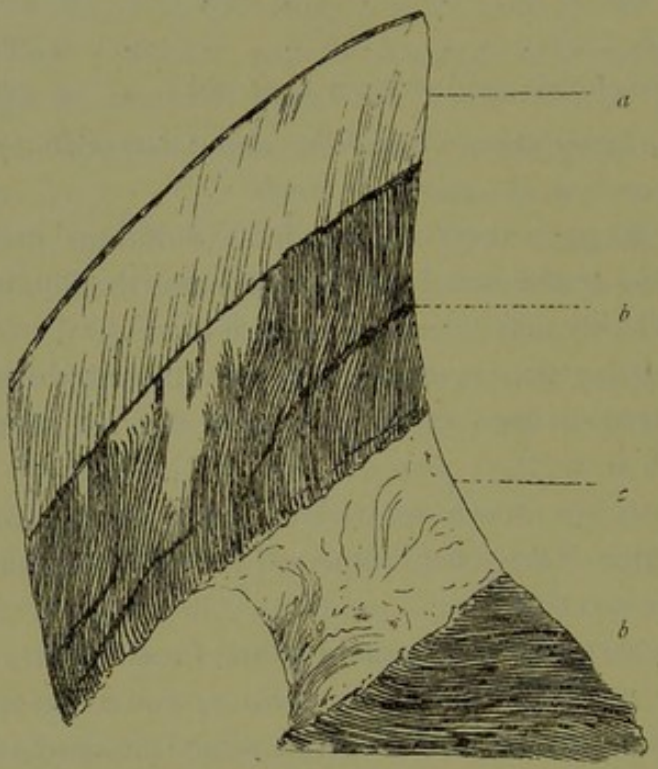


FIG. 11.—Section of Marsupial tooth (Kangaroo), from a preparation by Mr. C. S. Tomes. *a*, Tubular enamel; *b*, Dentine; *c*, Secondary dentine filling pulp chamber.

outer surface of the cusps; and, lastly, that curvature of the prisms known as Schreger's lines.

Enamel of Animals and Fish

In longitudinal sections of the enamel of *rat*, OBSERVE the pronounced striation of the prisms and occasional pigmentation of the tissue; of *manatee*, their straight courses; of *squirrel*, their crossings in inner $\frac{2}{3}$, and their bendings in outer $\frac{1}{3}$, at an angle of 45° with their original course; of *porcupine*, their serpentine

course and flexuous outlines. Notice the great variations in the structure of the enamel of fishes,—*Sargus*, for instance, possessing a regular system of tubes, in a manner similar to that found in marsupials.

Tubular Enamel

The structure of the latter is well brought out by a plan of preparation, first used by Mr. C. S. Tomes ('Philosoph. Trans. Roy. Soc. of London,' 1898, p. 109). Sections are ground down in the ordinary way until they are about 5 μ thick. Then they are 'slightly washed in acid,' and afterwards stained with nigrosine. The outer prismatic substance is acted on quickly by the acid and stain, and the prisms and tubes become remarkably pronounced.

In the *Dentine of Man*:

Human Dentine

OBSERVE.—The primary and secondary curves in the courses of the tubules, the first being more pronounced in the crown than in the root, and the latter seen best in the root; their branchings, comparison being made between those in the crown and those in the root, where the branches are very numerous and come off at right angles to the main trunk; the endings of the dentinal tubules either in (i.) fine points or tiny culs-de-sac; (ii.) loops to anastomose with their neighbours; (iii.) interglobular spaces; (iv.) canaliculi and lacunæ of cementum; and (v.) rarely in the substance of the enamel. Observe also the interglobular spaces beneath the enamel, with here and there tubules crossing them; the 'granular layer' beneath the cementum; the lines of Owen and Schreger. In stained decalcified transverse sections, notice the fibrils as dark dots, surrounded by the sheaths of Neumann.

Specimens of the other varieties of dentine may be most readily procured by using teeth of *pike* as typical of osteo-dentine, the rostral teeth of *Pristis* (saw-fish) of plici-dentine, and of *hake* of vaso-dentine.

Laminæ

Laminæ in Dentine can be demonstrated by adopting *Mr. F. J. Bennett's* glycerine methods.[1]

These are briefly the following:—

(*a*) Freshly extracted teeth are ground down until very thin. The sections are then polished, and suspended in pure glycerine, or glycerine and bicarbonate of soda, for one to six months. Then they are washed and mounted in glycerine; or,

(*b*) Freshly extracted teeth are immersed whole in pure glycerine for a similar period. Then ground down, polished, and mounted; or,

(*c*) Whole teeth are placed in a very diluted solution of glycerine, whose strength is daily increased until pure glycerine is used. Then they are kept in this for one or two months, and treated as in (*b*).

Cementum, etc.

The structure of *Cementum*, *Interglobular spaces*, *Striæ of Retzius*, *Owen's* and *Schreger's lines* are all best demonstrated by grinding-down and polishing, and, generally speaking, leaving the tissue unstained.

In *Human Cementum*:

OBSERVE.—The lacunæ, resembling those of bone, but more variable in shape, and in the great number and length of their canaliculi, which run towards the periphery of the tooth; the inter-cemental lines of Salter; the perforating fibres of Sharpey; and the structureless characters of thin cementum, the outermost layer of which has no lacunæ. Notice that the cementum of the temporary teeth is very thin, and contains few cells. 'Encapsuled lacunæ' or osteoblasts may be obtained from the cementum of the teeth of horse by careful decalcification.

Schreger's lines are well seen in fairly thick unstained sections of walrus's ivory, and also unstained longitudinal sections of ground-down human enamel.

[1] See 'Transactions, Odontological Society of Great Britain,' November, 1888.

Fishes' Teeth

Fishes' Teeth and Jaws of Animals can be prepared by either grinding-down or decalcifying them. Small teeth and jaws are difficult to grind down, and sections are therefore better prepared by immersion in acid, viz., either a 5 per cent. solution of chromic acid, or 10 per cent. of hydrochloric acid, and after washing and neutralisation, impregnation with gum or paraffin prior to their being cut on a microtome.

Table of Hard Tissues suitable for Preparation by

Grinding or Rubbing Down.	Decalcification.
1. Human teeth (adult), to show normal relationship of Hard Tissues.	1. Individual isolated Hard Tissues, *e.g.*, enamel prisms, sheaths of Neumann, etc., dentine affected by caries, etc.
2. Jaws of animals, *e.g.*, rat, monkey, etc.	2. Alveolus and bone of jaw.
3. Teeth of larger animals, horse, elephant, etc.	3. Jaws with temporary and permanent teeth, *in situ*.
4. Fossil teeth.	4. The teeth of fish, pike, hake, etc.
5. Certain pathological conditions of the Hard Tissues, *e.g.*, caries of dentine, exostosis of cementum, etc.	5. The teeth and jaws of small animals.
6. Cases in which it is necessary to retain the enamel.	6. Cases in which it is unnecessary to retain the enamel.
7. In connection with Weil's process.	7. In connection with the author's process.

CHAPTER III

ON THE PREPARATION OF SECTIONS OF THE SOFT TISSUES

THE difficulties attendant on the preparation of the soft dental tissues, for microscopical purposes, differ considerably from those discussed in the last chapter, and are of quite another nature. Here the great aim is to treat soft and delicate organs in such a manner that their individual cells, fibres, and other important elements shall undergo no appreciable change in shape or appearance, either by shrinkage, swelling, or *post-mortem* disintegration. It is obvious, therefore, that, in the first place, the tissues themselves must be perfectly fresh, and that the action of reagents on them must commence immediately after removal from their living condition; it is also clear that the simpler the method of preparation the better the results: the shorter the time spent on preliminaries, *cæteris paribus*, the truer and more faithful to nature will be the specimens under the microscope. It would be impossible, with a razor or knife, to satisfactorily cut a piece of soft tissue, the parts of which had not previously been treated and properly prepared; the friable cells and fibres would, in that case, be broken up and hopelessly destroyed by the movements of the cutter alone. Hence, various reagents have to be employed.

To prevent Shrinkage, &c.

The first steps are concerned with the performance

of what are known as the processes of fixing and hardening the tissues. These generally occur simultaneously, but sometimes it is necessary first to fix, and afterwards to harden, the specimen. This is done (*e.g.*, in Weil's method) where mercury perchloride is used for fixing the soft parts, and alcohol for hardening them.

REAGENTS AND THEIR USES

Fixing

Fixing the Tissues.—This means the coagulation of the albuminoids and gelatins of a living tissue, which occurs when it is brought into contact with a certain solution. The coagulating action fixes the delicate elements in the same position that they occupied during life. Many reagents may be used for this purpose, but of these the most serviceable for dental sections are mercury, osmic acid, and copper. The first mentioned may be used either as a cold concentrated alcoholic solution of the perchloride, or a 5 per cent. aqueous solution; osmic acid, a 1 per cent. aqueous solution; and, third, a saturated aqueous solution of acetate of copper. Alcohol in varying strengths is also an extremely valuable fixing agent. For mere fixing purposes, mercury is the best, although it leaves behind a troublesome precipitate, which requires removal by iodine. The disadvantages of employing osmic acid are that it does not penetrate well (because only the external portion of the tissue is affected), that it stains, fixes, and hardens simultaneously, and that the operations have to be conducted in the dark.

Whatever agent has been used, it is most important to remove it thoroughly from the tissues by immersion in alcohol, after complete fixation has taken place.

Hardening

The '*Hardening*' of a piece of tissue has for its object the prevention of swelling or other changes in cells when placed in water or the staining solutions; and

it gives such great consistency to specimens that the thinnest possible sections can be cut and be easily and safely manipulated.

The chief 'hardening' reagents are Müller's fluid, formalin, alcohol, picric acid, and picric with sulphuric acid when made up according to *Fol's* formula. Chromic acid, too, is sometimes used, alone or combined with osmic acid. *Dr. Weil*, of Munich, in his 'Histologie der Zahn-Pulpa,' recommends as a useful fluid—a 1 per cent. solution of chromic acid, 100 c.c., with osmic acid (2 per cent. solution), 24 c.c., to which are added, afterwards, 6 c.c. of iced vinegar. 'The tissue is then ready in a week.' *Dr. Sudduth* considers that chromic acid alone is best. He uses a 1 per cent. solution, made by adding 30 grains of chromic acid to a quart of water.[1]

Weil's Hardening Fluid

Sudduth's System

Of all these, the most useful and satisfactory for ordinary dental purposes are Müller's fluid, formalin, and alcohol, the former being especially suited for soft developmental tissues, as well as other structures, the latter for fully-grown specimens.

Müller's Fluid consists of potassium bichromate 2 parts, sodium sulphate 1 part, and distilled water 100 parts. The salts are generally sold mixed together as a reddish crystalline powder in bottles, and instructions are given for making the solution. If the student, however, wishes to make the solution himself, this can be done by mixing together and thoroughly incorporating $3\frac{1}{2}$ drachms of potassium bichromate and $1\frac{1}{2}$ drachms of sodium sulphate, and placing in a jar containing 1 pint of distilled water. Many advantages may be claimed for this reagent: it possesses great penetrating power, does not cause shrinkage of the cells or fibrous tissues, and hardens uniformly. Sections are easy to manipulate, and are,

Müller's Fluid

Its Composition

Advantages

[1] 'Dental Cosmos,' 1884.

as a rule, not brittle. Its sole disadvantage is that it slowly tinges the specimen a yellow colour, but this is invisible in microscopical sections, and does not interfere with the staining. Its use is imperative if it is the student's intention to adopt for his sections Weigert's, Marchi's, Golgi's, or other special methods of staining. Müller's fluid may be sometimes usefully combined with methylated spirit, in the proportion of 3 of fluid to 1 of spirit. Specimens to be hardened must be cut into small pieces and placed in the fluid, which should be contained in large well-stoppered bottles or jars, after they have been washed for a few minutes in normal salt solution (common salt ·75 parts, and water 100 parts). The washing rids them of blood and other extraneous matters. About 20 times the bulk of Müller's fluid must be employed for each specimen. It must be changed on the second and fourth days, and then at the end of every week, the bottle or jar meanwhile being kept in a cool place. After a fortnight or three weeks have elapsed, the tissue is transferred to a bottle containing methylated spirit or rectified spirits of wine, in order that the hardening process may be completed and the colouring matter removed.[1]

And Methods of Using

As a hardening agent for dental work, the use of *Formalin* has lately received considerable attention from histologists. Perhaps it is even superior, as a fixer and hardener of soft tissues, to Müller's fluid. Not only is it an ideal germicide, but it possesses properties of much greater value than the chromates. Formalin contains 40 per cent. of formic aldehyde. It is miscible with all proportions of water, and colourless, and, above all, very rapidly hardens the tissues which are brought into it, although there is a tendency to render them

Formic Aldehyde

[1] Some histologists prefer Erlicki's solution to Müller's fluid. Its constituents are the same as the latter, except that sulphate of copper is substituted for soda. It hardens a little more rapidly than Müller's, but seems to possess no additional merit.

brittle. It penetrates well, especially if mixed with other reagents. The writer uses a 40 per cent. solution, and has found that fresh small pulps (bicuspids, for instance) can be splendidly hardened *in situ* in 70 to 100 hours, and that it is unnecessary to further harden them in alcohol. It may be used as a 40 per cent. solution, but a 10 per cent. is very convenient. The capillaries retain their corpuscles, the shrinkage of the cells and fibrous tissues is reduced to a minimum, and altogether highly satisfactory results are attained.

Combined with chromium salts, formalin gives more rapid penetration still, whereby the time for hardening is further shortened *Freeborn*, of the Columbia University,[1] recommends a mixture of

Freeborn's Mixture

Potassium bichromate . .	2 grammes
Sodium sulphate . . .	25 grammes
Formalin (2 per cent. solution)	100 c.c.

Pulps at the end of fifty hours are ready for cutting.

Uses of Alcohol

Alcohol, in the form of methylated spirit or rectified spirits of wine, will be found of great service in dental microscopy for the hardening of structures and organs less delicate than embryos. It is particularly of use in many pathological cases. It does not stain the specimen, and hardens more rapidly than the bichromates. It causes a certain amount of shrinkage, and must not be used if blood-corpuscles are to be retained in the capillaries of a part. The quantity of spirit should exceed the bulk of the specimen by about ten times, and need not be changed until it becomes a little cloudy.

The ordinary methylated spirit contains mineral naphtha, and becomes turbid when mixed with water.[2] This form must, of course, be avoided, for valuable speci-

[1] 'New York Medical Journal,' 1896, p. 771.
[2] Squire, 'Methods and Formulæ,' 1892, p. 3.

mens may easily be ruined by it. The best methylated spirit, free from naphtha, may be obtained from wholesale houses.

Other 'hardening' reagents might be mentioned, but the above are all that are requisite for dental microscopy.

HISTOLOGICAL CLASSIFICATION OF SOFT TISSUES

The soft dental tissues may be conveniently classified as—

(i.) Developmental, including those found in the early or late ante-eruptive periods; and

(ii.) Completed or post-eruptive.

For both these groups of tissues, methods similar to those already described are to be followed; although modifications in imbedding and staining may be introduced, the general principles of fixing and hardening must be adhered to. Details as to cutting sections will be given later.

A

To Obtain and Prepare Specimens of the Soft Developmental Tissues

Treatment of Embryonic Specimens

Remove several embryos and fœtuses from the uterus of some animal, viz. pig, cat, dog, rabbit, which has been killed with a dose of bi-cyanide of mercury administered by the mouth, or, perhaps better, by bleeding from the common carotids and jugulars, after chloroform anæsthesia. Decapitate the embryos, and rapidly wash the heads in plenty of warm normal salt solution to remove all traces of blood. This should be done while the animal remains warm. It will be noticed that the heads will show various stages of growth.

If practicable, with all the fœtal heads, pass a sharp

scalpel into the temporo-mandibular articulation on each side, and cut right back. The lower jaw will then be removed *in toto*. Divide this in the middle line, and then with a razor subdivide it still further, vertically, into small portions.

Hardening

Each of these pieces is then again to be washed in salt solution, and immersed in Müller's fluid, freshly made.

The upper jaws may be treated in the same manner, care being taken not to cut away the base of the skull.

Imbedding

After hardening is completed and the jaws decolorised by a fortnight's immersion in constantly changed alcohol, the pieces are allowed to soak for an hour in distilled water ; they must then be transferred to a solution of gum mucilage prepared according to the formula of the British Pharmacopœia. It is advisable to add to this medium a little pure carbolic acid—10 drops to 1 ounce of mucilage. Here the pieces of tissue may remain for a lengthy period without deterioration. For sections so prepared see plate III.

For *very* early embryonic jaws, fixing and hardening with mercury and alcohol, and imbedding in celloidin or paraffin are distinctly indicated. As the jaws are too soft and small to be disarticulated, vertical sections should be made through the entire head.

The above remarks apply only to the earlier stages of developmental life, embryonic pigs, from one to six inches long, being the most suitable animals to obtain.

Fœtal Specimens

Jaws of animals *at birth* may be treated as above, with the addition of the following precautions :—The tissues covering the mandible, lips, cheeks, etc., must be carefully stripped off, leaving nothing save the oral epithelium and flange of gum. Great care must be exercised not to use undue pressure on the soft parts ; it is important that the scissors and scalpel should be very sharp. Best results can be obtained from vertical

sections of the canine and bicuspid regions; because here the cap of dentine and enamel which is being formed is very thin and but semi-calcified, and the movement of the microtome cutter or razor does not disturb the normal relations of the parts.

The jaws of kittens, rabbits, or pups at birth are most useful for this purpose.

Professor Stirling, in his 'Outlines of Practical Histology,' gives the following directions for preparing tissues to show the various stages of development of teeth:—

Stirling's suggestions

'What may be called the first stage is to be obtained from the mandible of a sheep's embryo about 7 c.m. in length. At this period only a very little bony matter exists. Harden the whole jaw in corrosive sublimate, and decalcify in dilute hydrochloric acid. Stain in bulk in borax-carmine, imbed in paraffin, and make transverse sections across both rami of the jaw and the tongue. Or harden and decalcify at the same time the jaw of a fœtal kitten by placing small pieces containing embryonic teeth in Flemming's fluid,' made thus:—

Flemming's Solution

'To 45 c.c. of 1 per cent. chromic acid, add 12 c.c. of 2 per cent. osmic acid, and then 3 c.c. of glacial acetic acid. It "fixes" the tissues in from a few hours to twenty-four hours or longer, but small pieces must be used. A weaker mixture may be employed with advantage to that just given. It contains:—

Osmic acid (1 per cent.) . .	10 c.c.
Glacial acetic acid (1 per cent.) .	10 c.c.
Chromic acid (1 per cent.) . .	25 c.c.
Water	55 c.c.

'The specimens must be thoroughly washed for twenty-four hours, and then hardened in 70, 80, and 90 per cent. alcohol each for twenty-four hours. Stain with

safranine, hæmatoxylene or methyl violet as soon as possible after sections have been cut in paraffin.

'The second stage is obtainable from the upper jaw of an embryo sheep 15 c.m. long. It is treated similarly.

'The third stage is obtained from the mandible of a dog from one to six days old or thereabouts. Treatment is identical with the above.'

B

Hardening

The Second Group of Tissues—including gum, pulp, periodontal membrane, etc., may be treated as above, or with alcohol. If the latter is used, the specimens must be placed successively in 30, 50, and 70 per cent. spirit in watch-glasses, with twenty-four hours' immersion for each. They can then be kept in rectified spirits (84 per cent.) until required for imbedding and cutting.

Other Methods

Campion's Method

Mr. G. G. Campion uses chiefly perchloride of mercury and spirit as a fixing and hardening reagent for the preparation of specimens of pulp.

In a communication to the author he describes his method as follows:—'Immediately after extraction, wrap the tooth in a duster or piece of rag, and crack it in the jaws of a strong vice so as to expose the pulp thoroughly, then drop it into a saturated aqueous solution of mercury bi-chloride. This *fixes* the tissue, killing the cells and other tissue elements, so that they are but little affected by the other reagents to which they may afterwards be subjected. The time required for fixing varies with the size of the pulp and the completeness of its exposure to the fluid. The process is completed when the tissue has become thoroughly whitened;

The use of Mercury

it may take from one to twelve hours. Use glass or wood instruments to manipulate specimens in the mercury solution, as iron or steel produces a precipitate which may injure the tissue. When fixed, it is necessary to remove the bi-chloride entirely from the tissue, and complete the hardening by successive strengths of alcohol. Both these processes can be carried on at the same time by dropping the tooth, after removal from the bi-chloride solution, first into a tube filled with 30 per cent. alcohol, to which a couple of drops of iodine liniment (or four or five drops of tincture) has been added, and then into a tube (Wolrab's bottle) containing 50 per cent. alcohol, in which three or four pea-sized lumps of iodide of potassium have been dissolved. Leave the specimen for twelve hours in each tube, and afterwards, for the same time, in successive tubes containing 70 per cent., 90 per cent., and absolute alcohol.

Its removal

'The iodine in the second tube throws down the mercury in the tissue as a red iodide, which is readily dissolved by the potassium iodide and alcohol in the third tube. After remaining for twelve hours in absolute alcohol, the pulp must be carefully removed from the broken tooth with a scalpel, and placed in a tube or small dish containing cedar oil, on the surface of which a little absolute alcohol has been gently poured. These two fluids do not easily mix, and when the pulp has sunk to the bottom of the cedar oil it is ready for imbedding in paraffin, according to Mr. Mummery's method. The above is a modification of the method of hardening used in the Weil process.'

Final treatment

Mr. Cyril Marson, in the 'Journal of the British Dental Association,' Vol. XIV., No. 7, recommends the following:—'A large bottleful of a solution of chromic acid in water, of a strength of $\frac{1}{6}$th per cent., must be made. For two or three specimens, two wineglassfuls of this and one of methylated spirit must be mixed

Marson's Method

in a bottle or jar. The specimens are left in this for twenty-four hours, and the bottle occasionally shaken; the liquid is now poured off and a fresh supply given. In this they must remain thirty-six hours; two more supplies of solution must be given, in each of which the specimens must remain forty-eight hours. After this they may be transferred to methylated spirit and kept until required. During this process the specimens ought to be kept in a cupboard or dark room. Should they contain any calcified teeth or bone, hydrochloric acid in the proportion of about five or six drops to the ounce must be added to the two last hardening solutions before placing in the methylated spirit.'

Rothmann's Methods

Dr. Rothmann,[1] of Buda Pesth, proceeds in the following manner, in the case of investigating diseases of the pulp and periosteum.

(*a*) Tooth is washed in distilled water, and placed in absolute alcohol.

(*b*) Periosteum is detached from cementum by a sharp gouge or chisel.

(*c*) Tooth is split with a chisel or forceps, and pulp carefully removed in its entirety.

(*d*) Pulp is then stained, imbedded in celloidin, hardened in alcohol, cut on a microtome, and mounted in balsam.

DEMONSTRATION OF SPECIAL SOFT TISSUES

Absorbent Organ

The Absorbent Organ can be obtained after the removal of a loose temporary molar, by snipping off with fine pointed scissors the soft tissue observed on the summit of the permanent tooth beneath. This is washed in salt solution for a moment, and placed in Müller's

[1] 'Patho-Histologie der Zahnpulpa und Wurzelhaut,' Stuttgart, 1889.

fluid for a week, then in alcohol for a fortnight. It should be imbedded in celloidin or paraffin.

Another Method

The cells of the absorbent organ *in situ* are best demonstrated by treating a temporary tooth by Weil's method (plate VI., fig. 4).

Under high powers:

OBSERVE.—The great numbers of the blood-vessels, and the soft connective tissue stroma in which they are imbedded; the large multi-nucleated giant cells situated in close apposition to the Howship's lacunæ which they have excavated.

Vascular Supply

The Blood Supply of developing teeth can be shown by the methods of injection of capillaries (see Chapter XI.); the jaws are then removed, hardened in Müller's fluid and alcohol, and imbedded in gum or celloidin (see plate VII., fig. 3).

Dental Follicle

To obtain specimens of the *Dental Follicle* and so-called '*Gubernaculum.*' Purchase the lower jaw of a young heifer which has just been killed, place it in a vice, and make vertical saw cuts between contiguous teeth in the incisor and canine regions: permanent teeth will be discovered enclosed in the follicle in the substance of the jaw. Cut slices from this soft tissue, wash, immerse in methylated spirit, and then transfer to rectified spirit for a month. Wash again and imbed in gum mucilage.

Dental Gum

The Dental Gum can be sectionised after similar treatment to that just mentioned. It is better to prepare specimens *in situ* by the author's process.

Methods for Obtaining Sections of the Dental Pulp

Dental Pulp, and its Chief Elements

(i.) Crack a tooth longitudinally in a vice or with excising forceps, or fracture it with a hammer, and gently remove the soft organ with a pair of fine forceps

or a needle-point. Treat it with Müller's fluid, formalin, or alcohol.

Nerves

(ii.) Sections can be quickly made, by immersing a removed pulp in a 1 per cent. solution of osmic acid, which hardens the tissue and stains the medullated nerve bundles. The organ should remain in the acid for twenty-four hours in the dark, or until such time that delicate dark lines—stained nerve fasciculi—are visible on its surface. Then wash with distilled water, and transfer to 70 per cent. alcohol till convenient to soak in gum solution.

The pulp nerves may be teased out by means of needle points, and then mounted at once, in normal salt solution. For a method of holding the needles, see fig. 12.

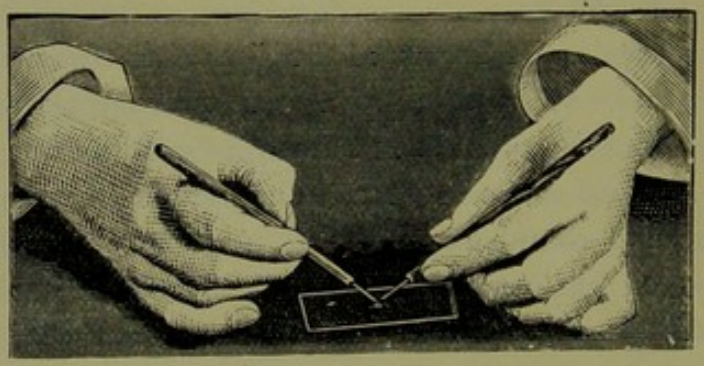


FIG. 12.—Method of holding the needles for teasing out specimens.

If the structure of the medullated nerves is to be demonstrated, it is more convenient to use the incisor pulps of the ox.

(iii.) Pulp sections are always better when cut *in situ*, either by Weil's or the author's special process.

Odontoblasts

Isolated odontoblasts may be well studied by (i.) teasing them from the cap of dentine which is being produced in developmental specimens. A section having been cut on the microtome, is placed on a glass slide and its parts carefully separated by means of needle-points. (ii.) Odontoblasts may also be obtained

by splitting a tooth in a vice, removing the pulp, and scraping them, with the point of a sharp knife, from the surface of the pulp cavity, to which they closely adhere.

The mass so obtained should be laid on a slide separated with needle-points, a drop of normal salt solution or glycerine jelly added, and a cover-glass applied and pressed down flat. They should be stained with solutions run under the cover-glass.

To demonstrate the long central processes of these cells, place an isolated dental pulp for 24 hours in a ·6 per cent. solution of potassium anhydro-chromate, and tease out in picro-carmine small fragments of its periphery.[1]

Much information of an interesting nature concerning the morphology of the *odontoblasts when observed in situ* may be gained by studying pulps of varying ages.

(i.) They are seen in their most active state in vertical sections made through the canine region of the mandible of pup at birth.

(ii.) In transverse sections of adult human teeth whose roots are only partially developed.

(iii.) In transverse sections of adult canine teeth taken through the cervical region.

In the last-mentioned instance the marked difference in shape is noticed, if the odontoblasts situated at the periphery of the long diameter of the pulp be compared with those in the short diameter.

Pulp Tissues

Prepare specimens of (i.) by hardening in Müller's fluid and alcohol, and cutting on a microtome; of (ii.) by Weil's process; of (iii.) by the author's process.

[1] Aitchison Robertson, 'Trans. Odonto.-Chirurg. Soc.,' Nov. 1892.

Table of Soft Tissues suitable for Preliminary Treatment with

Müller's Fluid and Alcohol, or Formalin.	Alcohol (84 per cent.).	Mercury Perchloride, and Alcohol.
1. Jaws of fœtal animals	1. Dental pulp, and periodontal membrane.	1. Dental pulp, *in situ*, in young teeth, with incompleted roots.
2. Dental pulp, and certain of its pathological conditions.	2. Soft tissues in connection with large animals' jaws and teeth.	2. Isolated dental pulp.
3. Periodontal membrane and its pathological conditions.	3. Dental gum.	3. Periodontal membrane.
4. In cases where special stainings are to be subsequently used.	4. Dental follicle when fully formed.	4. Cells of absorbent organ.
5. Absorbent organ isolated.		5. Very early embryonic jaws of animals.

CHAPTER IV

ON THE PREPARATION OF SECTIONS OF THE HARD AND SOFT TISSUES COMBINED

Retention of soft parts *in situ*

DENTAL histologists have of late years begun to recognise the importance of studying sections, where the soft parts are retained in their normal relationship to the harder tissues, with which they are so closely associated. Much time and labour have been spent in working out plans to attain this end; and, while considerable advancements have recently been made, one is still bound to admit that, of all the processes known for this special purpose, not one satisfies the critic in every particular. This is easily understood when the remarkable density of bone, dentine, and cementum, and the extreme delicacy of structures, like the pulp or periodontal membrane, are taken into account. Reagents applied for one purpose to a tissue react often on other tissues, and imperfect results are obtained.

There are, however, three chief methods of preparing specimens, the details of which, if carefully followed, lead to results which are approximately correct, and, as such, one must be content to adopt these plans untii some newer and more perfect suggestions are put forth. These are known as The chromic acid process, Weil's balsam process, and Hopewell Smith's process.

THE CHROMIC ACID PROCESS

For developmental Tissues

This, the oldest process, is one in which chromic acid is used in the dual capacity of a hardening and softening reagent. It is very slow in its action; but it serves the purpose well when the student desires to make sections of embryonic jaws, of developmental growths in which hard tissue is present, and of fully grown teeth *in situ*. Its details are as follow :—

The jaw—preferably the lower—of an animal, such as a cat, dog, or monkey, is removed while still fresh, and washed in normal salt solution. It is then subdivided into vertical pieces, and immersed in a solution containing—

Chromic acid . . .	45 grains
Nitric acid . . .	1½ drachms
Water	1 pint

Simultaneous Softening and Hardening

The fluid should be kept in a stoppered jar or bottle, and renewed at the end of the second day, a large quantity being used for a small piece of tissue. The latter presents, after some time, a green appearance, due to the formation of chromic sesquioxide; but this colouring is invisible in microscopical sections.

When the tissue has become completely flexible and soft, removal and well washing for a lengthy period under a tap are indicated, and then immersion for one or two hours in a neutralising solution of bicarbonate of soda, 1 drachm to 1 pint of water. The object is again washed, and finally transferred to gum mucilage, whence it is taken for microtome cutting.

In this way, if double staining be practised (see pages 89 and 94), very beautiful and instructive slides may be made of those developing teeth in which the cap of dentine is thick, and also of temporary and permanent teeth *in situ*,

to demonstrate the method of eruption (plate VI., fig. 1). The enamel becomes, however, softened and removed *in toto*.

WEIL'S PROCESS

Weil's Method

The late *Dr. Weil*, of Munich, elaborated a plan in which the main feature is impregnation by balsam, after special preparation (according to details first given by *Koch*), followed later by grinding on a stone. These details were published[1] in England in 1888, and Mr. J. Howard Mummery introduced the process to the Odontological Society of Great Britain in 1890.

Advantages

The process attains very nearly to perfection; and if it is carried out in minute detail, highly satisfactory results are obtained.

Disadvantages

Nevertheless it is open to several objections, amongst which may be noticed its tediousness and length of performance which requires constant attention, its inadaptability to numerous important nuclear and specific stains, and its prolonged treatment with many varied reagents at different degrees of temperature.

Details of the Process

Modus operandi

(i.) Freshly extracted teeth are divided into several pieces with a sharp fine fret-saw (watch-spring saw), by being held between the fingers under cold water trickling from a tap, or squeezed from a clean sponge. This division enables reagents and stains to penetrate quickly and evenly into the pulp.

Fixing

(ii.) The pieces are then laid in a concentrated aqueous solution of corrosive sublimate for some hours, six or eight being necessary as a rule. The mercury 'fixes' the soft tissues, through coagulation of their albumens and gelatins.

[1] 'Journal Royal Microscopical Society,' 1888, p. 1042.

(iii.) They are then placed in a dish, and water from a tap is allowed to wash them for one hour.

(iv.) Removal to 30 per cent. alcohol is the next step. Here they remain for twelve hours. After transference to 50 per cent. and 70 per cent. alcohol for corresponding periods of time, they are put into a bottle containing 90 per cent. alcohol, to which has been added 1·5 or 2 per cent. of tincture of iodine. The iodine removes the precipitate in the tissue by the production of iodide of mercury, which in its turn is eliminated from the specimen by a prolonged immersion in absolute alcohol. The teeth then appear quite white.

Removal of Mercury

Staining

(v.) Again the pieces of teeth are well washed under the tap, and stains are now used, Weil recommending borax-carmine. Grenacher's is the best for staining in bulk; but the alcoholic and not the aqueous solution must be used, as it penetrates well, and does not gelatinise, which the latter is apt to do after a time. Mr. Mummery has found that aniline blue-black stains efficiently in this process, and that it is particularly useful for ulterior photomicrographic purposes. Immersion in the colouring solution is prolonged. The time varies as to the thickness of the pieces, but from three to seven days will generally suffice.

'Fixing' Stain

(vi.) In order to fix the stain, it is necessary next to pass the pieces into acidulated alcohol, viz., 100 c.m. of 70 per cent. alcohol, to which 1 c.m. of hydrochloric or acetic acid has been added. Here they remain for twenty-four or thirty-six hours. If an aqueous stain has been used, half that time will be sufficient.

(vii.) The pieces are now transferred to 90 per cent. alcohol for fifteen minutes, and afterwards to absolute alcohol for half an hour. This prepares them for clearing.

Clearing

(viii.) Oil of cloves or oil of cedar wood is to be used, and pieces remain here for twelve hours.

(ix.) The oil is next quickly washed off with xylol, and the pieces suspended in pure choloroform.

Balsam Impregnation

(x.) Twenty-four hours later they are saturated in a chloroform extract of dried or desiccated Canada balsam, made about the consistency of treacle. They should be kept in this chloroform balsam for a day, and then more dried balsam is added to the solution until the chloroform can no longer take it up. Only a little is to be added at a time. This stage is reached in three or four days.

(xi.) The prepared pieces are finally placed in a china jar or other receptacle over a water-bath kept at the temperature of 90° C., or 194° F. They remain here for two or three days or more, until the balsam in which they are imbedded, when quite cooled, cracks like glass on the introduction of a needle point into it.

Grinding

(xii.) Thin sections are now made, by again cutting each piece into halves with a fret-saw under water, each piece being then ground down on a corundum or carborundum wheel on the lathe, and lastly rubbed on a Washita stone with the finger. Chloroform balsam is to be used as the mountant.

Mr. Mummery recommends Wolrab's gold cylinder bottles, labelled, as being the most convenient for holding the various reagents through which specimens have to pass during the stages of the process. If a note of the stage they have reached be made on the labels of the different bottles, and the stages begun at different times, the process is not so complicated or troublesome as might at first be imagined.

Precautions

With reference to the process itself, special care should be taken in keeping the specimens sufficiently long over the water-bath, because if this is not done, when the student begins to grind down the pieces, portions of the pulp, if not the whole organ, will be dragged away. On the other hand, prolonged heating leads to brittleness; the temperature should

therefore never exceed 90° C. Rapid grinding should not be attempted; a slow cutting stone gives best results, and does not fill the tissue with detritus. The preparations seem to grind down more easily if they are left for some weeks exposed to the air previous to cutting on the wheel. The use of an indiarubber finger-stall has been found by *Mr. Sydney Spokes* to be extremely satisfactory in finishing the rubbing down of tolerably thin sections during the final stages of Weil's process. The indiarubber seems to 'surround' the specimen better and hold it more firmly than do the finger-tips. It also prevents the latter from coming into contact with the stone.

Spokes's Methods of Finishing

In cases where trouble may be threatened by the different rate of wear when grinding hard and soft dental tissues, such as sections of jaw with temporary or permanent teeth *in situ*, the same worker adopts the following ingenious precaution:—One side of a somewhat thick specimen is ground smooth, and it is then sunk, finished side downwards, into a piece of softened A 1 composition, the other surface of the composition being pinched up to make a kind of 'handle.' The surface of the section to be still further ground is turned on to a glass slab, whilst the composition becomes cold. Both slab and section should be first well wetted, so that the composition does not adhere to them. One is thus enabled to make a matrix filling the irregular outline of the section, in which the latter may be conveniently ground until very thin.

