NOTES ON THE PRACTICAL COURSE IN NORMAL HISTOLOGY; GIVEN IN THE LABORATORY OF THE ALUMNI ASSOCIATION OF THE COLLEGE OF PHYSICIANS AND SURGEONS, NEW YORK CITY

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Notes on the Practical Course in Normal Histology; Given in the Laboratory of the Alumni Association of the College of Physicians and Surgeons, New York City by T. Mitchell Prudden

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BY

T. MITCHELL PRUDDEN, M.D.

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PREFACE.

THE full course for classes in Normal Histology in this laboratory comprises forty lessons, of from one and a half to two hours each. Students are furnished with microscopes, reagents, and all necessary apparatus, with the exception of razors, slides, cover-glasses, and boxes for the preservation of specimens. So little time is usually at the disposal of medical students for collateral reading, and so necessary is it to occupy as little of the laboratory time as possible in oral descriptions of tissues and methods, that these notes have been prepared with the expectation that students will anticipate each lesson by reading beforehand the brief section devoted to its theme, and thus be ready to commence the practical work of the lesson hour without loss of time.

It is not to be expected that epitomized descriptions of structures as elaborate as are many of those with which we have to deal in histology, will be in all cases perfectly clear and intelligible without the aid of figures; but the actual specimens prepared, and the sketches from them which are made in the laboratory by the students themselves, will make good, it is hoped, the lack of illustration in the text.

There are many points in this as in every developing science which are still unsettled—opinion in regard to them changing or being modified as new facts and investigations are recorded. These have been treated, for the most part, very briefly in the text, it being left for the supplementary oral instruction to enlarge upon and explain them as the light thrown upon each by new researches may seem to require.

T. M. P.

LABORATORY OF THE ALUMNI ASSOCIATION, COLLEGE OF PHYSICIANS AND SURGEONS, NEW YORK, Sept., 1879.

INTRODUCTION.

GENERAL METHODS FOR PRESERVING TISSUES AND PREPARING THEM FOR STUDY.

ANIMAL tissues must conform to certain physical conditions before they can be subjected to a satisfactory microscopical examination. Portions of them subjected to study must be sufficiently thin to allow the light to pass readily through them, and transparent enough to permit the determination of the form, character, and relations of their structural elements. At the same time the refractive power of the different elements should not be too nearly alike, since upon differences in this respect the form and characters which microscopical objects present to the eye are largely dependent; or, in case they are so, the different elements must be rendered visible by staining them with coloring agents. Certain tissues naturally undergo rapid changes of structure after death; these are to be prevented by the application of preservative agents. Some are too soft to permit the preparation of thin sections and must be hardened; others are too hard and must be softened. In some specimens one, in others another structural feature, is to be brought into prominence. All of these indications in the histological technique are to be met in such a way as to leave the structures under investigation in as natural a form as possible. Finally, specimens suitably prepared for examination are, in many cases, to be rendered permanent for future reference and study.

We will now consider briefly some of the methods by which these indications may be fulfilled. Most histological specimens are laid either with or without some enclosing fluid medium, on a glass plate, and covered with a very thin slip of glass, before being brought under the instrument. One of the simplest methods of studying tissues is to place them, when quite fresh, and after they are reduced to a condition of suitable tennity, on a slide with some fluid which alters their physical condition but little or not at all, or at least very slowly, and examine them at once. Such fluids are called *indifferent fluids*, and among the best and most commonly employed are the aqueous humor, blood-serum, amniotic fluid. These organic fluids, however, although well suited for this purpose, are not always readily obtained, and are moreover liable to undergo more or less rapid decomposition; and since for most purposes a dilute solution of common salt, one-half to three-quarters per cent., answers very well, we shall generally employ

this when, in the following lessons, we have occasion to use an indifferent fluid in the study of fresh tissues.

The examination of fresh tissues is very important, not only because it enables us to follow the vital phenomena in certain elements, but because we are able by comparison to determine the amount of change which tissues undergo when prepared by more elaborate methods. Still this simple mode of examination is in many respects unsatisfactory. In the first place, it is not always easy to procure fresh tissues for every observation, and even in the indifferent fluids the tissues sooner or later undergo very considerable structural alterations, so that they cannot be permanently preserved. Again, fresh tissues are frequently not sufficiently hard and firm to allow the necessary preparation of specimens. A still more important difficulty which this method presents is the lack of clearness in the details of structure of fresh tissues. A very considerable proportion of the fresh animal tissues are nearly transparent, in thin pieces, and their structural elements possess so nearly the same refractive power, that we see through them, but do not see them; or, if we do see them indistinctly, it is not generally with that definiteness which our purposes demand. Now, these difficulties are usually met by the employment of agents which harden and preserve the tissues and at the same time render the details of their structure visible, by changing the refractive power of one or other of their elements; or we employ, as above indicated, certain coloring agents, which, being taken up with different degrees of avidity by different parts, permit the recognition of details by differences in color; or, such agents are used as both harden and stain at once; or, finally, which is the most common method, we employ two or more of the different classes of agents one after the other. We shall consider here only some of the most commonly employed of the hardening, preservative and coloring agents.

Alcohol is one of the most valuable of the preservative and hardening agents. It causes a considerable shrinkage of the tissues by the withdrawal of water from them, and, like many of the preservative agents, causes a precipitation of certain of their albuminoid constituents, thus diminishing their transparency.

