NOTES ON TECHNIQUE.

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In many of the modern articles, the methods by which certain pathological structures are demonstrated, if mentioned at all, are frequently so meager in the description of important details as to be practically useless to many workers, unless a certain amount of their time is devoted to experimentation. A person, who has obtained fairly successful results with his older methods, is loath to forsake them, especially if his first few attempts with the new are failures. Each investigator may have certain laboratory conveniences; reagents of the best quality and dyes that have been well tested, all of which will enable him to obtain results much superior to his less fortunate colleague. It is difficult, therefore, to work successfully unless details are carefully attended to, and the reasons for the various steps understood. The methods following have been well tested, and have been attended with uniformly good results, which in some cases, it is believed, would have ended in failure with the older methods.

FIXATION.

The fixation of pathological tissues, with strong alcohol for histological study, is very commonly employed for the double purpose of killing at once any microörganism that may be present and at the same time to preserve the structure of the part. With many tissues this caused a too rapid withdrawal of the contained water or lymph, so that the specimen becomes hard and gives unsatisfactory results when it comes to the cutting process.

Some experiments with different reagents, upon known pathological material, were of service in formulating a mix-

ture, which obviated the defects of strong alcohol when used alone. This mixture, while quickly killing the bacteria, also preserves most faithfully the histological structure. Various solutions of formalin, including the undiluted, were employed, and gave good results, particularly the presentation of the bacteria, after the usual staining methods. The tissues were more or less swollen by the weaker solutions, in marked contrast to the contraction caused by alcohol. Various combinations of formalin with alcohol were also tried, and that which seemed to be most completely satisfactory for quick penetration and convenience, bacteriologically and histologically, was as follows :

95 per cent. alcohol	100 parts.
Commercial formalin (40 per cent. formic aldehyde) .	10 parts.

Pieces of tissue, $\frac{1}{2}$ centimeter square, are well fixed in from twelve to twenty-four hours, after which it is well to leave for a few hours in 95 per cent. alcohol before clarifying for the paraffin bath. Specimens, transferred directly from the fixing mixture, have been clarified in chloroform or cedar oil, but it requires a longer time.

The addition of the formalin is advantageous, because in a way it brings about a state of equilibrium. The alcohol alone shrinks the tissue, while on the other hand formalin swells it, so that in this respect the one reacts against the other.

ADHESION TO THE SLIDE.

After the infiltration and imbedding of the tissue in paraffin, the question of the treatment of the sections is one of some importance. If they are to be carried through a series of reagents in watch glasses, and not placed upon the slide until they are mounted, the sections must necessarily be rather thick, in order to withstand the manipulation. Very much thinner sections, if adherent to the slide, and consequently supported by it, can be carried through the different steps of the process without injury, and show the structural elements to much better advantage.

The albumen or collodion adhesive, usually employed for this purpose, however, possesses the disadvantage of taking the aniline colors used in bacteriology, sufficiently to disfigure the preparations. If a clean slide be coated with a thin film of glycerine and then rubbed very nearly dry with a cloth or the hand, and a drop or two of 35 per cent. alcohol be placed upon it, the section, if curled, will tend to flatten itself when placed on the alcohol. If the slide now be placed in a thermostat for a few hours, at a temperature near the melting point of paraffin, the heat will cause any wrinkles or irregularities of the section to disappear; the alcohol slowly evaporates and when the slide is thoroughly dry the albumen molecules of the tissue adhere quite firmly to the slide, as noted by Gaule. After this the slide may be heated gently over a flame until the paraffin begins to melt. If any moisture remains the section will be quite likely to loosen during the latter stages. Thick sections do not adhere so firmly as thin ones. The slides may then be immersed in a jar of turpentine or any solvent of paraffin and carried through the various grades of alcohol to water.

A shorter method, in which there is as firm adhesion of the section to the slide, is to bring the slide in contact with aniline oil for a few minutes after the treatment with the turpentine, absorbing the superfluous turpentine with filter paper. The aniline oil is also removed by means of filter paper. The section is then thoroughly washed in distilled water which removes the oil, and the tissue is then stained and washed in water. If aniline stains are used, a hurried rinsing is sufficient. Drain or absorb the water and again apply the aniline oil. Besides clearing the section the oil tends to remove the aniline stain and care must be exercised in not letting this process go too far. Displace the aniline oil with xylol and mount in balsam. The color ought not to fade if the aniline oil has been thoroughly removed.

With certain stains, or combinations of them, the aniline oil may not succeed in preserving the sharp definition of the color. Under such conditions the section, after staining,

may be treated directly with absolute alcohol to dehydrate and remove any superfluous stain. Some aniline dyes are not as soluble in absolute alcohol as in the weaker grades. Clear in xylol and mount in balsam.

The use of aniline oil in the treatment of the sections will be recognised as having been recommended by Weigert for bacterial purposes. It likewise gives most excellent results in ordinary histological work and is a saving of time and material.

MOUNTING.

Many valuable specimens are ruined for the want of sufficient precaution in the preparation of the balsam. In its commercial state it contains many volatile principles and traces of acids, which, in the course of time, act upon the specimen and diminishes or entirely removes the color. All this may be lessened, if the balsam be heated sufficiently to drive off the volatile constituents, or more thoroughly obviated if a little potassium carbonate or mild alkali be added to neutralise the acid just before the balsam is heated. When the balsam becomes hard it can be broken into flakes and stored. When wanted for use dissolve in xylol to the desired consistency and filter through absorbent cotton. Specimens stained with the Biondi-Ehrlich mixture (which fades so easily) have at the end of a year shown no signs of losing their pristine clearness.