The use of a Matrix

MODIFICATION OF WEIL'S PROCESS

Must-Rose Modification

The drawbacks and disappointments attendant on the employment of Weil's original method have stimulated several histologists to devise other plans, which, by adopting the general principles of the process, yield

similar but even better results. *Messrs. W. H. Must* and *S. Rose* have together worked out a very convenient and highly satisfactory method of dealing with the hard and soft tissues. Their modification takes up less time in its performance, admits the use of practically all the best known and most important staining reagents, and sections, when finished, never present the annoying deposit of granules in the pulp tissues so frequently observed after they have undergone fixation by means of mercury perchloride. Advantages

Modus Operandi

The steps of their process are as follow :—

(*a*) Fresh teeth, cleansed from all extraneous matters, are ground down in such a manner that both sides of the pulp cavity are exposed at two or three points. This is easily done on a large grindstone, or carborundum wheel, on a lathe, copious supplies of water being allowed to moisten them at the same time. Caution must be exercised, or else the pulp will be torn away from the hard parts. If the teeth cannot be ground down at once after extraction, they should be placed in a jar of absolute alcohol. The history of the specimens having been recorded in a notebook, should be numbered, and corresponding numbers marked with lead pencil on the surfaces that have been cut. By doing this, the specimens can be readily identified at the end of the process, because the pencil marks show quite plainly through the clear hard balsam. Preparation

(*β*) The specimens are now placed in absolute alcohol for a fortnight.

(*γ*) They are then stained *en masse* with Grenacher's alcoholic borax-carmine for a fortnight. Staining, etc.

(*δ*) This stain is fixed by immersion of the tissues in acid alcohol (rectified spirits of wine with 1 per cent. hydrochloric acid) for twenty-four hours.

(ε) Dehydration in absolute alcohol next takes place.

(ζ) Clearing with oil of cloves is the following step, and its excess removed by washing off with xylol.

(η) The teeth are then soaked in chloroform, which should be renewed if cloudiness appears.

Impregnation

(θ) Finely powdered Canada balsam must next be added to the chloroform till a saturated solution is produced. This should be done by degrees, taking three or four days, or more perhaps, to attain this end.

(ι) The teeth are now transferred to the water-bath at a temperature of 90° C. At first they should be well covered with the desiccated balsam, to allow all air bubbles to escape from the specimens; but this is unnecessary at the end of two or three days. When the balsam is quite dry and still warm, the teeth can be removed with a pair of tweezers and placed on a slab of glass. In a few minutes the imbedding medium will be quite hard, and should then be ground off the surface of the teeth. Thus the specimens in this condition will keep indefinitely, and can be finished at leisure.

Finishing Sections

(κ) Sections are made by grinding on a carborundum wheel on the lathe, using all the time plenty of water. When quite thin they may be finished between two surfaces of ground glass with a free supply of water and nothing else. When the ground glass becomes smooth, its surface should be scoured and roughened by means of silver sand.

(λ) Sections are finally washed, dehydrated, cleared, and mounted in xylol balsam in the usual manner.

NOTE.—If preferred, the soft tissues may be hardened in Müller's fluid or formalin, and then passed through the process.

Alternative Staining

It will be observed that following out the lines above suggested, staining with carmine alone is admissible.

Any of the selective stains may, however, be used as already stated, by somewhat altering the *modus operandi.*

The method is proceeded with as far as (β), and the four succeeding steps omitted.

Powdered Canada balsam is then added to the chloroform, the specimens are thoroughly dried over a water-bath, and, lastly, ground down thin enough to mount.

The sections (unstained) are now suspended in chloroform, which must be changed until all the balsam has been dissolved out of the tissues. Slightly warming the mixture will do no harm, and facilitates the process.

The resulting sections exhibit the hard and soft parts *in situ*, and they can then be treated with ordinary stains. Iron and tannin, and chloride of gold have been found by experience to be perhaps the best reagents to employ. If the latter is used, the sections must be mounted in glycerine jelly, and not balsam.

ANOTHER MODIFICATION OF WEIL'S PROCESS

Choquet's Modification

Mons. Jules Choquet, of Paris, has recently (1899) conceived a new modification of Weil's process, by which sections of hard and soft tissue in combination may successfully be procured by balsam impregnation, on the lines suggested by *Koch*, in the space of a week. The rapidity of the method depends on the use of formalin, in place of Müller's fluid or perchloride of mercury, and in dispensing with chloroform as a solvent for the desiccated balsam.

Steps of the Process

1. A newly extracted tooth is cut on a corundum or other wheel in the lathe, under water, until the pulp chamber is almost opened. The pulp may be exposed by careful grinding.

Fixing and Hardening

2. It is now placed for

Six hours in formic aldehyde, 15 per cent., and alcohol, 10 per cent. (or without alcohol).

Staining

3. Six hours in the stain—viz. Grenacher's borax-carmine in alcoholic solution.

4. Without washing, it is transferred for

Half an hour to alcohol, 50 per cent., 100 c.c.; hydrochloric acid, 3 drops.

5. Then for

Preparing for Balsam

Half an hour to alcohol, 70 per cent.
Half an hour to alcohol, 90 per cent.
Half an hour to absolute alcohol.
One hour to absolute alcohol, 10 c.c., and xylol, 1 c.c.
One hour to absolute alcohol, 10 c.c., and xylol, 3 c.c.
One hour to absolute alcohol, 10 c.c., and xylol, 5 c.c.
One hour to absolute alcohol, 10 c.c., and xylol, 10 c.c.
One hour to pure xylol.

6. Add powdered Canada balsam, in small quantities at first and larger quantities afterwards, till the solution becomes thick.

Hardening preparatory to grinding-down into Sections

7. Place the slice of tooth in a watch-glass in the thick balsam solution, with its cut surface on the top, over a water-bath, till the balsam is hard on becoming cold. Entire hardening requires two or three days, according to the amount of balsam used for the section. The xylol is much more volatile than chloroform, and apparently does not change the structure of cells.

'By this method,' as Mons. Choquet writes, 'a

longitudinal section of a tooth, with pulp and its ligament *in situ*, can be ready for microscopical examination in four or five days, the rapidity of the work belonging to the xylol.'

HOPEWELL SMITH'S PROCESS

This method, like the preceding, has for its object the retention, *in situ*, of the hard and soft tissues. It was brought before the Odontological Society of Great Britain, in November, 1891, and a complete account of it can be found in that Society's transactions. Its advantages are, briefly:—the retention of the soft parts in close juxtaposition to the hard tissues (viz., pulp to dentine, periodontal membrane to cementum or bone), the great ease and rapidity of its performance, its extreme simplicity, and its adaptability to any method of imbedding in gum, celloidin, or paraffin, thus allowing, if desired, serial sections to be made. In addition, any number of sections can be cut in any direction from the same tooth, and by using a microtome these sections can be made thinner than by grinding-down methods. There is a quick and permanent penetration of stains, which may be of any kind. Its great disadvantage is that the enamel is not preserved, as the acids rapidly soften and remove this tissue entirely. It is certain, too, that the hard tissues are chemically changed during the removal of their lime salts; but no appreciable alteration in structure can be noticed, this compensating in a great degree for the chemical differences that have occurred. M. Choquet,[1] of the École Dentaire de Paris, speaks in praise of the acid solution and this process, but thinks that it has 'a dissolving action on the nuclei of the cells (of the soft parts).' He suggests the addition of a 1 per

Advantages

Disadvantages

[1] 'Traité Technique des Préparations Microscopiques,' 1895, p. 80.

cent. solution of chloride of palladium to obviate this 'inconvenience.'

Preliminary Treatment

Details of the Process.—A newly extracted tooth is well washed in normal salt solution for some minutes. In order to allow reagents to penetrate into the pulp, it is often necessary to enlarge the apical foramen or foramina with a fissure bur on the dental engine, and to make a counter opening through the crown. As a rule it is better to divide the tooth into halves, by cutting through the cervical region with a sharp fret-saw, allowing meanwhile a good supply of salt solution to moisten the dentine. In those cases where the pulp chamber is opened by the progress of caries, these precautions need not be taken.

Hardening

The pieces are now placed in a stoppered jar containing freshly made Müller's fluid, the volume of the latter being about twenty to thirty times the bulk of the tooth. Fresh fluid should be used on the fourth day, and this changed again at the end of a fortnight. At the end of three weeks pieces should be removed to alcohol (84 per cent.) for ten to twenty days or more. Rectified spirits of wine may be used in place of Müller's fluid from the first, and is preferable in some cases. Thus the delicate soft tissues are fixed and hardened.[1]

Protection of Soft Parts

The portions of tooth are now taken from the hardening fluid and well washed. The soft parts and the apices of the roots must be dried on a cloth, and a large drop of flexile collodion or celloidin placed on them, in such a manner that in a few moments a thick film covers them over, and will protect them from the action of the acid reagents.

Decalcifying

The tooth is now placed in 12 c.c. of a 10 per cent. solution of hydrochloric acid, freshly

[1] Since the introduction of formalin, the author prefers the use of it to that of Müller's fluid, except under certain conditions. Pulps may be 'fixed' and hardened in seventy or eighty hours when a 40 per cent. solution is employed.

made. A Wolrab's bottle is very useful for holding this decalcifying fluid. It should be labelled, and the hour of immersion and character of the specimen noted. At the end of fifteen hours, 1·5 c.c. of strong pure (non-fuming) nitric acid are added to the hydrochloric acid solution, and this repeated at the end of forty-eight hours. In about three or three and a half days (seventy to eighty hours) the whole of the dentine and cementum should be completely decalcified. Temporary teeth and molars will require a shorter or longer acid immersion. The student must of course be guided by the amount of softening that has already taken place. This can be easily ascertained by trying to bend the specimen with the fingers or piercing it with a needle point.

If sufficiently decalcified, the tooth must be washed and placed in a solution of bicarbonate of soda or lithium (5 grains to the ounce) for half an hour, after which it is to be further subdivided by a sharp scalpel or razor, and the pieces well washed and put into gum mucilage solution. Here they remain for at least fifteen hours, care being taken that the pieces of tissue are not cut too large for complete saturation by gum. Removal of the film of collodion is best effected by allowing the tooth to remain in a watch-glass of ether for about five minutes, and carefully picking or rubbing it off with a brush. This should be done before it is passed into the neutralising solution. The pieces of tissue are finally placed on the stage of an ether freezing microtome and cut in the ordinary manner. For the gum solution there may be substituted celloidin or paraffin—the former must be used if the tissues are exceedingly delicate and friable, and the latter if serial sections are required.

Saturating

Cutting Sections

Sections having been made, are next washed, stained, and mounted in the usual way.

Special Points.—The hard parts must not be too

Precautions

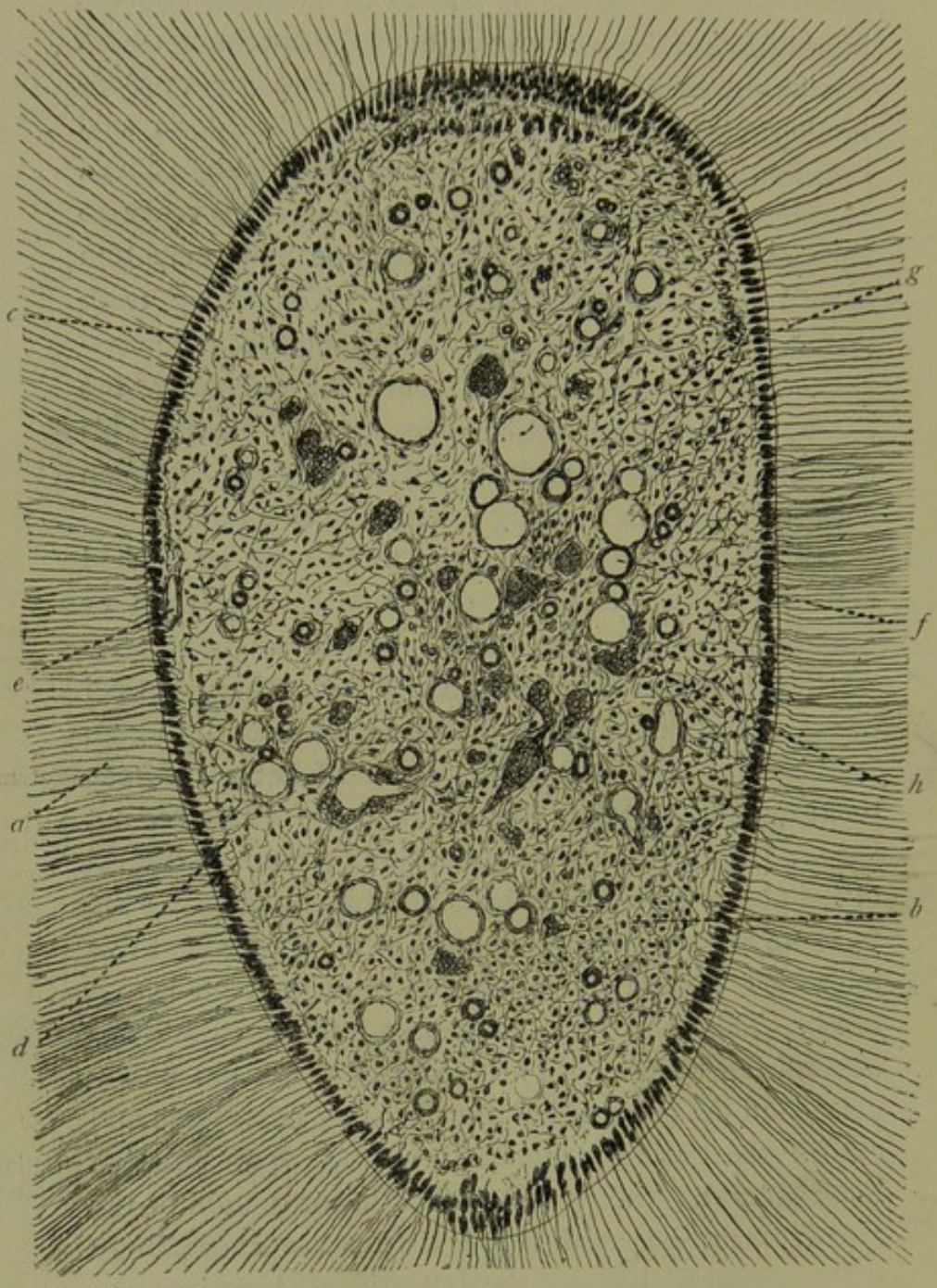


FIG. 13.—The pulp *in situ*, transverse section (Author's process). Mag. 45. *a*, Dentine; *b*, Pulp; *c*, Odontoblasts; *d*, Basal layer of Weil; *e*, Blood-vessels (longitudinally cut) in *d*, *f*, artery; *g*, vein; *h*, Nerve bundles.

much decalcified, the collodion film must cover up all the soft tissues, and the specimens must remain for a sufficient length of time in the gum solution. If the latter has been used as a saturating agent, it is advisable occasionally to dehydrate sections in the following manner :—

Special Dehydration

They should be transferred from a watch-glass containing 30 per cent. spirit, to 70 and 90 per cent., and absolute alcohol. The period of immersion in the varying strengths of alcohol should be one minute. This is to prevent the delicate pulp tissue from shrinking from the softened dentine walls, which often occurs if the sections are at once placed in absolute alcohol. All risks of tearing the pulp from the walls of its cavity may, however, be avoided, if sections after staining be washed, and at once mounted in Farrant's medium or glycerine jelly. When aniline dyes have been used, they must perforce undergo the special precautions of dehydration just alluded to.

Coloured Formalin

The use of a stained solution of formic aldehyde, when associated with this process as a hardening reagent, has been recently (1899) suggested by Mr. Rayson Sears, who adds a concentrated aqueous solution of borax-carmine (Grenacher) to a 40 per cent. solution of formalin, until a deep red coloration takes place. Thus sections of pulp *in situ* are obtained, the pulp being coloured pink, and the mass of dentine, except the fibrils near the pulp cavity, unstained.

Caush's Method

Mr. D. E. Caush, in the 'Journal of the British Dental Association,' suggests 'a simple method of staining and cutting hard and soft sections combined.' He writes :—

'Take a freshly extracted tooth, and if it has a live pulp, place in alcohol for twenty-four hours to harden the pulp. On taking the tooth out of the alcohol, place it in a stain "made by dissolving any of the usual dyes

in alcohol," for two or three days. On removing the tooth from the dye, grind on the flat side of a corundum wheel, until the pulp is *almost* exposed; afterwards grind the opposite side until you have a section of the tooth, with a slight covering of hard tissue on either side of the pulp. Now finish grinding down between two pieces of ground glass, with a small quantity of pumice powder moistened with alcohol or methylated spirit, until the section is as thin as required; towards the end of the grinding use plenty of the liquid with little or no pumice powder. When ground down, wash thoroughly in distilled water; dry off the *surface moisture*, and mount in Canada balsam. With ordinary care, sections may be made with the hard and soft tissues in position. If we want to show the tubuli of the dentine, or blood-vessels, lacunæ, etc., of alveolus, place at once in the stain, and in the case of a tooth the stain will pass up the pulp canal and permeate the dentine by passing through the tubuli; after the tooth has remained in the stain for a day or two, prepare as above. Sections so prepared are especially adapted for examination with $\frac{1}{6}$ or $\frac{1}{8}$ inch objective.'

For what available

The method seems to answer very well for making specimens of alveolar bone with the soft tissues retained *in situ*, but it is not conducive to best results to place the tooth in alcohol as directed, until an opening has first been made into the pulp chamber, so that the spirit can easily reach the soft tissues.

Summary of Weil's Process[1]

Summary

Fresh teeth cut under water with watch-spring saw.

Concentrated corrosive sublimate solution for some hours.

Running water, one hour or more.

[1] See, for this and the following summary, 'Transactions Odontological Society,' Vol. XXII. p. 222, and Vol. XXIV. p. 20.

Summary

30 per cent. spirit, twelve hours.

50 per cent. spirit, twelve hours.

70 per cent. spirit, twelve hours.

90 per cent. spirit and 2 per cent. iodine, twelve hours.

Absolute alcohol till teeth are white.

Running water, half an hour.

Stain, borax-carmine, etc., three to seven days, according to stain used.

70 per cent. spirit (and 1 per cent. HCl if borax-carmine), twelve to thirty-six hours.

90 per cent. spirit, fifteen minutes.

Absolute alcohol, half an hour.

Etherial oil, twelve hours.

Wash this off with xylol.

Chloroform, twenty-four hours.

Thin solution of dried Canada balsam in chloroform.

Thick solution of dried Canada balsam in chloroform.

Water-bath at 90° C. till hard.

Summary of Author's Process

Summary

Immerse a newly extracted tooth, after division with a fret-saw, under salt solution, in Müller's fluid for three to four weeks, and remove to spir. vini rect. for ten to twenty days. Alcohol (84 per cent.) may be used instead of Müller's fluid.[1]

Remove, wash in water, and seal up apical foramen and soft parts with collodion.

Place tooth in 15 c.c. of following solution :—

HCl, 12 parts (pure).

HNO_3, 30 parts (non-fuming).

Aq. dest., 108 parts.

[1] Formalin, 40 per cent., may be substituted for Müller's fluid, or alcohol. Time of immersion varies from fifty to one hundred hours.

Summary

Thus :—Take 12 c.c. of 10 per cent. solution of HCl, and at end of fifteen hours add 1·5 c.c. of HNO_3, and at end of forty-eight hours add 1·5 c.c. of HNO_3 from commencement of immersion in acid solution.

Remove tooth at end of seventy-five to eighty hours or more, and wash in a solution of lithium carb. (5 grains to an ounce) for half an hour. Wash thoroughly with distilled water.

Divide tooth with razor into several pieces, and wash again in water. Place each in gum mucilage (B.P.). Leave in mucilage twelve to fifteen hours or more.

Transfer to stage of freezing microtome, cut, wash sections, and stain with orange-rubine, or gold chloride, or borax-carmine, or Weigert's solutions.

Dehydrate in absolute alcohol three minutes, 'clear' in cedar oil one and a half minutes, and mount in Canada balsam.[1]

Table of Tissues suitable for Preparation by

Chromic Acid Process.	Weil's Process.	Author's Process.
1. Jaws of human and comparative embryos which contain large areas of hard tissues.	1. Pulp *in situ*.	1. Pulp *in situ* in both temporary and permanent teeth.
2. Jaws of animals with temporary and permanent teeth *in situ*.	2. Pulp in connection with semi-calcified dentine.	2. Periodontal membrane *in situ*.
3. Fully developed human teeth.	3. Calcification of dentine.	3. Dental gum *in situ*.
	4. Teeth with uncompleted roots.	4. Teeth of fish and animals where soft tissues are to be preserved.
	5. Adult teeth *in situ*.	5. In all pathological conditions except affections of the enamel.
	6. Periodontal membrane *in situ*.	
	7. Absorbent organ *in situ*.	
	8. Absorption occurring in adult teeth.	

[1] It is considered more satisfactory to clear and mount in Farrant's medium or glycerine jelly, shrinkage of the soft parts being thereby prevented.

CHAPTER V

ON IMBEDDING AND CUTTING SECTIONS

THE chief modes of preparing the various dental tissues for histological examination having already been described, it is now necessary to consider the means at the disposal of the student, whereby all soft and softened tissues may be imbedded and cut. The object of the preliminary treatment of specimens by fixing, hardening, and decalcifying reagents has been to render them fit for imbedding, prior to cutting them into sections on a microtome. When a piece of tissue is imbedded, it is placed in a suitable medium of proper consistency, which is intended to run into and fill all the interstices, not only saturating and impregnating it throughout, but holding its delicate structures in position until a razor or cutter divides it into the thinnest possible sections.

Object of Imbedding

General Principles

There are two methods of imbedding—(i.) simple, and (ii.) interstitial.

In the former, tissues are simply fixed in another medium, and mechanically retained until cut. It is useless for dental work, and need not be further considered here.

Simple Imbedding

Interstitial imbedding implies that the substance penetrates into, and is retained within, the tissue; and

Interstitial Imbedding

there are three important media for achieving this purpose—gum mucilage, celloidin, and paraffin. Their nature, advantages, functions, and methods of using must now be detailed in particular.

THE EMPLOYMENT OF GUM MUCILAGE

Gum Mucilage

The British Pharmacopœial form of gum solution is most convenient. It can be bought already made, or obtained by dissolving 4 ounces of 'picked' gum acacia in 6 ounces of water. If carbolic acid, in the proportion of 10 drops of a saturated solution to the ounce, is added, tissues prepared for cutting can be kept in the mass all the year round, without undergoing deterioration, loss of water by evaporation being occasionally renewed. A combination of 5 parts of syrup (1 pound of lump sugar to 1 pint of boiling water) to 3 parts of mucilage is said to make the impregnation more complete.

Advantages

Of all imbedding media, gum mucilage is found to be the most useful for ordinary dental microscopical work; it is suitable for nearly every class of tissue. Its merits are many. The preliminary steps—such as it is necessary to perform when specimens are about to be imbedded in celloidin or paraffin—are reduced to a minimum, much time and labour are saved, and the procedure is simple, rapid, and clean.

Method of Using

Small pieces of tissue having been soaked in water for some hours, to remove all traces of the 'preparation' reagents, are placed in a large quantity of mucilage. Here they should remain from ten to fifteen hours or more, according to the size of the tissue. The criterion for complete saturation is afforded by the fact that the specimen falls to the bottom of the bottle or jar when it cannot take up any more of the medium. It is then in a fit state to be frozen and cut into sections.

Microtome

The student, at this point of his work, should obtain the use of an ether-freezing microtome. There are several useful varieties; but the beginner cannot do better than use a Cathcart's microtome. It may be said that with this, as with other instruments, practice only will lead to satisfactory results. When once the knack is attained, section cutting becomes a simple and easy matter.

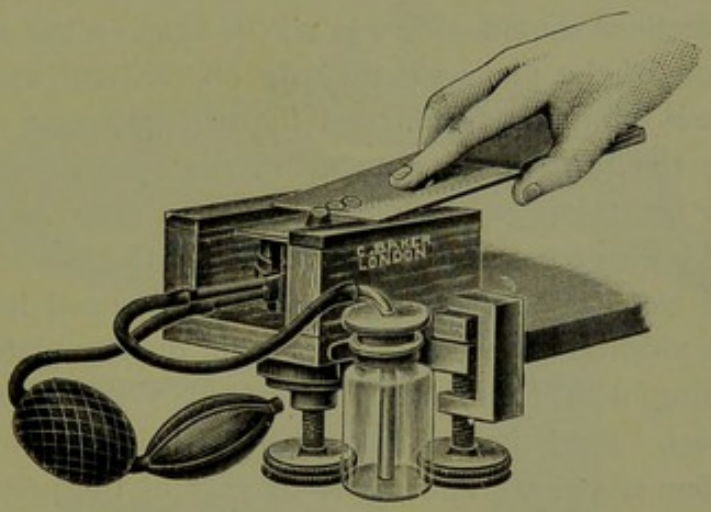


FIG. 14.—Cathcart's Microtome, showing the Method of using the Cutter, and the way in which Sections are made.

Cathcart's Microtome.

The accompanying figure exhibits the chief points of Cathcart's instrument. It will be seen that the tissue is raised by means of a large differential screw beneath, and that the cutter moves from behind forwards and is not automatic.

To Cut Sections

Method of Using.—The microtome is clamped to the edge of a firm table, and the bottle half filled with ether. Ether sulph. meth. sp. gr. ·735, and ether rect. opt. (Ætheris Purus B.P.), sp. gr. ·720, answer equally well for freezing purposes. A slice of the specimen, not more than $\frac{1}{8}$ inch thick, is now placed in the middle of the plate, and a drop or two of gum mucilage allowed to fall on the top and run down its sides

equally in all directions. An assistant should manipulate the bellows, and direct a continuous spray of ether on the under surface of the stage. It may be necessary to add more gum, until at length the object is frozen right through in a solid mass. The plane must be held firmly with the right hand, and rapidly pushed through the specimen, while the left hand slightly moves the milled head of the screw at every stroke of the cutter. The sections should collect in a little heap on the upper surface of the plane; if they fly off, or curl up, the tissue is too much frozen, and the assistant must cease using the bellows for a moment, the operator meanwhile breathing gently on the object. The sections should then be carefully removed from the plane by a small wet camel's-hair brush, and be dropped into a black vulcanite tray—a photographic quarter-plate developing dish filled with water makes an excellent receiver. Here they will separate of their own accord in a few minutes. The whole or part of the prepared tissue having been thus sectionised, a piece of ordinary glass is placed over the vulcanite dish to protect the floating sections from dust. They may then be examined at leisure, and the thinnest, *i.e.* the most transparent, chosen, and placed in a bottle containing 30 or 50 per cent. alcohol until the student has time to stain and mount them. The thinnest, and therefore the most useful sections can be most readily recognised and selected for staining, by placing the black tray containing them in the rays of direct sunlight.

Their subsequent Treatment

The cutter should be set and stropped before each time of using, and at the end of the operation washed with alcohol, and wiped on a rag which has been smeared with vaseline. The microtome also should be wiped dry, and kept in a box to protect it.

The apparatus can be obtained from Baker, of High Holborn.

Swift's Microtome

A larger and more useful form of apparatus is shown below. This is Swift's modification of Williams's microtome. Here, the cutter, similar in size and shape to a razor, is fastened into a frame, provided with three delicate screws, which regulate the thinness of the sec-

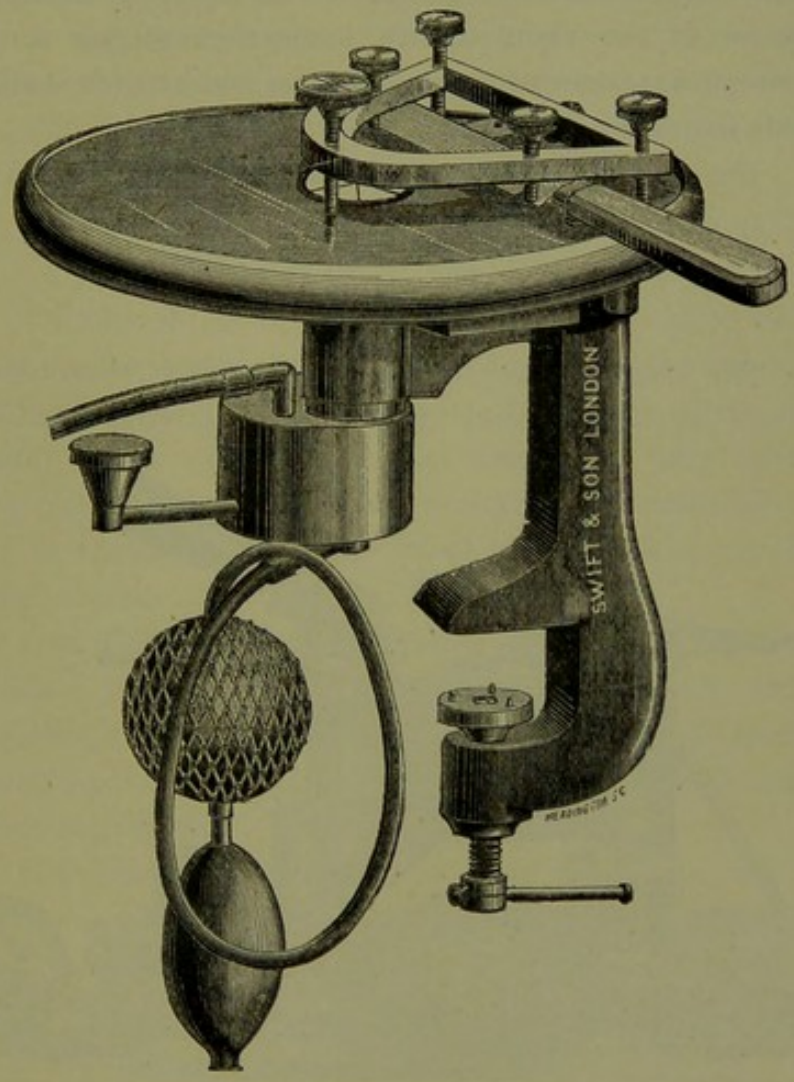


FIG. 15.—Swift's Ether-Freezing Microtome.

tions. The movements are in an opposite direction to those of Cathcart's knife, viz. towards the operator, who has thus greater control over the cutter. The

tissue is firmly fixed, and cannot be heightened or lowered.

Method of Using.—Before using this instrument, moisten its upper glass surface with a little water; this makes the cutter frame run quite smoothly. Place the tissue on the stage and freeze as before directed.

How to hold the Frame

Hold the frame very firmly with the fingers of both hands, the thumbs being towards the operator; and by means of the right thumb move the anterior screw through a quarter of a revolution or less at each stroke. This requires practice.

The sections collect on the knife, and are placed in a tray of water as already described.

Roy's Microtome

Advantages of Roy's Instrument

The chief feature of Roy's Freezing Microtome, which is the best and most convenient ether-freezing

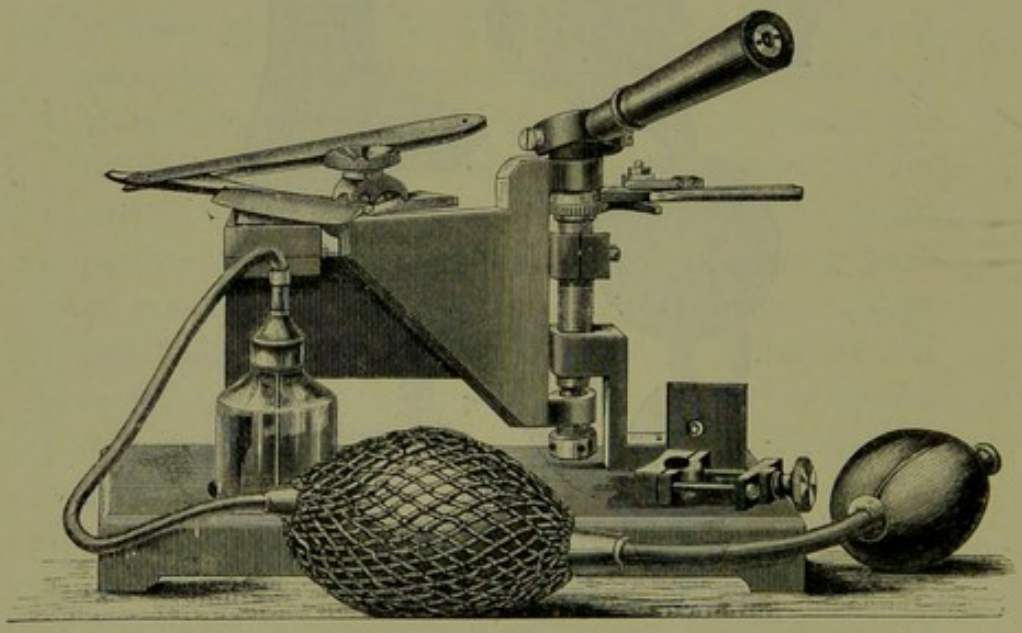


FIG. 16.—Roy's Improved Freezing Microtome.

apparatus made, is that it is automatic. It can also be used for cutting specimens imbedded in celloidin, the slicing movement of the razor being well adapted for this purpose.

Roy's Microtome

Method of Using.—The razor should be clamped quite near its end, as shown in the cut, and raised to its highest position above the plate. This is done by moving the horizontal brass handle to and fro, the ratchet click engaging with the teeth of the ratchet wheel on the further side of the instrument. The reverse of this will lower the razor and razor-holder. Sections are made by moving the wooden handle backwards and forwards, their thickness depending on the distance that the brass handle is moved at each stroke.

The microtome is made by the Cambridge Scientific Company, and can be highly recommended.

IMBEDDING IN CELLOIDIN

The use of Celloidin

The employment of celloidin as an imbedding medium for dental tissues has not received the support of histologists that it deserves. It is an extremely useful agent. It possesses, at once, not only great penetrative power, and equal uniformity of permeation, but is remarkable for the ease with which it can be manipulated.

Application

One finds it most invaluable for imbedding frail delicate organs, whose parts are but loosely held together, such as early embryonic jaws and teeth, decalcified fishes' teeth, certain decalcified teeth with pulps *in situ*, etc.

Nature

Celloidin is a preparation of pure pyroxylin, and is merely a patented collodion. Schering's celloidin is the best. It is obtained in the form of thick plates of a tough, gelatinous, semi-transparent substance, which should be cut up into shavings before using.

Steps of the Process

Modus Operandi

(*A*) *Infiltration.*—Have ready four glass-stoppered bottles, labelled, and containing (i.) ABSOLUTE ALCOHOL, (ii.) ALCOHOL AND ETHER, (iii.) THIN CELLOIDIN,

and (iv.) THICK CELLOIDIN. For the second bottle make a mixture of equal parts of absolute alcohol and methylated ether. It is difficult to give the exact proportions of the celloidin solutions; they are both made by adding the shavings of celloidin to varying quantities of alcohol and ether. The 'thick' solution should be of a thick syrupy consistence, the 'thin' being the same diluted with absolute alcohol and ether. The object of using these two solutions is to make certain of getting a complete impregnation of the tissues.

The object having been dehydrated 'by immersion in absolute alcohol for twenty-four hours, is placed in the second mixture for the same period. It should then be removed and suspended in the thin celloidin, and finally in the thick solution, remaining in each from one day to one week. The length of time depends on the size of the object.

Paper Cell for Imbedding

(*B*) *Imbedding.*—After thorough infiltration the tissue is imbedded. Twist a piece of stout writing-paper round a small cork in such a manner that it projects from one end and makes a collar. Stick a pin through the paper into the cork, and paint the line of junction with celloidin solution. After this is set fill the paper thimble with thick solution and suspend the object in the middle of it. Expose it to the air to dry. In a few minutes a film will form over the surface of the celloidin, and then the paper thimble, with its contents, should be placed in a jar of pure chloroform,[1] free from water. Here, in one or two days, the celloidin will be thoroughly hardened.

Next remove the imbedded object from the chloroform, and tear off its paper support, leaving a clear block of celloidin. Then either place the block in a

[1] Many workers use methylated spirit and water instead of chloroform to harden the celloidin block.

phial of white oil of thyme, as suggested by Bumpus,[1] to clear it, or put it at once into the clamp of a microtome. Use for this purpose the clamp supplied with Roy's freezing microtome (see fig. 16). Keep the razor well moistened with spirit or oil of thyme, and take off the sections with a camel's-hair brush.

Staining

It is better, in the majority of cases, to stain the tissue *en masse* before imbedding, using borax or lithium carmine (Grenacher's). But some workers[2] prefer to stain the sections after cutting. The writer, however, considers the other method the better. In any case, sections must be dehydrated again in absolute alcohol, and cleared in cedar-wood oil or xylol, and *not* oil of cloves. They are to be mounted subsequently in Canada balsam.

Alternative Plan

Celloidin-imbedded specimens may be cut into sections by freezing on an ordinary Cathcart's microtome, provided they have been hardened in alcohol. The block should be kept in running water for at least one day to remove the alcohol, and then transferred to gum mucilage, and cut after some hours.

Much better sections, however, can be obtained by using Roy's instrument, because of the mowing movements of the razor.

