IDENTIFICATION OF MICROFILARIAE ENCOUNTERED IN WEST AFRICA

BY

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THE standard morphological descriptions of microfilariæ tend to gloss over several very real difficulties in identification. Satisfactory staining of the dead organism sometimes seems impossible, measurement of distances along the organism (see, e.g., Belding, 1952) is often impracticable because of its irregular disposition, and the tip of the tail, recognised as the site of certain distinctive features, is frequently hidden under another part of the organism. In addition, sheaths are not always stained sufficiently to be recognisable, and the characteristic rhythmicity of certain species is not absolute, so that microfilariæ may be found "out of hours," particularly in heavy infections. Supra-vital staining with methylene blue, which brings out certain other characteristics, has not been found adequate for final identification in many cases.

These notes are based on work done in evolving satisfactory methods of species identification, and it is hoped that they may be useful especially to those unaccustomed to these organisms. The microfilariæ concerned are those of *Wuchereria bancrofti*, *Loa loa* and *Acanthocheilonema perstans* in the blood, and *Onchocerca volvulus* and *A. streptocerca* in the skin.

METHODS

Blood

Using capillary blood, wet and dry preparations are made. The latter is rather thicker than the thick film made for malaria diagnosis, and it is allowed to dry while the wet preparation is examined and is discarded should that examination prove negative. If microfilariæ are found, however, the thick film is stained for species identification. When drying is complete, the preparation is dehæmoglobinised by flooding the slide with tap-water and allowing it to stand until the colour has run out of the drop (one change of water may help). The water is then poured off, and the preparation allowed to dry again.

For routine work, 1 per cent methylene blue in water is a simple and satisfactory stain. The preparation is covered with it, and allowed to stand for at least ten minutes (temperature 25°-30° C.) The stain is then washed off with tap-water, and the slide after drying is ready for examination. The whole area of the preparation itself is covered with immersion oil, and surveyed systematically under the low power. The one-twelfth objective is used for detailed study of microfilariæ as they are found. It is important to examine all the microfilariæ in the preparation, and not just the first one encountered : a mixed infection may be present.

While most microfilariæ (imf.) found in the blood in West Africa will stain well, some, usually those of *L. Loa*, do not, and a better preparation will be required before a positive diagnosis can be made. The hæmatoxylin method of Fülleborn (Faust, 1949), modified by counterstaining for two minutes with 1 per cent eosin, was found to give good results, even with mf. of *L. loa*. It was used on dried films, dehæmoglobinised as already described. For permanent preparations, mounting the dried film after staining in Canada balsam has been found satisfactory, the formal dehydration of Fülleborn's method being unnecessary.

With practice, mf. of *A. perstans* can sometimes be identified in the wet preparation, as its breadth is distinctly less than the diameter of the red cells. Furthermore, the sheaths of those species which have them can sometimes be made out. However, the movement of the organisms and the numerous red cells often make these observations difficult and unreliable.

Concentration methods, using haemolysed venous blood and centrifuging, may be used when suspected cases of filariasis prove persistently negative with the capillary blood method. The methods described by Harris & Summers (1945) primarily for quantitative studies, using saponin, and by Whitby & Britton (1950) using acetic acid, are satisfactory. The re-suspended deposit is dried on a slide and stained as already described. Similarly, a deposit from centrifuged urine or hydrocele fluid may be stained in the same way.

Skin

A snip of clean, unanæsthetised skin is taken by raising the epidermis on the point of a needle and slicing off a fragment with a sharp razor or scalpel-blade. The fragment should include some dermis. It is then teased out in a drop of water on a slide, and examined under the low power for motile microfilariæ. Distinction between mf. of *A. streptocerca* and that of the much commoner *O. volvulus* can usually be made at this stage (see below), but other species may be present if there is much blood in the preparation, in which case it is more satisfactory to have a stained preparation. This is made by allowing the wet preparation to dry, and then staining as for blood.

The site of the skin snip is determined by clinical considerations, e.g., near the outer canthus if ocular involvement is suspected, or over a nodule suspected to be onchocercal. For routine purposes, however, and in the absence of other special indications, the upper part of the buttock or the iliac crest is most satisfactory.

