DENTAL HISTOLOGY

BY

JAMES AITCHISON

H.D.D.(Glas.), L.D.S.(Glas.)

Director of Dental Education Glasgow University; Director of Glasgow Dental Hospital; Lecturer in Dental Surgery

Author of "DENTAL ANATOMY FOR STUDENTS"

(*Formerly Lecturer on Dental Histology, Glasgow Dental Hospital—Assistant Lecturer on Dental Anatomy—Assistant Visiting Surgeon, Glasgow Dental Hospital—Dental House Surgeon, Glasgow Dental Hospital—Demonstrator in Orthodontics—Demonstrator in Dental Bacteriology—Demonstrator in Dental Histology—Coach for H.D.D.(Glasgow)—Dental Surgeon to Royal Hospital for Sick Children, Glasgow*)

AND

JOHN S. DICK

L.D.S.(Eng.)

(*Formerly Demonstrator in Dental Histology, Guy's Hospital and Dental House Surgeon, Guy's Hospital*)

S T A P L E S P R E S S

STAPLES PRESS LIMITED — *Mandeville Place, London, W1*

STAPLES PRESS INCORPORATED — *70 East 45th Street, New York*

FIRST PUBLISHED 1943

Made and printed in England by
STAPLES PRESS LIMITED
at their Great Titchfield Street, London, establishment

PREFACE

This book has been mainly compiled from lectures delivered to students. The contents cover the required curriculum for entrants for the B.D.S. or L.D.S. of any of the British Universities or Dental Schools.

The authors wish to thank the writers acknowledged in the bibliography: and Messrs. W. Watson and Sons, Ltd., of High Holborn, for permission to use their illustrations of microscopes and accessories.

JAMES AITCHISON.
J. S. DICK.

LIST OF PLATES

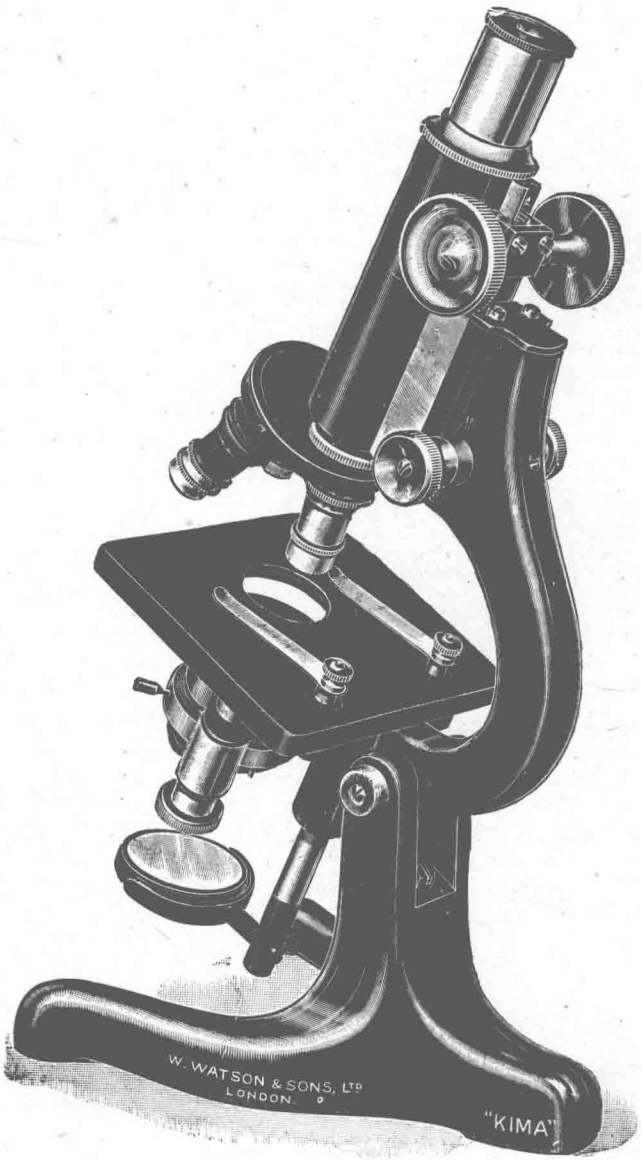

FIG. 1.—The Microscope.