SERIAL SECTION IMBEDDING AND CUTTING

For what applicable

If the student intends to do research work, he will find it often desirable to make sections of an organ in series. In dental microscopy this applies chiefly to developmental tissues, and pathological conditions of the pulp, or periodontal membrane. From what has been already said, it will be at once seen that it is a difficult thing to do serial section cutting if gum or celloidin is

[1] 'Amer. Natur.' Vol. XXVI., 1892, pp. 80, 81.
[2] Stirling's 'Practical Histology,' 1893, p. 45.

used as the imbedding medium. Paraffin and a special form of microtome have therefore to be employed.

Imbedding in Paraffin

Advantages

The trouble and care and time involved in the employment of paraffin as an imbedding agent are amply compensated for by the beauty of the sections and their value for subsequent research. There is little risk of destroying or injuring tissues if moderate care and attention be given to details, and the following plans can be well recommended. The various steps may be pointed out by taking a typical case.

A typical Case

A small portion through the incisor region of the mandible of a fœtus affords a convenient piece of tissue for purposes of practice.

The jaw having been previously decalcified, if necessary, in chromic and nitric acids, has been subsequently kept in 30 per cent. alcohol.

Hydration

A slice is taken from the mandible, and hydrated for a quarter of an hour, in order to get rid of the alcohol and prepare the mass for staining.[1]

Staining

It is then stained in bulk with borax-carmine (Grenacher) or Kleinenberg's hæmatoxylene for twenty-four to thirty-six hours. If the former has been used, it must be fixed by soaking the tissue in acid alcohol (70 per cent. alcohol with ·5 per cent. pure hydrochloric acid). This must be changed constantly until no further colouring takes place.

Dehydration and Clearing

The tissue is next soaked in absolute alcohol for eighteen hours, then 'cleared' in xylol or oil of cedar for four or five hours. The former is said to cause slight shrinkage of the cellular tissues, but it penetrates them splendidly. Incomplete dehydration on clearing is indicated by the presence in the tissues of tiny

[1] It is unnecessary to hydrate if an *alcoholic* solution of a stain is subsequently chosen.

bubbles. These can be removed by agitating the liquid in which the organ is immersed, by shaking the bottle, or by removal of it into the air for a few moments, or by very carefully expressing them from its interior with a pair of fine forceps. If many bubbles exist the mass should be entirely discarded.

Method of Imbedding

Condition of Paraffin

The next four or five hours are occupied by its being soaked in a mass of melted hard paraffin. This medium, which melts at 60° C. (Stirling), acts better when it has been previously mixed with equal parts of soft paraffin (melting point 45° C.). If it is still too hard, a little pure vaseline may be added ; if too soft, the addition of more hard paraffin only is requisite. A little experience will soon enable the student to judge when the wax is of the right consistency.

The paraffin should be melted in beakers which are kept at the right temperature by being placed in a regulated water-bath or chemical oven. (Figs. 17 and 18.)

Precautions

It is a good thing, sometimes, to warm the xylol slightly at first, as this prepares the tissue for its immersion in the hot melted wax, and so reduces to a minimum its chances of shrinking—an annoying and destructive complication. This shrinkage takes place very often, and in the majority of cases matters but little, as the various parts of the organ contract slightly but uniformly. It is obvious, however, that with regard to decalcified pieces of teeth the pulp shrinks more rapidly than the softened dentine. Under these circumstances, therefore, special care must be taken.

Thorough impregnation having occurred, a suitable receptacle for containing the melted paraffin must be made or obtained.

Circular Cell

The simplest consists of a cell made by rolling a piece of stiff white note-paper round the end of a

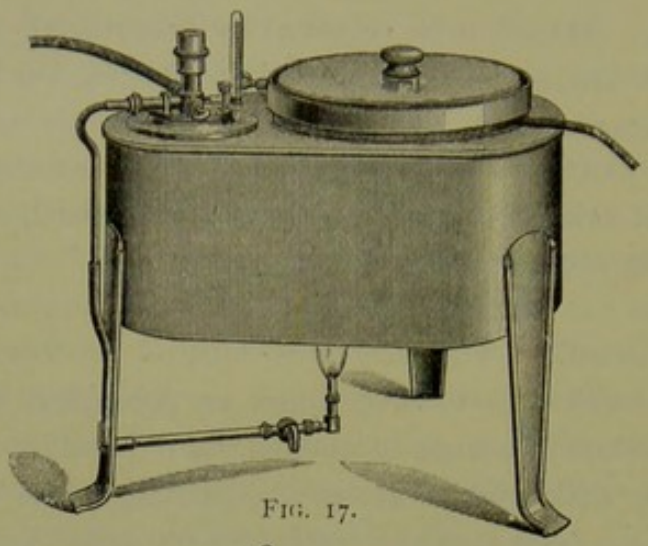


FIG. 17.

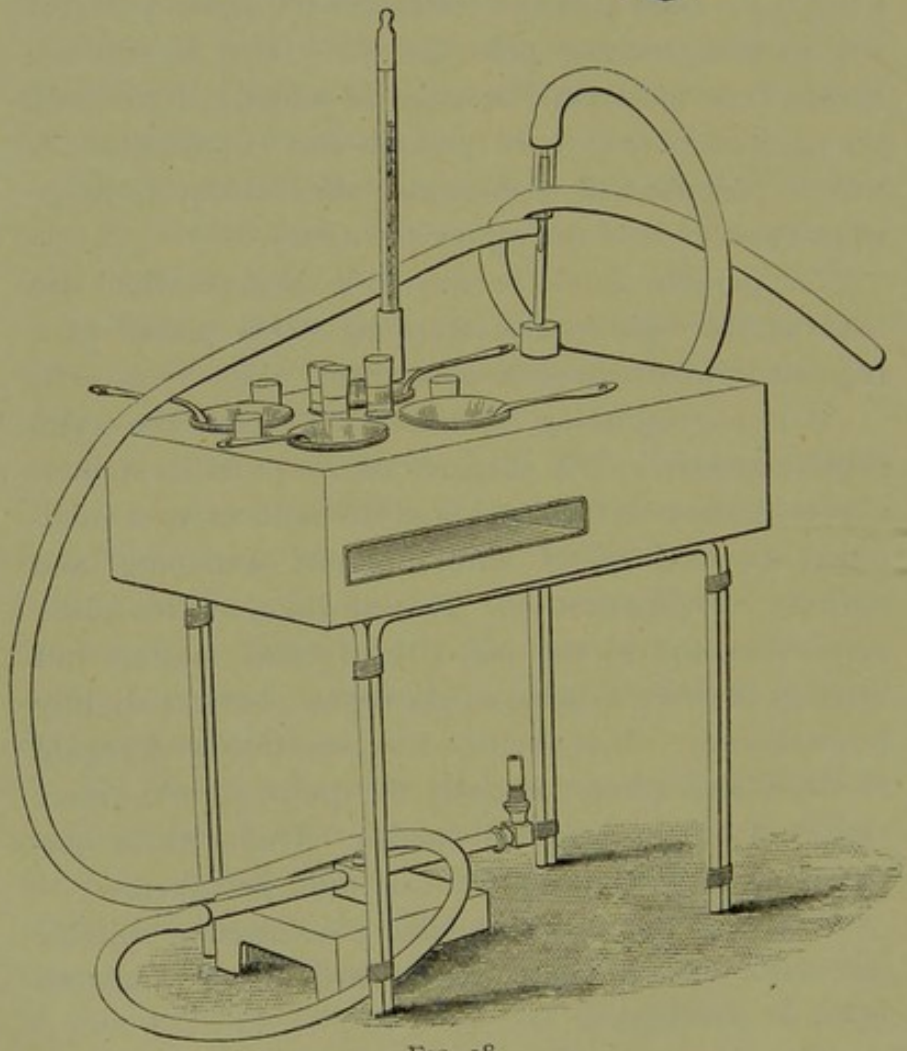


FIG. 18.

FIGS. 17 and 18.—Two forms of water-baths for imbedding in paraffin. The latter has also a warm chamber in which slides and preparations may be dried, chloroform evaporated, &c.

suitably sized cork, and fixing it with a pin. A circular cell results.

A paper-box may be constructed in the following way :—

Rectangular Cell

An oblong piece of stiff paper is first doubly folded over and under along the lines A and A′. These lines form the long sides of the base of the box. The process is repeated along the lines B and B′. These form the ends of the base of the box, the folds of paper being twice or three times the diameter of the first two. B and B′ are again folded on themselves C and C′. The box is then formed by bending up the walls and bringing them to right angles with the floor of the box, which is completed finally by bending the long flaps over the sides of the ends of the box in an outward direction. Thus :—

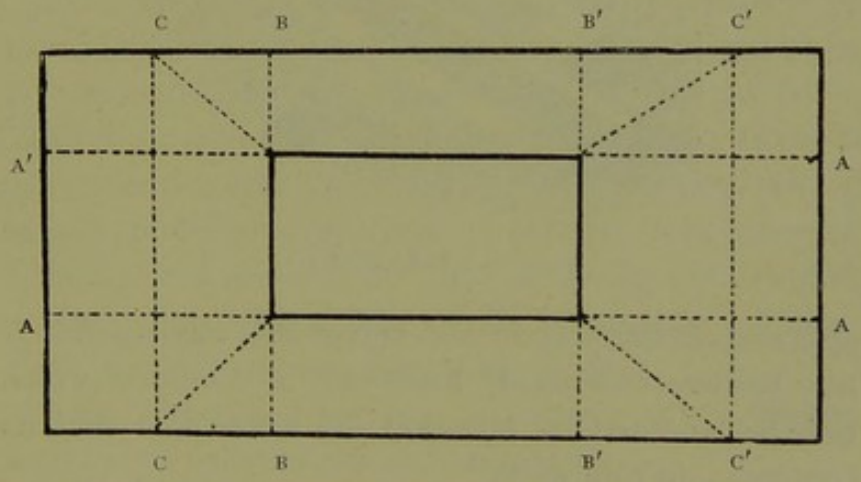


FIG. 19.—Diagram for making a paper imbedding cell. The centre, marked in a thick line, forms the floor of the box, the sides the walls, and the ends the flaps. The exact size is very useful.

Other Methods

In addition, special imbedding tubes, or imbedding **L**'s of lead or brass, may be purchased, and are exceedingly useful. (Fig. 20.) With the latter, a piece of glass receives on its surface the imbedding blocks, and thus forms the bottom of the trough. The object

having been transfixed by a pin, as shown in the figure, and molten paraffin having been poured into the mould, the object, as the wax begins to set, is placed in position at one end. When cool, the whole block can be removed from the mould.

Details

The box is placed on a cold surface, and the wax poured in. As the lower part begins to set, the tissue should be suspended in the middle of the medium, either by being dropped into place, or held in position by means of a pair of forceps or a mounted needle. The arrangement of the specimen must be noted as a guide to trimming up the wax into a rectangular block, for

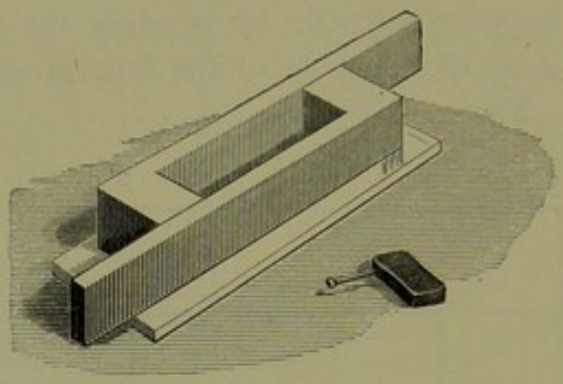


FIG. 20. – Imbedding L's.

applying to the wax in the microtome carrier, after it has become thoroughly hardened—a condition which may be hastened by plunging the whole box and its contents into cold water.

To produce a good ribbon, softer wax should be placed above and below the block, so that as each section is cut it will cohere to its neighbours, by opposite edges.

This being done, and the screw set to the desired thickness of section, a few slices of wax alone should be removed, till the ribbon is continuous, when the whole or parts of the tissue may be cut.

Imbedded specimens keep for any length of time.

Subsequent Treatment

When the sections have been cut either on the 'Rocker' or the Choquet microtome, four or five should be placed side by side on a glass slide, and all moisture removed by drainage (*see* p. 107). Sometimes it is most important that the sections be specially fixed on the slide; but, under ordinary circumstances, and if great care is exercised, they need not be so treated.

Fixing Sections on Slide

It is found in actual practice that if the sections are very thin indeed, they will, of themselves, *when absolutely dry*, adhere quite suitably to the glass slide, and may then be passed through the various processes for the purpose of removing the paraffin by dissolving it out, clearing and mounting in the usual way.

The foregoing remarks apply to all ordinary developmental tissues; the same principles should of course be also extended to fully erupted and completed teeth. With these it is, however, advisable to remove as much of the dentine and cementum as possible before staining in bulk, or before preparing for paraffin imbedding, by grinding on a stone on the lathe in the case of calcified, or by paring with a sharp knife in the case of those teeth which have been already decalcified.

To prevent Shrinkage

Mr. Howard Mummery advocates the following methods of dehydration and imbedding:—

Mummery's Method of Imbedding

After the tissue has been fixed and hardened it is put into 50 per cent. alcohol for two hours, then into 70 per cent. for twenty-four hours, followed by 80 per cent. for twelve hours, and 95 per cent. for two hours, complete dehydration being finally produced by a short immersion in absolute alcohol. Wolrab's bottles, well corked, are very useful for this dehydration process.

'Clearing' is the next step, and cedar-wood oil or turpentine is to be used. Pour some of the medium

into a test tube, and on the top put a little absolute alcohol. Carefully place the object in the alcohol, and allow it to sink to the bottom of the test tube, afterwards drawing off the alcohol with a pipette.

The clarifying oil has prepared it for the imbedding medium, which is hard paraffin. A lump of paraffin should be placed in a water-bath and kept at a tem-

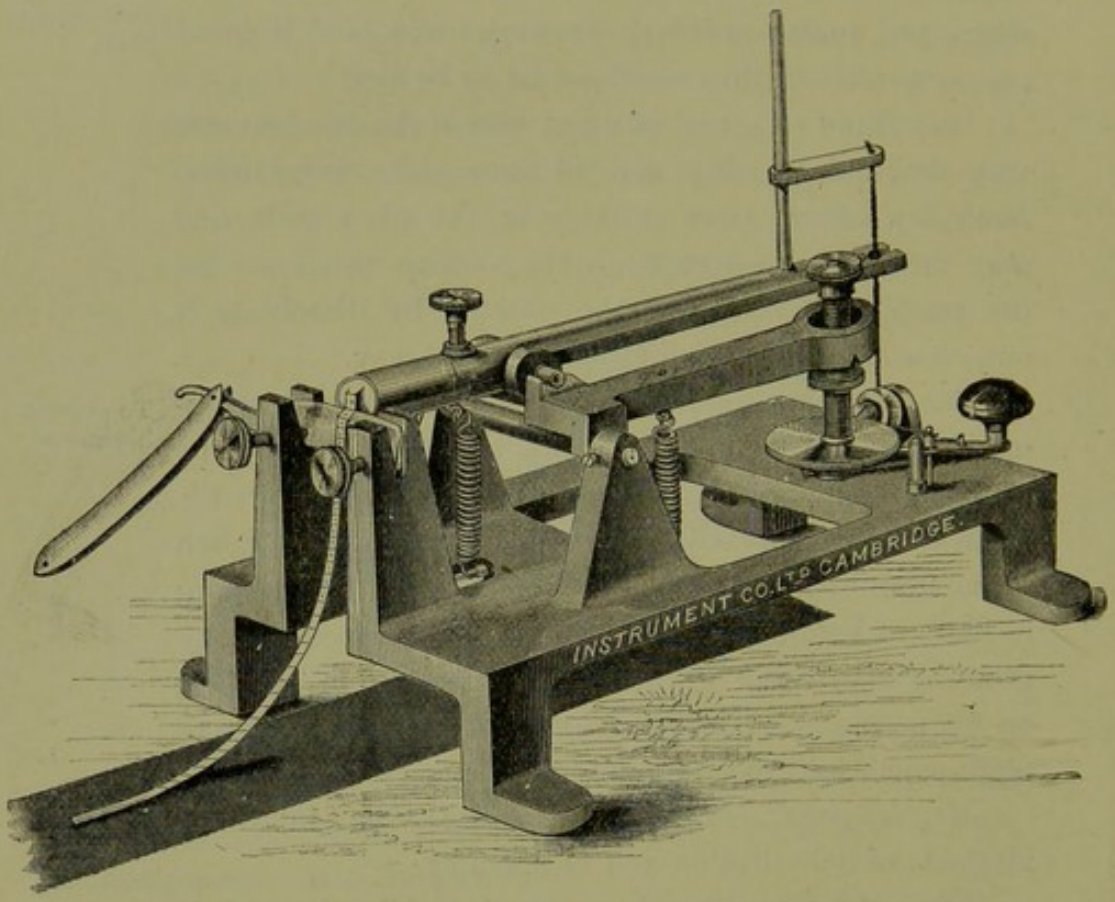


FIG. 21.—Rocking Microtome.

perature of 45° C.—its melting point. The object is placed therein, and it should remain there for one or two days. The paraffin at the end of that time is allowed to cool, and when it becomes hard it can be cut out of the water-bath dish or tray.

Fixing the Block

The block is fixed on to the object carrier of a Rocking Microtome by melting with a hot knife the

surface of the block and the paraffin already attached to the carrier. When set again, and in position, pare the edges of the block square, and close to the object, set the knife—which should be quite dry—square, and cut sections by moving the handle of the instrument rapidly in a lateral direction. A ribbon of sections is thus produced. (See illustration.)

Cutting Serial Sections

The imbedded sections should be allowed to gently fall on to the surface of warm water in a flat dish, where they quickly flatten out, but still cohere at opposite edges. They can then be stained and mounted by special methods.

The Rocking Microtome

The Cambridge Rocking Microtome is automatic, and will cut sections from $\frac{1}{40000}$ of an inch to $\frac{1}{2500}$ of an inch in thickness, these figures, of course, being only approximate. It can be obtained from the Cambridge Scientific Instrument Company, St. Tibb's Row, Cambridge ; or Swift, of Tottenham Court Road.

The Choquet Microtome

The recently designed microtome of Mons. Choquet must not be omitted from these pages, as the inventor is the only dental surgeon who has introduced to science an apparatus of this kind. It is automatic, and adapted for paraffin-imbedded specimens. Consisting of a heavy base with an iron pillar and horizontal table, on which the razor is fixed, sections are made by means of two movements—a vertical, holding the imbedded object, and a horizontal. The differential micrometer screw is situated at its base, and is connected to the driving wheel by an eccentric lever. It somewhat resembles the Minot microtome, but whereas in the former the object

advances to the knife, the opposite occurs in the latter. It will cut much larger sections than the Rocking microtome, and, on adjustment, sections varying from $\frac{1}{80}$ to $\frac{1}{400}$ mm. ($\frac{1}{2000}$ to $\frac{1}{10000}$ inch) can be readily made. Messrs. Baker, of Holborn, manufacture the instrument.

Table of Tissues suitable for Imbedding in

Gum Mucilage	Celloidin	Paraffin
1. The majority of dental tissues, including all soft and softened organs.	1. Early developmental tissues. 2. Decalcified fishes' teeth. 3. Pulps *in situ*, especially those of molars and other large teeth.	1. Embryonic tissues, pulps, and periodontal membrane, when serial sections are required.

CHAPTER VI

ON STAINING AND MOUNTING SECTIONS

In general histology it is found that unstained sections do not impart so much knowledge to the student as those specimens the structures of which have been differentiated by means of chemical stains or aniline dyes. It is necessary to colour the protoplasm of cells and the outlines of fibrous tissues, because, otherwise, they would be lost in the general transparency of the section. In dental histology, however, many sections of the hard tissues, which have been prepared by grinding, are full of interesting information, even if they have not been subjected to the action of a dye. The histology of dentine and cementum is a case in point. But here the normal markings are rendered more or less prominent by the accumulation of detritus, water, and air, which get into the interspaces of the tissues whilst grinding down the tooth specimen. Consequently, a preparation showing well-marked degrees of contrast—a study in blacks and greys, it might be termed—results.

The Advantages of Staining

Yet, in connection with the soft and hard dental tissues generally, it is all important to employ staining fluids, all sections having first been examined under the microscope before the choice of stain is determined upon.

The various reagents used in microscopy may be classified as General and Specific.

Classification of Stains

The General Stains include all colouring matters that act on the plasma or ground-substance and nuclei of cells and tissues. They may therefore be spoken of as *Nuclear* and *Plasmatic*. No exclusively nuclear stain exists: the colouring of these parts of the cells depends on the comparatively greater affinity that some dyes have for them, than for the plasma in which they are imbedded.

The Specific Stains are those produced chiefly by the action of the salts of certain chemical compounds on certain tissues. Several aniline dyes are also included under this head.

The Two most Valuable

Of all the general stains, the two most important are hæmatoxylene and carmine, and their derivatives; and for all ordinary purposes the former is, beyond doubt, the more useful, either as a nuclear or as a plasmatic stain.

GENERAL METHODS OF STAINING, DEHYDRATION, AND CLEARING

Washing

Sections are removed from the preservative alcohol by means of a section lifter, and are dropped into a dish containing distilled water. Curled-up sections flatten out, twisted ones become unfolded, and presently thorough hydration takes place. A gentle stream of water from a glass pipette is useful for moving the objects about and washing them.

Immersion

(i.) *Staining by Immersion.*—Three watch-glasses are placed on the table and half filled with reagents. The first contains the staining solution, the second absolute alcohol, the third oil of cedar wood, or other clarifying medium. A glass capsule, of large dimensions, filled with distilled water, is also required.

The washed section is placed in the stain, in which it remains for a length of time variable according to the

nature of the specimen and the character and strength of the stain used. When the object is completely coloured, it is lifted out on a section lifter, and excess of colouring matter removed by well washing in distilled water.

Dehydration

It is now ready for dehydration, which is done by transferring the section to the watch-glass containing alcohol. After about three minutes' immersion, the tissue is made to float on to a clean section lifter, which removes it from the alcohol.

Clearing

Allow excess of spirit to drop from off the lifter, and finally place the specimen in cedar wood oil. Leave it here about one or two minutes. This 'clears' it, *i.e.* renders it transparent. The action of the alcohol is to get rid of all water from the tissue, and make it fit for immersion in oil.

The section is finally removed from the clarifying medium, laid on a glass slide, and immediately mounted.

For teased-out Specimens

(ii.) *Staining by Transfusion.*—This plan is only adopted when certain tissues have been teased out, and are, therefore, not suitable for transference from reagent to reagent. Specimens of enamel prisms, sheaths of Neumann, isolated odontoblasts, etc., may all be stained by placing a small portion of the teased-out mass in the centre of a glass slide, and covering it with a drop or two of normal salt solution or glycerine, and a cover-glass, and applying an aqueous solution of borax-carmine, or rubine, to the side of the cover-glass, by means of a glass rod. The dye immediately runs beneath, and soon stains the tissues, all excess being removed by means of blotting-paper held to the opposite side of the cover-glass. It is then washed with distilled water, to which a small quantity of a 1 per cent. solution of acetic acid has been added, if the stain has to be fixed as is the case with carmine. More salt solution is added, and in this or in glycerine the

tissues are mounted, the cover-glass being, at once, 'rung' round with cement.

General Stains

Hæmatoxylene

Of all the general stains, *Hæmatoxylene* is the most useful. There are numerous varieties of this stain—Ehrlich's, Delafield's, Kleinenberg's, Weigert's, &c., all differing in their formulæ. It is advisable, however, to make a point of using one kind for general purposes, and a special variety for special work.

The alcoholic solution of hæmatoxylene has, for its colouring principle, hæmatëin, and it may be used as a nuclear or as a plasmatic stain.

To Stain Cell Nuclei

As a Nuclear Stain.—Place in a glass capsule 6 c.c. of distilled water, and add three or four drops of a strong 'ripened' alcoholic solution, such as Squire's ammoniated hæmatoxylene. Stir the mixture well. Place in the fluid three or four sections of soft or decalcified specimens. Leave them for a quarter or half an hour. Remove and wash thoroughly, first with distilled water, then ordinary (slightly alkaline) tap water. The stain is thus rendered free from precipitate,[1] and the nuclei are tinged a clear dark blue.

Contrast Staining

Counter-staining may be accomplished by dehydrating sections stained as above in absolute alcohol, to which an alcoholic solution of eosine has been added in sufficient quantity to deeply colour the absolute alcohol. The sections remain in the alcohol for two minutes, and are then passed through cedar wood oil, and mounted in Canada balsam. Thus most beautiful results are obtained. All kinds of developmental specimens are good objects for thus doubly staining in blue and red.

[1] A heavy blue precipitate seems to form in most hæmatoxylenes, apparently due to the conversion of alum into free sulphuric acid, and a basic compound of alumina. See Bolles Lee's Manual 'Microtomist's Vade Mecum,' 1893.

As a Plasmatic Stain.—Proceed as just described, but leave sections in the hæmatoxylene from 18 to 24 hours. Do not counter-stain. Overstaining may be discharged by immersing sections in 70 per cent. alcohol to which one-tenth per cent. pure hydrochloric acid has been added. To neutralise the acidity of the specimen, place it in a solution of bicarbonate of soda, 1 gramme to 2 ounces, to prevent the subsequent fading of the stain.

For Cell Protoplasm and Nuclei

To remove Overstaining

Hæmatoxylene Staining Solutions.—The following formulæ will be found to be useful to the advanced student :—

(i.) Delafield's Stain—

To make Delafield's Stain

Hæmatoxylene (crystals) . .	4 grammes
Absolute alcohol	25 c.c.
To be added to	
Ammonia alum (Sat. aq. sol.) .	400 c.c.

Expose to light and air for several days, then filter and add

Glycerine (pure)	100 c.c.
Methylic alcohol	100 c.c.

A sufficient quantity of this mixture is to be added to distilled water to make a very dilute solution.

This is altogether the most powerful and precise of all the hæmatoxylene stains yet introduced.

(ii.) Ehrlich's Stain—

To make Ehrlich's Stain

Hæmatoxylene (crystals) . .	2 grammes
Absolute alcohol	100 c.c.
To be added to	
Glycerine (pure)	100 c.c.
Ammonia alum	2 grammes
Glacial acetic acid . . .	10 c.c.
Distilled water	100 c.c.

The stain retains its properties for years if kept in

well-stoppered bottles, and can be used for staining *en masse*. It is excellent for class use.

(iii.) Kleinenberg's Stain—

Hæmatoxylene (crystals) . .	2½ grammes
Rectified spirits of wine . .	240 c.c.

To be added to the two following solutions :—

Calcium chloride (crystals) . .	20 grammes
Distilled water	10 c.c.

And

Alum	3 grammes
Distilled water	16 c.c.

The above is Squire's modification of the original formulæ. It is highly recommended for the certainty of its results.

Carmine

Borax-Carmine is useful for staining in bulk, and as a nuclear or plasmatic stain.

Staining in Bulk.—For early embryonic jaws which are to be stained *en masse* and imbedded in celloidin, Grenacher's alcoholic solution of borax-carmine is valuable.

Place the tissue in the stain for one to four days ; remove to 70 per cent. alcohol plus one-half per cent. of pure hydrochloric acid for one day ; transfer to 90 per cent. alcohol for twenty-four hours, and finally keep in absolute alcohol before imbedding.

As a Nuclear and Plasmatic Stain.—Immerse sections in borax or lithium carmine for from half an hour to two hours. The stain may be removed from the ground substance of cells by placing in acidulated alcohol for ten minutes. Plasmatic stains are 'fixed' by washing in one per cent. acetic acid in water for five minutes, and then washing in distilled water. Sections are afterwards dehydrated, cleared, and mounted in the usual way.

Fixing

Contrast Staining

As a Contrast Stain to carmine, alcoholic picric acid

may be used. Treat the sections as just described, but do not place them in acidulated alcohol. After rinsing the sections for two minutes in 70 per cent. spirit, place them in picric acid for five minutes; then dehydrate, clear, and mount. This combination is useful for sections of the pulp and the periodontal membrane, the cells being stained pink and fibrous tissues yellow.

Additional Combinations

Extremely beautiful effects may be obtained by staining vertical sections of fœtal jaws with teeth *in situ* —similar to those figured in plates III. and VI.— with borax-carmine in the usual way, and counter-staining by immersing for a few minutes in an alcoholic solution of sulph-indigotate of soda. If this is done, the fibrous, connective, and other soft tissues are coloured violet, while bone and dentine are pink, and enamel (when present) a darker shade of pink. Another variation, producing equally striking results, occurs if eosine and methyl green are used in place of the carmine and indigo stains.

Other carmine stains comprise those known as lithium and ammonium carmines, and Beale's, Merkel's, Orth's, and Grenacher's solutions.

Carmine Staining Solutions:—

To make Beale's Stain

(i.) Beale's Stain—

Carmine	10 grains
Strong solution of ammonia	30 minims.

To be added, after boiling and evaporation, to

Glycerine (pure)	2 ounces
Alcohol	½ ounce
Distilled water	2 ounces.

A useful general stain.

To make Grenacher's Alcoholic Stain

(ii.) Grenacher's Stain—

Carmine	3 grammes
Borax	4 grammes
Distilled water	100 c.c.

To be added, after gentle heating, to

Alcohol (70 per cent.) . . . 100 c.c.

Caution

The student is cautioned against the use of Grenacher's *alum* carmine stain, as it has a dissolving action on all calcareous structures, and therefore soon destroys the hard parts of his dental sections. By the employment of the above, this inconvenience is avoided.

To make Orth's Stain

(iii.) Orth's Stain—

Carmine . . . . . 2½ grammes.

To be added to 100 c.c. of

Carbonate of lithium . . . 7 grammes

Distilled water . . . . 700 c.c.

This is both a nuclear and plasmatic stain. But if acidulated alcohol is used after staining, in the manner described on page 88, the colouring of the cell substance is removed, but that of the nucleus is still retained.

Useful Aniline Dyes

Other general stains are rubine, eosine, fuchsine, methylene blue, gentian violet, safranine, nigrosine, etc. These may be used as the fancy of the student dictates: for sections of decalcified teeth with the pulp *in situ*, the three first-named will be found of great value.[1]

Specific Stains

For what useful

These possess an affinity for certain elements in the tissues, and are used for demonstrating the outlines of cells, nerve filaments, etc., either singly or combined with plasmatic stains.

Varieties

In this group there may be mentioned, as suitable for dental microscopy, chloride of gold, osmic acid, chromic acid, salts of iron, and nitrate of silver.

Chloride of Gold

Chloride of Gold.—This is an important reagent for differentiating the course of nerve fibres, and marking out the soft tissues in connection with dentine and cementum.

[1] Accidental staining of the fingers with aniline dyes may be removed by an application of soap and pumice.

It is not necessary for the tissues to be absolutely fresh: those that have been previously hardened, and have passed through various stages of decalcification, may be stained equally as well as any fresh section.

There are several methods in vogue, but the most convenient is that introduced by *Mr. Underwood.* He proceeds as follows :—

Underwood's Method

'(*a*) Wash the sections in a solution of bicarbonate of soda. (5 grains to the ounce.)

'(*b*) Put some 1 per cent. solution of chloride of gold in a watch-glass, test it with litmus paper, and, if it be acid, add bicarbonate of soda by drops till it is neutral; place the sections in the solution, and cover the watch-glass with a lid to keep it in the dark—a lid of a china pot such as is used for potted meat serves very well—for from half an hour to an hour, until the sections look straw-coloured.

'(*c*) Remove the sections from the staining fluid to distilled water, and leave them covered over—they must not be exposed to light for more than a few seconds—for a few minutes.

'(*d*) Put some 1 per cent. solution of formic acid in a watch-glass, float the glass in hot water, put the sections in the acid, cover them over, and keep them in the dark, and fairly hot, until they turn crimson. This generally takes about an hour, but the operator must be guided by the tint of the sections, which he must look at from time to time. A simple way to do this is to fill an old china anchovy-paste pot with hot water, place it on a stove, float the watch-glass containing the acid and the sections in it, and cover it up with its own lid.

'(*e*) When stained, immerse the sections in cold distilled water for about half an hour.

'(*f*) Dry the sections and mount them in glycerine jelly. Avoid Canada balsam.'

Precaution

During the manipulations with this and the following

stains, it is advisable to use bone or wooden section lifters and other non-metallic instruments. It is not necessary to keep the bottle containing the gold solution in the dark.

Osmic Acid

Osmic Acid is valuable (i.) as a Specific; (ii.) as a Pigmentation stain: for the former purpose, it is useful for colouring the myelin sheaths of medullated nerves; for the latter, the interglobular spaces in dentine.

(i.) Place a fresh isolated pulp in a 1 per cent. solution of this acid for twenty-four hours in the dark. Wash with distilled water, imbed in gum, cut sections, counter-stain with eosine if desired, dehydrate, clear, and mount.

(ii.) Ground-down sections of dentine are first stained, for a few minutes, in the ordinary solution of hæmatoxylene, and then partially decolorised by means of very dilute acetic acid, in such a manner that the interglobular spaces only retain the colour. Wash the sections in distilled water, and then place them in a 1 per cent. solution of osmic acid for one hour. Finally, wash, clear, and mount. Thus, pigmentation of the interglobular spaces occurs, due, as Black has shown, to their being filled with a fine black amorphous deposit, which consequently renders them remarkably conspicuous.

Robertson's Modification of Heller's Stain

Heller's method of using osmic acid as a nerve stain, as modified by *Dr. Ford Robertson*, has been adapted with much success by Mr. Storer Bennett to the tissues of the pulp. 'The nerves stand out as a vivid black on a very pale background.'

Isolated dental pulps must first be hardened in Weigert's chrome-alum-copper solution, made up as follows:—

Chrome alum . .	2½ per cent.
Copper acetate . .	5 per cent.
Acetic acid . . .	5 per cent.
Formalin . . .	2 per cent.

Here they remain for ten days or more, as they will not be rendered brittle by the formalin. After removal and washing they are imbedded in gum or celloidin, and cut in the ordinary way.

Sections are then passed through the following reagents :—

Osmic acid . . .	1 per cent. (in the dark), half an hour.
Pyrogallic acid . .	5 per cent., half an hour.
Potassium permanganate	·25 per cent., three or four minutes.
Oxalic acid . .	1 per cent., three or five minutes.

Of course, sections must be freely washed between the use of each stain ; they are then dehydrated, cleared, and mounted. Eosine makes a good counter-stain.

Chromic Acid can be used as a $\frac{1}{8}$ per cent. solution for staining the peripheral nerves in a fresh pulp (Boll).[1] Chromic Acid

Iron and Tannin Stain.—Place the section, after washing in distilled water, in a capsule containing Liquor ferri perchloridi (B.P.) for twenty-four hours. Wash quickly in distilled water, and pass into a solution of tannic acid, 2 grains, and distilled water 6 c.c. for five or ten minutes. Remove and again wash in water. Iron

Mr. Howard Mummery (after Polaillon), in whose hands this iron stain has been very successful, has succeeded in tracing numerous fine fibres from the nerve bundles in the pulp.

Nitrate of Silver is said to be of service for bringing out the epithelial nature of Nasmyth's membrane by staining black the intercellular cement substance of the tissue. A $\frac{1}{2}$ per cent. solution in distilled water must be used, the membrane remaining in the stain for half an hour in the dark. After washing, it must be Silver

[1] Tomes's 'Manual of Dental Anatomy,' p. 45. 1894.

mounted in glycerine or Farrant's medium, and kept in the dark. This is the least useful of the specific stains, and cannot be recommended.

Contrast Stains

Other Contrast Stains

In addition to those already mentioned, the following make good counter-stains :—

Hæmatoxylene (Ehrlich's) and orange rubine.

Gentian violet and benzo-purpurine.

The Ehrlich-Biondi mixture—orange, fuchsine, and methyl green.

Borax-carmine (Merkel's) and borax-indigo-carmine.

For the last-named, use oxalic acid (a saturated aqueous solution) for washing sections. It fixes the indigo-carmine.

The above may be applied to specimens of developing tissues, pulp, and periodontal membrane and dentine.

For double staining soft tissues *en masse*, combine alum-carmine with osmic acid.

To variously Stain Developmental Tissues

Sections of fœtal jaws showing the development of teeth when stained with the Ehrlich-Biondi mixture and cut in celloidin make very beautiful objects. Their parts are variously stained : dentine and alveolar bone are rose-pink ; enamel, orange ; pulp tissues, pale brown ; odontoblasts, darker brown ; stellate reticulum, pale heliotrope ; muscles of jaw, dark brown ; nerve bundles, nearly black ; capillaries and contents, russet brown ; epithelium of gum, vandyke brown ; and ameloblasts, orange.

Special Stains

Need for Special Stains

When it is necessary to investigate certain tissues during original research, it is most desirable to employ more than one method of staining. There are several special stains that are suited for dental work, and they

must not be omitted here. A brief description of these special methods will be found useful.

Golgi's Stain.—There are three variations. The following is, however, convenient :— Golgi's Stain

Place fresh sections of dentine in bichromate of potash (2 per cent. solution) 8 parts, osmic acid (1 per cent. solution) 2 parts, for 24 to 36 hours.

