Wuchereria bancrofti: The staining of the microfilarial sheath in giemsa and haematoxylin for diagnosis.

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(Received for Publication: 3rd March 1977).

SUMMARY

Indigenous cases of bancroftian filariasis have recently been identified in the country for the first time. The diagnosis of such infections depends on demonstrating the characteristic sheathed microfilariae of Wuchereria bancrofti in the blood. Giemsa, a stain recommended by many authors, was found to be unsuitable as the microfilarial sheath, an important distinguishing feature, does not take up the stain. In contrast, the sheath stains clearly in haematoxylin. The differences in the staining reaction of the microfilariae in the two stains is demonstrated. The need to establish the presence of the sheath is emphasized, especially since the microfilariae of Dipetalonema perstans, the widespread filarial infection in the country, are unsheathed.

INTRODUCTION

The laboratory diagnosis of filariasis is based on identifying the larval stage, the microfilariae either in blood or skin. The species diagnosis though is based on the characteristic movement, general shape, size, a detailed examination of the distribution of the body nuclei, the shape of the tail and the presence of nuclei therein. However, it is the presence or absence of the sheath, a delicate membrane which is so closely fitting that it is only detectable as it projects beyond the the head or tail in stained specimens, that is crucial in preliminary identification.

There has been no comprehensive survey of filarial infections in Zambia but limited studies (Buckley, 1946; Hira, unpublished data) have established the endemicity of Dipetalonema perstans, the microfilariae of which are sheathless and diurnal in blood. Recently Wuchereria bancrofti, the microfilariae of which are sheathed and nocturnal in blood, have been identified in an indigenous Zambian in the Eastern Province (Hira, 1975); since then further autochthonous cases have come to light (Hira, unpublished data).

During investigations of the endemicity of bancroftian filariasis in the country the first step in identification, the basic problem of assigning the microfilariae to the sheathed or sheathless groups, arose. Several textbooks (Watson, 1960; Brooks, 1963; Belding, 1965; Faust et al, 1970; Galindo, 1971; Faust et al, 1975; Brown, 1975) specialist monographs (WHO, 1974) and references in the literature (Gratama, 1970) recommended giemsa as a stain of choice to demonstrate the microfilariae in blood films.

This communication reports on the use of giemsa to stain microfilariae of W. bancrofti, the consequent pitfalls in diagnosis and reports on the advantages of haematoxylin to demonstrate the larval forms. This is especially essential now as the presumed absence of W. bancrofti in the country is no longer valid; because there is need to distinguish such sheathed microfilariae from sheathless ones of D. perstans and finally, this is mandatory to identify further cases thereby delineating the limits of the occurrence of the filarial infection in the country as a whole.

METHOD AND RESULTS

Routine hospital laboratories are generally presented with a thick and thin blood film for parasites; these are stained in dilute giemsa according to table 1. Alternatively, a thick and a thin film is made of sequestrinated blood in the laboratory, stained in giemsa and examined under the microscope. The blood may be subjected to a concentration technique for microfilariae or trypanosomes if a firm clinical diagnosis is

TABLE 1

Staining blood films in Giemsa (The "Merck" brand stocked by the Government Medical Stores, Lusaka).

A. Dilute the concentrated giemsa 1:10 in buffered water adjusted to pH 7.2.

B. Fix the thin film in absolute alcohol. Thick films should be dehaemoglobinized only.

C. Stain for 35 minutes.

D. Rinse in water (1 minute), allow to dry, mount in DPX if necessary, examine.

made and the laboratory is requested. Eventually a film of the concentrate is also made, stained in giemsa and examined.

A specimen positive for microfilariae, stained in Giemsa (table 1) gives a result as shown in figure 1.

TABLE 2

Destaining Giemsa-stained blood film for restaining.

A. If the slide has been mounted, immerse the slide in Xylol until the coverslip detaches of its own accord. This may take 24 to 48 hrs or even longer depending on how long ago the slide was mounted.

