layer forms in the chromatographic chamber incorrect results will be obtained.

With phenol prepared in this way extraneous colors are completely eliminated. The amino acid bands are sharply defined and full advantage of the color range is obtained. It should be mentioned that the sample should also be free from heavy metals, particularly Cu, Zn, Fe and Mn. All chromatograms run in this laboratory are run against gravity as described by Horne and Pollard (*3*). An ammonia atmosphere is always used with phenol. With these procedures Rf values confirm very exactly those obtained by Dent (*2*).

References

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A Method of Automatic Dehydration for Histological Technique[1]

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Heretofore the mechanics commonly involved in the process of dehydration have consisted of manually or mechanically transferring tissues through a series of dehydrating chambers containing the dehydrating agents, or of leaving the tissues in a single chamber and periodically changing the surrounding dehydrating fluids.

A new method is now introduced whereby the tissues are continuously and automatically bathed in a continuous flow of anhydrous dehydrating agent. This is accomplished by the use of an extraction apparatus equipped with a Soxhlet Extractor having an overflow drainage rather than the usual siphon type drainage (see Fig. 1). The tissues are placed in the modified Soxhlet Extractor, which is then filled with dehydrating fluid. A funnel placed in the extractor is used to carry the condensate to the bottom of the chamber to insure a continuous change in fluid throughout the depth of the extractor. The flask is filled approximately three-fourths full of dehydrating agent, and 80–100 g of calcium carbide (depending upon the moisture content of the dehydrating agent and tissues) is also added to insure a completely anhydrous condensate. A thermostatically controlled hot plate with a variable temperature control is used as the source of heat. Thus, as the dehydrant is refluxed, the condensate (anhydrous dehydrating agent) enters the bottom of the extracting chamber, circulates through the tissues, and finally spills out through the overflow drainage back down into the flask. The apparatus should be operated under a hood, or else a rubber hose should be attached to the top of the condenser to carry off the fumes.

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This method offers several definite advantages. Dehydration is more complete. A flow of circulating fresh anhydrous dehydrant completely surrounds the tissues at all times. This is an important consideration, since incomplete dehydration is a frequent cause of shrinkage and hardening of tissues within paraffin blocks after they have been cut off and put away. If a gradually increasing concentration of the dehydrant is desired, one need only fill the extraction chamber with the desired

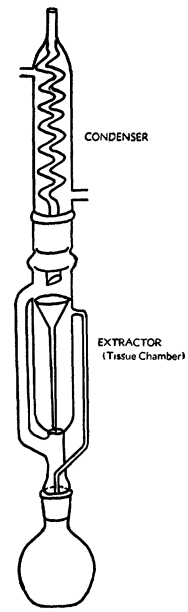

Fig. 1.

starting concentration of dehydrant at the beginning of the procedure. This dehydrant then will be very gradually replaced by completely anhydrous dehydrating agent, giving the effect of transferring the tissues through a finely graded series of reagents.

It is automatic. A manual transfer of the tissues is not necessary, nor is a manual change of dehydrating fluid. The tissues may be placed in the apparatus towards evening and left unattended until the following morning, when they will be dehydrated and ready for further processing.

It is inexpensive. Only small amounts of dehydrating agent are necessary, since the dehydrant is being continually refreshed as the process proceeds. The apparatus itself is of simple design, and any glass blower can make it at a nominal cost simply by altering a regular Soxhlet Extractor as indicated.

Although most of the commonly used dehydrating agents are adaptable to this procedure, it is preferable to use those having relatively low boiling points, so that the tissues may be safeguarded against any heat damage. We have had good success with acetone with the above method and at present are testing various other reagents, particularly those which can be used both for the dehydration and the clearing process, thus extending the automaticity of the procedure.