Remove to a ·5 per cent. solution of silver nitrate for one day. The tissues should be kept in the dark during the latter part of the process. Dehydrate, clear, and mount. Cover-glasses may be used.

Marchi's Method for staining degenerate nerve fibres. Applicable to sections of dental pulp. Pulps are hardened for a week in Müller's fluid, and then for another week in a solution composed of Müller's fluid 2 parts, osmic acid (1 per cent. solution) 1 part. Large quantities of the solution should be used, and the tissues be very thin. Marchi's Stain

Weigert's Stain.—To blacken the medullated sheaths of nerves. Useful when pulps are prepared and cut *in situ*, having been previously hardened in bichromate of potash. Weigert's Stain

Sections are immersed in (i.) a saturated aqueous solution of acetate of copper with equal parts of water, kept at a temperature of 40° C. or 104° F. for two days. They are then washed in 90 per cent. alcohol, and placed in (ii.) a fresh solution made after this formula :—

Hæmatoxylene . .	1 part or 1 gramme
Alcohol	10 parts or 10 c.c.
Distilled water . .	90 parts or 90 c.c.
Saturated sol. of lithium carb.	1 part or 1 c.c.

Leave in the solution for twenty-four hours. They are again washed in distilled water for many hours, and finally decolorised in two hours by placing in (iii.) a solution of

Borax . . .	2 parts or 2 grammes
Potassium ferricyanide	2½ parts or 2·5 grammes
Water . . .	200 parts or 200 c.c.

Subsequently they are washed, dehydrated, and mounted in the usual manner.

Charters White's Stain

Charters White's Stain.—This method has for its object the differentiation of internal cavities and spaces in bone and teeth. It produces very beautiful specimens of the calcified tissues. Cut teeth into sections, having the thinness of about $\frac{1}{25}$ of an inch. Soak them in absolute alcohol for a short time, then place them in ether sulph. meth. Make a stained celloidin solution by adding fuchsine to alcohol 'until a dark port wine colour is produced,' mixing this with ether and adding celloidin till the required consistency is reached. Saturate the sections with this mixture for several days. Remove them and let them dry by evaporation. Finish by grinding on a wheel, and rubbing between plates of glass, and mount at once in balsam.

Methylene-Blue Injection Stain

The 'Intra-vitam' Methylene-blue Stain.—A method, variously modified, was introduced originally by Ehrlich for staining peripheral nerves. *Dr. Huber*,[1] of Michigan, has found it extremely useful for differentiating the medullated and non-medullated nerves in the pulp of rabbit. It was applied as follows: A rabbit was killed with chloroform, and immediately a canula inserted into the exposed common carotid of one side of the neck. A 1 per cent. solution of methylene blue made up in normal salt solution was injected in sufficient quantities to deeply pigment the tongue and lips of the animal. After half an hour had elapsed, the mandible was severed, and wiped with a dry clean cloth. On being broken with bone forceps the teeth were removed carefully. A molar having been chosen, the dentine of

[1] 'Dental Cosmos,' p. 804, Oct. 1898.

its anterior and posterior surfaces was cut away, a fine needle inserted under one of the processes of the pulp, some slight traction made, and the organ 'hardly crushed or lacerated' thus removed. The isolated pulp was placed at once on a slide moistened with normal salt solution. In a few moments the axis cylinders of the nerves were found to be stained a deep blue, the other tissues scarcely colourised at all. It is necessary to use a fixative, as there is a great tendency for the stain to fade. A saturated aqueous solution of ammonium picrate, or a solution of ammonium molybdate, may be employed, the preparations being mounted in glycerol. It is advantageous to cut sections. This is done most serviceably by placing pulps between two flat pieces of elder-pith, and cutting down between them with a microtome cutter. Thus the general distribution of the nerves is seen as well as their finer endings. Dr. Huber concluded that these fine fibrillæ (non-medullated fibres) 'terminate in free endings between the odontoblasts or between these cells and the dentine, and are not in connection with the dentinal fibres directly or through the odontoblasts.'

Stroebe's Stain

Stroebe's Method of Staining axis cylinders of Nerves. The special feature is that a practically isolated stain of the axis cylinder is obtained.

(i.) Tissue is hardened in Müller's fluid, thereafter in alcohol if desired, and sections cut as usual.

(ii.) Stain in fresh saturated aqueous solution of aniline blue for ten minutes to one hour. Sections become blue-black in colour.

(iii.) Wash off, in water, excess of stain. Then place in porcelain dish of absolute alcohol, to which has been added twenty or thirty drops of 1 per cent. solution of alkali alcohol (1 gramme caustic potash to 100 c.c. of alcohol, allow to stand for twenty-four hours, then filter). In the

alkali alcohol sections turn a rusty red colour, clouds of reddish matter issuing from them. As soon as these cease to form, and the sections are of a light red-brown colour and transparent, differentiation is complete. (One to several minutes.)

(iv.) Wash in distilled water for five minutes. Sections acquire a deep blue tint.

(v.) Bring them into following contrast stain for quarter to half an hour: Concentrated aqueous solution of safranine diluted with equal parts of water.

(vi.) Place in absolute alcohol to remove excess of safranine and to dehydrate. Sections again look red with a tinge of blue.

(vii.) Clear in xylol, and mount in xylol balsam. Thus axis cylinders appear dark blue, medullary sheaths, cell protoplasm, ground substance, and cell nuclei shades of red. Cell nuclei sometimes retain their blue colour.[1]

Freud's Process

Freud's Process.—(i.) Harden isolated pulps in Müller's fluid for four to six weeks.

(ii.) Place for five minutes in solution of

40 per cent. alcohol (not methylated), and

2 per cent chloride of gold.

(iii.) Place in very strong solution of caustic potash (saturated caustic potash diluted with five times the volume of water).

(iv.) Leave various lengths of time, to be decided by experiment; an isolated pulp requires five minutes.

(v.) Then bring into 10 per cent. solution of potassium iodide. Leave until pulp acquires a purple-red tint.

DEHYDRATING TISSUES

Special Dehydration

An immersion of two minutes' duration in absolute alcohol will thoroughly rid ordinary sections of all water, after they have been stained, and before 'clearing.'

[1] 'Centralbl. f. allgem. Path.' Band. iv. No. 2.

Very delicate tissues, however, shrink too much if placed at once in absolute alcohol. Therefore it is necessary, when dealing with these cases, to pass sections quickly through 50 per cent., 70 per cent., and 90 per cent. spirit, before finally immersing in absolute alcohol.

Table of Tissues suitable for Staining with

1. Hæmatoxylene and its varieties	All embryonic and fœtal soft tissues, pulp, periodontal membrane, gum, etc.
2. Carmine and its varieties	The same, and also enamel prisms, dentinal fibrils in young dentine, interglobular spaces. Fishes' teeth.
3. Fuchsine . .	Ramifications of dentinal tubules, cemental canaliculi, and the spaces in osseous tissue, etc.
4. Rubine, methylene blue, eosine, etc.	Dental pulp, and periodontal membrane, striæ of Retzius, dentine, dental gum.
5. Double stains as Ehrlich-Biondi fluid	Deve'opmental tissues.
6. Gold chloride . (Underwood's plan)	Interglobular spaces, odontoblasts *in situ*, nerves of pulp, dentinal fibrils, interprismatic enamel substance (Bödecker).
7. Osmic acid . .	Medullated nerves of pulp, interglobular spaces.
8. Chromic acid . .	The same.
9. Iron and tannin .	Dentinal fibrils, nerves of pulp.
10. Nitrate of silver .	As Golgi's stain—sheaths of Neumann *in situ*, interglobular spaces, layer of semi-calcified dentine.

CLEARING SECTIONS

Clearing

Several essential oils are used for this purpose, including the oils of cedar wood, cloves, bergamot, origanum, and also xylol or turpentine. Their functions are twofold—first, to render stained sections transparent, and, second, to prepare them for the balsamic mounting media.

The object is removed from absolute alcohol, and

floated on to the surface of the oil in a watch-glass. It should remain in the oil for 1–2 minutes.

Another Method

A newer and better plan is to put the clearing medium into a test tube, and carefully pour on its surface a quantity of absolute alcohol. Place the sections in the alcohol; they will shortly have sunk to the bottom of the test tube through the alcohol, which may then be drawn off by means of a glass pipette. Sections are then ready for mounting.

Special Precaution

Oil of cloves must not be used for clearing celloidin imbedded sections. It removes aniline dyes and causes a certain amount of shrinkage. Oil of bergamot clears celloidin, and does not dissolve it.

MOUNTING SECTIONS

The final stages of practical microscopy are concerned with the operations of permanently mounting sections, and 'finishing' slides by cementing their cover-glasses in such a manner that evaporation of the mountant cannot take place.

There are two chief methods by which sections may be mounted, viz. (A) Transference with a section lifter, and (B) Flotation. The former is the one more generally employed, being applicable to the majority of specimens which are to be preserved in Canada balsam or other media. Mounting by flotation is used when the sections are too thin or small or friable to be moved from reagent to reagent, as in the first method; and when they are to be mounted in aqueous media.

Reasons for using the Flotation Method

A

Mounting by Transference

Use of a Section Lifter

Sections having been stained, washed, dehydrated, and cleared as already indicated, are removed from the watch-glass of cedar oil by passing a clean section lifter

underneath them. Holding the blade horizontally with a considerable amount of oil and the section upon it, the student carries the section to a clean slide laid flat on the surface of the white porcelain tile or glass slab or a sheet of white paper lying on the table,[1] and slightly tilts the lifter, allowing the section, enveloped in oil, to run on to the slide, and guiding it with a needle-point towards the centre. Excess of oil is removed by tilting the slide and carefully absorbing what remains by means of a piece of clean thin blotting or filter paper. A drop of benzole balsam from the end of a glass rod is next placed on the top of the section, and a cover-

Mounting in Balsam

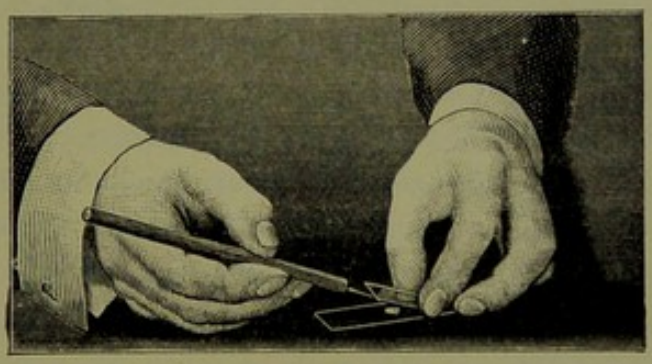


FIG. 22.—Method of applying the cover-glass to prevent the formation of air bubbles.

glass, which must be thoroughly clean and dry, having received a drop of the same medium on its reverse side (that is, the side which will shortly touch the slide), is gently lowered on to the section in the following way :—

Method of Applying the Cover-glass.—Hold the cover-glass by its periphery, between the left forefinger and thumb in a tilted position ; pass beneath it a needle, and gradually bring the needle closer and closer to the slide till the drops of medium have met (see fig. 22). Then slowly remove the needle, and the mountant will gradually fill the whole of the under surface of the cover-

To avoid Air Bubbles

[1] The black tile is used for the purpose of mounting *unstained* sections.

glass, at the same time that it excludes a ring of air from its centre. This method of placing the cover-glass in position is applicable to both aqueous and balsamic mounting media, and with a little practice it is thus possible to prevent any air bubbles from being retained between the glasses.

Removal of Air Bubbles

Should they, however, be present, the balsam should be slowly warmed over a spirit flame, and a mounted clip passed over the centres of both cover-glass and slide. The clip should remain in place for a few days.

If it is found after a section has been mounted that an insufficient quantity of balsam has been used, and that air *spaces* exist beneath the cover-glass, it is best not to warm the slide, but to apply a drop or two of thin balsam to the slide, near that edge of the cover-glass where the space is seen. The balsam, by capillary attraction, runs beneath the cover-glass, and efficiently fills the vacuum. In a week or two the mountant will be dry, and the slide can then be 'rung.'

Balsam in xylol becomes hard in a few days, and is often more useful than benzole balsam. Sections should have been clarified in xylol.

Final treatment

Any superfluous balsam that may have been pressed from beneath the cover-glass during or after mounting should not be removed until quite dry. If a penknife is used for scraping it away, and the edge of the cover-glass finally wiped with a clean rag moistened with xylol, the slide will present a neat appearance.

Other Methods of Mounting in Balsam

(1) *For Calcified Tissues.*—Dried, ground-down sections of teeth can be mounted in Canada balsam without previous dehydration or clearing, the object here being the retention of air and water in the dentinal tubules and other spaces. The highly refractive balsam

should not be thin enough to allow of its penetration into these spaces, or the structure of the hard tissues will become obliterated. For attaining this end, the plan of *Mr. Charters White*[1] is to be recommended.

Charters White's Method

The tooth having been ground while fastened on to the slide with Canada balsam, is unloosed from its position by long immersion in absolute alcohol, which may be wiped off with a camel's-hair brush. When quite clean, the section should be placed for a long period in distilled water till complete hydration occurs. The surfaces of the section should be then quickly dried on a clean thin cloth, and the specimen mounted in stiff balsam. The water and air in the dentinal tubules will thus prevent the balsam entering them.

Mr. J. E. Ady[2] adopts for the same purpose the following mode of procedure :—

Ady's Method

Finished sections are dipped for a moment in an alcoholic solution of white shellac, 'and withdrawn when a thin coating of the lac is left over its surface, occluding the spaces.' They are then mounted in the usual way.

A plan has been devised by *Mr. Mansbridge*[3] for giving the same results. He says :—

Mansbridge's Method

'Take a clean slide, place it upon a hot table with a small single lump of (desiccated) balsam upon it : use sufficient heat to slowly melt the balsam, which must not be made too hot. When sufficiently fluid, lay the section upon it, and cover with a hot cover-glass, which must be pressed down in such a way as to expel the air from beneath it. Remove the slide to a cool surface, and continue to keep pressure upon the cover-glass for a few minutes, when the balsam will be found to be quite hard.'

(2) *For Decalcified Tissues.*—Air may be well re-

[1] *Op. cit.* pp. 41 and 42. [2] *Idem*, p. 42.
[3] 'Transactions Odontological Society,' Vol. XXV., p. 176.

tained in interglobular spaces, tubules, etc., by treating sections of teeth with the method that *Professor Flemming*[1] recommends for decalcified bone.

Flemming's Method

They should be washed with alcohol and ether and laid flat on glass, and then covered with a double layer of blotting-paper under a heavy piece of glass. They can then be dried in the air, or better and more quickly in an oven. A glass slide having been prepared by putting a drop of warmed stiff balsam in the centre, and allowing it to spread out flat, receives the dried section. It is then covered with a similarly prepared cover-glass, a clip is applied, and the slide and section warmed over a spirit flame.

To remove the thick, hard, and perhaps dirty Canada balsam that sometimes occludes the clear interspaces of sections prepared by Weil's process, *Mr. Sydney Spokes* suggests the following :—

Spokes's Method

If the section has been sufficiently ground, it is well washed with water and scrubbed with a camel's-hair pencil. Next, it is placed in the centre of a glass slide, covered from dust, and allowed to dry. When about to mount the section, the student should have ready at hand a clean cover-glass with a drop of warm balsam in the centre. Then upon this section—which has not been moved from its position on the slide—must be dropped a succession of drops of chloroform, one drop at a time in such a manner that at no time does the chloroform become completely evaporated, or the section curled by drying. The solvent action of the chloroform will cause small *débris* to be thus finally floated to the circumference of the section without the parts being disturbed in their mutual relationship. Before the last drop of chloroform has evaporated, the cover-glass should be inverted and lowered gradually on to the specimen, in the way already described.

[1] 'Zeit. f. Wiss. Mik.,' 1886, p. 47.

B

Mounting by Flotation

The section, stained or otherwise, having been finally washed, is placed in a large dish of clean water, where it is flattened out, if necessary, by gently removing the creases or folds with a camel's-hair brush.

Modus operandi

A thoroughly clean slide is held between the fore-finger and thumb of the left hand at an angle of about 60°, and dipped for half its length in the water. The section is guided into the centre of the slide by means of careful manipulation with a mounted needle held in the right hand. If one end of the slide is tilted up a little further, and the needle-point fixes the section in position, the slide may be entirely withdrawn from the water, with the section lying flat upon its surface. Excess of water must be removed by the application of a piece of blotting-paper to the side of the section as the slide lies on the table top. A drop of an aqueous mountant having been put on the section, a cover-glass is applied, and the mounting completed.

Ordinary sections that are to be mounted in aqueous media are not necessarily always treated with the flotation process. In many cases the section is lifted out of the washing water and simply placed on the slide by means of a section lifter.

Aqueous Mountants

The Aqueous Mountants include, among others, glycerine jelly, Farrant's medium, normal salt solution, and soluble glass. Sections which are to be mounted in any of these fluids do not require dehydrating or clarifying, the media themselves performing these functions at the time of using. The two first-named are the most useful.

Glycerine Jelly is of service for mounting sections stained with chloride of gold, and for teased-out

Constituents of Glycerine Jelly

specimens, such as sheaths of Neumann, &c. It consists of French gelatine, glycerine, and distilled water, in the proportion of one, four, and six parts respectively. The slide, mountant, and cover-glass should be slightly warmed, and the slide 'rung' with cement as soon afterwards as practicable.

Similar in properties, but dissimilar in composition, is *Farrant's Medium*, which consists of equal parts of glycerine, powdered gum-arabic, water, and a saturated solution of arsenious acid.

It is useful, generally speaking, for all tissues, and can specially be recommended for mounting sections prepared by the author's process; but it must not be used for sections which have been stained by any of the aniline dyes.

Precaution

Normal Salt Solution is a valuable mountant after specimens have been stained by transfusion. It should be allowed to run under the cover-glass after excess of the stain has been washed away.

Soluble Glass has been found to be a fairly successful mountant by the writer. It does not possess any very special qualifications, and the foregoing media are certainly more reliable.

It is a mistake to mount more than one section on each slide, unless they are *extremely* thin.

SPECIAL METHODS OF MOUNTING

Special Slides for Ribbon Sections

For sections which have been cut in paraffin, and are to be arranged in series, special slides and cover-glasses must be purchased. The former should measure 6 by 1 or 2 inches, and the latter 5 by ¾-inch or 1½ inches. The sections are placed on a dry slide, one after the other, or in a ribbon, and the slide tilted to allow excess of water to drain away, ten minutes

To fix Sections by means of Shellac

being necessary for this. When nearly dry, drop some creosote-shellac on the section and place the slide over a water-bath for 24 hours. Dissolve the paraffin off with xylol, and the sections will be adherent to the glass. Clear them with oil of cedar, and mount in Canada balsam. Another way is to put a drop of water and spirit on each section, and evaporate carefully over a spirit lamp. Then use xylol, etc.

By means of Alcohol

Another way

A more convenient and satisfactory method is not to fix them on the glass by any special agent, but to allow them to dry in position on the slide, which has

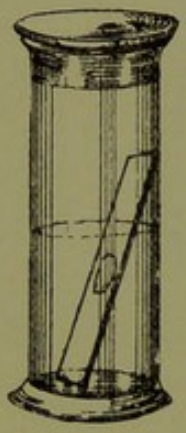


Fig. 23.

been tilted up on the surface of a piece of blotting-paper. When *all* traces of moisture have disappeared, each slide may be placed, still tilted, in a long glass 'specimen' jar, made for the purpose, containing xylol (see fig. 23). The paraffin having been dissolved out, the slide is next passed into absolute alcohol, and finally xylol, the reagents all being contained in suitable glass jars. Excess of the clearing reagent is now removed, and each section receives on its surface a drop of xylol balsam, and finally a large oblong cover-glass is carefully adjusted, and two cover-glass spring clamps applied.

SCHEME I

To show the Stages of making Sections of the Pulp in situ
(Author's method)

1. Fresh tooth
2. Wash saline solution
3. Open pulp cavity

4. Fix, harden, and stain in carmine-formalin
5. Close pulp cavity
6. Decalcify
7. Neutralise, wash in water
8. Gum mucilage
9. Cut sections on ether microtome
10. Wash

4. Fix and harden in formalin, or Müller's fluid and alcohol
5. Wash in water

If not stained *en masse*, then
11. Stain with hæmatoxylene, carmine, or aniline dye
12. Wash off excess, and fix, and again wash, if necessary

11. 50 % alcohol 13.
12. 70 % alcohol 14.
13. Absolute alcohol 15.
14. Xylol 16.
15. Mount xylol balsam 17.

11. Mount Farrant's medium 13.

SCHEME II

To show the Stages of making Sections of Developing Teeth

1. Fresh mandible (already fixed and hardened)
2. Divide vertically
3. Wash in water

4. Stain *en masse*
5. Stain fixed, if carmine
6. Water
7. 50 % alcohol

If to be stained after sectionised, then
4. 50 % alcohol

8. Absolute alcohol 5.
9. Xylol 6.
10. Molten paraffin 7.
11. Cut on microtome 8.

12. Place on slide
13. Dry thoroughly
14. Warm and dissolve off paraffin with xylol
15. Mount xylol balsam

9. Dissolve paraffin off with xylol
10. Absolute alcohol
11. 50 % alcohol
12. Stain hæmatoxylene or carmine, etc.
13. 50 % alcohol
14. Absolute alcohol
15. Xylol
16. Xylol balsam

UNMOUNTING SECTIONS

Need for Unmounting

It is sometimes necessary to remove a mounted section from the slide, and re-stain and re-mount it. This applies chiefly to sections which, having been kept some length of time, have faded, and to those for which a wrong stain has been used. Tissues dyed with hæmatoxylene, no matter how carefully it has been done, lose, in the course of years, a great deal of their beauty and their brilliancy.

If care is exercised, these old sections can be renovated with much success.

The process will vary with the nature of the mountant.

(i.) *Those Mounted in Canada Balsam.*—First remove the cementing ring around the cover-glass by soaking the whole slide in a small quantity of ether. A sharp fine knife-point will then scratch off the rest of the dissolved cement. The centre of the slide should next be held over a spirit flame, or placed on a hot brass table. Heat must be applied gradually, and at once stopped on any signs of bubbling of the balsam becoming noticed. Before the slide and cover-glass have become cold again, gently push the latter towards the side of the slide, and carefully lift it up with a pair of fine tweezers. Dissolve off the rest of the balsam by immersing the slide, with the section still adherent, in a bath of chloroform or xylol for five or ten minutes. The specimen is then removed from the slide by means of a small stiff brush, and placed on a clean glass slip and examined quickly microscopically, to ascertain if it has suffered by removal. If not, float it off on to distilled water, where it should be well washed for an hour. Then re-stain and mount in the ordinary manner.

(ii.) *Those Mounted in Aqueous Media.*—Place the

whole slide in a tray of hot water for about one minute. The heat will dissolve the mountant, and at the same time loosen the cover-glass, which should not be lifted up, but gently pushed along the surface of the slide.

FINISHING SLIDES

It is often quite unnecessary to 'ring' balsam preparations, though a black cement, neatly applied, always gives a slide a finished appearance. It is imperative, however, to cement cover-glasses having beneath them glycerine jelly or Farrant's solution.

Use of a Turn-Table

Fix the slide on a turn-table, by means of the clips, in such a position that the centres of cover-glass and the brass table correspond. Revolve the disc with the left

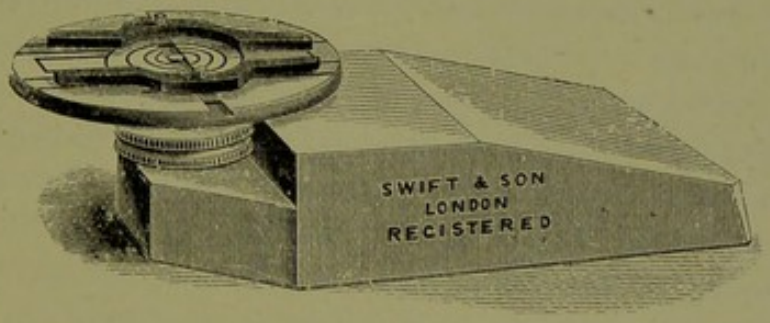


FIG. 24.

forefinger, and apply the medium with a long thin camel's-hair brush, a Rigger, No. 1, being best for this purpose. The brush can afterwards be washed with turpentine or soap and hot water.

About Cements

There are many cements made and sold for 'ringing' slides—Aspinall's black enamel gives very satisfactory results—black being preferred, because white or coloured cements soon begin to look dirty and a fresh 'ring' has to be applied.

Final treatment

Finally, a neat white label is affixed to one end of the slide, and the character of the specimen, the name of the stain, and mountant, and date noted

thereon, the slide being subsequently placed in the cabinet.

The student is advised to purchase his staining solutions, mountants, and cements ready made. They can be obtained of Messrs. Becker & Co., of Hatton Wall; Kanthack, of Berners Street; the Dental Manufacturing Co., of Lexington Street; and of all opticians and dealers in microscopical materials. The stains sent out from the laboratory of Dr. Grübler, of Leipzig, and sold by Messrs. Baker & Co., of Holborn, are perfectly trustworthy, and may be most highly recommended for the brilliancy of their results.

CHAPTER VII

ON THE PREPARATION OF SECTIONS OF THE PATHO-HISTOLOGICAL TISSUES

Patho-histology

THE attention of the student having been already directed to the consideration of various normal, human, and comparative dental tissues, he must now turn to the study of these same tissues when they have undergone morbid or retrogressive changes. A knowledge of pathology and patho-histology must necessarily follow the study of the structure of organs under natural and healthy conditions. Enough has been already said to indicate the general lines which must now be pursued. A few brief hints as to the methods of preparing and staining preparations need only be here discussed. The subject will be dealt with under the headings of morbid affections of (I.) enamel, (II.) dentine, (III.) cementum, (IV.) pulp, (V.) periodontal membrane, (VI.) gums and oral mucous membrane, and (VII.) bones of the jaws.

I. *Enamel*

Enamel, abnormal as well as normal, must be, as a rule, ground down on a carborundum wheel on a lathe, rubbed thin between plates of glass, and then polished, to remove scratches, on a leather strop, or an Alpmêr or other stone, and mounted, unstained, in balsam. If sections are very dense, in spite of being thin, then clearing in oil of

cedar or cloves, or in xylol is necessary. Thus, sections showing erosion, attrition, abrasion, nodes, and honeycombed and syphilitic appearances may all be treated. Caries of the hard parts will be specially described in a subsequent chapter.

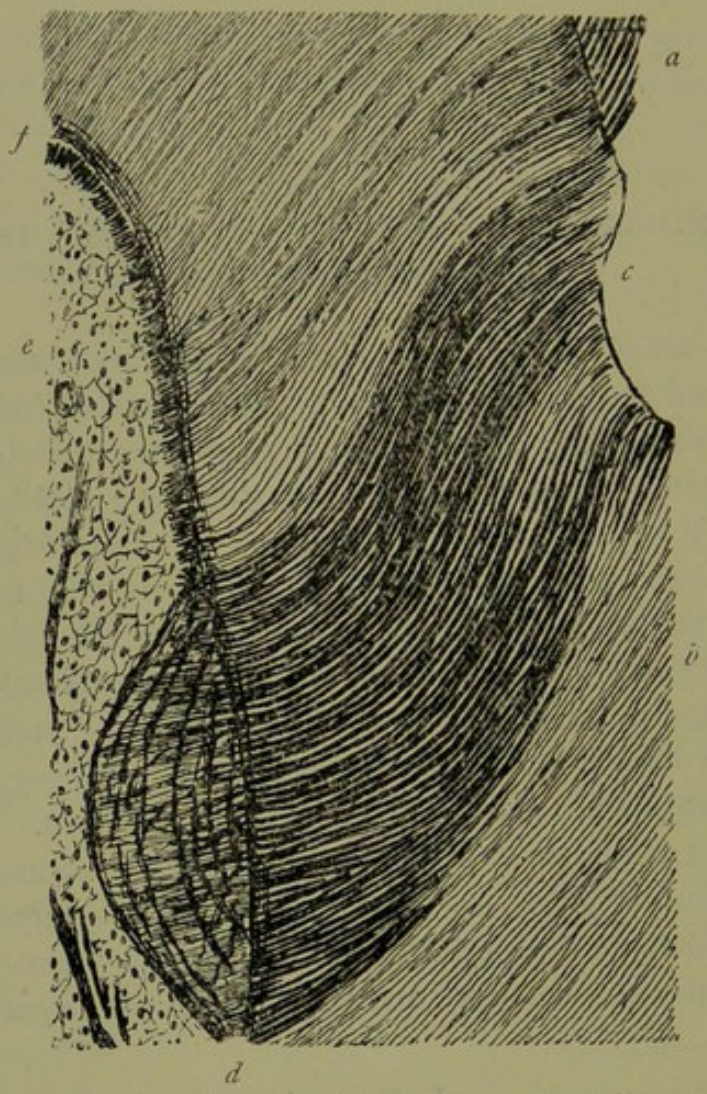


FIG. 25.—Longitudinal section of tooth affected by erosion, × 40. *a*, granular enamel; *b*, normal dentine; *c*, hyper-sensitive eroded surface with dark dentinal tubules; *d*, freshly deposited fine-tubed dentine (Salter's 'dentine of repair'); *e*, pulp tissue; *f*, odontoblasts.

In sections of (A) *Erosion* of enamel, and underlying dentine:

Structure under Low Powers

OBSERVE.—The marked granularity of the enamel prisms, with many developmental defects throughout its

substance, and the pronounced striæ of Retzius. When stained with borax-carmine, the parts underlying the eroded surface remain uncoloured. Sections show this when prepared by Weil's process, a thick band of tubules standing out dark and clearly defined against the pink coloration of the other tubules. Note a deposit of fine irregular-tubed adventitious dentine at the pulp end of this band, extending in a rounded shape

FIG. 26.—Longitudinal section of tooth presenting the so-called 'honey-combed' appearance. *a*, imperfectly developed enamel; *b*, dentine; *c*, pronounced striation of enamel prisms; *d*, interglobular spaces; *e*, pit or fissure on free surface of enamel.

into the pulp cavity. In sections made by the author's process, and stained with hæmatoxylene, the band is also visible.

In (B) *Attrition* and (C) *Abrasion*:

OBSERVE.—The tubules ending with large open mouths on the free surface of the tooth. Structure under High Powers

In (D) *Enamel nodules*:

OBSERVE.—The thick layer of badly, irregularly Structure under Low Powers

developed tissue situated on an eminence of irregular-tubed dentine.

Sections of enamel exhibiting developmental errors, termed the (E) '*Honeycombed*' *appearance*, must be ground down with great care, as enamel is very brittle. Stain with *Charters White's* stain, and

Structure under Low Powers

OBSERVE.—The faulty enamel prisms, and the broad dark bands running irregularly through it. The dentine is remarkable for its large numbers of inter-globular

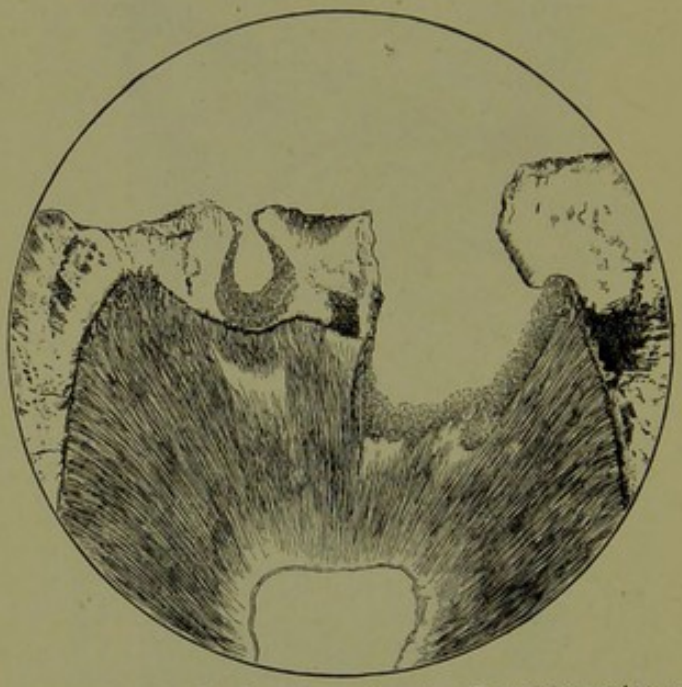


FIG. 27.—Longitudinal section of molar affected by caries, and exhibiting the 'Transparent zone.' (Mag. 10).

spaces, which may exist in several layers beneath the defective enamel.

II. *Dentine*

Dentine must be subjected to similar treatment to enamel. Unstained preparations are very useful, but the coloured celloidin stain of *Charters White* permeates well the ultimate ramifications of the tubules, all spaces and fissures, and yields very effective results.

The (A) '*Transparent Zone*' associated with dental

caries is best observed in teeth where the enamel only has been attacked by decay, and in teeth that have undergone partial arrest of caries. In longitudinal sections :

Structure under Low Powers

OBSERVE.—A cone-shaped zone extending between the carious and healthy parts, its apex placed towards the pulp cavity. Sometimes the zone appears as a white line only. In transverse sections, occasionally, islands of new dentine are seen surrounded by areas of normal dentine, which are bordered by irregular rings of translucency.

FIG. 28.—Areolar adventitious dentine, longitudinal section ; high power.

A (B) *Vascular Canal* passing from the pulp cavity to the periphery of the cementum occurs more or less frequently. Dentine should be prepared by Weil's or the author's process.

Structure

OBSERVE.—Running throughout its length the afferent artery and efferent vein, the latter sometimes blocked by a thrombus.

New growths of dentine or (C) *Adventitious Dentine*[1] —(i.) areolar, (ii.) cellular, (iii.) fibrillar, (iv.) hyaline, and (v.) laminar—are associated with many lesions of the enamel and pulp. They are seen in teeth affected

[1] The 'secondary' dentine of some authors.

by caries, in cases of exposure of the pulp, and in many hyperplasic conditions of that organ. Longitudinal sections are best for the exhibition of these varieties,

FIG. 29.—Cellular adventitious dentine, longitudinal section; high power.

made after treatment by the author's process, and subsequently stained with hæmatoxylene.

Structure

OBSERVE.—In (i.) an arcolation very similar in appearance to interglobular spaces;

FIG. 30.—Fibrillar adventitious dentine, longitudinal section; high power.

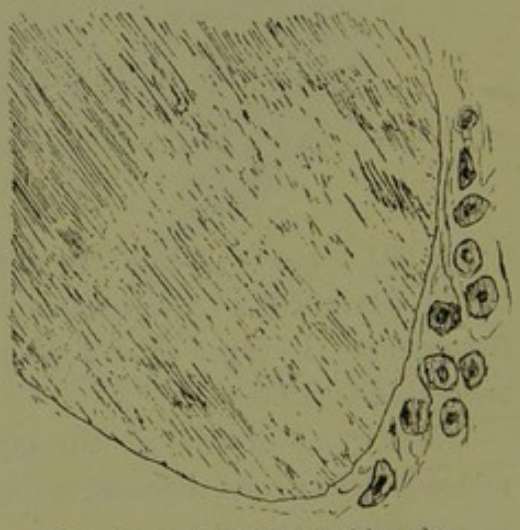


FIG. 31.—Hyaline adventitious den longitudinal section; high power.

in (ii.) cells with nuclei retained (suddenly caught, as it were) in the midst of the deposit;

in (iii.) fibrils, usually very fine and straight;

in (iv.) total absence of cells or apparent fibrification of the matrix, similar to the ground-glass-like matrix of hyaline cartilage ;

and in (v.) laminæ arranged concentrically around a cell or nucleus, similar to a section of an onion.

Two of these varieties are figured in the frontispiece.

(D) *Gemination* and *dilaceration* of teeth. The former is generally found as united mandibular temporary lateral incisor and canine.

OBSERVE.—The normal structure of enamel and Structure

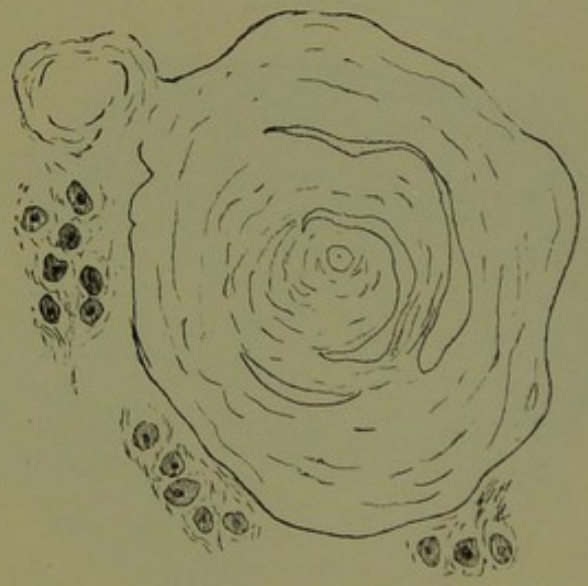


FIG. 32.—Laminar adventitious dentine, in chronic inflammation of the pulp, transverse section ; high power.

dentine, and the very slight increase in deposition of fresh cementum.

'False' gemination may be distinguished from gemination proper by the cementum being grossly hyperplasic, and the teeth themselves fairly regular in size and shape.