B. Destain by immersing the slide in two changes of absolute alcohol for 5 minutes each.

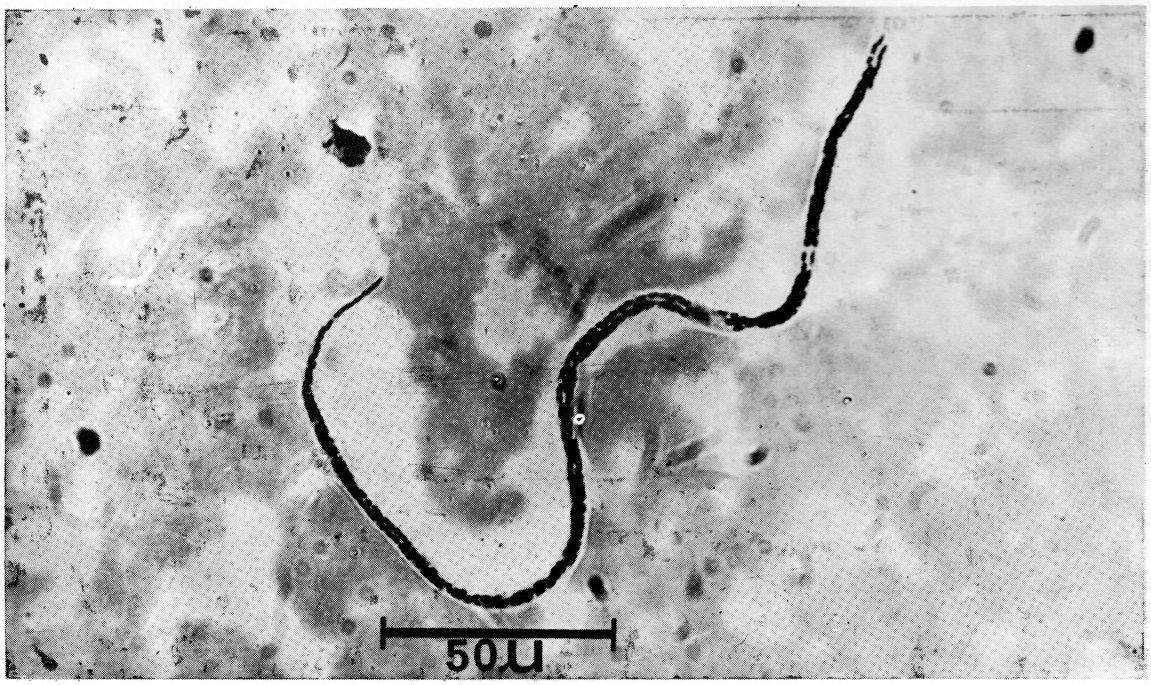
C. Transfer to 90% alcohol for 5 minutes.

D. Transfer to 70% alcohol for a further 5 minutes

E. Immerse the slide in 1% acid alcohol for 10-15 minutes until all the stain has been removed.

F. Wash in water for 2 to 3 minutes to remove the alcohol.

FIG. I



Microfilaria of W. bancrofti stained in giesma

Destaining the slide (table 2) and restaining in Harris' alum haematoxylin (table 3), gives a result as depicted in figure 2. Comparison of the two shows that the sheath, an important identifying feature, is not stained in giemsa but shows up clearly in haematoxylin.

However, it is fair to state that prolonged staining in giemsa – about 2½ to 3 hours – usually but not invariably shows up the sheath which stains light pink to pink. This, of course, is time consuming and impracticable for a hospital laboratory which is flooded with many slides and other clinical specimens each day, is generally under pressure to report quickly, does not have sophisticated manpower and uses the popular giemsa stain.

TABLE 3

Staining the blood film in Harris' alum haematoxylin*

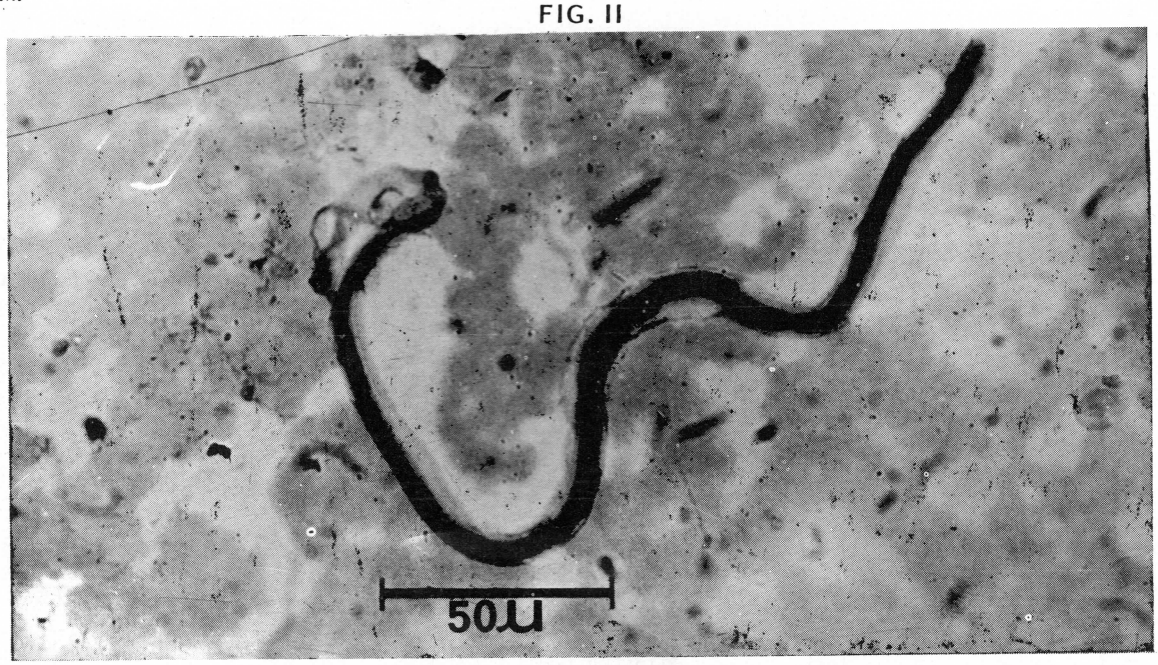
A.	Immerse destained giemsa slide in haematoxylin for 15 to 20 minutes.
B.	Blue in running tap water for 9 to 10 minutes.
C.	Dehydrate in 70% alcohol for 2 mins. 95% alcohol for 2 mins. Absolute alcohol for 2 mins. Absolute alcohol for 2 mins.
D.	Immerse in Xylol for 2 mins. Xylol for 2 mins.
E.	Mount in DPX and examine.