(From "Microscopes and Accessories," published by Messrs. W. Watson & Sons, Ltd.).

CONTENTS

ILLUSTRATIONS

CHAPTER 1

THE MICROSCOPE

Definition.

The microscope is an instrument for producing an enlarged image of a small object.

The enlargement or magnification is not the actual magnification of the object but its magnification as it appears when 10 in. from the human eye (i.e. normal focus). As everyone has not the same normal vision, microscopes are capable of focusing to suit the individual's focal length, and also the difference in thickness of cover slips, mounting media and slides.

Parts.

The modern microscope consists of 3 main parts : —

1. The magnifying apparatus, which consists of the *tube or body* and carries the *lenses*.
2. The stand or framework.
3. The illumination apparatus.

The body consists of an inner and an outer tube and carries a coarse and a fine adjustment.

The coarse adjustment is worked on a rack and pinion system, operating on the outer tube. The coarse adjustment is used first and is the only adjustment that need be used with the low-power objectives.

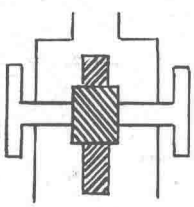

FIG. 2.—The coarse adjustment.

The fine adjustment is dependent upon a lever working on the outer tube by means of a screw with a very fine thread. It is only used with the higher power objectives and only after the rough focusing has been done with the coarse adjustment.

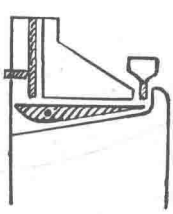

FIG. 3.—The fine adjustment.

These adjustments raise or depress the body. The image seen is not *real* but *virtual*, and is produced beyond the object.

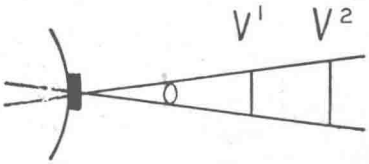

FIG. 4.—Real and virtual image.

Magnification.

If the magnification is 10 diameters with a 10 in. draw tube and the tube is extended to 15 in., the magnification will be increased to 15 diameters.

The outer tube carries a swivelled, triple nosepiece, so that the low-power, high-power and the oil-immersion objectives can be carried at the same time.

Objectives.

The objectives used are :—

1 in. or 2/3 in. (low-power objectives).
1/6 in. (high-power objective).
1/12 in. (oil-immersion objective for bacteriology).

These measurements do not represent the working distance of the object glass from the object, but the distance from the object to the centre of focus of the objective.

100 years ago simple bi-concave lenses were used. These gave an enlarged image which was surrounded by concentric coloured rings which confused the image. To overcome this fault different types of glass are combined in each lens to-day.

The modern objective consists of several lenses, making it compound in structure, and the higher the power of the objective the more complicated and numerous are the lenses.

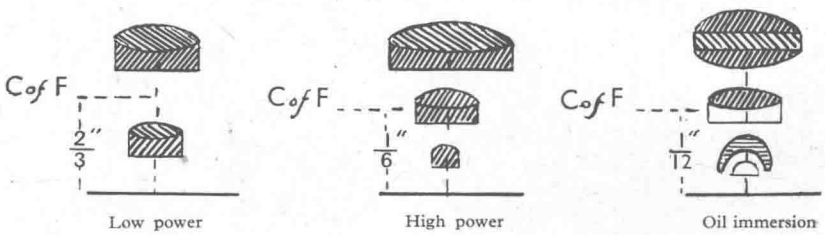

FIG. 5.—Diagrams of lenses in low power, high power and oil immersion objectives.

From the diagrams in Fig. 5 it will be seen that the 2/3 in. (or low power) objective has 2 lenses, with the centre of focus lying between them ; the 1/6 in. (or high power) objective has 3 lenses and the centre of focus passes through the middle one ;

the 1/12 in. (or oil immersion) lens has 4 lenses and the centre of focus passes through the third lens.