Dilacerated teeth deviate in their position from the line of the dental arches. They may be found, *e.g.*, high up in the dental arch.

OBSERVE.—The irregularity in amount and structure Structure

of enamel and dentine. The cementum is but little altered.

Longitudinal sections should always be made. It is unnecessary to stain such specimens.

III. *Cementum*

Pathological conditions are best studied when transverse sections have been made.

Exostosis, or cemental hyperplasia, makes very

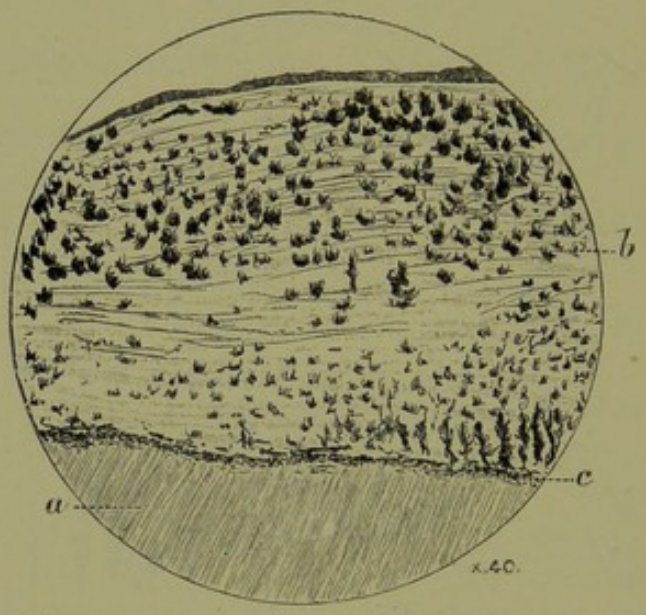


FIG. 33.—Transverse section of tooth affected by cemental hyperplasia (mag. 40): shows *a*, normal dentine; *b*, cementum, enlarged and thickened (exostosed); and *c*, 'granular' layer.

beautiful sections, especially when treated by *Charters White's* or *Caush's* method.

The latter, called 'surface staining' by the originator, proceeds as follows:—

Caush's Method of Staining

After the section has been rubbed down to the required thinness, it is placed in distilled water for a short time, and then transferred to an aqueous solution of fuchsine or similar aniline dye, where it remains from one to thirty minutes, according to the strength of the

stain employed. A rapid examination under the microscope will show if the proper length of time has been given to it. If this is so, any soft tissue that may be adherent to the section will be coloured very deeply ; the cementum, with its exostosed tissue, stained, but not so deeply ; whilst the dentine should retain its normal whiteness. Overstains can be easily removed by rubbing very slightly between ground-glass plates. Sections are then dried, cleared if necessary, and mounted in balsam.

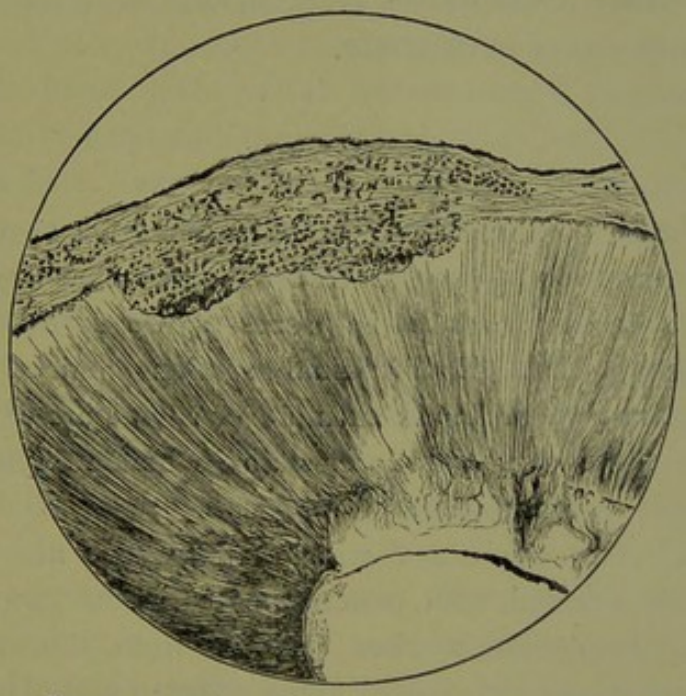


FIG. 34.—Transverse section of tooth (× 10) : shows a portion of the periphery of the dentine absorbed and fresh cemental tissue deposited therein, 'inostosis.'

Still more variegated sections may be obtained by combining these two stains, and using methylene blue for 'surface staining.' As a result the tubules are pink or brown, dentine matrix uncoloured, lines of Salter blue, and the rest of the exostosed tissue violet. Double Staining

OBSERVE.—The largeness and coarseness of the lacunæ ; the canaliculi running towards the periphery of the section ; the prominence of the intercemental lines of Salter, with clear intervening laminæ. The granular Structure

Exostosis

layer is unstained. In some instances observe, too, absorption of the primary dentine (Howship's lacunæ), and that corresponding re-deposition of cemental tissue termed 'inostosis.' Vascular canals frequently exist. Three varieties of new cementum may sometimes be observed according to Mr. E. Lloyd Williams, (α) a granular, nearly structureless form; (β) the familiar lacunar form; and (γ) a laminar variety which occurs in connection with teeth whose pulps are alive, and in third molar teeth whose eruption has been attended with any degree of difficulty.

IV. *The Pulp*

The patho-histology of the pulp, like other tissues, is most suited for study and research when that organ is cut *in situ*, by means of the processes already described in Chapter IV. Longitudinal sections stained with hæmatoxylene or borax carmine or rubine are of most value. Eosine as a counter-stain to hæmatoxylene is desirable in the majority of instances.

(A) *Hyperæmia and acute inflammation* are found in teeth affected with penetrating caries and exposed pulps. Immediately after removal from the mouth, they are washed in normal saline solution and placed in formalin, afterwards being treated either by Weil's or the author's process. If the latter is employed, sections may be coloured by any staining method as desired.

Dr. Black, in 'The American System of Dentistry,' 1887, pp. 841 and 842, recommends the following plan for obtaining specimens of pulp hyperæmia. It is here given *in extenso*.

Impregnation with Gum for Hyperæmia of the Pulp

'When a suitable case is presented, first examine the condition of the tooth itself as seen in the mouth. Then obtain its history, the symptoms it has presented from the first painful impressions until the present. If

the pain has been paroxysmal, find, if possible, what has been the disturbing cause that has ushered in the paroxysms, the duration of the paroxysms, the occurrence of soreness on closing the mouth, and, in short, a full history of the case. The condition of the tooth at the moment of extraction, especially as to pain, is a matter of prime importance in this study.

'Now extract the tooth, and drop it at once into Müller's fluid. It should not be handled nor disturbed in any way. It should lie in the fluid for at least one week, at the expiration of which time it will be found that the blood in the vessels has become so hard that it will not be displaced if carefully handled, and that the red globules have preserved their form perfectly, and will do so during the subsequent handling. Hardening

'After the expiration of this period the tooth should be cracked in the vice, as recommended by Salter. This is done by wrapping it in muslin, and placing it in the jaws of a powerful vice, and bringing them together steadily until the tooth cracks open. If it is skilfully placed, the line of fracture will generally follow the long axis.

'Then place the tooth in clear, freshly filtered Müller's fluid, and carefully remove the pulp from its bed. In some instances the layer of odontoblasts will remain adherent to the walls of the pulp-chamber, in others it will remain with the pulp, and often the dentinal fibrils will be pulled out of the dentine to a considerable length.

'The pulp is now to be placed in a thin solution of gum-arabic, to which some gum camphor has been added to prevent mould. The strength of this solution is very important ; *it should in no case be strong enough to float the pulp*. If the fluid be of greater specific gravity than the pulp, its tissue will shrink, otherwise not. Strength of Gum Solution

Evaporation

'The gum solution should now be slowly evaporated in any convenient way, so that it is not done too rapidly, to the consistence of very thick jelly. This requires three or four days, and it will be found that the impregnation of the pulp-tissue with the gum will keep even pace with the thickening of the solution, and that the tissue will remain at the bottom of the vessel.

Hardening the Mass

'When the solution has become as thick as is consistent with handling, the pulp should be taken up with as much mucilage as will adhere to it, and be placed in such a position as may be desirable for cutting on a bit of fine cork, which is then floated on alcohol with the side on which the pulp is placed down. In from twelve to thirty-six hours the surface will become hard from the abstraction of the water by the alcohol. It should not be allowed to become too hard, or the tissue will be injured.

Cutting

'When the drying has reached the right point, the tissue, cork and all, should be invested in the microtome in the proper position for cutting, using paraffin or other suitable substance for imbedding, and allowed to stand for twelve or twenty-four hours. The moisture remaining in the mass will by this time have become evenly distributed, so that it will be of equal consistence throughout. It should now be just hard enough to cut smoothly when kept wet with alcohol.

Mounting

'The sections may be mounted directly in glycerine without dissolving out the mucilage, and every cell retained in position, or the mucilage may be dissolved out in tepid water, and afterwards the section may be stained or prepared in any way desirable, just as can those obtained by any other process; and it will be found that the blood will remain in all but the largest vessels.'

To Stain the Red Corpuscles

This may seem to be a somewhat tedious plan, but it is reliable. The author uses hæmatoxylene; but if a

one per cent. aqueous solution of methyl-green be combined with a weak, watery solution of eosine, most beautiful effects will be produced, the latter being a specific stain for the hæmoglobin of the red blood corpuscles, turning them a coppery-red colour.

In hyperæmia, accompanied by inflammation:

Structure under High Powers

OBSERVE.—The enlarged tortuous vessels, in many places still retaining their blood corpuscles; the enormously increased number of pulp-cells, due to proliferation of pre-existing connective tissue and other cells; the migration of leucocytes; the increase in number and layers of the so-called odontoblasts; and the presence in the centre of the tissues of isolated cylindrical dentine formations in the neighbourhood of the vessels. Notice in slight cases, partial or 'regional'[1] hyperæmia (dilated vessels crowded with blood cells, many of which have passed through the vessel walls into the tissues around); the rest of the pulp presents features differing but little from its normal aspect.

In (B) *Acute Inflammation*:

Structure

OBSERVE.—The various forms of adventitious dentine, previously described; the altered appearance of the odontoblasts; the large masses of calcoglobulin deposited in cylindrical forms and stained more intensely than the other parts, the nerve bundles having lost their definite structure. Notice, too, under high powers, several small translucent, structureless, non-laminated nodules similar to calcospherite spherules interposed here and there between the odontoblasts, at the dentine border, and sometimes in Weil's basal layer. (These are not constant.) In suppuration, the cells in the neighbourhood of the abscesses are broken down

[1] 'Regional hyperæmia' is a term used to indicate a localised partial hyperæmic condition of the blood-vessels. According to its situation, one may speak of 'coronal' or 'cornual,' 'cervical,' and 'radicular hyperæmia.

altogether, and changed to indifferent cells with squarish nuclei; escaped leucocytes crowd the tissues, and, surrounding the abscess, undergo fibrification, thus attempting to heal the lesion.

Suitable Staining

(C) *Chronic Inflammation* of the pulp, incorrectly termed 'polypus,' should be stained with picro-carmine, the fibrous tissues and superficial epithelial cells with the

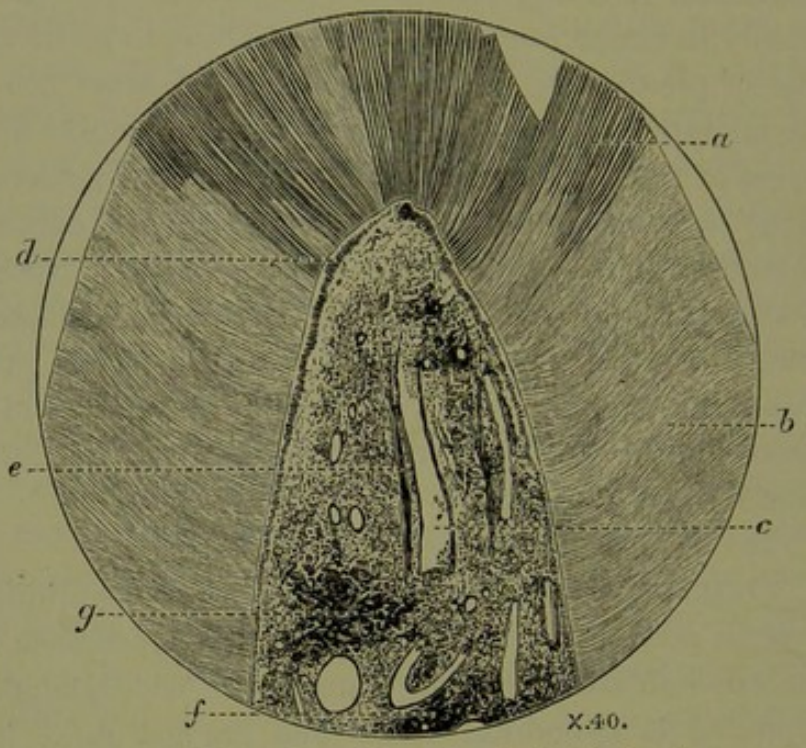


FIG. 35.—Acute inflammation of the pulp, longitudinal section prepared by author's method, pulp *in situ*: shows *a*, carious dentine; *b*, normal dentine; *c*, hyperæmic capillaries; *d*, degenerate odontoblasts; *e*, nerve bundles; *f*, pulp tissue filled with inflammatory products; *g*, commencement of formation of abscess.

dentine being stained yellow, and the cellular elements pink. (See figs. 37 and 38.)

Structure

OBSERVE.—The large, squarish, nucleated cells, together with numbers of new blood-vessels imbedded in a firm fine stroma of connective tissue fibres; and in many instances a layer of squamous and cylindrical epithelial cells in the free surface of the growth. Often these cells dip into the substance of the growth, forming

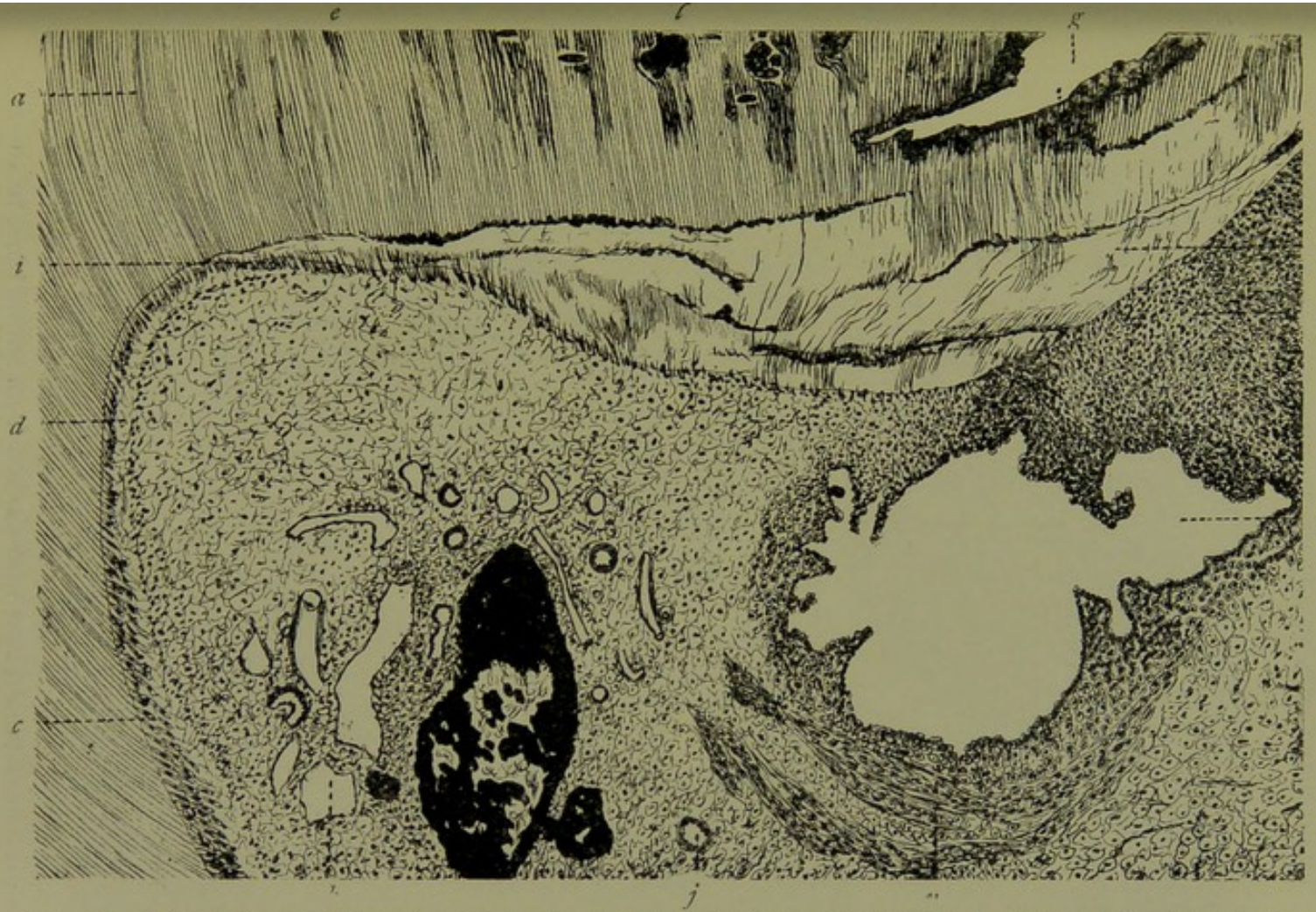


FIG. 36.—Inflammation and suppuration of the pulp, vertical section (mag. 45) : shows *a*, dentine ; *b*, pulp tissue ; *c*, odontoblasts multiplied in size and number ; *d*, basal layer of Weil ; *e*, micrococci in dentinal tubules ; *f*, liquefaction foci ; *g*, carious cavity in dentine ; *h*, fibrillar adventitious dentine ; *i*, degenerate odontoblasts ; *j*, artery ; *k*, vein in cornual hyperæmia of the pulp ; *l*, inflammatory cells and products ; *m*, abscess cavity ; *n*, fibrification of cells, attempting to heal the abscess ; *o*, mass of freshly deposited calcoglobulin.

appearances identical with the simple and compound papillæ of the oral mucous membrane. Occasionally laminar and cellular adventitious dentines may be found.

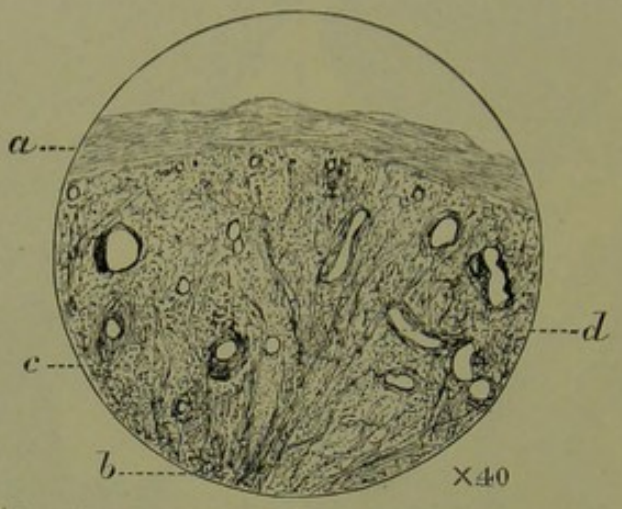


FIG. 37.—Chronic inflammation of the pulp, longitudinal section: shows *a*, stratified epithelium, in this case not assuming the form of papillæ; *b*, fibrous stroma; *c*, inflammatory cells; *d*, blood-vessels.

FIG. 38.—Chronic inflammation of the pulp, a more highly magnified portion of fig. 37. A, fibrous stroma; B, cells which make up the great part of the new tissue.

Whence obtained

Specimens of (D) *Fibroid Degeneration* of the pulp may be obtained by a rare chance from sound loose teeth in aged persons. Rubine is an excellent stain.

OBSERVE.—In longitudinal sections the great re- Structure
semblance to retiform tissue (minus cells); and, in transverse sections, minute chains of areolæ stretching

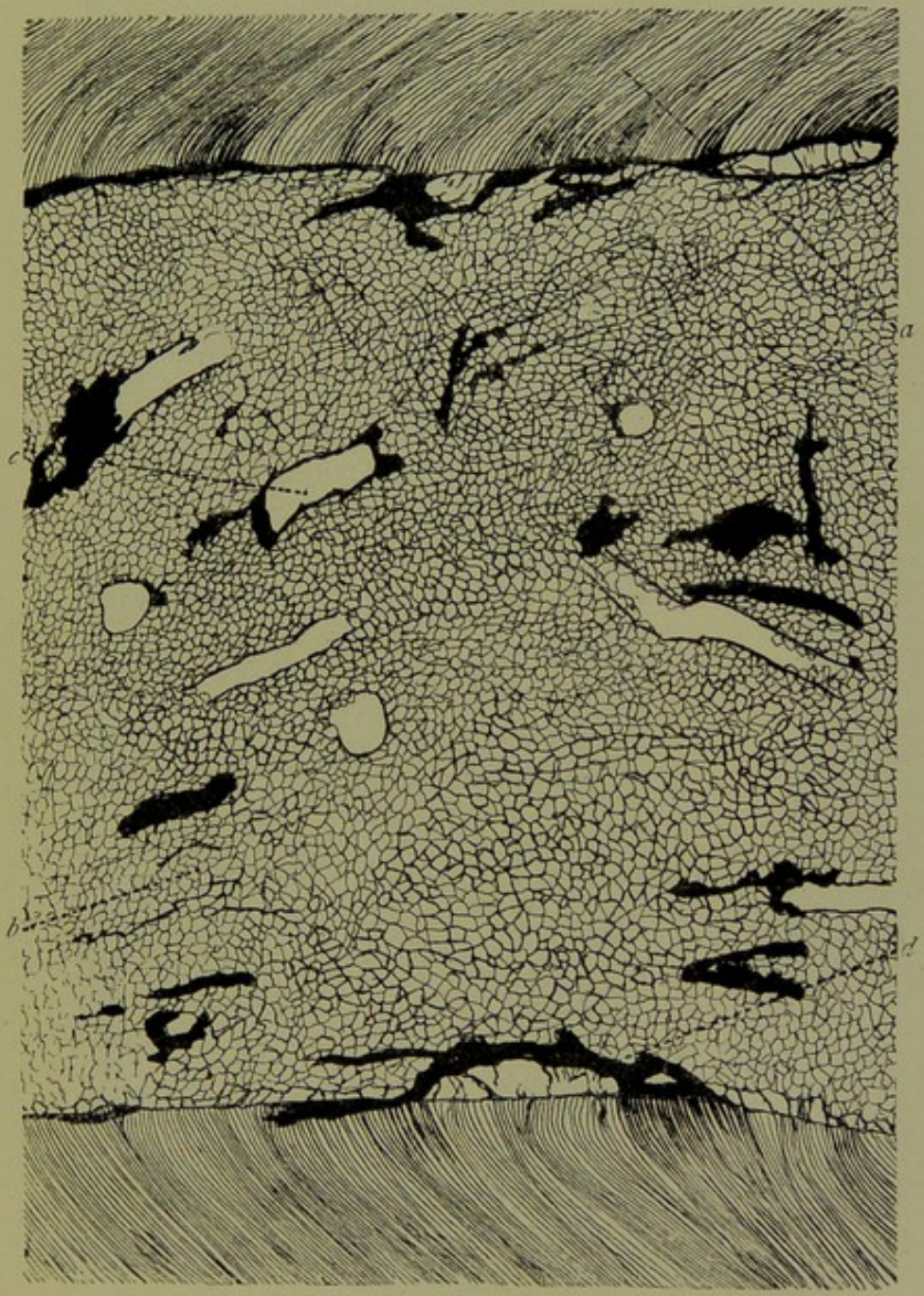


FIG. 39.—Fibroid degeneration of the pulp, longitudinal section (mag. 45): shows *a*, dentine; *b*, reticular appearance of pulp tissue; *c*, areolæ; *d*, shrunken odontoblasts.

across the pulp cavity. Notice the absolute disappearance of all cells, blood-vessels, nerve bundles, etc., nothing being present but a firm fibrous connective tissue.

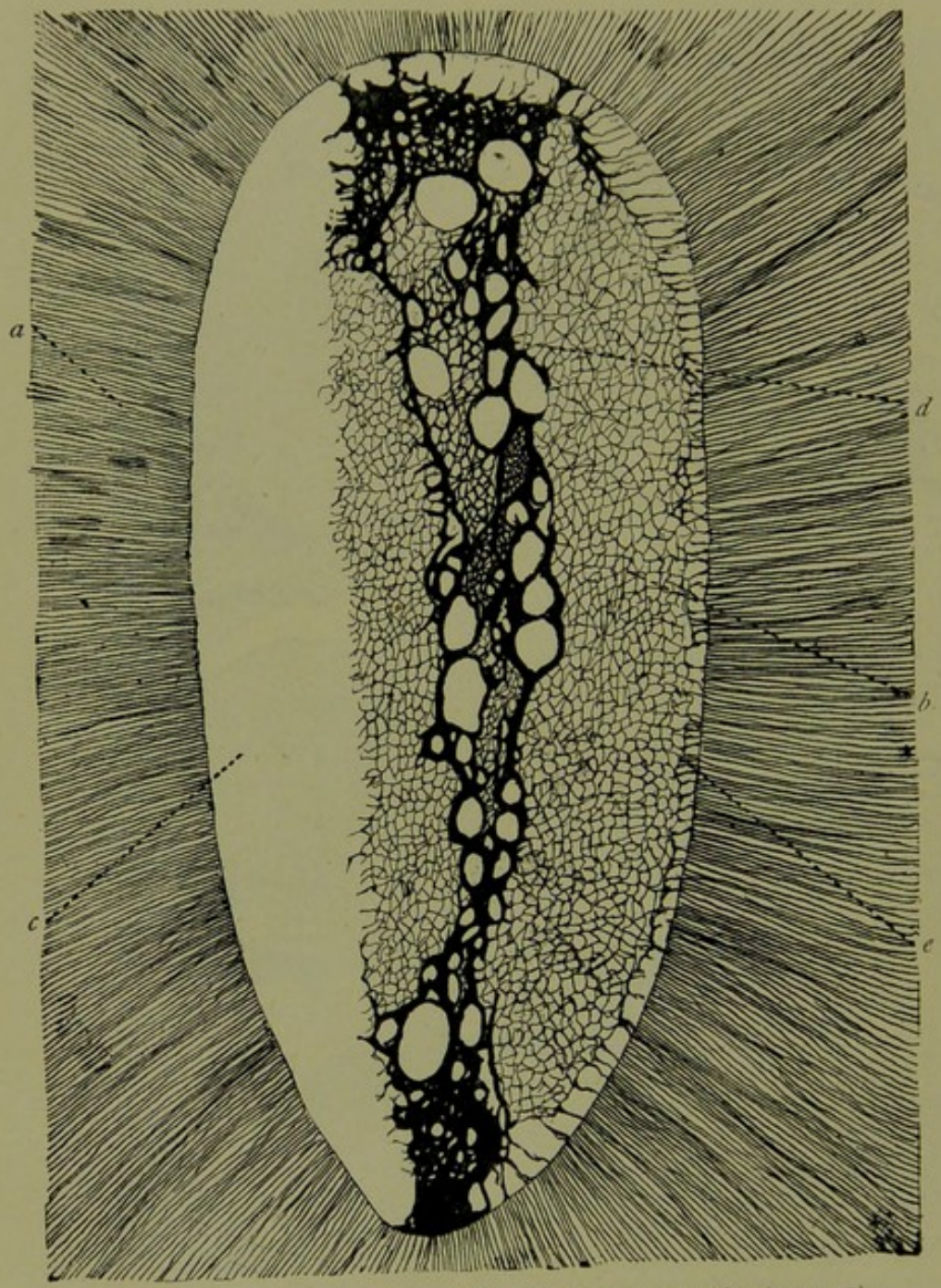


FIG. 40.—Fibroid degeneration of the pulp, transverse section (mag. 45): shows *a*, dentine; *b*, retiform connective tissue of pulp (minus all cells, nerves, and vessels); *c*, pulp cavity, the space produced by the fibroid shrinkage of the tissues; *d*, areolæ; *e*, fibroid odontoblasts.

(E) *Atrophic and Fatty Degeneration* may be seen sometimes in senile teeth.

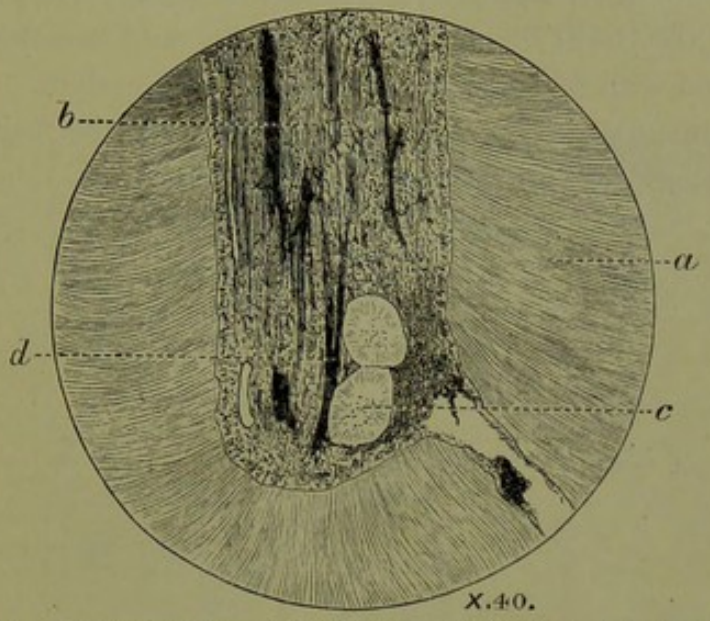


FIG. 41.—Calcareous changes in the pulp, longitudinal section of pulp *in situ*: shows *a*, dentine; *b*, normal pulp tissue; *c*, pulp nodule; *d*, nerve bundles.

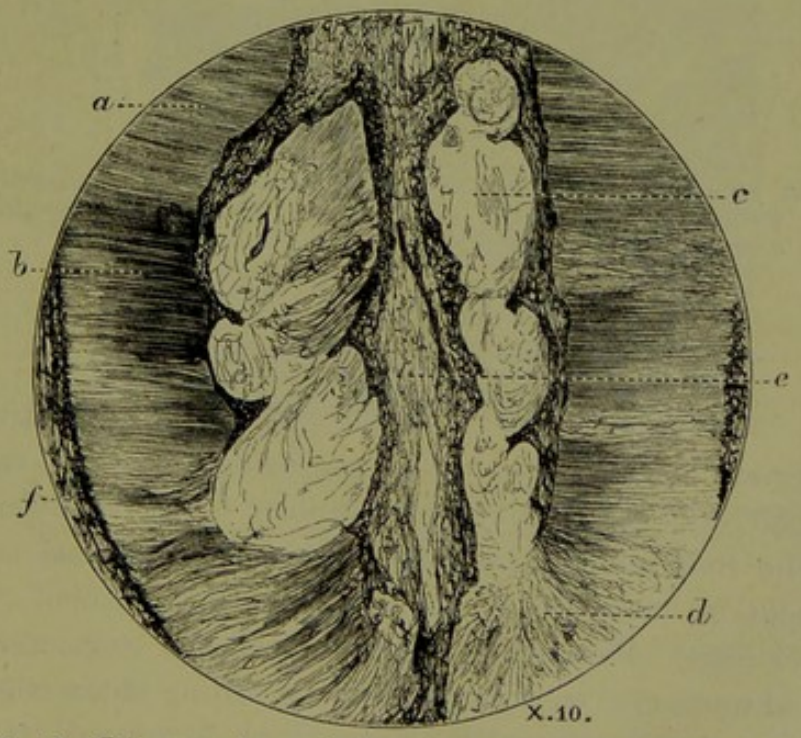


FIG. 42.—Calcareous changes in the pulp, longitudinal section: shows a large number of calcareous deposits. *a* and *b*, normal dentine; *c*, pulp nodule; *d*, irregular tubed dentine; *e*, pulp tissue; *f*, cementum.

Forms in which Calcareous Degenerations may occur

(F) *Calcareous changes* in the pulp are often found, not only in young sound teeth, which have been the source of odontalgia, but also in senile teeth. They may take the form of nodules, or rods, and are highly stained with fuchsine or other of the aniline dyes.

OBSERVE.—The general structure and the fusiform and rounded cells situated at their peripheries.

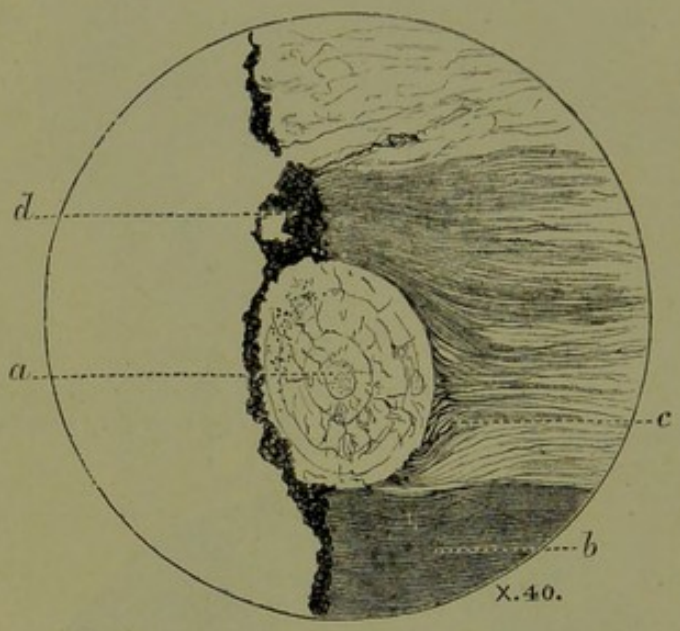


FIG. 43.—Nodule of calcoglobulin attached to the wall of the pulp cavity: shows *a*, 'pulp stone'; *b*, dentine; *c*, bent tubes of dentine; *d*, remnants of pulp tissue.

V. *The Periodontal Membrane*

Methods of Preparing

Morbid affections of this tissue are noticed after removal of teeth, the membrane being thickened, vascular, and in chronic cases purulent or semi-purulent. The roots should be carefully rinsed in normal salt solution, hardened in formalin, and then alcohol (84 per cent.) Finally they are decalcified, and longitudinal and transverse sections obtained by cutting on an ether-freezing microtome. Hæmatoxylene is a good stain, and eosine a counter-stain.

In (A) *Acute and Chronic Inflammation*:

Structure under High Powers

OBSERVE.—The tissue crowded with leucocytes; the 'principal fibres' separated by masses of inflammatory cells and products, many breaking down into pus

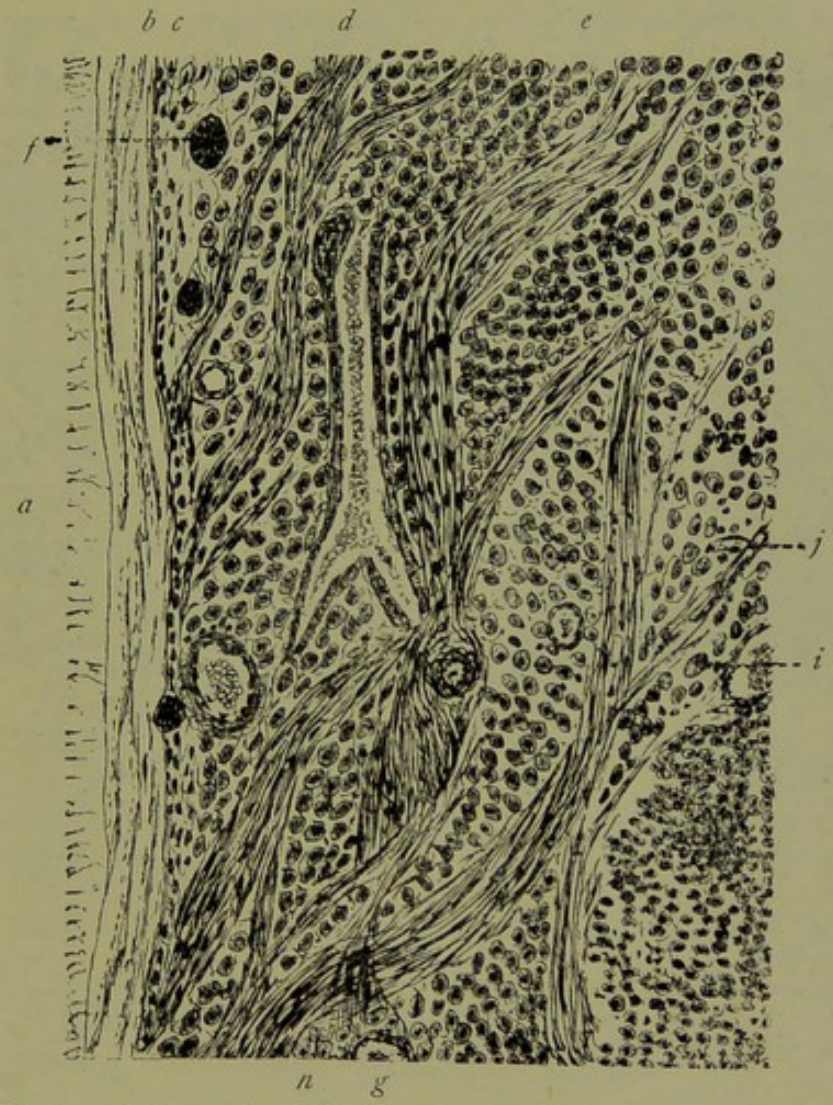


FIG. 44.—Inflammation of the root membrane, longitudinal section (mag. 150): shows *a*, dentine; *b*, cementum; *c*, cementoblasts; *d*, 'principal' fibres; *e*, proliferated connective tissue cells; *f*, lymphatic; *g*, blood-vessel with blood corpuscles; *h*, escaped leucocytes; *i*, cell undergoing karyokinesis; *j*, newly formed capillary.

corpuscles, and the perivascular tissues infiltrated and thickened.