*NOTE**
The stain is made up according to Culling (1974); the staining time in A has to be adjusted wtih each batch of newly made-up stain.

all blood slides should be stained in giemsa; if microfilariae are present, the distribution of the body nuclei in the tail should be noted. If there is reasonable doubt as to the species diagnosis the slide should be destained and restained in haematoxylin to confirm the presence of a sheath. Alternatively, the slide should be routinely restained in haematoxylin (tables 2 and 3).

It may well be argued that infact this is unnecessary since D. perstans microfilariae are diurnal whereas those of W. bancrofti are nocturnal. Experience in Zambia has shown that patients with D. perstans may present with either diurnal or nocturnal periodicity; in some cases there has been no periodicity is not absolute i.e. some microfilariae are always present throughout the 24-hour period but maximum

FIG. II



Same specimen as in figure I. destained and restained in haematoxyline showing the sheath clearly.

DISCUSSION

Thus the use of the widely recommended giemsa as a stain of choice for the diagnosis of filarial infections may be over emphasized and over estimated. Undoubtedly, in routine hospital laboratories in the tropics the stain is essential for malaria, trypanosomes and borrelia.

Therefore, a practical solution would be to retain the use of giemsa but at the same time adequate precautions should be taken so that sheathed microfilariae of W. bancrofti do not go undetected. As such, numbers appear between particular hours. It is therefore not unusual to find W. bancrofti microfilariae during the day; in a bancroftian filariasis foci in neighbouring Zaire, 50% of the infected individuals had a detectable microfilaraemia by the blood slide method during daylight hours but the maximum numbers were always during the night (Fain et al, 1974).

If laboratories in the country were aware of the staining reactions of the sheath with giemsa vis-a-vis haematoxylin and also of the periodicity of the microfilariae, it is not unlikely that more W.bancrofti infec-

tions may come to light. Furthermore, such microfilariae may also be detected in blood sent for other examinations as asymptomatic cases or unusual symptomatology may not be rare. Recent studies (Wijers and McMahon, 1976) show that in this broad geographical area the classical symptoms of bancroftian filariasis as detailed in textbooks are in fact a rarity. The commoner symptoms in males, for instance, are funiculitis, orchitis and eventually hydrocoeles rather than lymphangitis, lymphadenitis or elephantiasis.

It is essential to take a careful history including the period of residence in various parts of the Republic and indeed neighbouring countries. Only in this way would it be possible to identify endemic foci as so little is known about the infection thus far in Zambia, yet the consequences of bancroftian filariasis may be debilitating in the long term.

The staining reaction of the sheath, which was observed in innumerable cases, is a consistent feature. Whether this is peculiar to Zambia in the light of the stain preferred by so many authors in conjectural. There are references though which clearly state that the sheath of W. bancrofti microfilariae also stains best in haematoxylin (Davey Crewe, 1973) while others (Faust et al, 1975) mention that some workers prefer haematoxylin to giemsa.

ACKNOWLEDGEMENTS

I thank Mr. C.S. Cardozo for helpful discussions on the staining procedures and Mr. M.A. Ansary for assistance with the photomicrography.

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