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II. PHOSPHOTUNGSTIC-ACID-HÆMATOXYLIN FOR CERTAIN TISSUE ELEMENTS
III. A METHOD OF FIXATION FOR NEUROGLIA FIBRES

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(FROM THE SEARS PATHOLOGICAL LABORATORY OF HARVARD UNIVERSITY)

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(*From the Sears Pathological Laboratory of Harvard University.*)

Plate XLI.

A DIFFERENTIAL STAIN FOR AMŒBÆ COLI.

The recognition of amœbæ coli has depended in part on their rather characteristic morphology, but chiefly on their movements as shown by the extrusion of pseudopodia. Examination of the organism in the fresh state, therefore, has been indispensable, and a positive diagnosis has rested on the exhibition of the characteristic amœboid movements.

In hardened tissues amœbæ coli are usually recognized with difficulty. Their nuclei do not stain with the ordinary basic stains, such as methylene blue and alum hæmatoxylin. The following method not only stains the nuclei of the organisms, but brings them out in a color entirely different from that of the nuclei of other cells. It is believed that this differential stain renders possible the recognition of amœbæ coli in tissues in the same way and with the same certainty that the tubercle bacillus is recognized by its characteristic stain.

DIRECTIONS FOR STAINING.

1. Fix tissues in alcohol.
2. Stain sections (celloidin or paraffin) in a saturated aqueous solution of thionin for 5–20 minutes.
3. Wash in water.

4. Differentiate in a 2 per cent aqueous solution of oxalic acid for ½ to 1 minute.

5. Wash in water.

6. Dehydrate in 95 per cent alcohol.

7. Clear in oleum origani cretici or in oil of bergamot.

8. Wash with xylol.

9. Xylol balsam.

For paraffin sections use absolute alcohol and clear directly in xylol.

The nuclei of the amœbæ and the granules of mastzellen are stained brownish red (Plate XLI). The nuclei of mastzellen and of all other cells are stained blue. If the sections are washed out too long in alcohol the nuclei of the amœbæ assume a light and more brownish tint, and the protoplasm is colorless. By decolorizing the sections to a less extent the nuclei of the amœbæ are of a deeper red, and the protoplasm is often tinted bluish or purplish, so that it can be more easily seen. The nuclei of the amœbæ stand out in such marked contrast to those of other cells that they are readily recognizable with comparatively low powers.

In a case of amœbic abscess of the liver it was found easily possible to stain small fragments of the softened material which had been hardened in alcohol (Plate XLI, Fig. 4). The stained bits were teased into still smaller fragments after they were in balsam. In this way a positive diagnosis of the presence of amœbæ coli was quickly and easily made.

With hardened dysenteric discharges the results obtained with the same method of staining were only fairly satisfactory, mainly because many substances present in the fæces precipitate thionin in the form of reddish crystals. Attempts to stain cover-slip preparations proved wholly negative.

It is possible to obtain a similar differential stain for amœbæ coli by using Unna's method for staining differentially the protoplasmic granules of mastzellen. His method is as follows:

1. Harden tissues in absolute alcohol.

2. Stain sections in Unna's polychrome methylene blue solution (Gruebler) ¼ hour to over night.

3. Decolorize and differentiate in a small dish of water to which are added a few drops of glycerine-ether mixture (Gruebler).
4. Wash thoroughly in water.
5. Alcohol.
6. Oil of bergamot.
7. Balsam.

It is possible that this second method would give better results with intestinal discharges.

PHOSPHOTUNGSTIC-ACID HÆMATOXYLIN.

The following solution of hæmatoxylin is a more or less perfect differential stain for certain tissue elements and possesses the peculiar property of being polychromic:

Hæmatoxylin .....0.1 gramme.
Phosphotungstic acid (Merck) 1 per cent aqueous solution.....100 cc.

Dissolve the hæmatoxylin in a little hot water and add when cool to the dilute acid solution. The color, at first greenish, turns in a few minutes to a reddish brown of little intensity. The solution is ready for use at once, and will keep for several months if not exposed to too much light.

DIRECTIONS FOR STAINING.

1. Stain sections 2–24 hours.
2. Wash in water.
3. Dehydrate in alcohol.
4. Clear in oleum origani cretici.
5. Xylol balsam.

Although the time required for staining is long, a stronger solution has not been found advisable. Prolonged washing in alcohol will remove most or all of the pink color. Celloidin remains unstained.

After any fixative, nuclei by this method are stained blue, while connective tissue fibres and the intercellular substances of bone, cartilage and the cornea are stained a light to a deep pink. If the staining is prolonged the cell protoplasm is usually colored a light blue.

Besides the nuclei, certain other tissue elements and products stain blue. Fibrin, the contractile elements of striated muscle fibres and elastic fibres stain sharply after fixation in alcohol, less well after Zenker's fluid. Bile capillaries often stain sharply after fixation in Zenker's fluid. Possibly better results would be obtained from the method used for neuroglia fibres. Smooth muscle fibres sometimes stain after Zenker's fluid, but the results are not constant. Red blood globules generally take a dark greenish tint.

For the central nervous system the stain will be found useful after fixation by the method already published, and given here again slightly changed, for mordanting neuroglia fibres so that they will stain by the fibrin method. The mordanting with the chrome salt must be as long as when a stain of the myelin sheath is desired, otherwise the myelin will stain to some extent with this solution.

The method is as follows:

METHOD OF FIXATION FOR NEUROGLIA FIBRES.

