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A COMPARATIVE STUDY OF THE HARDENING EFFECTS OF VARIOUS FIXATIVES, DEHYDRATING, CLEARING, AND EMBEDDING AGENTS

KARL A. STILES AND THOMAS N. STEWART

One of the problems commonly encountered in the preparation of tissue for histological studies is the tendency for it to become excessively hardened before sectioning with a rotary microtome. The purpose of the investigation was to discover by as objective a means as possible not only the hardening effects of various agents on tissues, but also the influence of the time element.

The approach to this problem has been to test the hardness of the tissue with a standard penetration needle after each step in the various techniques used.

The instrument employed was a standard penetrometer, using a No. 1203 Standard Penetration Needle, Roberts No. 2, made by the Arthur H. Thomas Co. of Philadelphia, Pa. Since liver tissue tends to harden excessively in histological preparation and is relatively uniform in texture, it was used throughout the study. A piece of tissue 4 mm. thick was placed beneath the needle and force was applied until the needle completely penetrated the tissue. The force exerted was measured by means of a spring balance, and the reading was recorded as the data.

The fixatives tested were formalin, Zenker's, Bouin's, FAA, Gilson's, and Petrunkevitch's. Tissue in each solution was fixed for 24 hours, except in the case of Gilson's, where a shorter time was used. Formalin, Zenker's, and Bouin's hardened at about the same rate over a period of 24 hours, FAA considerably less, and Gilson's and Petrunkevitch the least of all.

Dehydration was done by the ethyl alcohol, n-butyl alcohol, and dioxan methods. In the dioxan method the rate of hardening per hour was rapid, but the time element was short so that the end hardness of this method was relatively low.

In the n-butyl method, it was found that a mixture of n-butyl alcohol and the lower percentage ethyl alcohols does not noticeably harden the tissue. However, when 95% ethyl alcohol was mixed with n-butyl alcohol, the increase was quite rapid. When pure n-butyl alcohol was used, some hardening was noticeable, but was not as rapid as in the mixtures of one of the higher ethyl alcohols and n-butyl alcohol.

In the ethyl alcohol method, no appreciable hardening was observed through the lower alcohols. In 83% alcohol, there was a slight rise, but it was in 95% and 100% alcohols that the increase in hardness is extremely rapid.

Xylol, cedar oil, and aniline oil were used to clear the ethyl dehydrated tissue. Mixtures of one of the higher ethyl alcohols and either xylol or cedar oil continued to harden the tissues. In the next step, pure xylol likewise hardened the tissues, but no appreciable hardening was observed with cedar oil. In the case of aniline oil, which is miscible with 83% alcohol, there was apparently no hardening.

Two mediums were used in infiltration. These were paraffin and Diglycol Stearate S. Tissue infiltrated with paraffin from dioxan hardened rapidly, but the time element was short, so that the end result was comparatively little hardening. Tissue infiltrated from n-butyl alcohol hardened quite noticeably, but in the case of tissue fixed in Gilson's, it was not too hard to section. Tissue infiltrated from ethyl alcohol and cedar oil has a more rapid rate of hardening, but still sections well. However, in the case of infiltration from ethyl alcohol and xylol, the tissue became too hard to section satisfactorily. Tissue infiltrated from aniline oil sectioned very well.

In general, the best results were had from infiltrating in Diglycol Stearate S. Since dehydrating in the higher alcohols and clearing is unnecessary, the tissue does not harden previous to infiltration. It was found that tissues fixed in one of the fixatives which harden the most were as soft after infiltration in Diglycol Stearate S as tissues fixed in the fixative which hardens the least when embedded in paraffin after treatment by means other than dioxan. It was found in this technique that if the tissue is placed in a mixture of 70% ethyl alcohol and Diglycol Stearate S for several hours before infiltration, it hardens less than if it was placed in pure Diglycol Stearate S directly from 70% ethyl alcohol.

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