A METHOD OF PRODUCING CHROMATIN STAINING IN SECTIONS.

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In a recent Report[1] upon the new parasitic bodies associated with tropical splenomegaly, Lieut. S. R. Christophers, I.M.S., published a method of producing chromatin staining in sections. This method, however, labours under the disadvantage that, after staining, the section must be dried in the air before it is cleared and mounted, since the chromatin reaction is lost if dehydration by absolute alcohol is attempted. The consequent shrinkage of the tissue, even in very thin sections, renders the method of little service for delicate work.

Prior to this I had made many futile attempts to produce this reaction in sections by employing my modified Romanowsky stain[2] and had come to the conclusion that the processes of hardening and embedding tissues for section cutting had induced chemical changes in the tissues which interfered with the reaction. Christophers' method, however, showed that this was not the case, and my further experiments have resulted in a certain measure of success, inasmuch as the method described below induces a chromatin staining of the tissues which resists the decolorising action of the absolute alcohol used for dehydration. There is, further, a certain convenience in the employment of a single staining fluid instead of separate solutions of eosin and 'ripened' methylene blue. The method in question is probably susceptible of further improvement and simplification, but, as some such method is much wanted in several branches of research work, it may at least be of temporary service.

Small pieces of tissue, after a short sojourn in spirit followed by absolute alcohol, are put through xylol and paraffin in the usual way—adherence to the narrow time limits given by Christophers is not essential. Sections as thin as are consistent with the nature of the

tissue are to be recommended, those which I employ are of an average thickness of 5μ. After fixation on a perfectly clean slide, by pressure, drying and heat, especial pains must be taken to dissolve out all the paraffin; this is secured by applying xylol while the paraffin is still liquid and by treating the section with alternate baths of absolute alcohol and xylol 3 or 4 times. After the final bath of alcohol, distilled water is poured on to the slide before the alcohol has evaporated and the section is well washed to remove all traces of the alcohol. The excess of water on the slide is now got rid of by blotting with tissue paper and, while the section is still moist, a drop or two of fresh blood serum is placed on it and allowed to soak into it for five minutes. The action of the serum I conceive to be of the nature of a 'refreshing' of the tissue, probably in the direction of a restoration of its normal alkalinity. The excess of serum is now removed by blotting and the remainder allowed to dry as a thin film on and around the section.

The staining fluid is now prepared by mixing 2 parts of my Romanowsky stain with 3 parts of distilled water. This mixture is poured on the section, the slide covered with the lid of a Petri dish and staining is allowed to go on for 1 to 1½ hours. I find the best results are obtained by pouring off the stain once or twice during this period and replacing it with a fresh supply.

The stain is finally washed off with distilled water and the section examined under a low power when the cell nuclei should appear very densely stained, in fact almost black. It will be found that the section and the part of slide covered by the serum are quite free from the deposit which so often results from prolonged Romanowsky staining.

The subsequent processes of decolorisation and differentiation are essentially the same as those which I have employed in ordinary section staining for the last year and of which I recently published the details[3]. Two solutions are required, 1–1500 acetic acid and 1–7000 caustic soda, freshly prepared with distilled water. The alkaline solution is employed to dissolve out from the tissue the excess of eosin, which would otherwise render it too opaque, besides forming a bad contrast colour to the red chromatin. The acid solution removes the excess of blue and at the same time brightens the red tint of the chromatin. These two solutions are used alternately, commencing with the acid, and the section is frequently observed under a low power until the desired colour contrasts are obtained. As a rule the acid solution is that which requires to be most used. When the cell nuclei of the tissue are seen to be of a deep Romanowsky red colour, and the rest of the tissue

either a very pale pink or a light blue, the section is washed in water and is ready for dehydration by absolute alcohol. This process is conducted very rapidly, and, to this end, the excess of water on and around the section is removed, by blotting with tissue paper and wiping, so that as little work is left for the alcohol as possible. A few drops of alcohol are allowed to run down the slide and over the section, the slide being then at once dipped in xylol and washed therein until the section is perfectly cleared. It is now mounted in Canada balsam which is dropped on when the xylol is on the point of evaporating.

The material employed in testing the above method consisted of the organs of rats and rabbits infected with *Trypanosoma brucei*, and in sections of these organs, stained as above, the parasites are readily recognisable by reason of the deep red staining of the macro- and micro-nuclei, while the bodies of the parasites are frequently coloured a pale blue; the flagella are unstained. The bright red reaction of the cell nuclei before dehydration may be modified to a purplish tint by the action of the alcohol, but this is of only secondary and aesthetic importance as the chromatin of the parasites is unaffected. In the same sections stained by many other methods no trace of the Trypanosomata could be detected.

A little experience is necessary in controlling the effects of the acid and alkaline solutions, but, once this is attained, and provided that the initial staining has been carried to a sufficient degree of density, the method gives constant and regular results.

REFERENCES.

(1) *Scientific Memoirs of Officers of the Medical and Sanitary Departments of the Government of India.* No. 8, 1904.
(2) *British Medical Journal.* Sept. 21st, 1901.
(3) *Journal of the Royal Army Medical Corps.* Vol. II. No. 6, p. 669. June, 1904.