A METHOD OF RAPID STAINING OF INTESTINAL FLAGELLATES

By H. N. RAY, M.SC., PH.D. (Lond.)

Research Officer (Protozoology), Imperial Veterinary Research Institute, Mukteswar-Kumaon, U. P., India

IT is recognized that the staining of flagella of flagellate protozoa is often no easy task. Fresh fæcal samples containing flagellates can be subjected to treatment with Donaldson's eosin-iodine solution in order to demonstrate the the flagella, but such a preparation is not of a permanent nature. Wet fixed films of flagellates can be stained with Heidenhain's iron-alum hæmatoxylin, but in this method too, one often experiences great difficulty in bringing about the proper differentiation of the different organelles and at the same time maintaining a proper depth of staining for the flagella. This is particularly so in the case of very minute flagellates.

Shortt (1923) fixed wet films of flagellates by exposing the film to the vapour of 4 per cent osmic acid solution and subsequently stained the dry film with Giemsa after the manner of blood films. This method produces excellent results, and the flagellates, however minute, stand out very clearly. This method, however, involves the use of an expensive material, *viz*, osmic acid. Noller's method, as quoted by Stitt *et al.* (1938), involves the use of a fixative and, after washing with water and saline, the film has to be immersed in clear sterile serum (*e.g.*, that of the horse) for 5 to 10 minutes and then fixed again in absolute alcohol before staining according to the blood film technique. This method takes time and, in addition, it is difficult to ensure that sterile serum shall be always ready to hand.

This article describes a process of staining which does not involve the use of an expensive reagent, but which produces excellent results. In less than half an hour the preparation is ready for microscopical examination. By this method one is able to count quickly the number of flagella, to see the disposition of the different organelles, and to ascertain the systematic position of the organism. The photomicrographs (*see* figures 1 to 12, plate V) illustrating this article are taken from preparations stained by the method described below.

Technique

With a long fine-bore pipette, remove the material (fæcal or culture) containing the flagellates, place it on a very small drop of dried mammalian blood* on a slide, allow the material to mix with the blood, and then draw it back into the same pipette. The sucked-up fluid should have a blood red colour; if it has not, the material may be pipetted back to another droplet of dried blood, and the process should

(*Concluded at foot of next column*)

*A worker's own blood can be used with great advantage. Prick the finger-tip with a sharp needle. Then press the bleeding finger on a clean slide so as to have several impressions of the blood on the same slide. Cover the slide and allow it to dry. This dried blood can be stored in some dry dust-proof place and can be used for a week or ten days without any appreciable change in the requisite reaction.

PROTEIN HYDROLYSATES AS TRANSFUSION MATERIAL

By E. K. NARAYANAN

and

K. V. KRISHNAN

Microbiology Department

All-India Institute of Hygiene and Public Health, Calcutta

DUE to the increasing demand for large quantities of transfusion material for combating shock and hypoproteinæmia, and due to the high cost and other difficulties associated with the

(*Continued from previous column*)

be repeated. Now take a clean grease-free slide, warm it gently over a flame, and, with gentle pressure on the teat of the pipette, squeeze out the contents in long streaks on the slide. Only a small amount of the fluid should come out of the pipette at one time, so that the material dries up immediately it comes in contact with slide. With a little practice, a steady flow can be maintained so as to give parallel edges to the streaks. To accelerate the process of drying, the slide may be kept exposed to a gentle breeze from a table fan while the operation is being carried out. In this case, warming of the slide is not necessary. Stain the slide with either Leishman or Giemsa.

A thin film of this material can also be made after the manner of a blood film and stained as above. In such a preparation, the flagellates are always found at the 'fish-tail' end of the film. The writer, however, is of the opinion that the fine streak method described above is more convenient for rapid examination than the thin film method. Moreover, in the thin film method, the flagellates are slightly distorted, since they are dragged in the process in making the film; while in the streak method the organisms are allowed to flow on to the side in a steady stream and are allowed to dry immediately. This precludes any chance of distortion. Flagella stained by this method, however, appear thicker than normal owing to the flattening that takes place during drying.

This method, though unsuitable for detailed cytological studies, is, nevertheless, a great aid to the proper understanding of the morphology of the organism. The flagella of the non-parasitic flagellates can also be stained by this method with great ease.

In conclusion I should like to place on record my indebtedness to my teacher, Rai Bahadur Dr. G. C. Chatterjee, who about twenty years ago initiated me into this method of staining intestinal flagellates, which I propose to designate 'Chatterjee's method'.

REFERENCES

SHORTT, H. E. (1923) .. *Indian J. Med. Res.*, **10**, 721.

STITT, E. R., CLOUGH, P. W., and CLOUGH, M. C. (1938). *Practical Bacteriology, Hœmatology and Animal Parasitology*. H. K. Lewis & Co., Ltd., London.

Fig. 1.

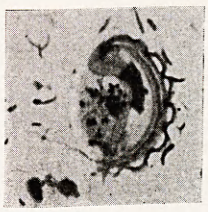

Fig. 2.

Fig. 3.

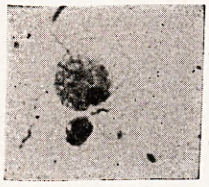

Fig. 4.

Fig. 5.

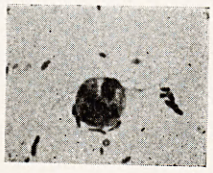

Fig. 6.

Fig. 7.

Fig. 8.

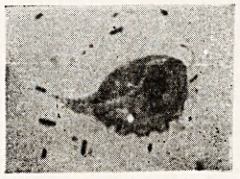

Fig. 9.

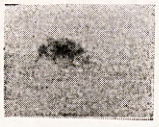

Fig. 10.

Fig. 11.

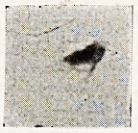

Fig. 12.

EXPLANATION OF PLATE V

Fig. 1.—Trichomonas from the cæcum of porcupine. Note the posterior axostylar chromatic ring.

Fig. 2.—Trichomonas from the cæcum of porcupine. Note the basal fibre and the rhizoplast.

Fig. 3.—Trichomonas from the cæcum of porcupine. Note the clear cytoplasm and the typical disposition of the nucleus.

(*N.B.*—In this trichomonas all the three free flagella were found to be directed posteriorly.)

Fig. 4.—Retortamonas from the cæcum of guinea-pigs.

Fig. 5.—Prowazekella from the gut of a lizard.

Fig. 6.—Hexamastix from the gut of a lizard.

Fig. 7.—Chilomastix from the rectum of a Himalayan toad.

Fig. 8.—Trichomonas from the intestine of a Himalayan lizard.

Fig. 9.—Trichomonas from the rectum of a Himalayan frog.

Figs. 10 and 12.—Pentatrichomonas from the gut of a Himalayan crow.

Fig. 11.—Retortamonas from the mid-gut of a cockroach.

(Photographs were taken with Spencer's vertical camera and incandescent lamp. Magnification × 715.)