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A rapid method of making permanent specimens from frozen sections by the use of formalin.

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A RAPID METHOD OF MAKING PERMANENT SPECIMENS FROM FROZEN SECTIONS BY THE USE OF FORMALIN.

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Any one who has hardened tissues in formalin will be impressed with the rapidity of its action, with the firm consistence of the tissue, and with the absence of the contraction of the specimen so often seen when alcohol is used as the hardening medium. Microscopical examination of a specimen hardened in formalin, as we all know, shows almost perfect preservation of the cellular structure. Recently it occurred to me that formalin might be used in the preparation of frozen sections.

One of the greatest difficulties experienced in rendering frozen sections permanent lies in the fact that when passed through alcohol the section frequently not only contracts but contracts irregularly, distorting the specimen; further, such specimens will often stain imperfectly. The use of formalin will obviate these difficulties, allowing one to make an excellent permanent specimen from the frozen section. My method is as follows: The tissue to be examined is frozen with carbonic acid or ether and then cut; the sections are then placed in 5 per cent. watery solution of formalin for 3 to 5 minutes, or longer if desired; in 50 per cent. alcohol 3 minutes, and in absolute alcohol 1 minute. The tissue is now thoroughly hardened and can be treated as an ordinary celloidin section, being stained and mounted in the usual way. On examining this mounted section one might readily take it for a well preserved alcoholic specimen. Supposing we stain with hæmatoxylin and eosin, the entire process is as follows:

a. Place the frozen section in 5 per cent. aq. sol. formalin for 3 to 5 minutes.

b. Leave in 50 per cent. alcohol 3 minutes.

c. In absolute alcohol 1 minute.

d. Wash out in water.

e. Stain in hæmatoxylin for 2 minutes.

f. Decolorize in acid alcohol.

g. Rinse in water.

h. Stain with eosin.

i. Transfer to 95 per cent. alcohol.

j. Pass through absolute alcohol, then through either creosote or oil of cloves, and mount in Canada balsam.

The blood is lost in frozen sections. To overcome this Prof. Welch suggested that the specimen be first fixed in formalin and then frozen. I tried this and found that we were able to preserve the blood, but that it did not stain very distinctly. For convenience this second procedure will be called method II. The essential factor is the same in each case. The latter process, however, requires at least two hours. A small piece of the tissue is thrown into 10 per cent. solution formalin for two or three hours. It is then put on the freezing microtome and thin sections can be readily made. The sections are stained in the usual way. The detailed procedure of method II is as follows:

a. A piece of tissue 1x.5x.2 cm. is placed in 10 per cent. aq. sol. formalin for 2 hours.

b. Frozen sections are made.

c. Left in 50 per cent alcohol 3 minutes.

d. In absolute alcohol 1 minute.

e. The sections are now run through water and stained in hæmatoxylin for 2 minutes.

f. Decolorized in acid alcohol.

g. Rinsed in water.

h. Stained in eosin.

i. Transferred to 95 per cent. alcohol.

j. Passed through absolute alcohol, then either through creosote or oil of cloves, and mounted in Canada balsam.

For ordinary use method I is all that is required. Given a piece of tumor from the operating room, it is possible to give as definite a report in 15 minutes as one would be able to give after examining the alcoholic or Müller's fluid specimens at the expiration of two weeks. Method II is of especial value in the examination of uterine scrapings. Instead of putting them in the 95 per cent. alcohol in the operating room, they

may be immediately dropped into 10 per cent. aq. sol. formalin. By the time the pathologist receives them, which is at least two hours afterwards, they are firm enough to be frozen without difficulty, and permanent sections can be immediately made. The second method is to be recommended for all delicate tissues. In employing these methods one must remember, as for example in epithelioma, that some of the cell-nests will drop out, there not being anything to hold them *in situ*, as there is when celloidin is used. We have, however, hardened and stained epithelioma of the cervix by this method without the slightest difficulty.