1. Fix thin pieces of tissue, not over ½ cm. thick, in a 4 per cent aqueous solution of formaldehyde (10 per cent solution of formaline) for 4 days or longer.

2. Saturated aqueous solution of picric acid, 4–8 days. Steps 1 and 2 may be combined by adding the formaldehyde to the picric acid in proper proportion.

3. 5 per cent aqueous solution of bichromate of ammonium, 4–6 days in the incubator at 37° C., or for four weeks at room temperature. Change the solution on the second day.

4. Transfer to alcohol without washing.

5. Imbed in celloidin.

Sections may be stained by Weigert's fibrin method (decolorizing, however, with equal parts of aniline oil and xylol), or with the phosphotungstic-acid-hæmatoxylin solution. With the latter solution the neuroglia fibres which have been properly fixed and mordanted stain a deep blue. The nuclei stain blue, the protoplasm of ganglion cells a pale bluish or purplish gray, elastic fibres and fibrin blue, axis cylinders a dull pink, connective tissue a deep pink, myelin sheaths yellow (from chrome salt).

The stain is useful because all the various tissue elements are stained. For the study of individual neuroglia fibres and their relation to the cell protoplasm the stain is not so good as the modified fibrin method.

A previous light stain with Van Gieson's picro-acid-fuchsin mixture is often useful, because it makes the red of the axis-cylinder more pronounced and the dendritic processes stand out in sharper contrast to the neuroglia fibres.

Very good stains of some of the neuroglia fibres can often be obtained with this solution after simple fixation in formaldehyde, alcohol or Zenker's fluid.

It is important to bear in mind that good results with stains for neuroglia fibres can be obtained only with tissue that is perfectly fresh at the time it is placed in the fixing solution. For example, nearly perfect results can be expected from thin pieces of tissue placed in formaldehyde within one hour after death. Up to twelve hours *post mortem* fair results may be expected. After twenty-four hours the results are practically nil.

The first (surface) sections will always give the best results, because in them the neuroglia fibres are soonest fixed, and the peculiar chemical property which quickly disappears *post mortem* and on which the stains depend is preserved.

The above stain succeeds with human neuroglia fibres only.

DESCRIPTION OF PLATE XLI.

Figs. 1, 2 and 3 show amœbæ in dysenteric discharges, Fig. 1 after hardening in alcohol, Figs. 2 and 3 after fixation in corrosive sublimate.

Fig. 4 is a teased fragment from the purulent material obtained from an amœbic abscess. Alcohol hardening.

Fig. 5 is from the muscular coat of the intestine and shows three amœbæ and three mastzellen.

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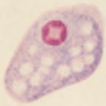

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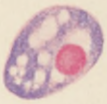

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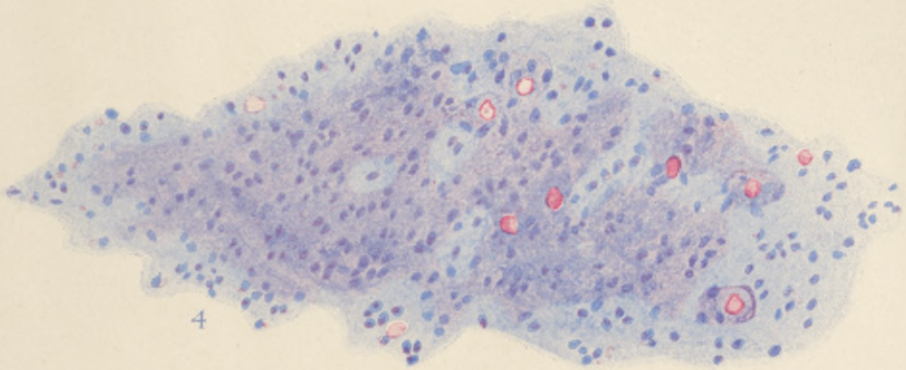

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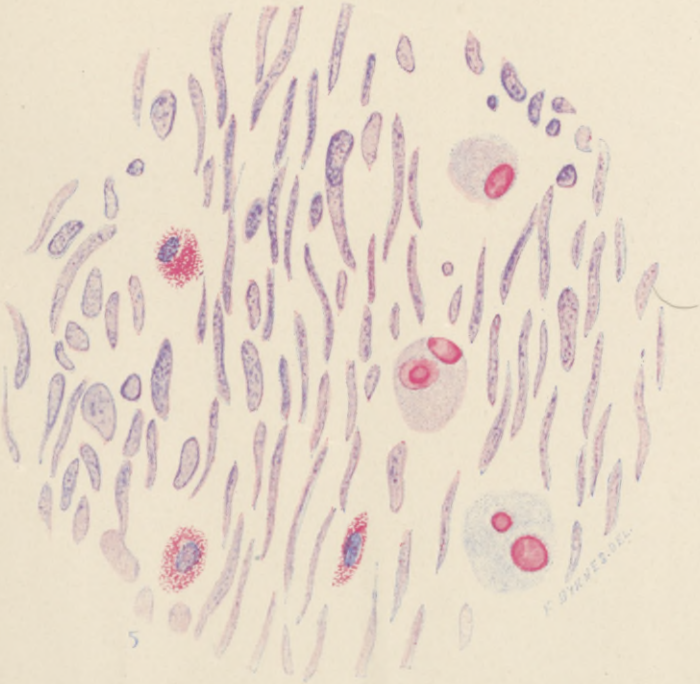

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