A METHOD FOR THE SIMULTANEOUS PASSAGE OF MANY PARAFFIN SECTIONS THROUGH THE MORE DIFFICULT STAINS.*

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NUMEROUS attempts have been made to find means whereby serial paraffin sections, or duplicate preparations intended for class-work, may be stained simultaneously; and, so far as regards stains that demand merely a transfer of the sections from one solution to another, these attempts have had success. Thus the Schmorl "celloidin-plate" method, by which the paraffin sections are incorporated in a film of celloidin and then manipulated, fulfils all the requirements for hematoxylin and eosin or other simple staining and counter-staining technic. When it is inadmissible—as, for instance, in clearing by turpentine, which affects celloidin—one may employ a glass or metal rack, such as that of Neumayer, to carry many slides on which the preparations have previously been fixed, or may, as recommended by Apathy, space the slides by means of bits of glass rod between each two, unite them all with rubber bands, and carry them through as one piece. But beyond the reach of these there remain a number of the processes for differential staining—Gram's stain, that of Weigert for fibrin, the amyloid and mucin stains, etc.—which, since they involve a careful differentiation of the dyes, blotting, and other detailed attention, have seemed to forbid the collective handling of many sections. To meet the conditions for simultaneous staining here involved some new method is needed. I have therefore devised the one that follows:

A clean glass plate is warmed over the burner and rubbed on one surface with a bit of paraffin of 36° C. melting-point, so that a thin coat of this, melted, is left. Cover-glasses to which the sections have been fixed in the usual manner are then dropped, with the free side down, on this coated surface, and at once adhere closely by reason of the film of paraffin. Since the paraffin about the sections themselves is of much higher melting-point than that between the cover-glasses

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and plate, the procedure need not alter it. Any excess on the coated surface may be wiped off from one corner of the plate, which is then allowed to cool, and next placed in xylol. This removes all exposed paraffin, that of the sections with the rest, but cannot affect such as is between the plate and cover-glasses, by reason of their close apposition; and the plate is now ready, with the sections temporarily part of it, for the solutions necessary to the stain. Though no special instrument is required wherewith to handle the plate, a wire "holder" like that figured will be found convenient. Differentiation and blotting are controlled as for a single section, with results as precise as though attention had been given to but one.

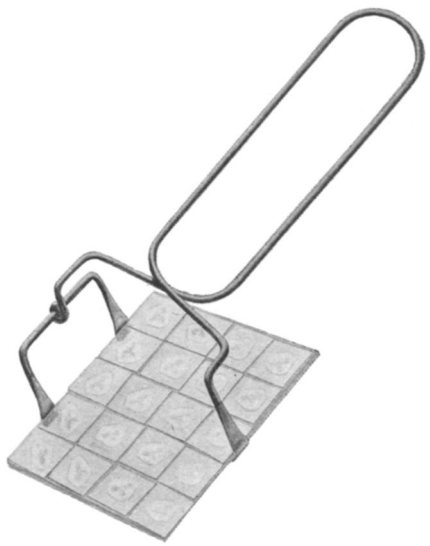

FIG. 1.—Plate in wire "holder" bearing 20 cover-slips with paraffin sections. The cover-slips are attached to the plate by means of paraffin melting at 36°C., and are thus ready to be passed as one through the staining solutions.

To loosen the cover-slips from the plate for mounting it is only needful that the last medium before the balsam be at the body temperature. With the paraffin thus melted, the cover-slips become individuals once more, and may be picked off, blotted, and mounted. As xylol or another medium dissolving paraffin is ordinarily the last one through which sections are "run up" into balsam, the film on the reverse of the cover-slips is dissolved off as soon as they are freed from the plate; but should such a medium not be used, it will be found necessary to clean this film from the cover slips when they have been mounted.

In such way it is feasible, with trays, to stain on one 4×5 glass plate 20 paraffin sections fixed to separate cover-slips of ordinary size; or, if jars are employed, 40 such sections, since both sides of the plate may be utilized. The Gram stain and other stains "in the cold" for bacteria, the Weigert fibrin stain, muchaematin for mucin, the cresylecht violet for mucin and amyloid,

and Weigert's elastic tissue stain lend themselves well to the procedure.

The limitations of the method are obvious. The newly coated plate with cover-slips applied cannot be left more than five or ten minutes in xylol; else the paraffin will slowly dissolve from under the margins of the slips, and the spaces so afforded will later prove a drawback in that they carry portions of one reagent into the next. More important, should the final fluid before the balsam be such as will take out the stain rapidly, the last section of a batch, mounted from this fluid, will differ somewhat in color from the first, owing to its longer sojourn therein. In this case the medium just preceding may be warmed to 36°, and the cover-glasses, thus loosened, passed on separately. The possible alteration in stain caused by a short stay of the preparations in a fluid heated to the body temperature may be cited as an objection, but in practice this difficulty is inconsiderable. During summer heat paraffin melting at 36° C. may not prove sufficiently stable to use in attaching cover-slips to the plate, and one of slightly higher melting-point may be adopted instead.

The method is, of course, valid for the routine stains of a laboratory, but seems, as already mentioned, to have a special field in those procedures more difficult technically.