Resolution.

Owing to the difference between the refractive index of glass and air a great deal of light is lost. This does not matter much in the lower powers where the resolution (i.e. the depicting of detail) is not required to such a high degree. With the 1/12 in. objective the structures to be examined are so minute that the utmost resolution is required. For this purpose cedarwood oil is used, as it has the same refractive index as glass. The objective is immersed in a drop of the oil, which is placed on top of the slide, completely occupying the air space between the slide and the objective, thus giving the effect of a homogeneous mass and conserving the rays of light.

Magnification of Objectives.

With a 10 in. draw tube

the 1 in.	objective	magnifies	10	diameters.
2/3 in.	,,	,,	15	,,
1/6 in.	,,	,,	60	,,
1/12 in.	,,	,,	120	,,

Eyepieces.

The eyepieces most generally used are A eyepiece and C eyepiece, which give magnifications of 3 and 9 respectively. Other eyepieces may be supplied with the microscope, but their magnification is always clearly marked on the upper surface.

The eyepieces have each two lenses, an upper and a lower, with a diaphragm between them, like the diaphragm in the draw tube.

These diaphragms prevent reflections of light from the inside of the tubes entering the eye and distorting the image.

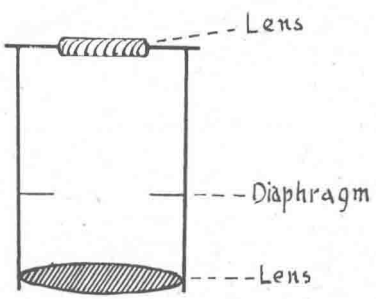

FIG. 6.—Diagram of arrangement of lenses in an eyepiece.

Mirrors.

A double mirror, flat on one side and concave on the other, is situated on a swivel below the substage, to reflect the light into the objectives. The concave mirror condenses the rays of light and is the only one used with the low-power objectives.

Abbé Condenser.

This is used with the flat mirror for the high-power objectives and should be removed or swung out of the path of light when studying dental histology.

Care of the Microscope.

Extreme cleanliness must be practised in the care and use of the microscope. Lenses are expensive and easily damaged. Every student must possess a fine silk cloth that will not scratch the glass surfaces and a soft chamois cloth for dusting the metal parts of the instrument. Before use, the oculars and objectives should be carefully wiped free of dust with the silk duster and the metal parts dusted with the chamois cloth. After use the same routine technique should be followed before placing the instrument in its case. If cedarwood oil or Canada balsam are found on the lenses, the silk cloth should be moistened in xylol, a solvent of both these reagents, and gently rubbed over the surfaces until they are absolutely clean and finally cleared with a drop of alcohol on a fresh portion of the silk cloth. The metal parts should be cleaned if necessary in the same manner, using the soft chamois cloth.

CHAPTER 2

Section Preparation

Before describing any of the methods of preparing sections for microscopic examination it is necessary to impress on the student's mind that the various processes employed in elementary histology are not part of a mysterious and exact science to which he must devote years of study and practice before he can produce reasonably good microscopic slides. On the contrary, it has been the author's experience that many students produce excellent slides of hard tissues from the beginning of their classes in dental histology.

The preparation of sections of soft tissues or of combined hard and soft tissues requires a little more care and experience. Any keen student, however, can procure a small amount of the various agents specified in the following pages, either from his lecturer or from a chemist, and can practice preparing dental histology slides at home, up to the stage when his embedded specimen is ready for cutting on a freezing microtome. His lecturer will gladly permit him to use the hospital microtome to cut his sections.

The time spent in such practice will amply repay the student. It will not only prove interesting but will teach him more in a short time of the various processes of section preparation than he can possibly learn from books or notes or in the most careful study of prepared or partially prepared sections. Once the full processes of preparing microscopic slides have been demonstrated to him in his dental histology class, the student should carry out at least one complete process for himself, by extracting a tooth and preparing it for microscopic examination of both hard and soft tissues.