(B) *Abscess of the Periodontal Membrane.*—Occasionally after extraction of a carious and dead tooth,

To make Sections

a rounded or pyriform mass of soft tissue may be seen depending from the apex of the root, and closely associated with its membrane. The root should be carefully

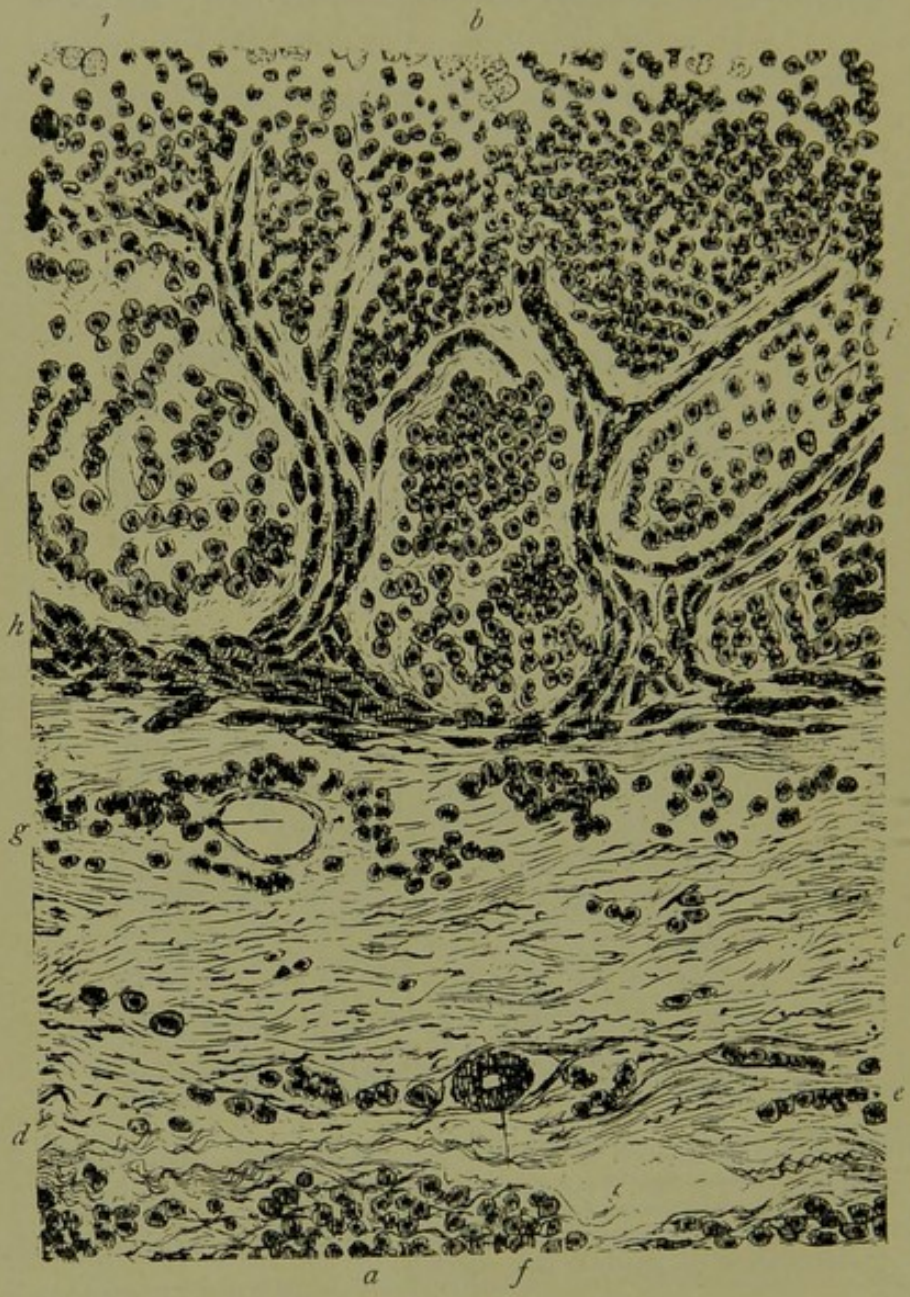


FIG. 45.—Abscess of the periodontal membrane, longitudinal section (mag. 250): shows *a*, external portion; *b*, internal portion, or part bordering the abscess cavity; *c*, connective tissue fibres of outer portion; *d*, white connective tissue fibres; *e*, inflammatory cells; *f*, arteriole; *g*, venule; *h*, spindle-shaped cells forming trabeculæ and dividing interior into compartments; *i*, inflammatory cells; *j*, pus corpuscles.

excised until a small portion of the dentine and cementum remain, then washed in salt solution, and placed in formalin or Müller's fluid, and finally cut on a microtome, and stained with hæmatoxylene. It is difficult to retain the central constituents of this new formation, as the act of removal from the mouth tears the covering, and they escape.

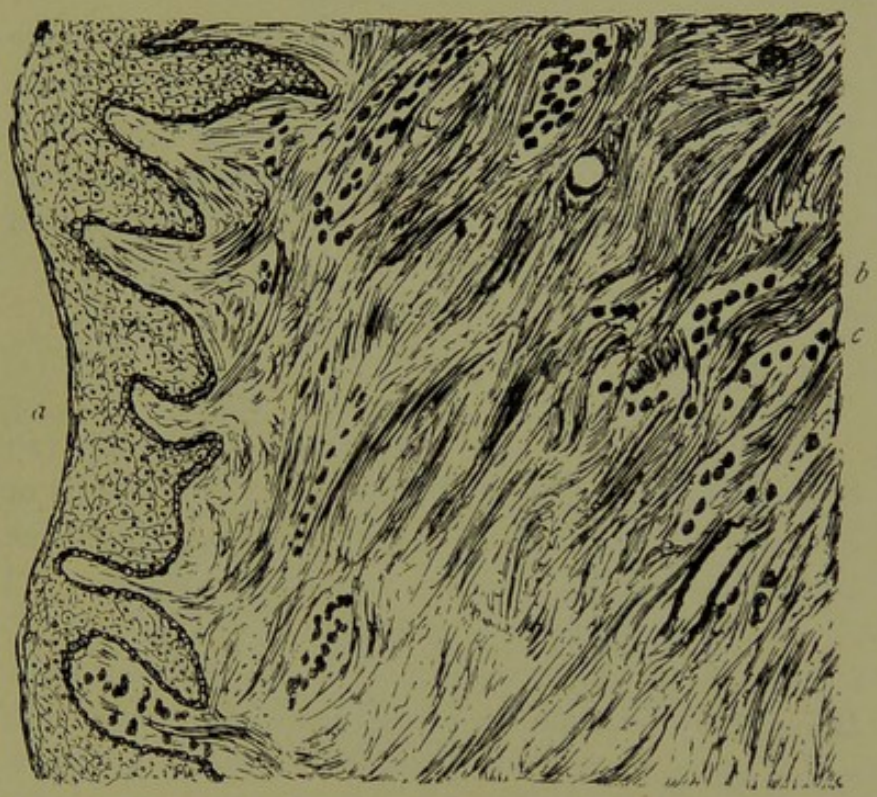


FIG. 46.—Fibroma of the palate, longitudinal section (mag. about 80): shows *a*, epithelium of surface, forming papillæ; *b*, connective tissue fibres; *c*, large connective tissue cells.

Structure under High Powers

OBSERVE.—An outer firm thick envelope of fine white connective tissue fibres, their long axes corresponding to that of the root. Between these principal white fibres many inflammatory cells are visible, some arranged in columns. Further in, trabeculæ of spindle-shaped cells spring up from this sac-like tissue, dividing the interior into compartments, which are filled with large numbers of roundish cells with prominent nuclei.

Still further in, a few pus cells remain adherent to the tissue.

Degeneration

(C) *Dental Cysts.*—An abscess, as above described, may undergo cystic degeneration. *Dr. Baker* and *Mr. J. Turner* have found ciliated cells in some instances in connection with these dental cysts, the cilia being probably obscurely concerned with the movements of the fluid or semi-fluid contents of the cyst.

(D) Attached to the alveolo-dental ligament there are sometimes found *innocent* and *malignant growths.* These may contain myeloid cells imbedded in a connective tissue matrix of low development, or they may present the well-known appearances of round and spindle-celled sarcomata.

VI. *Dental Gum*

To prepare the Tissues for Examination

Sections of *Gum and Oral Mucous Membrane* can be prepared by fixing and hardening in Müller's fluid or formalin, and cutting on a microtome. Hæmatoxylene is the most valuable stain.

In (A) *Hypertrophy of the Gum*:

Structure

OBSERVE.—The dense mass of strong firm interlacing fibres of connective tissue with a few round cells which go to make up the growth. The free surface is covered with thin layers of epithelial cells above, and mucous and submucous tissues beneath.

In (B) chronic inflammation, or so-called '*Polypus of the gum*':

Structure under High Powers

OBSERVE.—The superficial epithelial layers forming numerous simple and compound papillæ dipping into the submucous layer, the greater part of the growth composed of white wavy connective tissue fibres, large inflammatory cells and leucocytes, and numerous large blood-vessels. From the proliferation of the epithelium *Dr. A. W. W. Baker* suggests the possibility of these

small tumours being a connecting link between a papilloma and an epithelioma.

(C) *Fibromata* (fibrous epulides) usually arise from the periosteum covering the septum between two contiguous teeth, or from the mucous membrane of the palate, and (D) *Myeloid epulides* frequently exist.

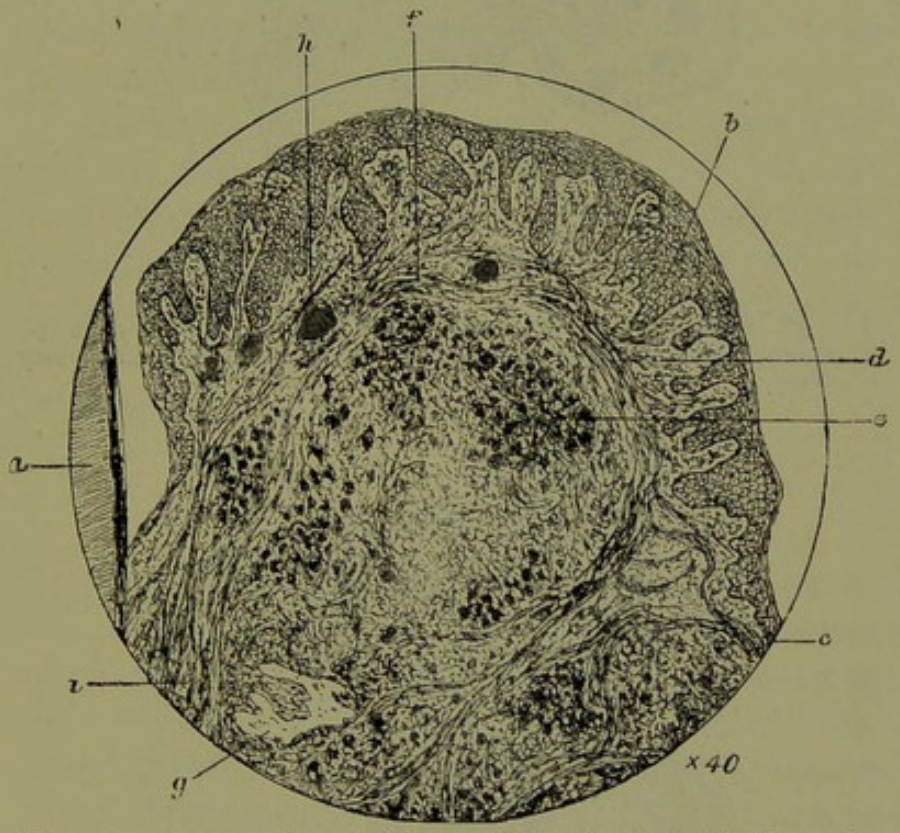


FIG. 47.—New growth, containing myeloid cells attached to the periodontal membrane and to the gum, longitudinal section: shows *a*, dentine and cementum; *b*, epithelium dipping into the submucous tissues to form papillæ; *c*, basement membrane; *d*, submucous tissue; *e*, masses of giant cells; *f*, fibrous septa; *g*, nodule of bone; *h*, spaces filled with colloid material; *i*, fibrous bands attaching the tumour to the cementum.

In the former:

OBSERVE.—The dense fibrous characters of the tissue with a few elastic fibres and connective tissue corpuscles intermingled. Notice the scantiness of the blood supply and the epithelial structure of the marginal tissues. Structure

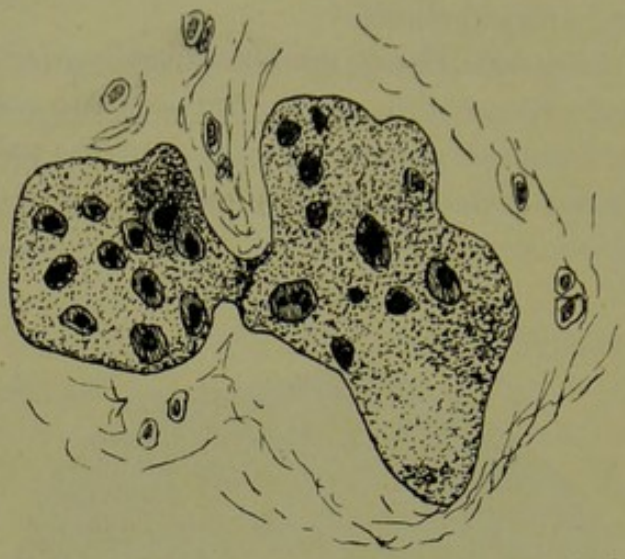


FIG. 48.—Two multi-nucleated giant (myeloid) cells from the preceding figure, under high powers. They show the nuclei and nucleoli very clearly

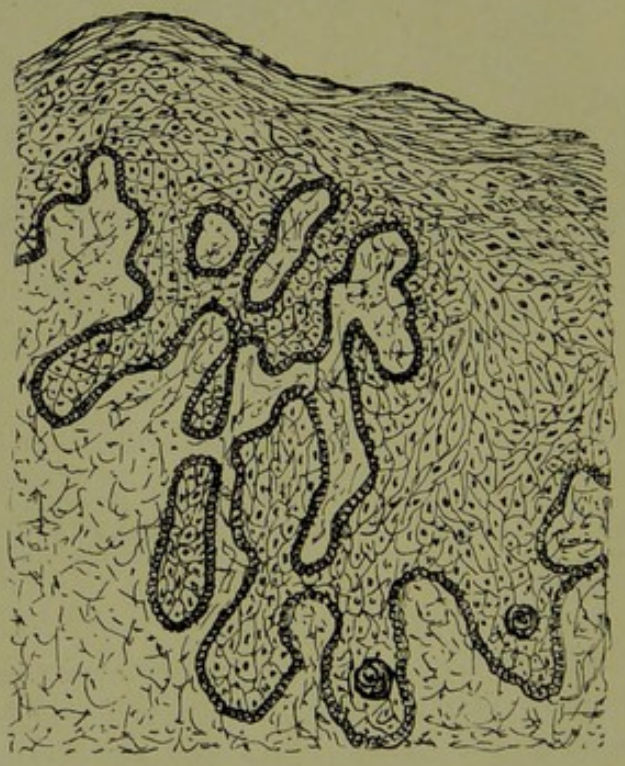


FIG. 49.—Epithelioma of the gum, longitudinal section, high powers. The columns are seen invading and penetrating the submucous tissues, and two cell-nests, which are absolutely pathognomonic of epithelioma, are shown.

In the latter :

Stain with Hæmatoxylene

OBSERVE.—The spindle or round cells and large irregularly shaped masses of protoplasm (giant cells), containing many oval distinct nuclei. Also the epithelial papillæ ; the fibrous septa ; and sometimes tiny nodules of bone, and spaces filled with colloid material.

(E) *Epitheliomata* (squamous-celled carcinomata) should be stained with hæmatoxylene. They spring from the mucous membrane of the gum.

Structure under High Powers

OBSERVE.—The solid columns of epithelium piercing the basement membrane and growing into the underlying tissue ; the columns surrounded by a small-celled infiltration in such a manner as to form an irregular epithelial network ; around and within the columns the small aggregations of crescent-shaped cells called epithelial cell nests.

VII. *Affections of the Bones of the Jaws*

Odontomes are as a rule preserved in their entirety, their microscopical examination on occasion only being of value if sections must be made. The mass should be ground down and treated like the hard parts of teeth. Staining should not be practised. *Mr. Bland Sutton* thus describes their patho-histology : '. . . These tumours are composed of branching and anastomosing rods or columns of epithelium, portions of which form alveoli. The stroma is composed of fibrous tissue, when abundant embryonic tissue in various stages is present. The cells occupying the alveoli vary in form ; the outer layer may be columnar, whilst the central cells degenerate, and give rise to a reticulum of stellate cells, resembling in structure the stratum inter-medium of the enamel organ.'

Structure

[1] 'Transactions of the Odontological Society of Great Britain,' Vol. XX.

CHAPTER VIII

ON BACTERIOLOGICAL APPLIANCES

INTRODUCTORY

Introductory

PROGRESSIVE dental science is bound to include in its range the ever-opening field of oral and dental bacteriology. The importance of its study is readily conceded by those who have given the matter their attention.

It is a common error to suppose that the subject is surrounded by innumerable difficulties only to be surmounted by a few experts. Those versed in histological methods can easily extend their knowledge, so that a firm grip of the fascinating science can be rapidly and successfully acquired and practical methods and results easily attained. Of course, the student's armamentorium must be augmented by special apparatus and a set of staining reagents; but, guided by his previous experiences, he may soon possess a full cabinet of interesting and valuable bacteriological preparations.

In a work of this character it is impossible to give complete descriptions of all the apparatus, media, and methods of cultivating and isolating micro-organisms which are used in fully equipped bacteriological laboratories. A succinct and clear outline only will be attempted, sufficient being said to point the way to the subsequent perusal of standard text-books and current literature, through a fair comprehension of the elements of the science.

Object attempted

A

Histological Apparatus and Reagents

Microscope.—An instrument of any pattern may be used; it is unnecessary to obtain any special form. It should, however, be fitted with a nose-piece attachment, for the rapid changing of the objectives, of which 1 inch and $\frac{1}{12}$ inch are the most useful. The former quickly traverses the area of the cover-glass preparation or section, and picks out the most typical spot for critical examination with the oil-immersion lens. A 2-inch objective is valuable for the examination of plate cultures.

Most useful Powers

The employment of a sub-stage or under-stage condenser is imperative. An iris diaphragm is also needful.

The microscope is generally placed vertically, and the clips attached to the stage for holding the glass slide in position are not required.

Cover-glasses.—A point of great importance is always to work with chemically cleaned cover-glasses and slides. The first are of the usual size and shape. They are rendered free from dust, grease, etc., by boiling carefully for two minutes in a 10 per cent. solution of carbonate of potash, washed for five minutes in dilute nitric acid (25 per cent.), washed in distilled water, and kept in a glass-stoppered glass jar filled with methylated spirits. The slides are treated similarly and kept in absolute alcohol. A few special slides should be obtained. These are hollowed out in the centre, either circularly or ovally, and are to be used for making and examining 'hanging-drop' preparations.

To cleanse Cover-Glasses Chemically

Hollow Slides

Platinum Needles or Osës.—Each consists of a glass rod, 9 or 10 inches long, into the end of which is imbedded a platinum wire, either thin (·4 mm.) for

ordinary work, or ·7 mm. for making 'stab' cultures. Each is twisted into a tiny loop at the end. Before using, the wire must be sterilised thoroughly (*i.e.* made red hot) in a Bunsen flame, and it should not be put aside until the 'flaming' has again been done (fig. 51).

Precautions

Test Tubes.—A supply should be obtained, some of which are empty, others sterilised, containing the various culture media, and plugged with sterilised cotton wool. It is better for the student to purchase these tubes of media, as the apparatus for sterilising is expensive, and

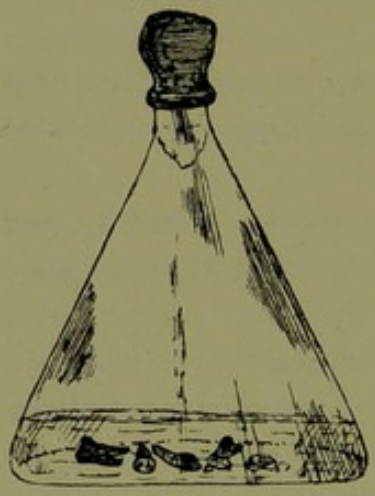


Fig. 50.

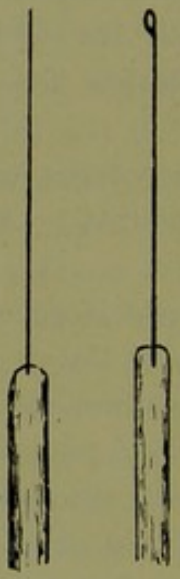


Fig. 51.

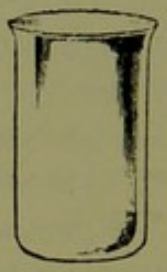


Fig. 52.

the operations connected therewith tedious and lengthy. They can be obtained at most of the dealers in bacteriological apparatus, and at many hospitals. In the agar and gelatine tubes the media should present a slanting surface.

Beakers, Flasks, etc.—The ordinary beakers are useful. They should be of various sizes, for containing water, and washing preparations, etc. (fig. 52). Two or three Erlenmeyer's flasks will be required for the performance of artificial caries (fig. 50).

Petri Dishes.—These are flat, shallow, glass dishes,

3 or 4 inches in diameter, provided with glass lids. They are used for plate cultures, and should be used cold after sterilising in the hot-air steriliser.

Minor apparatus consists of glass funnel, capillary pipettes, wash-bottle for holding distilled water, watch-glasses, a few glass capsules, test-tube racks, forceps, mounted needles, clean linen rag, Bunsen burner and gauze, tripod for holding reagents, etc., black and white glass or tile slabs for mounting specimens on, filter papers and glass-writing pencils, and the following set of reagents, stains, etc. :—

Stains, etc.

Aniline gentian violet :—

Saturated alcoholic solution of gentian violet, 3 per cent., in aniline water.

Carbol-fuchsine :—

Fuchsine, 1 part ; absolute alcohol, 10 parts ; and 100 parts of a 5 per cent. solution of aqueous carbolic acid.

Löffler's Blue :—

Concentrated alcoholic solution of methylene blue, 3 per cent., in a ·01 per cent. solution of caustic potash.

Eosine :—

Aqueous solution.

Gram's solution :—

Iodine, 1 part ; potassium iodide, 2 parts ; distilled water, 300 parts.

Nitric acid :—

25 per cent. solution.

Alcohol :—

Methylated spirits free from naphtha.

Alcohol absolute.

Xylol.

Canada balsam (xylol).

Oil of cedar-wood, thick but clear, for immersion purposes.

Vaseline (pure), and

An abundant supply of distilled water.

It is convenient to keep the reagents in similar-sized glass bottles, the first five being provided with capillary pipette stoppers, the next four corked, and the last three capped. The whole should be placed in a suitable wood rack.

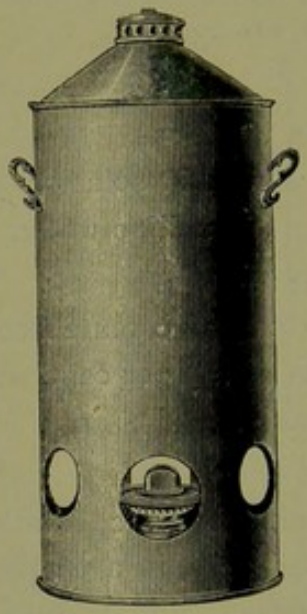


FIG. 53.—STEAM STERILISER.

B

Sterilising and Incubating Apparatus

Need for cleanliness

Too much stress cannot be laid upon the absolute necessity for the strict observance of cleanliness in bacteriological work. All apparatus, media, etc., must be rendered sterile, hands constantly washed in carbolic acid solution (1–40), and dust and dirt removed from the student's environment.

Steam is most used for sterilising instruments, etc., and a steam steriliser employed for this purpose.

Steam Steriliser.—This is a vessel of tin or copper so arranged as to sterilise at 100° C. all culture media and glass apparatus. An ordinary kitchen saucepan makes an admirable substitute for the more expensive article. (Fig. 53.)

A Biological Incubator is a most necessary piece of

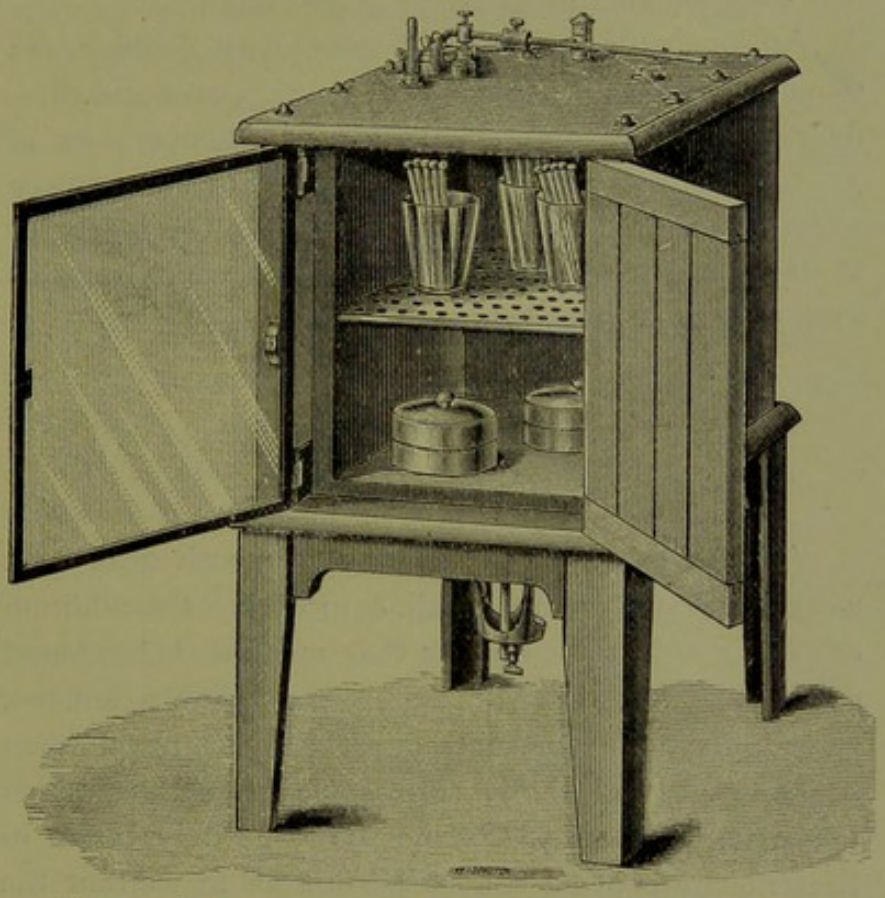


FIG. 54.—A warm Biological Incubator for the cultivation of micro-organisms. It shows the thermometer and thermostatic 'Capsule.' Test tubes plugged with wool, and cultivating dishes are seen in its interior.

apparatus for the cultivation of micro-organisms on suitable media at a temperature approximating to that of the body: for it is impossible to give a name to the micro-organisms of the mouth when viewed solely by the microscope. One can say whether such-and-such an organism is a micrococcus, or a bacillus, or a sarcina, whether it is motile or non-motile, but it cannot

Necessity of cultivating Micro-organisms

be classified until its methods of growth and cultivation have been observed and compared with similar micro-organisms. For instance, the typhoid and colon bacilli are practically indistinguishable from each other under a $\frac{1}{12}$-inch lens; but when cultivated, their varied appearances clearly and easily differentiate them. Thus it is with the micro-organisms of the mouth.

An incubator—a box or chamber with double walls of copper or iron—supplied with gas regulator and thermometer, is used, and maintained at a temperature of 37° C., or blood-heat, or 22° C., the heat of an ordinary warm room. All media may be put into the incubator at the former temperature, save gelatine, which remains solid only at degrees below 22° C.[1]

Cool Incubator for Gelatine Cultures

C

Culture Media

Alkaline Media to be used for Dental Bacteria

All the micro-organisms of the mouth grow best when cultivated on an alkaline medium; the addition of caustic soda 1 per cent. to that medium being found suitable. They seem to have a strange predilection, too, for gelatine. These points must be remembered when ordering tubes of sterile culture media—a thing which the writer advises to the novitiate, until his work in bacteriology is of such a proportion as to warrant the purchase of special appliances, such as autoclaves, exhaust pumps, etc.

Cultures

Dental bacteria may be cultivated on beef broth, nutrient gelatine, potato gelatine, agar, litmus agar, milk, and least successfully potato. Other media, such as blood serum, are unnecessary at the beginning of the study.

Beef broth should be made with shin of beef (it con-

[1] For fuller descriptions, students are referred to standard text-books of bacteriology.

Various Cultures

tains a certain percentage of gelatine), peptone, common salt, and sodium carbonate and hydrate. It is very useful for cultivating organisms directly from the mouth, and *Streptococcus longus* is best observed in this medium.

Nutrient gelatine contains the above, plus gelatine and white of egg. Bacteria grow slowly, but their colonies are more clearly discerned than on agar.

Potato gelatine (raw potato, gelatine, white of egg, and 1 per cent. of sodium hydrate) is highly recommended by Mr. Goadby[1] for the cultivation and isolation of *Bacillus buccalis maximus*, and the hitherto uncultivable *Spirillum sputigenum* and *Spirochæte dentium*.

Agar is one of the most valuable culture media known; it remains solid at blood-heat (37° C.), and bacteria grow very readily on its surface.

Litmus agar is nutrient agar with the addition of neutral litmus, to demonstrate the production of acids by the bacteria. Blue litmus agar becomes in a few days converted into a pink colour if 'streaked' with an osë of the micro-organisms of the mouth.

Milk sterilised by steaming is an important medium.

Potato finally yields, as a rule, only poor results with dental organisms.

[1] See 'Trans. Odonto. Soc.,' June, 1898.

CHAPTER IX

ON BACTERIOLOGICAL METHODS

Introductory

THE making of microscopical preparations of the bacteria found in the mouth is usually a very simple matter; even the more difficult and troublesome methods of staining spores and flagella, with a little practice, become quite easy. Still, it is advisable always to begin at the beginning.

Bacteriological methods may be considered under the headings of (1) Making hanging-drop preparations; (2) making cover-glass specimens; (3) staining tissues; (4) cultivating micro-organisms; (5) isolating them; and (6) making permanent preparations of the most important dental and oral bacteria. These now given will be the most useful for all ordinary work, but are not detailed absolutely sufficiently for original researches.

The entrance to the domain of practical bacteriology may be said to be in the construction of a 'hanging-drop' preparation to show casually the general characteristics and the motility or non-motility of the organisms.

1. *Hanging-Drop Preparations*

Modus operandi

A hollowed-out glass slide is placed on the black glass slab, and a ring of vaseline applied, by means of a glass dipper, round the margin of the hollow. A square, dry cover-glass held between the left forefinger

and thumb receives on the centre of its upper surface the droplet which is to be examined. This may be removed from the mouth either in the form of a drop of salivary mucus or a scraping from the oral mucous membrane or surface of the tongue ; or in the form of a drop of broth which has been previously inoculated with bacteria. A sterilised platinum needle places the drop on the cover-glass. This is at once inverted and put into position on the slide, and carefully pressed flat on the vaseline ring, using a clean rag in order not to smear the lens surface of the cover-glass.

To examine a Preparation

A small quantity of cedar-wood oil is next dropped over the preparation ; the microscope placed vertically ; the slide clipped to the stage ; the iris diaphragm two-thirds closed, and finally the $\frac{1}{12}$-inch oil immersion objective focussed over the ' hanging drop.'

After some careful focussing, micrococci and a few bacilli will be seen dimly outlined, some possessing a greater degree of motility than others. This movement is not the Brownian oscillation.

2. *Cover-glass Preparations*

These include preparations made (*a*) from the mouth direct ; (*b*) from cultures.

Have ready several chemically cleaned cover-glasses, sterilise the thin osë in a Bunsen flame, place cover-glass on black slab, and apply to its centre a droplet of distilled water.

Preparations direct from the Mouth

(*a*) Remove from the mouth with any convenient instrument a small quantity of material to be examined, either the surface scrapings from the tongue, the cheek, the sulcus between the gum and cheek, or the white deposit around the cervical margins of the teeth (*Materia alba* of Leeuwenhoeck), or the carious contents of their cavities.

And from Cultures

(*b*) Hold the test tube containing the culture hori-

zontally in the left hand. With a pair of forceps remove the cotton-wool plug from the mouth of the tube, using a twisting or screwing movement, and place it between the third and fourth fingers of the left hand. Now flame the platinum osë, the end of which has been twisted into a loop, and pass it down the tube ; lightly touch the medium, then gently scrape the edge of the culture, as here the micro-organisms are youngest, and consequently stain better than the old ones. A tiny mass of micro-organisms will adhere to the loop, and should now be brought into the water on the cover-glass, where it is spread out and evenly distributed by means of the loop. The same plan applies to (*a*).

Precautions

To dry the Film

The film is next dried by holding the cover-glass between the fingers over the Bunsen flame, then 'fixed' in the following way : Grasp the edge of the cover-glass with a pair of forceps, and pass rapidly with a large circular sweeping motion *through the flame* three times. This requires great care, or otherwise the bacteria will be injured.

To stain and mount it

The film being dry, it should be stained as desired, by placing the cover-glass, film side uppermost, in watch-glasses which hold the staining reagent.

The staining being complete, excess must be washed off in tap water, then in distilled water, and the cover-glass ultimately dried by propping it up against a ledge of filter-paper. Drying may be further hastened by using the filter-paper as a blotting-paper.

Now put a drop of balsam on the glass slide, another one on the film, and apply the cover-glass.

3. *Staining Tissues*

Most valuable Stain to employ

The employment of gentian aniline violet, followed by iodine, known as Gram's method of staining, is almost as universal in dental as in general work. The great

majority of organisms are thus beautifully stained, the following being the exact method of using this stain:—

Cover-glass specimens remain in a watch-glass of gentian aniline violet solution for five or ten minutes, sections from ten minutes to half an hour. They thus become over-stained. Excess of stain is drained off, and the preparations are immersed for one or two minutes in

A Mordant Solution

Iodine	1 part
Potassium iodide . . .	2 parts
Distilled water	300 parts

When they have assumed a dirty brown colour, specimens must be removed, drained, and transferred to methylated spirit. Here they should remain until practically decolorised, but the stain must not be *entirely* removed. The purple colour of the gentian violet having been discharged, films should be washed in water, dried, and mounted in the manner already indicated. Eosine or bismarck brown may be used as counter-stains. Sections on their leaving the methylated spirit are passed through absolute alcohol and xylol, and mounted in xylol balsam. Eosine or bismarck brown may be used as counter-stains; but in this case they must again be passed through methylated spirit, absolute alcohol, and xylol.

Treatment of Films

And of Sections

This is the best method of staining specimens of dental caries, and also some species of oral micro-organisms, but aniline gentian violet is useful *per se*. Films are stained in two or three minutes. Carbol-fuchsine, when used for films, should be diluted with two to six parts of water, and thus stains them in about three minutes. Sections should be left for half an hour in a warmed diluted solution. Löffler's alkaline methylene blue is most suitable when the organisms are mixed with other material, and when the cultures are quite

Films

Sections

fresh. Films are stained in ten minutes, and sections half an hour or more.

Thus it will be seen that Gram's method is valuable for demonstrating bacteria in carious dentine, and in the tissues of the pulp and periodontal membrane, and the other staining reagents for cultures or for micro-organisms from the mouth direct.

Utility of Löffler's Blue Stain

Some bacteria are greatly decolorised by Gram's method ; Löffler's blue is then a good stain. Sections should remain here from fifteen minutes to six hours. After staining they are well washed, and slightly decolorised by rinsing for half a minute in a watch-glass of distilled water, in which two or three drops of glacial acetic acid have been placed. The subsequent treatment consists in again washing, passing rapidly through alcohol and xylol, and mounting in balsam. When Löffler's blue is used, dehydration must be rapid, or the colour will become abstracted *in toto*.