MORPHOLOGY OF MICROFILARIAE

Mf. of *A. perstans* (Fig. 1). This is the commonest variety in West Africa and may be found in the blood by day or night. It is small, its breadth being half or less that of the near-by white cells. It is often coiled on itself in a characteristic way like a piece of rope that has been thrown down on to the ground. There is no sheath. Its nuclei stain well with methylene blue, and present several characteristic features: they appear closely packed and rather coarse and angular, as though mutually compressed, and occupy most of the width

of the organism, the body itself on either side of the nuclear column being inconspicuous and staining poorly. The anterior nuclei have a characteristic arrangement, one, sometimes two, being in front of the rest and laterally placed. The "nerve ring" appears as a sharply defined but narrow gap in the column. A round nucleus occupies the tip of the rounded tail.

Mf. of *W. bancrofti* (Fig. 2). The feature of periodicity is well known, but distinction from mf. of *L. loa* cannot always be made solely on this basis: an occasional mf. of *W. bancrofti* may be found during the day in capillary blood, and in other fluids, e.g., hydrocele fluid, or urine, periodicity may not be demonstrable at all. This microfilaria is large, its breadth being not much less than the diameter of a white blood cell. Its disposition in smooth sweeping curves, and its sheath, are well known. It usually stains well with methylene blue but occasionally unaccountably does not. The nuclei tend to be rounded, small and well spaced, and give the impression of orderly arrangement. The excretory pore is small, but much easier to appreciate is the very characteristic arrangement of the tail nuclei. There is a distinct interval between the pointed tip of the gently tapering tail and the end of the nuclear column ; the nuclei, as it were, only exist as far down as the width of the organism will permit. The terminal four nuclei are somewhat elongated and are arranged in single file in the midline of the organism. The next three more rounded nuclei usually conform to the same line, but the most proximal may be laterally placed. The next two nuclei are conspicuously laterally placed, on opposite sides. There are then between two and five more nuclei in single file, before the start of an irregular double column. Minor modifications of this pattern may be met with (as would be expected in a two-dimensional view of a cylindrical organism containing some eccentric nuclei), but the general appearance is very characteristic and the pattern shown in Fig. 2 and described above is the most common.

Mf. of *L. loa* (Fig. 3). Fairly strict diurnal periodicity is exhibited, but the remarks made in this connection, under the heading "Mf. of *W. bancrofti*," are relevant also here. Mf. of *L. loa* is large, being on the average broader and longer than mf. of *W. bancrofti*, and is sheathed. Its disposition tends to be characteristic ; its curves are multiple, irregular, short and often sharp. In preparations made by concentration methods, however, this feature is often absent, the curves resembling those of mf. of *W. bancrofti*. Staining with methylene blue is often poor, the nuclei being indistinct even after prolonged staining, and the sheath may not be visible at all. Carefully performed hæmotoxylin and eosin staining, however, brings out nuclear detail well, and shows the large excretory pore. The nuclei are coarse and rather large and show much overlapping, and those in the tail present distinctive features. In addition, the shape of the tail itself is characteristic. There is a rather sudden narrowing where the body becomes the tail, and the tail itself, often sharply curved on the body (mf. of *W. bancrofti* may also show this particular feature), does not narrow much more before the tip, which close inspection will show to be rounded rather than pointed. The general appearance of the tail is that of a loose appendage to the body instead of (as in mf. of *W. bancrofti*) an even continuation

Fig. 1.—Mf. of *A. perstans*

Fig. 2.—Mf. of *W. bancrofti*

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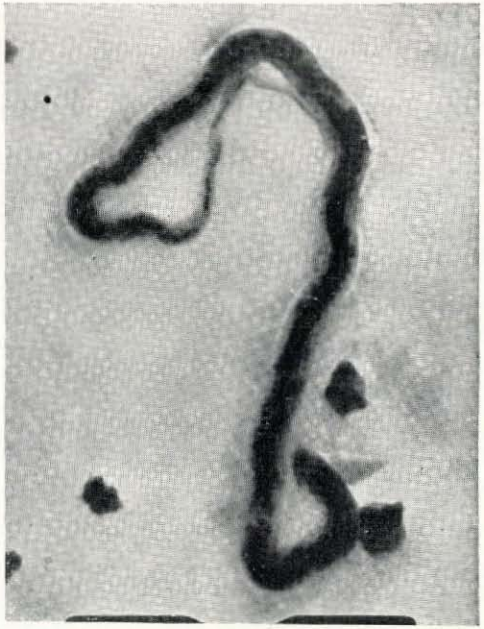

Fig. 3.—Mf. of *L. loa*

Fig. 4.—Mf. of *O. volvulus*

Fig. 5.—Mf. of *A. streptocerca*

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of it. In the tail are five large and markedly elongated nuclei in single file, the last reaching to within its own width of the tip. Occasionally one of them may be replaced by two smaller nuclei. The last one or two nuclei of the body itself may lie separately, and in line with the tail nuclei, but they are rounded and quite small.