It must also be emphasised from the commencement of dental histology studies that all the structures which are accredited to a tissue do not show in every good microscopic slide of that tissue. Some structures require special processes and stains to demonstrate their presence, some can only be seen in transverse sections, others in longitudinal sections and a few structures can only be seen when the section passes through special areas of the tissue.

Fixing and Hardening Soft Tissues

It is necessary to know something of the actions of the various chemicals in use for the preparation of microscopic slides.

Fixing.

The first step in section preparing, where a soft tissue is to be examined, is known as fixing.

The fixing agent is a mordant solution which rapidly kills the cellular elements so that they do not have time to alter the form they possessed during life. This is accomplished by the fixing agent coagulating the albuminoids and gelatins in the various cells and thus rendering insoluble certain cell elements which otherwise would be dissolved, and thereby fixing their positions (e.g. Boil an egg and break the shell and the fluid albuminous contents retain their shape and relative positions).

Hardening.

The second step in treating a soft tissue for microscopic examination is hardening the fixed tissues. By this means the tissues

1. Withstand the action of the various agents with which they are subsequently treated.

2. Offer sufficient resistance to the mechanical strain of section cutting.

(e.g. Boil the egg a little longer and sections of it can be cut without altering the relative positions of the white and the yolk. Hardening is therefore practically a continuation of fixing.)

Fixing and Hardening Agents.

Most of the reagents used are both fixing and hardening reagents.

Formaldehyde.

One of the best reagents for fixing dental tissues is (H.COH), formic aldehyde or formaldehyde, a gas obtained by the oxidation of methyl alcohol.

Formalin.

A 40% saturated solution of formaldehyde in water is known as formalin. Mummery recommends 10 parts formalin to 90 parts H_2O or normal saline as a fixing and hardening agent. (Normal saline is 80 gr. salt in a pint of water, or 0·75 parts salt to 100 parts water.)

Formalin is an excellent fixative but has not sufficient hardening effect. A high percentage concentration of alcohol is used to dehydrate the tissues afterwards and this completes the hardening.

The penetration of formalin is so rapid and extensive that it obviates the necessity of cutting away the apical portion of the root of a tooth when this reagent is to be used for fixing and hardening a dental pulp. With other reagents it is essential to remove the apex of the root and to expose the pulp in the coronal

portion in order to admit free access of the reagent to the pulpal tissues before fixing and hardening.

A large amount of formalin should be used and the time allowed for fixing varies according to the size and nature of the tissue being treated. A small piece of soft tissue, 2 mm. to 4 mm. thick, should take 12 hours; a young human premolar tooth with a large open apex, about 36 to 48 hours; an older tooth with a small apical foramen, about 4 to 7 days. When fixing the dental pulp of a salt water animal it is necessary to double the strength of the fixing agent or to prolong the fixing time.

When placing a specimen in a fixing fluid it should be suspended in the fixing fluid to permit the fluid completely to surround the specimen and so increase its penetrative power. Specimens should be kept in a coloured bottle to prevent sunlight reacting on the formalin, and there is no limit to the time that the specimen can be left in the formalin, provided the latter is changed regularly.

After fixing in formalin it is not necessary to wash the specimen. It can be placed immediately in alcohol to complete the hardening and is then ready for embedding.

There are many other fixing agents used in histology. The commoner ones in use for dental tissues are: (1) Mercury, (2) Chromic Acid; (3) Osmic Acid; (4) Alcohol.

Mercury.

The best of these latter reagents for mere fixing purposes is mercury, which may be used either as a cold concentrated alcoholic solution of the perchloride or as a 5% aqueous solution.

The objection to mercury is that it leaves a very persistent precipitate which can be removed only by the use of iodine.

Chromic Acid.

Chromic Acid is no longer used alone, but generally in combination with some other fixative such as osmic acid or mercury. It renders the tissues brittle unless combined with glycerine.

Potassium Bichromate.

Potassium bichromate is the commonest of the chromates. It is an excellent hardening agent and does not render the tissues brittle, no matter how long they may be immersed in it. It is slow in action and should be used in solutions of 2 to 4 per cent, beginning with the weak solution and increasing the strength. When the hardening is complete the specimen should be well washed in water and passed through increasing strengths of alcohol. The latter process must be performed in the dark or, as with all chromic acid solutions, a fine precipitate forms on

the surface of the specimen which prevents further action of the alcohol.