Moeller's Stain

Spore Staining is sometimes necessary to ascertain the manner in which micro-organisms multiply. It is rather difficult to accomplish at first, the patience of the student being somewhat exercised. *Moeller's* method is at once fairly simple and satisfactory. The steps are as follow :—

1. Film preparations are made as usual.

2. Immerse them in absolute alcohol, and chloroform, each for two minutes.

3. Wash, and treat with chromic acid, 5 per cent. solution, for two minutes.

4. Wash, and stain with warm carbol-fuchsine for ten minutes.

5. Wash, and carefully decolorise in sulphuric acid, 5 per cent. solution.

6. Wash, and counter-stain with Löffler's blue for one minute.

7. Wash.

8. Dry with filter-paper, and mount in balsam.

Thus are demonstrated the spores of *Bacillus buccalis maximus*, and other bacilli, which become deep red.

Flagella and Capsule-staining.—Pittfield's method is a simple one, and yields good results, rendering prominent as many as six flagella in the case of the *Bacillus buccalis maximus*. Prepare two solutions :— Pittfield's Stain

I. 10 c.c. of a saturated aqueous solution of alum with 1 c.c. of a saturated alcoholic solution of gentian violet ; and

II. 1 grain of tannic acid with 10 c.c. of distilled water. Use cold water, filter, and keep in separate bottles.

When about to stain, mix equal quantities of both. Films on cover-glasses are saturated with the mixture, and held over the Bunsen flame until the stain nearly boils. They are then allowed to cool, and washed in water. Aniline gentian violet is once more used for about a second ; the preparations are again washed, dried, and mounted.

But the impregnation *Process of Van Ermengen*, when better known and more frequently used, is destined perhaps to supersede all other plans of coloration. It renders prominent such delicate structures as the flagella of many bacilli, and the capsule of Friedlander's pneumococcus.

Van Ermengen's Stain

The process briefly is as follows :—

A small quantity of the culture containing the micro-organisms having been diluted with a drop or two of distilled water, is placed on a clean slide.

Evaporation of the water is allowed to take place, and then the culture is fixed by means of a mixture of

Ether sulphuric Alcohol absolute	Equal parts

The slide being again dry, several drops of a fixing-bath are placed on the surface of the culture, and left for half an hour in the cold, or five minutes in a temperature of about 60° C. The bath is made of

Fixing-bath

Osmic acid (2 per cent. solution) . .	1 gramme
Tannic acid (10 to 25 per cent. solution)	2 grammes

When fixation has occurred, the slide is carefully washed in distilled water, and then placed, for several seconds, in a 0·5 to 0·25 per cent. solution of silver nitrate. It is then removed, and, without washing, passed into a reducing-bath composed of

Reducing-bath

Gallic acid . . .	5 grammes
Tannic acid . . .	3 grammes
Melted acetate of soda .	10 grammes
Distilled water . . .	350 grammes

The preparation remains here for a few minutes, and is then again transferred to the silver nitrate solution. It is next removed and carefully washed in distilled water. The slide is finally dried, a small quantity of Canada balsam added, and a cover-glass applied.

Final Treatment

4. *Cultivating Micro-organisms*

Bacteria may be cultivated in several ways on several different kinds of media, as has already been pointed out. To begin, *shake cultures* should be made, then sub-cultures, by the processes of 'streaking,' 'stabbing,' and 'shaking.'

To make a 'Shake' Culture

The following experiments may be performed :—

From a healthy mouth remove, by means of a sterilised looped osë, a tiny scraping of the mucous

membrane of the (i.) buccal sulcus, (ii.) and the superior surface of the tip of the tongue, and (iii.) also a minute portion of the *materia alba* from the interdental spaces in the maxillary molar region.

Have ready in a test-tube rack some tubes of sterile peptone broth. 'Flame' the mouth of one of them for a second, and with a 'flamed' forceps, loosen and partially withdraw, with a rotatory movement, its wool plug. 'Flame' the osë by holding it nearly vertically in a large Bunsen flame, and heating it to redness. Hold the needle between the right finger and thumb; remove the plug, and place it between the left third and fourth fingers, and pick up, with the loop of the osë, a particle of the material which contains the micro-organisms. Pass this down the tube to the fluid medium, rub the loop on the side of the tube near the margin of the broth, and wash it down by tilting the tube, all traces of the bacterial mass being ultimately got rid of by rapidly rotating the osë. Having completed the inoculation, replace the plug quickly, and reheat to redness the osë. It is a good plan to pass the plug rapidly through the Bunsen flame, and to replace it, while still alight, in the mouth of the tube. This should always be done if it has dropped on to the table or brushed against any extraneous substances. Cap the tube finally with a rubber cap which has been soaked in 1–500 perchloride of mercury, and place the tube in an incubator.

To make a 'Streak' Culture

In making a 'streak' culture ('line' culture of some authors), all of the above precautions must be adopted. The loop of the osë must be very tiny, and a single light streak made on the sloping surface of the agar, or gelatine, or potato medium, from the bottom to the top, without penetrating the medium. 'Stab' cultures are produced by using the thick osë, and plunging it steadily, in a straight line, into the centre of

To make a 'Stab Culture

the medium, nearly to the bottom of the test tube, and carefully withdrawing it in the same straight line.

After the period of development is complete, microscopical preparations are made from the various media, at various times, in the manner already given on p. 149, under paragraph (*b*).

These experiments may be varied by incubating in sterile peptone broth at 37° C. a fragment of soft carious dentine immediately after removal from a tooth. A 'shake' culture is made, and from it sub-cultures can be obtained in the way about to be described.

5. *Isolating Micro-organisms*

This is necessary to be done when the student desires to obtain *pure* cultures of any organism. A mixed infection occurs when the methods previously described have been followed; that is to say, in the growth on the medium, on microscopic examination, it will be found that rods, cocci, curved and spiral forms, are confusedly mixed. Preparations consisting of one, or at most two, species can be isolated either by streaking in tubes or by plate cultivation.

To obtain Pure Cultures

'Streak' Cultures

The simpler is the first named.

After a peptone broth tube has been inoculated for twenty-four hours, a cloudy flocculent precipitate will be noticed on removal from the incubator at 37° C.

Three agar streak cultures should be made in separate tubes: (1) by means of a platinum loopful from the peptone broth; (2) by means of the same loopful; and (3) by means of the same loopful, without any intervening 'flaming.' Thus it is obvious that in the third tube a fewer number of bacteria will develop than in the first and second. The tubes are all returned to the hot

incubator, and Number 3, after a lapse of a day or two, examined, macro- as well as microscopically.

Should a mixed infection still exist, the process must be repeated, using instead of the original peptone broth a particle of the edge of the growth of Tube 3.

While in the incubator, tubes of culture from the mouth emit a foul smell after forty-eight hours' incubation; but this passes off in a few days.

Plate Cultures

Petri dishes are used for plate cultivations, and sterile nutrient gelatine is the medium employed.

To obtain Colonies

Three tubes of sterile gelatine—1, 2, and 3—are melted at a low temperature in a beaker of water over the Bunsen flame. The temperature should not exceed 45° C.

Tube Number 1 is inoculated with the bacterial mass from the mouth, direct from the peptone broth tube, or from the edge of an agar streak, as may be wished. This mass is thoroughly incorporated with the melted gelatine. Number 2 tube is inoculated with two or four loopfuls of the gelatine in Number 1, and is also thoroughly mixed up. A further dilution must be obtained by treating the melted gelatine in Number 3 in a like manner.

Three petri dishes are sterilised in the hot air steriliser, and allowed to cool. The melted gelatine from each tube is poured into a dish, which is tilted to distribute a film of gelatine evenly over the flat surface of the bottom of the dish. The gelatine is cooled by placing the dish on a flat cool surface, and, finally, all are placed in the cool incubator in order that colonies may develop.

Examination of Colonies

In a few days longer than if agar were used, colonies, with probably a few moulds, will appear, and can then be examined with a hand lens or the 2-inch on the

microscope. Films are made from them in the usual manner.

Perhaps agar plate cultures are more suitable for dental work than those made on gelatine.

To describe Colonies

Thus, twenty-four hours after inoculation on an agar medium of the *materia alba* there are seen, to give an example, colonies which may be described as numerous, large, rounded, whitish, discreet (*i.e.*, separate), raised, thick, and opalescent, and also tiny pin-point colonies between the former.

A microscopical examination of films of the above, when stained with Gram and also carbol-fuchsine, shows that the pin-point colonies consist mainly of bacilli and cocci, the thick colonies of pure micrococci (Staphylococci, etc.), and the transparent part of the same of Diplococci pure.

6. *Methods of Making Microscopical Preparations of the most Important Dental and Oral Bacteria*

A few Principal Varieties

Authorities differ as to the number and varieties of the various species of mouth organisms. It is not within the province of these pages to discuss these questions. A few brief outlines of methods of preparing specimens only need claim the reader's attention.

Cocci Forms

Micrococci.—(i.) Take a small portion of the edge of the thick colonies on the surface of agar medium, seventy-two hours after inoculation from the peptone broth 'shake' culture, which (twenty-four hours old) was made from the *materia alba.*

Stain Gram's method.

A practically pure culture results.

(ii.) Obtain micrococci from the scrapings of the surface of the tongue or mucous membrane of the mouth direct.

Diplococci are chiefly obtained from the region of

the buccal sulcus in healthy and properly cleansed mouths, massed round the edges of the squamous epithelial cells, many of which are partially destroyed by the action of the bacteria generally. Found also in great quantities in pus from an alveolar abscess. By cultivation, the diplococci grow into *Streptococcus brevis.* Stain, carbol fuchsine.

M. Tetragonus is rare, and found sometimes in the sputum surrounded by a delicate capsule. Stain Gram or Pittfield's method.

Cocci in Chains

Streptococcus brevis and longus.—Inoculate agar with a loopful of peptone broth, inoculated from the healthy mouth. Then take an osë of a thick colony and make a sub-culture in broth. Smear gently for fear of breaking the chains, on a cover-glass. Stain Gram. Long chains, some containing seventy or eighty individuals, are seen, with a few *Sarcinæ* in packets. Pure cultivations are obtained by further sub-culturing on agar.

Thread Forms

Leptothrix may be obtained (*a*) direct, or (*b*) cultivated.

(*a*) is made from scrapings from the tongue, or, better, the gum margins beneath masses of salivary calculus, in aged as well as young people.

(*b*) Cultivate in broth seventy-two hours. Apply a droplet of broth to cover-glass, acidulate this slightly with acetic acid or lactic acid, and then irrigate with tincture of iodine, removing excess with blotting-paper.

Leptothrix innominata of Miller is obtained by bringing a small quantity of the *materia alba* into a drop of iodine in iodide of potassium, slightly acidulated with lactic acid. Its irregular threads with other bacteria assume a yellowish colour. Thick bacilli and chains of cocci are distinctly coloured blue-violet. These are the *Bacillus buccalis maximus* and *Iodococcus vaginatus* of Miller.

Leptothrix buccalis maxima, occurring as long, thick,

straight, or curved filaments, is found in the mucoid deposits on the teeth. It closely resembles *B. buccalis maximus*, but gives no iodine reaction.

Rod Forms

Bacillus buccalis maximus.—Obtained from a mouth in which caries is very prevalent, or from the inflamed gum margins, or *pyorrhœa alveolaris*, this micro-organism must be inoculated on broth direct from the mouth, and films prepared.

Goadby uses the following method: Inoculate from a very carious mouth direct on to slant potato gelatine, made by 'extracting one kilo grated raw potato with a litre of water for two hours, and after adding gelatine and boiling, two eggs are added (whites only), reboiled, neutralised, and 1 per cent. normal caustic soda added.'

Plating is successful at the end of four or five days, and thence microscopical preparations can be made. It consists of large-jointed bacilli which stain purple on the addition of iodine and lactic acid.

Comma and Spiral Forms

Spirillum sputigenum and *Spirochæte dentium*, hitherto uncultivable outside the mouth, can be obtained in the same way by inoculating from the margin of unhealthy gums and in cases of *pyorrhœa alveolaris*. The former species are curved rods, and are motile when examined in a fresh unstained condition in a hanging-drop. The latter stain faintly with aniline dyes, are shaped like tiny corkscrews, and are slightly pointed at their extremities. The organism has been found in the discharge from a suppurating empyema of the antrum.

Groups or Masses

Several varieties of *Staphylococcus*, such as *albus*, *citreus*, and least commonly *aureus*, occur in unclean mouths. Microscopical preparations of pure cultures may be obtained by plating from broth inoculated with *materia alba* of an unhealthy mouth. The colony appears on gelatine as whitish in colour, and possesses a crinkled irregular outline. A minute particle is

removed by means of an osë loop, and a film made. Stained by Gram's method, a permanent slide of *S. albus* can thus be prepared.

FIG. 55.—Chart of the chief varieties of micro-organisms found in the oral cavity, the teeth, beneath the gums and in the jaws (after Miller). *a*, Leptothrix innominata from the mouth; *b*, Diplococci, × 1000; *c*, Staphylococcus pyogenes aureus, pure culture × 1000; *d*, Bacillus buccalis maximus, × 400; *e*, Streptococcus longus, × 1000; *f*, Streptococcus brevis, × 1000; *g*, Saccharomyces albicans (thrush), × 350; *h*, Spirillum sputigenum; *i*, Spirochæte dentium; *j*, Leptothrix buccalis maxima; *k*, Micrococcus nexifer, × 1100; *l*, Actinomyces as seen in the tissues, × 1000; *m*, Actinomyces when cultivated, × 750; *n*, Leptothrix gigantea, × 400; *o*, Iodococcus vaginatus, × 1000; *p*, Micrococcus tetragonus, × 500; *q*, Iodococcus magnus, × 800; *r*, Coccus of sputum septicæmia, × 1200; *s*, Micrococcus gingivæ pyogenes, × 1000; *t*, Bacterium gingivæ pyogenes, × 1000; *u*, Bacillus dentalis viridans, × 1000; *v*, Bacillus pulpæ pyogenes, × 1000; *w*, Vibrio viridans; *x*, Ascococcus buccalis, × 1000.

The opaque, round, yellowish, clean-cut-outline colonies are masses of nearly pure *Sarcina lutea*, easily recognisable under the lens by their large size, compared with those of micrococci, their arrangement in twos or fours, and in hanging-drops by their non-motility.

Micro-organisms that produce Colours

Chromogenic bacteria, *i.e.* those micro-organisms that give rise to pigmentation, are found in unhealthy mouths. Thus the *Bacillus fluorescens non liquefaciens* (*motilis*) may be obtained by cultivation from cases in which tartar, stained a greenish colour, is present. Goadby has described also a short motile bacillus which produces a fine pink colour on agar streak. This pink coloration becomes eventually red. On potato, red beadlike colonies are seen.

To make Permanent Cultures

Agar, gelatine and potato cultures may be preserved for many months, without much alteration, by the aid of formalin. A few drops of a 40 per cent. solution should be poured into the tube, so as not to touch the growth, and excess poured off at the end of five minutes. The wool plug is moistened with mercury perchloride (1—1000) and replaced in the mouth of the tube. Molten paraffin is then poured on the top of the plug, and an india-rubber cap finally seals the culture.

Plate cultures are similarly treated, and the lids of the dishes cemented in position.

CHAPTER X

ON THE PREPARATION OF SECTIONS OF THE CARIOUS DENTAL TISSUES, NATURAL AND ARTIFICIAL

Introductory

THE microscopical phenomena attending dental caries in their various stages may be, on the whole, very easily observed. The preparation and staining of the tissues present no great difficulties, especially if the instructions recorded in the preceding chapters are followed, and if the principles which underlie dental microscopy generally be applied to them.

Brief directions for pursuing authentic methods of dealing with these tissues are now to be considered.

A

Caries of the Enamel Cuticle

To obtain and stain Nasmyth's Membrane

A carious tooth is placed immediately after extraction in a mixture of phloroglucin and nitric acid (see page 25), and in ten minutes the membrane begins to be separated. This is neutralised and well washed. Fragments stained in carbol-fuchsine should exhibit thread, round and rod-shaped bacteria, and sometimes these are so crowded and massed together that the membrane is obscured almost entirely.

Pigmentation of the membrane occurs also, but is only noted in unstained preparations. 'Sections of enamel in an early stage of caries, after being stained with fuchsine, clearly show that the membrane is

loosened from the enamel on the decayed point, thickened and invaded by means of bacteria. The membrane in this condition affords a matrix, that is, a point of retention for bacteria, as well as for very minute particles of food, and thereby accelerates the progress of decay.'—(*Miller.*)

B

Caries of Enamel

The preparation of satisfactory sections of carious enamel may be very difficult, owing to the natural friability of the tissue, and its chalky character when partially disintegrated by disease. For more knowledge may be obtained from ground sections than from enamel when decalcified, although specimens of the latter are useful. These plans of treatment are now described.

Ground-down Sections

(*a*) A carious tooth is split down the middle by means of a pair of excising forceps, and each half is ground thin on a rough corundum wheel, till it is about 1 mm. thick, under plenty of running water. These pieces are then ground thinner on a fine corundum wheel on the lathe, until they are as thin as possible. A firm cork or piece of Stent's composition is useful for holding them against the wheel.

Leon Williams's Method

Mr. Leon Williams's name will always be associated with the production of some of the most magnificent sections of carious enamel ever seen, his work having been conducted with much skill and remarkable assiduity. In order to get bacteria attached to the surface of enamel and its membrane, he places freshly extracted carious teeth in a 2 per cent. solution of formalin for two days. Sections are then made by grinding under water on a fine corundum wheel on a lathe, being held in place by means of a wedge-shaped piece of very hard boxwood. If the finger or a cork is used, the bacterial mass is very readily detached. Sections are finally

polished on a fine stone, a free supply of water keeping them moist. They are then mounted in balsam. The

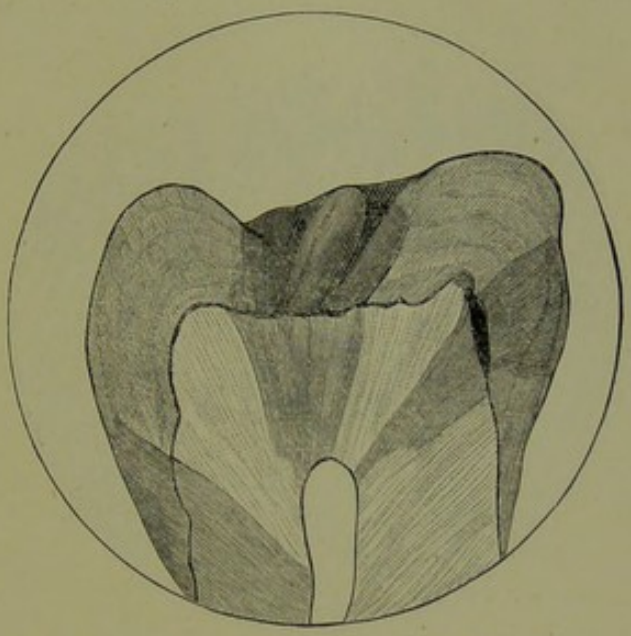


FIG. 56.—Caries of enamel, diagrammatic, longitudinal section, lower power. This and the following figures are from drawings by Leon Williams.

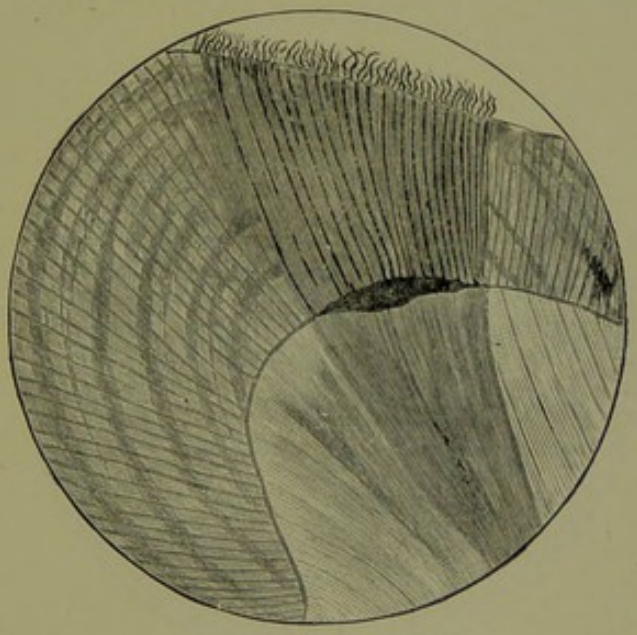


FIG. 57.—The same, more highly magnified; the micro-organisms are shown on the free surface of the enamel.

best photomicrographs and specimens that Mr. Leon Williams obtained were made from unstained tissues,

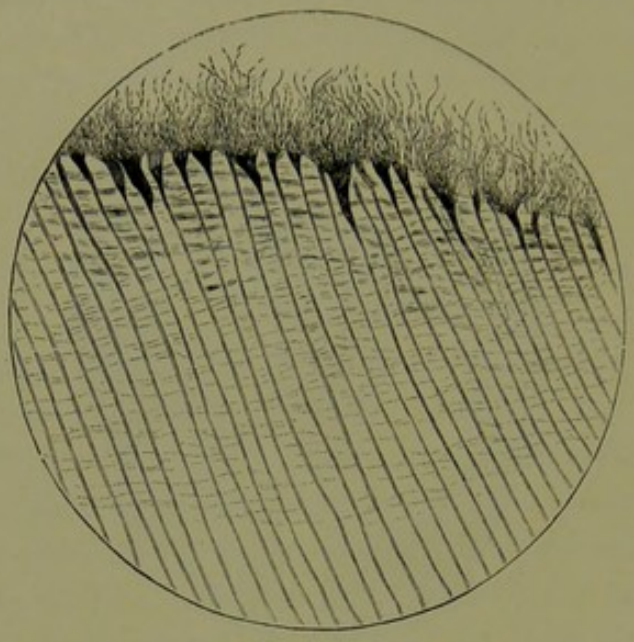


FIG. 58.—The same, more highly magnified; showing the separation and disintegration of the enamel prisms by the adherent bacteria.

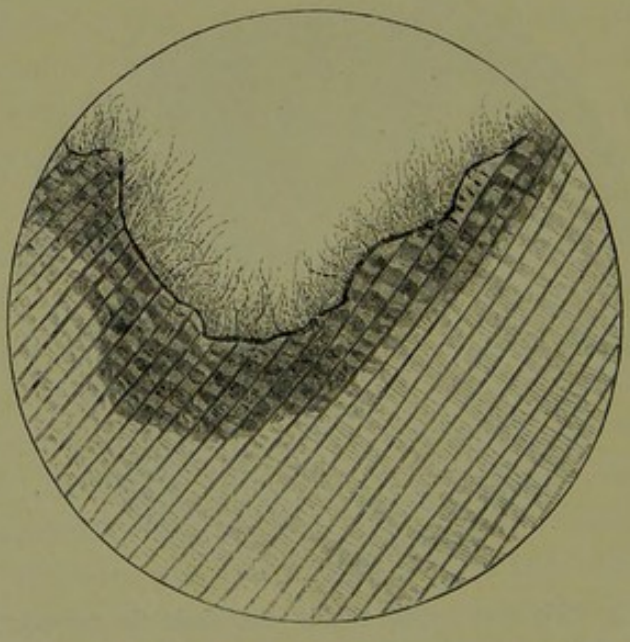


FIG. 59.—The same. Shows the decalcification of the enamel surface.

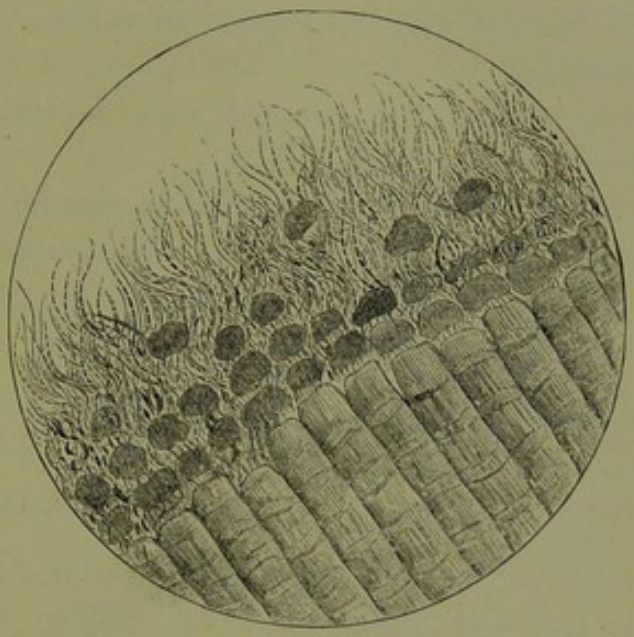


FIG. 60.—The same, very highly magnified.

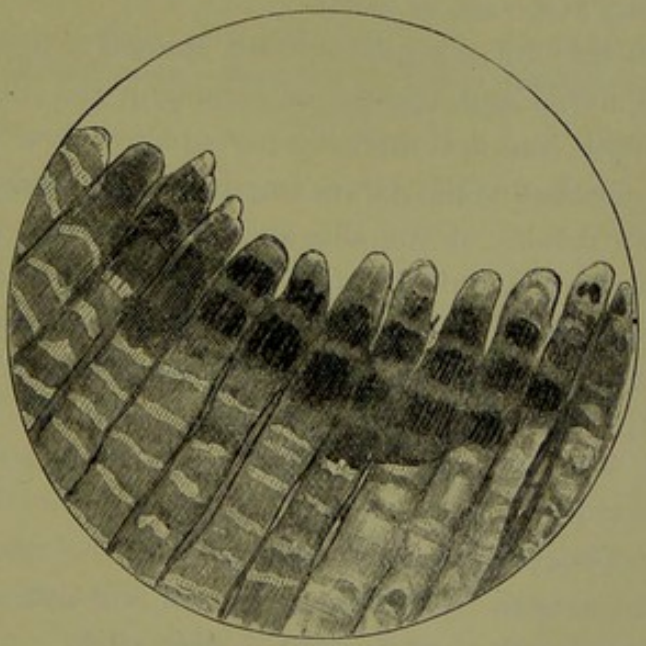


FIG. 61.—The same, showing the disintegration of the free surface of the enamel prisms.

although Gram's solution or carbol-fuchsine yields excellent results.

Weil's method may be applied to carious enamel with advantage.

To stain

Sections, having been washed, may be stained in several ways.

(i.) Picro-carmine or picro-lithium-carmine is recommended by Miller. From a concentrated solution of carmine where they have been immersed for a quarter of an hour, they are transferred to

Alcohol	70 parts
Hydrochloric acid . . .	1 part
Water	29 parts

for a period varying from fifteen minutes to five hours.

They are now dehydrated by passing through absolute alcohol, which has been coloured yellow by having had added to it a few crystals of picric acid. The succeeding steps are as usual.

Thus the micro organisms are stained a light red, dentinal fibrils and sheaths of Neumann red, dentinal matrix pink, and decomposing parts pale yellow.

Another Method of Staining

(ii.) Gentian-violet (Gram's method) or fuchsine yields the best results of all the aniline dyes, the former particularly if Günther's modification of decolorising be tried. Alcohol alone, as used in Gram's method, abstracts the colour from the tissues, sometimes with difficulty. They should then be treated for a few seconds with a 3 per cent. solution of hydrochloric acid.

Counter-staining

(iii.) Vesuvine (Bismarck brown) may be employed as a counter-stain, but the student will find that it is, as a rule, a good plan not to use a double stain.

Natural Decalcification of Enamel

(β) Micrococci and bacilli amongst enamel prisms may be seen in experimenting with methods for the production of artificial caries. Five days after immer-

sion in the saliva mixture, at a temperature of 37° C., the enamel assumes a chalky opaque appearance (see p. 170). If a small portion be removed from the crown of a tooth, and transferred as a film to a cover-glass,

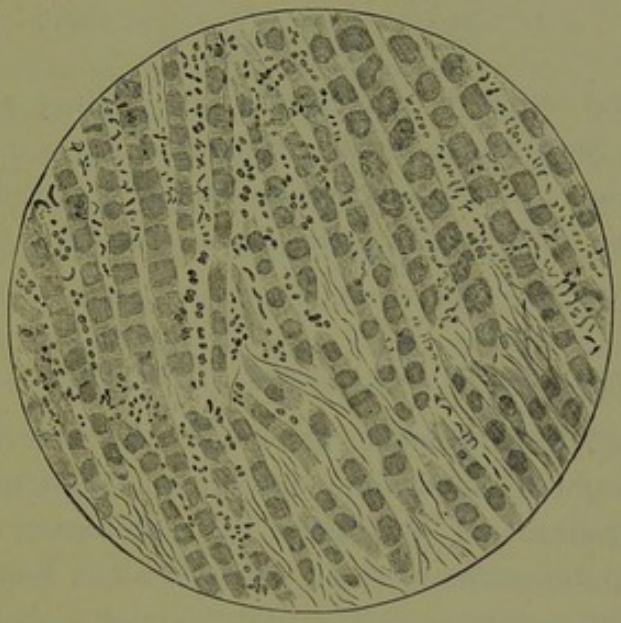


FIG. 62. – Caries of enamel, very highly magnified; longitudinal section; shows masses of micro-organisms between and separating the disintegrated enamel prisms.

an interesting preparation can be quickly made. Gram's method of staining is to be employed.

C

Caries of Dentine

How to obtain Carious Dentine

Sections showing the tubules filled with bacteria are easily made by removing with a sharp excavator from a carious cavity in a tooth a particle of softened decayed dentine. This is put at once into a little gum mucilage (B.P.) and frozen, and cut on an ether microtome.

Sections thus made are stained in any of the ways already mentioned. In some sections, thread forms are

seen attached to the superficial portions of the dentine, which at one time had formed the margin or edge of the cavity.

D

Caries of Cementum

Natural caries is not often found; when present, however, the tissues should be decalcified for some days in a 10 per cent. solution of hydrochloric acid, cut on a microtome, and stained with any of the basic aniline dyes.

THE PRODUCTION OF ARTIFICIAL DENTAL CARIES

Caries produced outside the mouth has been successfully brought about by many observers—Magitot, Miller, Sewill, Underwood, Mummery, Baker, Foerster, etc.

Of all the methods given, the writer has found, and can highly recommend for rapidity and certainty of results, the following plan, based on the methods first suggested by *A. W. W. Baker*, of Dublin, and *Miller* of Berlin. By carefully following out all details, in less than eight weeks sections may be cut.

Baker's Method modified

Sound teeth, whole or in part, are placed in a mixture of mixed human saliva, a small quantity of glucose, and a fragment of bread and uncooked meat. The teeth are first well washed in saline solution. If they possess marked pits or fissures, so much the better. The roots of two or three may be covered with a film of collodion, or the whole teeth may be enveloped in this film, and spots laying bare the hard tissues made in places.

The mixture is contained in a sterilised Erlenmeyer's flask—a fairly large one—half a litre is useful. About four ounces of the fluid is the right proportion for four teeth.

On testing the mixture it will be found to be faintly alkaline or neutral.

Into the mouth of the flask there is placed a sterilised wool plug, and a sterilised caoutchouc cap fitted over it.

The whole is placed in an incubator at 37° C.

Examined at the end of twenty-four hours, the mixture will be found to be slightly acid.

This acidity is marked at the end of forty-eight hours, and not until the lapse of several weeks are there any signs of the fluid becoming again alkaline.

In five days, as already stated, the enamel becomes disorganised, and cover-glass films can be made.

At the end of seven or eight weeks the teeth should be removed and broken into small fragments, the film of collodion being, of course, removed.

The fragments are decalcified in a 10 per cent. solution of hydrochloric acid, then neutralised, washed, imbedded in gum mucilage, and cut on a microtome. The very thinnest sections are to be stained after *well* washing, by Gram's method, or in any other way, as the fancy of the student may dictate.

In this manner, then, the periphery of the hard parts of the teeth become carious.

In order to obtain further disintegration, the teeth should be washed, and placed in a fresh mixture under like conditions. Ultimately 'liquefaction foci' and the formation of cavities, to a limited extent, occur, the process being identical with caries produced naturally, and the resulting microscopical specimens differing in no particular when similar methods of staining have been pursued.

Miller's Method

Miller's Original Method for producing Artificial Dental Decay. A number of sound teeth, of varying density, were cut up into pieces of different size, and placed in a mixture of saliva and bread at 37° C. In

three months, after repeated renewal of the mixture, some of the dentine fragments were softened through, and secondary enamel decay was noted in places. Since 1884 he has modified the conditions by adding meat to the contents of the saliva flask, and by changing it every second or third day. If the experiments are not interfered with by the formation, as a white, thick, felty skin on the surface, of great numbers of *Saccharomyces mycoderma*, which will ultimately produce an alkaline reaction and disturb the process, in five weeks sections may be cut without further decalcification.

Sewill's Method

Sewill,[1] in conjunction with *Pound*, used an ounce of a mixture of bread and saliva, in the proportion of one to eight parts, for several teeth. These were placed in glass-stoppered bottles at 37° C., the bottles being unstopped once a day for the admission of air. After three weeks 5 per cent. acetic and ·5 per cent. lactic acids were found. Sections were stained in aniline violet, and counter-stained with orange-rubine. Thus the micro-organisms are coloured violet, softened dentine light yellow, 'zone of translucency' faint pink, 'pipe-stems' red, with lighter centre, and the normal uninfected dentine deep red.

In a report on dental caries contributed to 'The Dublin Journal of Medical Science,' 1892, *Baker* writes:

Baker's Original Method

'All the appearances which have been described as occurring in caries under natural conditions I have been able to produce artificially by placing sound teeth in a flask containing a mixture of bread, sugar, meat, and saliva, the flask being kept in a brood oven at 37° C. Some of the teeth I cut into pieces, others I[2] covered with wax, except at one point, so as to produce cavities

[1] 'Trans. Odonto. Soc. of Great Britian,' 1891.

[2] This experiment was first successfully carried out by Professor Miller, and has subsequently been repeated by other observers.

similar to what we meet with in the mouth. At the end of a few days, decalcification had so far advanced that I could penetrate the specimen with a needle, and at the end of fourteen days I was able to cut sections from the slices of teeth. The mixture in which the teeth were placed showed a strongly acid reaction by the end of the first day; it was occasionally renewed, and never at any time became putrid. Hanging drop preparations showed various micro-organisms, many of which were motile. On the addition of iodine and iodide of potassium solution, some of the micro-organisms stained blue, but it did not interfere with their movements in the least. . . . The existence in these specimens of large decalcified portions of dentine, into which micro-organisms had not yet penetrated, seems to support Professor Miller's view as to the existence in natural caries of softened but non-infected areas of dentine.'

CHAPTER XI

ON THE INJECTION OF CAPILLARIES, AND ON THE MEASUREMENT AND DELINEATION OF OBJECTS

Introductory

THOUGH falling outside the scope of dental microscopy, the injection of the blood-vessels of organs is of great interest to the enthusiastic student. The study of the relationship which exists between the vascular supply and the dental tissues themselves is also of importance, since it may throw occasional light on the causes of deviations or aberrations from, and suppressions of, certain typical forms. This study cannot be followed without a knowledge of the methods of injecting the vessels.

Natural and Artificial Injections

Vascular injection may be either natural or artificial: by the former is implied the retention of blood corpuscles within the capillary walls—best observed in sections of fishes' teeth (vaso-dentine), the pulps of which have been hardened in Müller's fluid, and cut *in situ* (see plate VIII., fig. 3). Artificial injection means the method of filling the vessels with an extraneous medium.

This medium may have for its basis water, glycerine, or gelatine combined with a colouring matter. When employing fluids, of which gelatine is the vehicle, it is necessary that the animal should be kept at the temperature of the body throughout the manipulations: and this is obviously inconvenient.

For dental work, perhaps the most suitable method is the following :—

Modus operandi

Procure a small animal, such as a kitten, or rabbit, and subject it to the vapours of chloroform, in which a capsule containing three minims of nitrite of amyl has been crushed.

Preparation of the Animal

Immediately after death, lay it on its back, and fasten its stretched-out limbs on to a board, which should be placed in a large shallow tray. Remove the sternum with its attachments, and expose the pericardium. Snip this through with scissors, and finally cut off the apex of the heart, opening up the right and left ventricles.

On a shelf, at the height of five or six feet above the table, place two Wulff's bottles, one containing an abundant supply of warm normal salt solution, and the other the injection mass, made according to the following formula : [1]

Injection Mass

Pure glycerine	4 ounces
Tincture of sesquichloride of iron	20 drops
Ferrocyanide of potassium	6 grains
Strong hydrochloric acid	5 drops
Water	2 ounces.

To a syphon, in each of the bottles, attach a piece of long, thin, flexible, india-rubber tubing which has been previously rinsed through with a stream of water. On the free end of each tube fasten a stop-cock. Exhaust the air in the dependent tubes by suction, thus causing a stream of salt solution in the one, and of injection fluid in the other. Stop the flow by turning off the stop-cocks. The weight of fluid in the two tubes will insure a continuous and uniform flow through the

[1] This is a slight modification of Dr. Beale's Prussian blue solution, as suggested by Mr. Charters White.

blood-vessels of the animal, this being sufficient to overcome the normal resistance of their walls.