Alcohol is, in general, to be used at first diluted with one-third water, and after the bits of tissue have lain for twenty-four hours in this they are transferred to commercial alcohol (eighty-five per cent.), in which they may be preserved indefinitely, losing in time, however, somewhat of the first clearness and naturalness of structural detail.

Bits of tissue to be preserved in alcohol, as in other hardening agents, should be quite small—not larger, as a rule, than 1 or 2 cms. on a side—and the quantity of fluid should be abundant. Certain structures are best preserved by plunging them at once into strong alcohol.

Chromic acid in solution and solutions of potassium and ammonium bichromate are very frequently employed to preserve and harden tissue, and many structures are more perfectly preserved in these fluids than in alcohol. The hardening process proceeds more slowly in the

chromic solutions than in alcohol, and the structures do not shrink as much. The tissue seems to be hardened and preserved by a slow process somewhat analogous to that of tanning.

It is a common practice to commence the hardening process with one of the chromic fluids, and complete it with alcohol.

Pure chromic acid is usually employed in solution of from one-sixth to one-half per cent. Potassium and ammonium bichromate are usually used in 2 per cent. solutions. A very valuable and much employed preservative solution is the so-called *Müller's Fluid*, consisting of—

Sodium Sulphate	1
Potassium Bichromate	2
Water	100

The ingredients are simply dissolved in water and the solution filtered. Chromic acid dissolves the lime salts in bone, and is often used to soften them in preparation for section-cutting. Tissues which contain fat are usually better preserved in the chromic fluids than in alcohol, since the fat is readily dissolved by the latter.

Picric Acid.—This agent hardens tissues, preserving in many cases their structural features most perfectly, and at the same time staining them intensely yellow. It is generally necessary to complete the hardening process with alcohol.

Picric acid is one of the best agents for the decalcification of bone, although it acts very slowly. It is commonly employed in cold, saturated, aqueous solution.

Osmic Acid.—This substance has the power, in dilute solutions, of fixing and hardening the tissue elements in a nearly normal form, and is one of the most valuable of this class of agents. It gives tissues a gray or brown appearance, and stains fat and certain allied substances deep black. It is a very expensive substance, and hence its use is at present somewhat limited. It is generally employed in a one per cent. aqueous solution, and the tissues should be quite fresh when immersed in it, and, as a rule, should remain for twenty-four hours. Specimens hardened in osmic acid, although very perfect at first, commonly become quite granular and black after a time, and nearly worthless.

The preservative fluids are sometimes brought into more perfect and immediate contact with the tissue elements by injecting them into the blood-vessels of the part before cutting them in pieces and immersing them in the fluids. This method is of special value when alcohol is used for hardening small organs like the kidney.

Indications as to which of these agents are best adapted for the preservation of different tissues, and the more exact details of the methods of employing them, will be given as we proceed with our practical study.

Hæmatoxylin is one of the most generally useful of the staining agents. It has the power of staining certain parts, as the nuclei of cells, deeply, while other parts are much less or not at all stained. It

can be employed for staining tissues which have been hardened by any of the above agents.

The following is Prof. Delafield's method of preparing the solution:

To make 200 c.c. of the solution, take 150 c.c. of saturated solution of ammonia alum; prepare a saturated solution of hæmatoxylin in absolute alcohol, and add 4 c.c. of it to the alum solution. This at first produces a violet, or sometimes a dirty red color, which, on exposure to the light in an open vessel, usually assumes in a few days a deep violet color. If the color does not become deep enough, a few drops more of the hæmatoxylin solution are added and the fluid exposed anew to the light. After standing for at least a week, and when the desired color is obtained, the solution is filtered and 25 c.c. each of glycerine and wood naphtha are added. Such a solution is to be diluted with several times its bulk of water before using, the exact amount of dilution depending upon the rapidity with which we wish the specimen to be stained. As a rule, slow staining with a dilute solution gives the best results, and is less likely to cause shrinkage of the specimen. In staining, bits of tissue are simply placed in a small dish of the solution, so that they are bathed on all sides by it and allowed to remain until sufficiently colored. The time required will depend, of course, upon the strength of the solution, and also to a considerable degree upon the character and previous preparation of the tissue. The excess of coloring fluid is to be thoroughly washed out of the specimen by water before mounting and studying.

Carmine.—This is employed in the same manner as hæmatoxylin, and like it stains different tissue elements with different degrees of intensity. Tissues preserved in chromic acid solutions do not stain as readily in carmine as in hæmatoxylin.

Frey's method for its preparation is the following: take of powdered carmine 0.30 grm., and add a sufficient quantity of aqua ammoniæ to dissolve it, mix with 30 c.c. of distilled water, filter, and add glycerine 30 grms., alcohol 4 grms.

Eosin.—This substance stains tissues somewhat more uniformly than those just mentioned, and is especially valuable when used in connection with other coloring agents, such as hæmatoxylin, which stain the cell nuclei more deeply, since by this method of double staining we have certain structural elements exhibiting one color, others another. Eosin may be conveniently used either in aqueous or alcoholic solutions of 1 to 100.

Picro-carminate of Ammonia or Picro-carmine.—This substance is for many purposes superior to the simple carmine. It usually stains more rapidly than the latter, and we can obtain with it at once the yellow color from the picric acid in certain elements, while others are stained red by the carmine.

It is prepared by adding to a saturated solution of picric acid a strong ammoniacal solution of carmine to saturation, evaporating the mixture to one-fifth its bulk, allowing to cool, filtering from the deposit, and evaporating the filtrate to dryness over a water-bath. The picro-carmine is left in the form of a crystalline ochre-red powder.