The common potassium bichromate processes are given below.

Zenker's Fluid.

Zenker's Fluid may be used for rapid fixation where time is an important factor, as in the case of a diagnosis of the condition of a dental pulp where pulpstones are suspected as the cause of a patient's severe neuralgia.

It consists of the following :—

Bichromate of potassium	2·5 gm.
Perchloride of mercury	5 to 8 gm.
Water	100 ml.
Glacial acetic acid	5 ml.

The potassium and mercury are dissolved in the water with the aid of heat and kept as a stock solution. When a quantity of the fixative is about to be used the glacial acetic acid is added in the proportions given. The tissues to be treated will be fixed in 12 to 24 hours, according to their size and nature. They must then be washed in running water for the same period and transferred to 80% alcohol.

Helly's Fluid.

This is an improvement on Zenker's Fluid. The 5 ml. of acetic acid are replaced by 5 to 10 ml. of formalin.

Müller's Fluid.

Müller's Fluid is used for soft developmental tissues. It consists of the following :—

Potassium bichromate	2 parts
Sodium sulphate	1 part
Distilled water	100 parts

This reagent has great penetrating powers, hardens uniformly and does not cause shrinkage of the cells or fibrous tissue. Sections prepared in Müller's Fluid are easily manipulated and are not brittle, but it tinges the specimen a yellow colour, which however is invisible in microscopic sections and does not interfere with staining.

Osmic Acid.

Osmic acid is a watery solution of osmium tetroxide. It is easily reduced if the slightest trace of dust or organic matter is present, but not when absolutely pure. The objections to its use alone are that it must be used in the dark to prevent reduction taking place, although the addition of a small quantity of potassium permanganate or sodium iodate will prevent this : that it stains, fixes and hardens simultaneously and too rapidly, thereby hindering its own penetration and sometimes only affecting the

external portions of the tissues. It fixes the cytoplasm of cells perfectly, but not the nuclei. It is generally used in combination with formic acid or acetic acid as a nuclear fixative. It is then used as a vapour fixative and not as an immersion fixative.

The tissue to be fixed is suspended in the neck of a bottle containing the mixture and retained there until it becomes brown. It is washed quickly and stained with hæmatoxylin or picro-carmine. Prolonged washing is not essential, as the specimen has not been immersed in the fixing solution.

Alcohol.

Alcohol is used for rapid hardening of adult tissues only. If any of the other fixing agents are used, the specimen must be washed in alcohol to remove the fixative. Commercial methylated spirits contains naphtha and must not be used in histology, as it becomes turbid when mixed with water and it is impossible afterwards to clean the specimen.

The objection to alcohol is that it causes shrinkage and cannot be used if blood corpuscles are to be retained in the vessels.

Acetic Acid.

Acetic acid is frequently used in combination with other fixing reagents as it causes the tissues to swell and counteracts the shrinkage effect of the other fixatives. It is never used alone because of this action, which affects especially fibrous tissue and other delicate, loosely knit tissues.

Fixing by Injection.

The above methods of fixing are known as immersion or block methods, with the exception of the osmic acid method described above and known as a vapour immersion method. Any of these methods may be employed by students in their classes. There is another type of fixing, known as fixing by injection, which they may see demonstrated. It is the ideal method of fixing. The fixing fluid is injected into the blood stream of the animal whose tissues are to be examined immediately after the animal is killed. It is not a difficult method to employ and it possesses many advantages over the immersion methods. It was first used by Golgi, using 0·5% potassium bichromate, for fixing the brain and spinal cord. The special advantages of the injection method in dental histology are :—

1. The fixation is rapid, and within one minute every cell in the body is perfectly fixed.

2. The internal and peripheral portions of the dental pulps or other soft oral tissues are equally fixed.

3. The blood-vessels are seen in their normal state and not shrivelled, collapsed or distorted, as they may be in the immersion methods of fixing.