Washing out the Vessels

Pass the nozzle of an injection syringe, through the left ventricle of the heart, into the orifice of the arch of the aorta, and, when in position, tie it firmly to the vessel walls by passing a threaded ligature needle underneath and around it. Insert the stop-cock attached to the tube containing the saline solution into the nozzle, avoiding the entrance of air, and turn the tap. The fluid should immediately commence to flow into the arterial system, and at the end of about half an hour run out with a clear stream from the right ventricle, showing that it has circulated through the whole of the

Their Injection

arteries, capillaries, and veins of the animal. Again avoiding the entrance of air, rapidly substitute the second for the first stop-cock, and the gradual diminution of the injection mass in the Wulff's bottle will denote the rate at which permeation of the blood-vessels is taking place. Examine the tongue and conjunctivæ of the animal, and, when they assume a bluish tinge, the operation may be stopped. On the completion of injection, turn off the stop-cock, ligature the artery,

Final Treatment

tying a reef knot, and remove the nozzle. Finally, lay the animal in a deep dish of cold water for an hour. Excise the mandible, divide it at the symphysis, and place the halves, after further division, in Müller's fluid till they are hardened. Complete the process by immersing the pieces in alcohol, afterwards decalcify if necessary, imbed in gum mucilage, and cut on an ether-freezing microtome. Stain the sections with carmine.

Precautions

There are two points of importance to notice in connection with the injection of the blood-vessels of *young* animals. First, during the act of excising the sternum and its attachments, unless great care is exercised, the knife will open the large venous trunks lying closely behind the manubrium sterni. It is well, there-

fore, to allow the sterno-clavicular articulations to remain. Secondly, it is very easy, when tying the reef knot, to draw the ligature so tightly that it cuts completely through the delicate walls of the aorta, with the result of spoiling the whole operation. Otherwise the method is quite simple, but nevertheless very successful.

THE MEASUREMENT OF OBJECTS

For purpose of research

It is often convenient, in giving descriptions of histological specimens, to be able to make a note of the dimensions, as well as the shape and general appearance of the object. This would be very difficult without an acquaintance with the several methods which have been devised. The size of cells, distance between dentinal tubules, length and width of interglobular or other spaces, etc., may be obtained by using a stage micrometer, singly or else combined with either a camera lucida or an ocular micrometer.

The Stage Micrometer

Instrument for Measuring Objects

This consists simply of an ordinary glass slide, ruled at its centre with microscopic diamond-cut lines, which are separated from each other by fractions of an

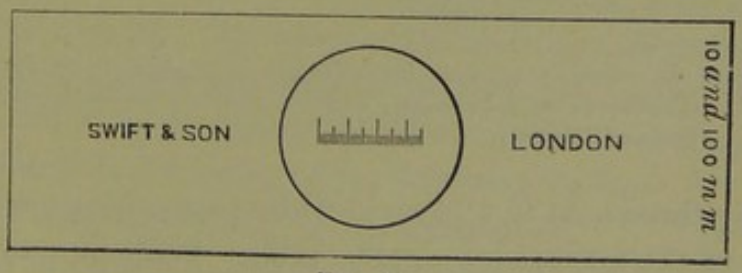


FIG. 63.

inch or millimetre. The stage micrometer alone almost suffices for measuring objects, but, if used in combination with a neutral tint reflector or eyepiece micrometer, it will form a very valuable adjunct to the student's microscopical outfit.

The lines on the slide are equidistant, and are either hundredths or thousandths of an inch apart in the English form, or tenths or hundredths of a millimetre in the Continental model, and when focussed and viewed as a transparent object, they appear sharp and clear in the field of vision.

Modus operandi

Method of Using.—Put the microscope in a vertical position, with the specimen on the stage. Focus the latter, and by keeping both eyes open, observe its faint

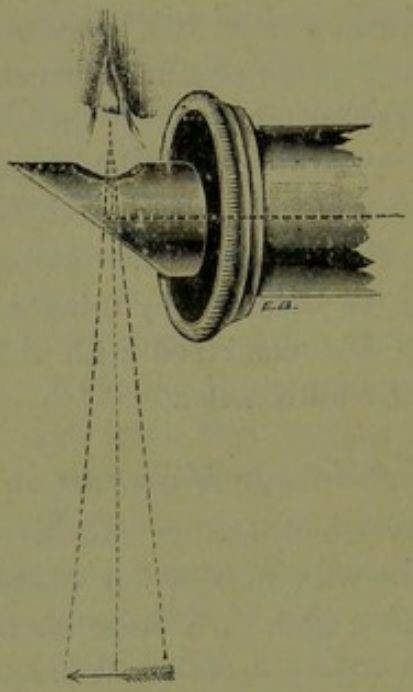


FIG. 64.—Beale's neutral tint reflector, in position over the eyepiece of the microscope, and the method of viewing the projected object.

image thrown on to a piece of white paper, lying on one side of the microscope on the table. Without moving the position of the eyes, trace the outline with a pencil. Remove the slide, substitute for it a stage micrometer, still keeping both eyes open, and the lines will appear faintly drawn across the sketch. Point off these lines with a pencil and rule them across. Note the combination of ocular and objective, the height of the eye-

piece above the paper, and the extent to which the draw-tube has been drawn out. Calculate the size of the cell by computing the number of spaces it occupies between the lines, the distance of which is known.

This is, at best, but a rough method of measuring the size of objects. More trustworthy data can be obtained by using a stage micrometer with a camera lucida or tint reflector. Beale's neutral tint reflector is a small circle of tinted glass mounted in a metal frame, and attached, by means of an arm at an angle, to a ring which is intended to be slipped over the tube of the eyepiece. The reflector does not magnify the object, but when in position, it projects the image on to a piece of white paper.

Beale's Tint Reflector

The Stage Micrometer and Reflector Combined

Method of Using.— The micrometer is fixed on the centre of the stage, and the microscope tilted horizontally, having the reflector at an angle of 45° to the eye-lens of the ocular. If the micrometer is well illuminated, and the observer's eye looks from above through the reflector, the lines will be seen projected on the paper underneath. These lines should be measured and pencil marks made on the paper, to be ultimately ruled in pen and ink. By subdividing the lines, thousandths or other greater fractions of an inch can be estimated, and these again converted into millimetres or micro-millimetres, according to the following scale :—

1 centimetre (cm.) = 10 millimetres.

1 millimetre (mm.) = 1,000 micromillimetres (μ).

25 micromillimetres (25μ) = $\frac{1}{1000}$ of an inch (approximately).

The distance between the centre of the eye-lens of the ocular and the paper being known, it is not a difficult thing for the student to make a chart bearing these lines upon it.

Micrometer Chart

Thus: If a $\frac{1}{4}$-inch objective and C ocular are used, and the paper is lying on a plane distant five inches from the centre of the eye-lens of the ocular, then on the paper—

$\frac{1}{100}$ inch will measure three inches nearly.
$\frac{1}{1000}$ inch will measure $\frac{3}{10}$ inch nearly.

The micrometer having now been removed from the microscope stage, and the section inserted in its place, it is only necessary to sketch in pencil the outlines of cells, distances between dentinal tubules, on the chart, over the lines, to easily make the desired measurements. These should only be taken at the centre of the disc of light thrown on the paper.

The Ocular Micrometer

Another Method

A still more easy and reliable method is to employ a stage and eyepiece micrometer together, the microscope body being, in this instance, kept in an upright position. The ocular micrometer consists of a single circle of glass, the scale of which is divided into hundredths of an inch. It is made to slip into the eyepiece between the eye and field glasses. If this is used instead of the tint reflector, two sets of lines will appear in the field of vision. When in focus, it is first necessary to determine how many divisions of the ocular micro-

FIG. 65.

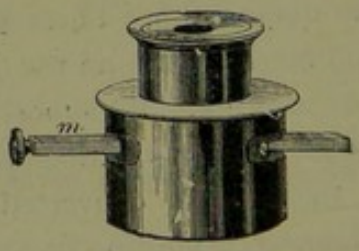


FIG. 66.—Jackson's eyepiece micrometer, with adjustable screw.

meter occupy one space of the stage micrometer.[1] The proportion they bear to each other must be noted, and when the section is placed on the stage and focussed, the size of its cells, or other constituents, can be estimated by remembering that so many of the lines seen over the section equal $\frac{1}{100}$ of an inch.

THE DELINEATION OF OBJECTS

The ability to reproduce on paper the chief features of his sections will be of great service to the student during his study of dental histology. Some workers can make drawings of their preparations with great ease, by mere inspection of their structure through the microscope; but the majority find this a difficult matter, and it certainly requires long practice before anything like perfection is reached.

Instruments for Drawing Specimens

But, by employing a camera lucida, or Beale's reflector, as already hinted, useful sketches of the tissues may, with a little trouble, be made by anyone.

Method of using

The arrangement of apparatus is similar to that just described—the microscope horizontally fixed, the reflector in position in front of the eyepiece, and the section well illuminated by means of lamplight. The intensity of the illuminant should be modified until a point is attained at which the image, seen through the reflector, is at its best.

There has, however, always been a certain amount of difficulty attending the use of the camera lucida, or Beale's neutral tint reflector, for the purpose of making microscopical drawings. The twisting of the head into an uncomfortable position, the great fatigue to the eyes, and the by no means easy task of viewing both image and pencil at the same time, add to the troubles of making a faithful reproduction of the object on paper.

[1] A rather more elaborate form of ocular micrometer is shown in fig. 66.

Alternative Method

A plan requiring no special apparatus

To those especially who do not possess a camera lucida, or Beale's instrument, and to students generally, the following arrangement of ordinary apparatus may be recommended: The microscope body is placed in a horizontal position, and the mirror removed from its sub-stage attachment. The microscopic slide having been placed on the stage, the illuminant (lamplight for choice) is 'condensed' on to the slide by means of a 'bull's-eye,' in the same way as for photo-micrography. Care must be taken to 'centre' the light. A concave mirror is then attached to the front of the eyepiece of the microscope by a piece of thin wood or a spring, having its surface at an angle of about 45° with the plane of the eye-lens of the ocular. The image is thus projected on to the paper beneath. No distortion will occur if the outer ring of light is *perfectly* circular. A dark cloth, such as photographers use, is thrown over the draughtsman's head and the body of the microscope, and all light excluded save that which passes through the microscope lenses. Any section can thus be easily, rapidly, and comfortably drawn, and fairly accurate representations obtained of objects magnified up to 500-600 diameters.

CHAPTER XII

ON PHOTO-MICROGRAPHY

THE drawing of an object made with the help of a camera lucida, or reflector, is obviously in many respects incomplete, no matter how skilful the draughtsman may be. Such a diagram is not useless, for its chief points being brought into prominence, by this very lack of detail, its clearness may be more serviceable for teaching purposes than would an elaborate reproduction. But when an exact copy of a section is required the outline sketch fails, and the application to microscopy of photographic processes can alone produce the desired effect. Photo-micrography is the term applied to the art by which enlarged images of microscopic specimens are permanently recorded.

Use of Diagrams

And of Photo-micrography

It is not possible, in a work like the present, to give a complete account of photo-micrography. The writer's aim, therefore, is merely to detail methods of working by which the beginner may obtain satisfactory negatives and pictures of his dental sections. To amateur photographers the combination of photography and microscopy will be simple ; but those who have no knowledge of the former will, at first, find it beset with many difficulties.

The subject may be considered under two heads : A, a description of the necessary apparatus, and B, methods of operating.

A

Photographic Apparatus

The room which the student intends to devote to photo-micrography should, if possible, be provided with arrangements both for taking photographs and developing plates. Spacious and well ventilated, it should be situated in such a position in the house that no vibrations due to disturbances out of doors can affect the instruments, rigidity of apparatus giving freedom from tremor being a *sine quâ non*. Some workers prefer a concrete floor on which to place their tables. All windows should be fitted with wooden frames which are covered over with two or three thicknesses of orange or ruby coloured medium. The doors should close in such a manner as to exclude all light. A firm deal table, measuring about seven feet long by two or three feet wide, standing in the centre of the room, supports the baseboard of the camera at the height of three or four feet from the floor. There should also be a plentiful supply of cold water, a large table on which to place the developing and other dishes, and a dark-room lamp.

Fittings of Room

A very important piece of apparatus is the photo-micrographic *camera*. This is not of the usual form, but has a long bellows body. It should be adapted for taking either $\frac{1}{4}$-plate or $\frac{1}{2}$-plate negatives—*i.e.* pictures measuring $3\frac{1}{4} \times 4\frac{1}{4}$ inches, or $6\frac{1}{2} \times 4\frac{3}{4}$ inches. The accompanying figure illustrates its chief parts.

Description of Camera

The bellows body extends 30 inches or more, on a mahogany baseboard, which has a scale let into it, so that the distance between the objective and plate may be accurately known, and the desired amplification of the object readily ascertained. The microscope, illuminant, and paralleliser (bull's-eye condenser) are placed on a projecting board at the fore part of the camera, the

Arrangement of Apparatus

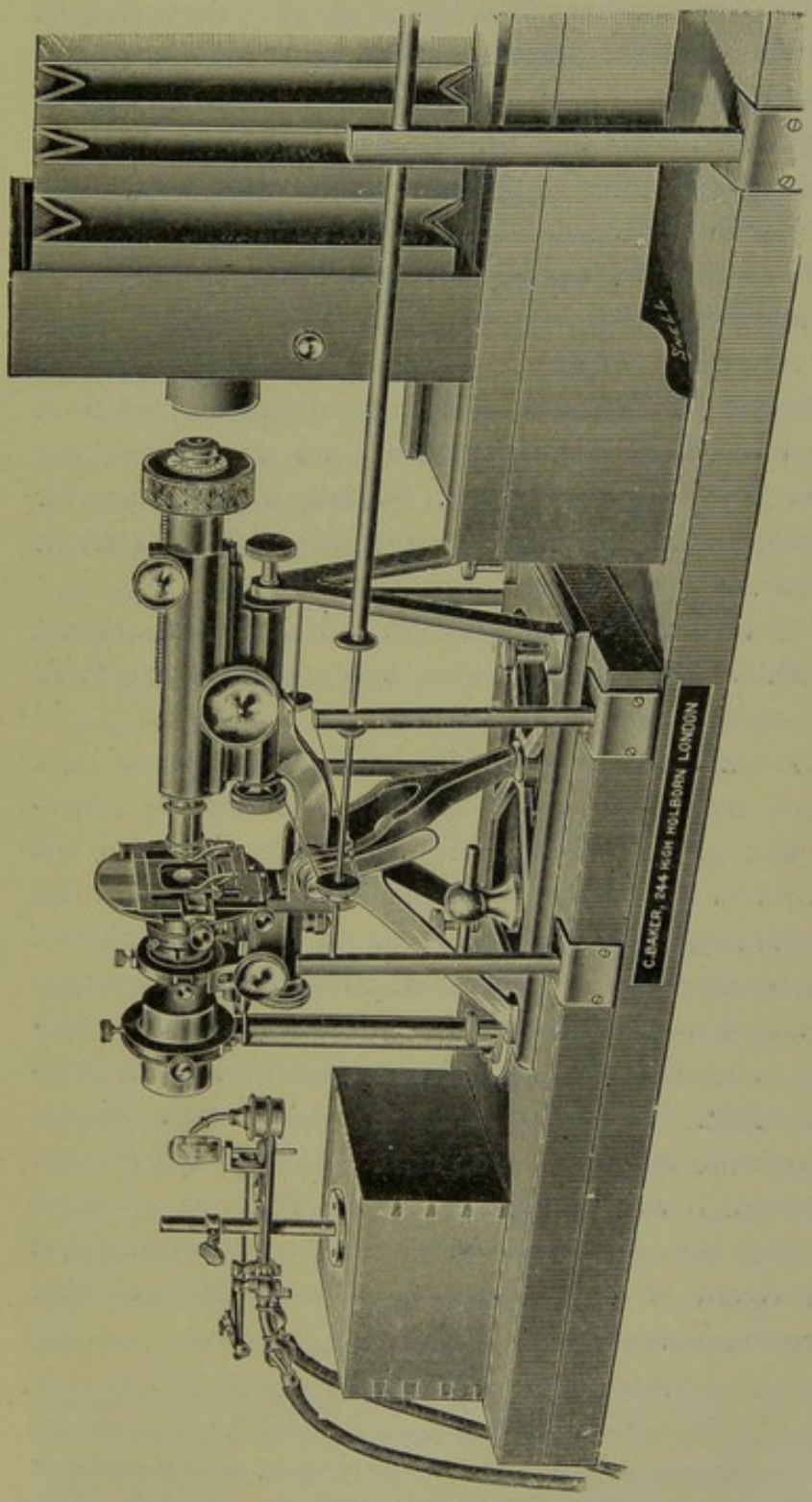


FIG. 67.—Complete photo-micrographic apparatus, with the arrangement of the parts—camera, long focussing rod, sub-stage, and bull's-eye condensers, and illuminant (limelight), in position.

sliding front of which allows any microscope to be fitted to it. A rod running from back to front of the apparatus is attached to the grooved milled head of the fine adjustment of the microscope by means of a thin india-rubber ring, and allows fine focussing to be done when the student is standing at the back of the camera. A dark slide with two carriers for ¼-plate negatives, and three masks—circular, square, and oval—for making neat pictures, comprise the rest of the camera outfit.[1]

Lenses

Any good *microscope* may be employed for photo-micrographic purposes. The advantages derived from the use of apochromatic lenses[2] are very great, but objectives that have not been specially manufactured for photographic work may be used, and will yield excellent results.

If the actinic (photographic) and visual foci are not perfectly coincident, they can be 'corrected' at a small cost by any competent optician.

Actinic and Visual Foci: definition of terms

Actinic is the name given to that property of light which chemically affects the film of a sensitive photographic plate. The term 'visual focus' means the sharpness and clearness of the image which is thrown on the camera screen when seen by the observer's eye. But it sometimes happens that although the image may appear sharply defined to the eye, it is not photographically in focus; the two foci are then said to be non-coincident. This occurs in 'uncorrected' lenses. Should it be found that the foci do not coincide, the uncorrected objective must be slightly moved from or advanced towards the object, the motion which is necessary and the extent of it being found by a carefully recorded actual experiment.

[1] The ingenious student can make, with the expenditure of but little time and money, a camera suited in most respects for all his requirements.

[2] In apochromatic objectives, the fringe of colour, or other aberration of light, seen in the field when ordinary lenses are used, is practically abolished.

Source of Lighting

The oxy-hydrogen limelight is by far the best *illuminant*, as the light which passes through the objective emanates from a mere point (approximately); but the student will find a paraffin microscopic lamp with a flat wick very serviceable, especially if a small piece of camphor is placed in the oil, to render the light whiter and more actinic.

A bull's-eye condenser throws very nearly parallel rays of light on the section, and, if centrally placed, illuminates it uniformly.

It is advisable not to use an eyepiece, the chief objections being a great restriction of the field of view and marked loss of light.

Other Apparatus

The accessory apparatus needed are glass-stoppered bottles to hold the developing and toning solutions, a graduated glass measure, three or four ½-plate porcelain developing dishes, and a larger one to contain the 'fixing' solution, and also several printing frames.

B

Methods of Operating

Preliminary Steps.—Having already placed a box of sensitive dry plates on the table and opened the dark slide, the student should light the dark-room lamp, and turn the flame down so low as to give only a very faint illumination. Then standing some distance from the light, he should, as quickly as possible, put a sensitive plate in one of the carriers of the dark slide. The edges only of the plate must be touched, and its shiny non-sensitive side lie uppermost when in position in the carrier. In the course of time it becomes quite easy to transfer the plates to the slide in absolute darkness, a touch of the fingers at one extreme corner of the plate indicating which is its film side. If this transference is accomplished in the dark, so much the better, as there

Placing Plates in the Slide

is then no risk whatever of 'fogging' or injuring the sensitiveness of the film. The operation is repeated with a second plate, and the dark slide tightly shut and wrapped for a few moments in a photographer's black cloth.

Kinds of Plates

The plates specially recommended for photo-micrography are Edwards's 'Isochromatic Instantaneous,' or the Ilford 'Isochromatic Medium' for using with stained sections. Ilford 'Ordinary' plates answer remarkably well for unstained objects.

Arrangement of Instruments.—The body of the microscope is placed horizontally and the eyepiece removed. A piece of thin dull black or brown paper is then inserted in the draw-tube, to prevent the production of what is technically known as a 'flare' on the sensitive plate. The draw-tube is fitted firmly to the front of the camera, the microscope stand being securely fixed to the projecting portion of the baseboard. The

Illuminant

lamp is then brought into position, and the wick having been trimmed to make the edges of the flame parallel, it is placed opposite the sub-stage condenser, with one edge of the flame turned towards it, in such a manner that the long axis of the flame and the optical axis of the microscope coincide—the centre of the flame exactly corresponding with the centre of the field lens of the objective. The mirror should be turned aside, and the sub stage condenser used with high-power objectives only. The student is recommended to employ nothing but his 1-inch objective, until the initial difficulties have

Condensing the Light

been overcome. A bull's-eye condenser is now interposed between the illuminant and the section which has been clamped on to the stage of the microscope; it is then centralised, and the whole section evenly illuminated. The importance of centralisation cannot be too much insisted upon. An examination of the ground-glass screen of the camera will show whether the lighting

of the object is uniform ; if it is not, the focus of the paralleliser must be altered, till the desired degree of brightness and uniformity is attained. The camera bellows are finally extended to the necessary distance, and a suitable mask placed in the end of the camera, so that the enlarged aërial image of the section is projected on the screen in the required form. Focussing is the next step ; and this is accomplished by slowly turning the long focussing rod. When the image appears well defined, the ground-glass screen should be removed, and replaced by a piece of transparent glass ruled with diamond cut lines, and a magnifier used to make certain that every portion of the image is perfectly clear and sharp. The circular edge of the image may appear out of focus ; this will necessitate the employment of a diaphragm which will cut off the scattered rays of light, and give it a better definition. To counteract the diminution of intensity of the light, a longer exposure must be given.

Focussin

Exposure.—The student having satisfied himself with the sharpness of the image, must exclude the passage of all light through the microscope by means of a thick screen of black cardboard, which is to be placed between the lamp and bull's-eye, or section and objective if there is room. This being done, the dark slide containing the sensitive plates is substituted for the glass screen, care being taken not to shake the instruments ; the shutter is opened, and one plate exposed by removing the cardboard screen. Fig. 67 shows the arrangement of camera and microscope just before this stage has been reached.

To prevent Blurring

The exact duration of exposure required to produce a strong negative is very difficult, at first, to estimate. No hard and fast rules can be laid down, though many attempts have been made ; the student's carefully recorded experience will prove to be the only sure guide.

Exposure

The author has obtained excellent negatives by using Edwards's 'Isochromatic Instantaneous' plates under the following conditions :—

Camera extended 24 inches, 1-inch objective used, no sub-stage condenser, no diaphragm, ordinary lamp-light : for hæmatoxylene stained sections—*exposure ten seconds* ; for sections somewhat feebly stained with carmine—*twenty-six seconds.*

Ilford 'Isochromatic Medium' plates will require, under circumstances similar to those first named, twenty to fifty seconds, and Ilford 'Ordinary,' four to ten seconds. It may also be stated that the image projected by a $\frac{1}{6}$-inch objective will require an exposure of fifty to sixty seconds, and $\frac{1}{12}$-inch oil immersion, three to six minutes. The beginner will be much assisted in his judgment of the proper length of exposure by first experimenting with a few plates. He may adopt this plan :—

To obtain Approximate Duration of Exposure

The shutter of the dark slide, on being drawn out a short distance, will allow only a portion of the plate to be influenced by the action of the light. This exposure must be timed in seconds by a watch. The shutter should be further opened and the time again noted, and the operation repeated at intervals of five or ten seconds, till the whole of the plate is exposed. On subsequent development *one* portion of the negative will probably be seen to have been correctly exposed, and the right length of time to give to similar sections thus ascertained.

Developing Negatives.—The plate having been exposed for the correct length of time, the cardboard screen must be again used to shut off rays of light, and the shutter of the dark slide immediately closed. The microscope lamp is then turned out, and the room faintly illuminated by the light from the ruby lamp.

Removal of Plate

The negative is carefully removed from the dark slide

and placed film-side uppermost in a developing dish, and then flooded with a developing solution.

Developing Solutions

Of developers there are many kinds—pyrogallic acid combined with soda or ammonia salts, hydroquinone, metol, amidol, etc. The formula given for use with Ilford plates is good, the only objections being that it stains the fingers if used carelessly, and it does not retain its properties for any long period of time. A stock solution consisting of pyrogallic acid 1 ounce and water 6 ounces, with the addition of 20 drops of nitric acid, should have been previously prepared, and two separate solutions, made as follows, contained in labelled, stoppered bottles :—

A	B
Stock solution, 2 ounces Water, 18 ounces.	Carbonate of soda (crystals), 2 ounces Sulphite of soda, 2 ounces Bromide of potassium, 20 grains Water to 20 ounces.

Immediately before using, 6 drachms of A solution are added to the same quantity of B solution. A colourless mixture results.

Removal of Stains

Stains from this developer can be removed by the application of a weak solution of citric or hydrochloric acid ; but as cleanliness of the hands and fingers is a matter of great importance to the dental student, he is recommended to use hydroquinone.

Hydroquinone : its Advantages

It has the advantages of not staining the fingers, can be used repeatedly, is suitable for plates and papers alike, and the negatives exhibit more detail, and are altogether softer than those obtained, under like conditions, with pyrogallic acid. A convenient formula is : —

A	B
Hydroquinone, 160 grains Potassium bromide, 30 grains Sodium sulphite, 2 ounces (avoirdupois) Water to 20 ounces.	Sodium hydrate, 100 grains Water, 20 ounces.

For use, take equal quantities of each solution.

Development of Plates

The developer is to be poured evenly over the plate so that all its surface may be covered at once. The solution is kept in motion by a rocking movement of the dish, which should be held at some distance from the light. An occasional rapid glance at the plate will suffice to tell how the development is proceeding, the criterion of complete development being afforded by the appearance of a faint black image seen *through* the glass, on the reverse side of the plate.

Caush's Method of determining the duration of Development

'To obtain the best results in photo-micrography, backed chromatic plates should always be used; and as this process of backing is a disagreeable operation to the amateur, these backed plates may be obtained from the makers. My experience has been chiefly with the Ilford chromatic backed plate, and the procedure is as follows:—

'After the plate has been exposed, take it into the dark room, "illuminated by a deep orange light," place the plate in the developing dish without removing the backing, pour over the developer, and cover the dish with a piece of fretwood or miliboard, at the same time having hung up the watch in such a position that the second hand can be easily seen. Note the time by the second hand. When the developer is poured over the plate, examine every fifteen seconds, until the faintest trace of the outline is seen, again cover up the plate, and multiply the number of seconds by five. At the end of that time, take the plate out of the developer, wash, and place in the fixing bath. In this way it can be developed in darkness, whilst there is a reasonable amount of light in the dark room. Thus, if thirty seconds elapse between the pouring on of the developer and the appearance of the high lights, then, multiply by $5 = 2\frac{1}{2}$ minutes. This is the time the plate will take to be properly developed.'—(*Caush.*)

Washing

Copious washing of the negative should next take

place, and the plate be afterwards put into a rack to dry.

Printing.—The clearness of detail observed in prints made on Ilford Printing-out Paper, or Eastman's 'Solio,' renders these classes of papers suitable for photo-micrography. The student is recommended to use these papers, and to follow the instructions, as to manipulation, suggested by the manufacturers. Toning in a bath consisting of chloride of gold, 3 grains; sulphocyanide of ammonium, 30 grains; and cold water, 18 ounces, should be allowed to proceed until marked contrasts between the blacks and whites are noticed. Toning Bath

Final Treatment.—A finished glossy appearance may be given to prints by adopting the following simple method:—A sheet of clear glass, free from scratches or other defects, should be thoroughly cleaned by brushing with soap and water. It is then dried, and French chalk powdered over it. This is removed by wiping with a dry clean rag, and the print, after having been soaked in clean water, is squeezed face downwards on to the glass, without excessive pressure, care being taken to remove all air-bubbles. If the print is then put into direct sunlight, it can be stripped off the glass, without sticking or tearing, at the end of two or three hours. Slip-in mounts are useful for holding the finished prints. Finishing Prints

THE CAUSES OF FAILURE

The chief causes that interfere with the production of satisfactory photo-micrographs, other than those arising from defective apparatus, may be here enumerated, and remedies suggested for the prevention of failure.

(1) The section may be too thick, or incorrectly stained, or improperly mounted. Only uniformly thin Sections

sections can be satisfactorily photographed—they must not be thick in some places and thin in others.

Correct Staining

The more commonly used stains for sections to be photographed may be divided into two classes—good and indifferent. To the former belong aniline blue black, bismarck brown, and hæmatoxylene, especially when this is of a clear dark blue colour. Of the latter class, picro-carmine, eosine, and rubine are perhaps the best. The staining must not be too dense.

The Use of Screens

In certain cases, when objects present only shades of a single colour, the interposition of a coloured screen (which can be purchased at opticians) between the illuminant and the section, produces better negatives than the employment of isochromatic plates alone. The tint of the screen should be the complement of the colour of the stain—that is, one which nearly reduces the colour of the image to a neutral grey. Thus an orange-tinted screen is to be used when a blue-stained preparation is to be photographed. Picro-carmine gives good results alone, but is improved by using a light green screen. The length of exposure will, under these circumstances, be necessarily prolonged.

Correct Mounting

The mountant should be colourless, or nearly so. Canada balsam sometimes acquires a yellow tinge after having been kept for a long period, but it is the best medium to use. The section must be perfectly flat. It is advisable always to use a strong clip to press down the cover-glass uniformly, immediately after mounting.

(2) There will be blurring of the image on the sensitive plate, if precautions are not taken to keep the apparatus firmly fixed and free from vibrations. The cardboard screen should always be used.

Under and Over Exposure

(3) Plates may be under or over exposed, by which is meant that negatives may either be lacking in detail, or 'flat,' that is, present no degrees of contrast. It is useless to endeavour to improve an improperly exposed

negative, either by intensification or any other method. Experience alone will enable the operator to judge the correct length of exposure; he must take into account the intensity of the illuminant, the size of the diaphragm, the power of the objective, and the staining of the section.

Developers

(4) The plates and developing solutions if old will not yield such good results as if they are new. Small quantities of both should be obtained at a time.

By careful attention to the foregoing details, the difficulties of making sharp, bright negatives should be removed.[1]

Lantern slides

Lantern slides may be easily made from negatives, either by direct contact as an ordinary print, or by reduction in a properly constructed reducing camera. Exposure is by gaslight, as a rule, though some German manufactured plates are to be exposed to daylight. In either case they are developed similarly to negatives. It is advisable to follow the instructions given by the makers as to the correct developing solution to employ.

And their coloration

Various coloured effects and combinations of shades can be produced by tinting with a brush the slide after fixing and mounting, but *before* drying, with eosine, indigo-carmine, chrysoidin, and patent tartrazene yellow. Uncoloured lantern slides, however, are generally of most service.

METHOD OF ASCERTAINING THE AMPLIFICATION OF AN OBJECT

Vereker's Rules

The approximate camera magnification of an object may be learnt by means of very convenient rules suggested by Mr. G. P. Vereker in the 'Photographic Quarterly,' Vol. III., No. 10. He writes: 'In using a

[1] For a fuller account of the art, the reader is referred to Mr. Andrew Pringle's excellent work, 'Practical Photo-micrography,' 1894.

microscope it must be looked upon as a double lens, or, if the objective alone is used, a single lens. Many different magnifications can be got out of one objective and eyepiece. The initial power of the lens is found by dividing 10 (the nearest average distance of distinct vision in inches) by the focus of the objective. Thus 10 divided by $\frac{1}{4}=40$, which is the initial power of $\frac{1}{4}$-inch objective. Multiply this by the power of the eyepiece, and the magnifying power of the combination results. Thus, if the eyepiece magnifies 5, the combination with the $\frac{1}{4}$-inch objective will equal 200 diameters.

If the eyepiece and objective are used :—

Rule

As 10 is to the camera length, so is the microscopic amplification to that of the camera. Thus, if $\frac{1}{4}$-inch objective and A ocular are used, and the camera extends 12 inches, then :—

As 10 : 12 :: 200 : 240 ; magnification = 240 diameters.

Rule

If the objective alone is used, the length of the microscope tube is added. Thus, if $\frac{1}{4}$-inch objective is used, and the microscope tube is 10 inches in length, and the camera extends 12 inches :—

As 10 : 12 + 10 :: 40 : 88 ; magnification = 88 diameters.

The rules above indicated are sufficient for the purpose of enabling the student to approximately compute the magnifications produced by any objectives he may employ.

CONCLUSION

It need only be added that in practical dental microscopy, as in other scientific pursuits, common sense, thoroughness, attention to detail, and perseverance, will lead the inquirer to the not-far-distant goal of success; and instead of becoming a mere *dilettante*, he will develop into an enthusiastic worker in the science and art of dental microscopy.

But a word of warning is necessary. In all his labours the dental catechumen must be imbued with a profound sense of honesty, conscientiousness, and truth, or his work will retard instead of advance the progress of Dental Science. 'The one test of scientific truth,' says an eminent American *littérateur*, 'is that it shall bear unlimited and untrammelled investigation. It must be not only verified, but always verifiable. It welcomes every trial, it recoils from no criticism—higher or lower—from no analysis, no scepticism. It challenges them all. It asks no aid from faith, it appeals to no authority, it relies on the dictum of no master. The evidence, and the only evidence to which it appeals or which it admits, is that which it is in the power of every one to judge—that which is furnished directly by the senses. . . . The only conditions that it enjoins are that the imperfections of the senses shall be corrected as far as possible, and that their observations shall be interpreted by the laws of logical induction.'

Keeping these high principles ever before him, the student will not only bring to himself the delightful satisfaction of having attempted and achieved something, but will also be adding lustre to the great world of Science generally.

APPENDIX

I.—Tables to show the Magnifying Power, in Diameters, of some Objectives with certain various Oculars, at the two positions of the Draw-tube of the Microscope, A *when closed,* B *when fully extended.*

A

Objectives	Eyepiece No. 1	Eyepiece No. 2	Eyepiece No. 3	Eyepiece No. 4
1 inch	23	35·5	46	58·5
$\frac{2}{3}$ inch	33	49·5	66	82
$\frac{1}{2}$ inch	58	87	116	145
$\frac{1}{4}$ inch	112	168	224	280
$\frac{1}{5}$ inch	142	213	284	305
$\frac{1}{6}$ inch	188	282	376	470
$\frac{1}{12}$ inch	316	474	632	790

B

Objectives	No. 1	No. 2	No. 3	No. 4
1 inch	45	67·5	90	112
$\frac{2}{3}$ inch	64	96	128	160
$\frac{1}{2}$ inch	107	158·5	214	267·5
$\frac{1}{4}$ inch	210	315	420	570
$\frac{1}{5}$ inch	256	384	512	640
$\frac{1}{6}$ inch	342	513	684	855
$\frac{1}{12}$ inch	550	825	1100	1375

II.—Table of Weights and Measures as used in Microscopy.

1 metre . . . . = 39·3704 inches.
1 centimetre (cm.) . . = 0·39 inch.
1 millimetre (mm.) . . = $\frac{1}{25}$ inch approximately.
1 micromillimetre (μ) . . = $\frac{1}{25000}$ inch approximately.
1 inch . . . . . = 2·539 centimetres.
1 fluid ounce . . . = 28 cubic centimetres.
1 fluid drachm . . . = $3\frac{1}{2}$ cubic centimetres.
1 drachm (Apothecaries') . = 4 grammes approximately.
1 gramme (grm.) . . . = $15\frac{1}{2}$ grains approximately.
1 cubic centimetre (c.c.) . = 16 minims approximately.
1 litre . . . . . = 35 fluid ounces approximately.

III.—Table to show Comparison between Centigrade and Fahrenheit Degrees of Temperature.

Centigrade	Fahrenheit	Centigrade	Fahrenheit
60	140·	30	86·
59	138·2	29	84·2
58	136·4	28	82·4
57	134·6	27	80·6
56	132·8	26	78·8
55	131·	25	77·
54	129·2	24	75·2
53	127·4	23	73·4
52	125·6	22	71·6
51	123·8	21	69·8
50	122·	20	68·
49	120·2	19	66·2
48	118·4	18	64·4
47	116·6	17	62·6
46	114·8	16	60·8
45	113·	15	59·
44	111·2	14	57·2
43	109·4	13	55·4
42	107·6	12	53·6
41	105·8	11	51·8
40	104·	10	50·
39	102·2	9	48·2
38	100·4	8	46·4
37	98·6	7	44·6
36	96·8	6	42·8
35	95·	5	41·
34	93·2	4	39·2
33	91·4	3	37·4
32	89·6	2	35·6
31	87·8	1	33·8

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PRINTED BY
SPOTTISWOODE AND CO., NEW-STREET SQUARE
LONDON