drop in the titer of the serum immediately after addition, but up to this time (5 months of preservation) none of the serum has showed any additional change. Specimens from all 3 methods have been used successfully in the routine Wassermann tests done in our college hospital for the last 5 months. The supply of hemolysin thus obtained is enough to last us for a number of years and the rates of deterioration in the various methods of preservation will form the subject for further study.

The results reported here suggest the conclusion that anti-sheep hemolysin of a satisfactory titer for use in the complement fixation reaction can be produced in a donkey having no natural hemolysin.

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Technique for the Complete Preservation of Supravital Stain of Neutral Red in Paraffin Sections.

C. H. HU. (Introduced by S. H. Liu.)

The technique for the preservation of the supravital stain of neutral red in paraffin sections has been described by McJunkin[1] and Forkner.[2] Their methods enable one to observe most of the neutral red present in the cells; but a certain amount of the stain is inevitably lost in the process of preparation, and they require rapid dehydration and strict limitation of the size of the tissue blocks. The method described below has the advantage over those previously described, in that the entire neutral red stain within the cells is faithfully preserved, that the size of the blocks can be reasonably large, and that the different steps of staining can be carried out in a more leisurely and comfortable manner.

In this technique, advantage is taken of the fact that neutral red is only slightly soluble in aqueous or alcoholic solution containing mercuric bichloride. After the minimal amount of the bichloride required in each solution for the maximal insolubility of the neutral red is determined, any solution which will check further dissolution of neutral red from the tissue can be easily prepared from it.

The tissue to be studied is best stained by the direct injection method described by Forkner with only a slight modification through replacement of his Zenker-formalin by solution A (see below).

[1] McJunkin, F. A., *Am. J. Path.*, 1925, **1**, 305.

[2] Forkner, C. E., *J. Exp. Med.*, 1930, **52**, 379.

For carrying out this technique we use the following 4 different special solutions:

Solution A. Fixing solution, prepared as follows:

Dissolve a small quantity of neutral red in 15 cc. of 40% formalin in a large flat dish. Add to it 85 cc. of Zenker's fluid. Most of the neutral red previously dissolved in formalin is now precipitated out. After a few minutes, during which time the mixture may be stirred with a glass rod, the mixture is filtered for use.

Solution B. Dehydrating solution, prepared as follows:

To every 10 cc. of absolute alcohol add 1.2 cc. of absolute alcohol saturated with $HgCl_2$. Add to this mixture enough neutral red powder so that some of it remains undissolved at the bottom of the bottle.

Solution C. Aqueous solution, prepared as follows:

To 100 cc. of distilled water add 2 cc. of saturated aqueous solution of $HgCl_2$ and then saturate it with neutral red. Filter and use the filtrate.

Solution D. Counter staining solution, prepared as follows:

Saturate the filtrate of solution C with methylene blue powder, filter and use the blue filtrate. This must be prepared fresh each time.

Method. (1) Cut blocks about 1x1 cm. in size and not more than 3-4 mm. in thickness. (2) Fix for 24 hours in solution A. (3) Dehydrate in 3 changes of solution B, one or more hours each time, according to the size of the block. (4) Transfer to xylol, 2 changes, 3-4 hours in each. (5) Imbed in paraffin, cut and attach sections on slides as usual. (6) Remove paraffin from the section with xylol, mount with balsam, or, if counter stain is desired, continue as follows: (7) Wash off xylol with filtrate of solution B. (8) Quickly wash off alcohol with solution C. (9) Counter stain the section with solution D for about 30 seconds. (10) Wash off the excess of methylene blue with solution C. (11) Dehydrate with solution B. (12) Xylol, mount.

From step (7) to step (11) the solutions can be most conveniently used from the drop-bottles.