How widely different are the appearances in the tail in mf. of *W. bancrofti* and mf. of *L. loa* is best seen by comparison of Fig. 2 with Fig. 3. The complete dissimilarity throughout the organism in typical specimens is also well shown.

Mf. of *O. volvulus* (Fig. 4). Two forms of this microfilaria are said to exist, a large and a small, but descriptions of their measurements show overlapping of the two groups and the effect is to indicate merely a wide range of sizes. There is no sheath. With practice, identification is possible in the wet preparation by noting the characteristic sharply pointed tail which is often smoothly curved back on the body. This can be confirmed after staining ; methylene blue is adequate for this, but hæmatoxylin and eosin enable other details to be made out. The zone free of nuclei at the head end is longer than it is broad, and is widest at about the level where the nuclear column starts. The body becomes a little narrower behind this point, and the result is that the head end has an outline reminiscent of that of a snake's head. The "nerve ring" is prominent, and the nuclei of the tail stop well short of the tip.

Mf. of *A. streptocerca* (Fig. 5). This is an unusual type, found in the skin. A well-known feature which may not be present, particularly in dried preparations, is the curved tail, the rest of the body being straight ; the appearance has been likened to that of a shepherd's crook. The tip of the tail is rounded. Hæmatoxylin and eosin staining shows a strikingly narrow microfilaria, tapering gradually at both ends. The "nerve ring" is relatively more posterior than in other microfilariæ. The anterior nuclei have a characteristic arrangement : the first is rounded and occupies most of the width of the organism and behind it is a group of three in an oblique single file. Dyce Sharp (1927) noted a characteristic arrangement of the first four nuclei, and described all four as being "in echelon." Behind these is a row of about six in straight single file before an irregular double column starts. None of the other four species has more than two nuclei in single file at the anterior end. Attention is more usually given to the nuclei of the tail of mf. of *A. streptocerca*, but these seem to show up less distinctly.

SUMMARY AND CONCLUSIONS

The usually described points of morphological difference between the species of microfilariæ, as found by staining after death, have often been found by personal experience to be unreliable in practice.

Laboratory methods found to be satisfactory are described, and the more distinctive features of the species found in West Africa enumerated.

Mf. of *A. perstans*, mf. of *O. volvulus* and mf. *A. streptocerca* are quite unlike each other, and readily distinguished with very little practice. In any case, the first occurs in the blood, and the others in the skin.

Mf. of *W. bancrofti* and mf. of *L. loa* may, however, be confused for the reasons given. With practice, many points of difference can be recognised, but it is suggested that the appearances of the nuclei throughout the tail (and not just features peculiar to the tip), being quite unlike in the two species, and easily seen in stained films, afford a reliable means of distinction.

Photographs illustrate these points, as it seems that photographs or accurate drawings to show practical points of distinction between microfilariæ are rare in textbooks or in the literature generally.

These notes are based on experience at the Military Hospital, Kaduna, Northern Nigeria, and I am grateful to Mr. A. E. Clark for the photomicrographs.

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TRIMEPRAZINE TARTRATE (VALLERGAN) AS A PREMEDICATIVE DRUG IN CHILDREN

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WHEN faced with the prospect of accepting nasal gas and oxygen for dental extractions, many children are frightened and consequently become uncooperative. To overcome this, many oral sedatives and tranquillising agents have been tried, including methylpentynol, seconal, and promezathine. The latter drugs, although satisfactory in many ways, have the disadvantage that, if given in adequate dosage to produce a contented and co-operative child, the recovery period is too long. Elixir seconal is unpalatable to some children and there is a definite incidence of vomiting of the drug shortly after it is given.

With a view to finding a more satisfactory drug, Vallergan (Forte) was tried here over a period of two months. During that time a total of 155 cases underwent dental extractions under nitrous oxide and oxygen delivered from a McKesson apparatus via a nasal mask.

The ages of the children varied between two and a half and nine years. They were mainly of British, Gurkha or Malay nationality, with an occasional Tamil and Chinese. It was found that the Malay children were always the most tearful and unconsolable. It was therefore deemed necessary to use a slightly larger dose of Vallergan (Forte) for children of that nationality.