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Acid Phosphatase Variations in the Microfilariae of *Dipetalonema perstans* and *Loa loa* from the Jos Plateau, Nigeria

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Introduction

The identification and characterization of microfilariae had been usually based on their morphological features. However, since Petithory (1966) showed the differences in the localization of acid phosphatase in the microfilariae of different species, many workers have used this method either separately or in combination with morphological characteristics in distinguishing different species and different strains of microfilariae (Chalifoux and Hunt, 1971; Redington *et al.*, 1975; Omar and Schulz-Key, 1976; Omar, 1977; Omar and Kuhlow, 1977; Omar, 1978; Omar *et al.*, 1982). Terwedow and Huff (1976) used this staining technique to characterize the Brazilian *Wuchereria bancrofti* microfilaria and observed that the discrepancy in the result of previous workers was likely due to the different enzyme patterns of the parasite strains. On the other hand, Yen and Mak (1978) were unable to differentiate the periodic *Brugia malayi* from the subperiodic, while they readily distinguished them from *B. pahangi, Depetalonema repens* from *D. immitis,* and *B. booliati* from *B. sergenti* in addition to separating histochemically the Malaysian strain of *W. bancrofti* from those of other regions. In the West African sub-region, Omar and Kuhlow (1977) demonstrated an uniform enzyme staining pattern in each of the microfilariae of *D. perstans* and *L. loa* most likely from forest region of Cameroon, and until now, acid phosphatase pattern in each of these species was considered species-specific and intraspecifically uniform in their microfilariae. In this paper, variations in enzyme distribution in the microfilariae of *D. perstans* and *L. loa* parasitizing rural inhabitants of Jos Plateau of Nigeria are reported.

Materials and Methods

The study area

The Jos Plaeau where the parasites used for this study were obtained from man is located in mid-northern Nigeria. Plateau State of Nigeria wehre the bulk of the highlands is located lies between latitudes 7° and 11°N, and longitudes 7° and 25°E with an area of 53,585 square kilometers and a population of 2,026,657 (1963 census). The Jos Plateau

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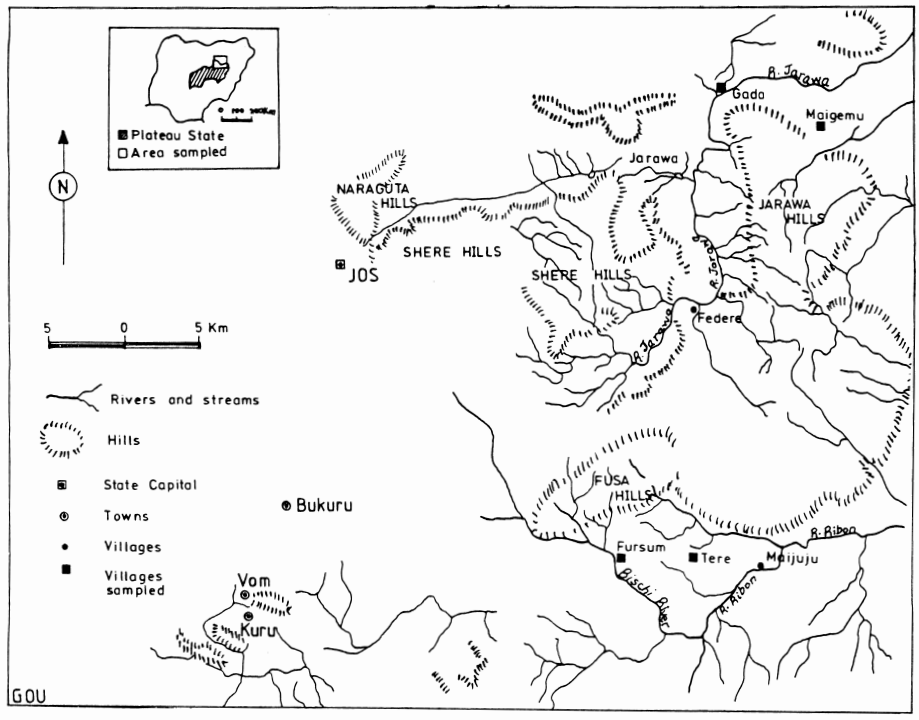

Fig. 1 Map of the northern area of the Jos Plateau showing locations (black squares) from where samples were obtained in relation to geographical features such as main rivers, streams and hills.

stands on an elevation of 1,000–1,500 m above sea level with peaks like Shere hills rising over 2,500 m. But this creates a rather high annual rain-fall of 1,600–2,000 mm especially in the western area of the plateau when compared with other parts of northern Nigeria (Crosskey, 1981). The vegetation is typically of northern Guinea savanna but that of the summit area (Kuru) is Montane grassland. The plateau is generally cool and mid-night temperature can fall to as low as 2°C in February. The villages, Gada (10°02′N, 9°07′E), Fursum (9°34′N, 9°06′E), Maigemu (10°01′N, 9°11′E) and Tere (9°46′N, 9°07′E), where the blood samples were collected lie in areas of endemic onchocerciasis in the warmer northern part (Fig. 1).

The main ethnic groups in this area are Jarawa, Berom and to a much lesser extent, Hausa and nomadic Fulani, the last grazing cattle on the tsetse-free plateau. The main

Fig. 2 A typical Fulani hut; in the background are Fulani women hawking fresh milk; also note the surrounding vegetation where vectors of filariasis (e.g. *Chrysops*) inhabit.

occupations are subsistence farming, petty trading, hunting and animal rearing. The Fulanis live in huts and sleep on raised platforms made of wood, and during the rainy season (April to November), the huts are

covered with large polyvinyl sheets (Fig. 2). They are found with their cattle on the outskirts of the village in clearings similar to the description of Gordon *et al.* (1950), thus rendering them susceptible to the attack of vectors of filariasis, *e.g. Chrysops.* Filarial infections are relatively higher among the Fulanis than among members of other tribes. The Jarawas, Beroms and Hausas live in villages and work on the farms.

Collections of samples

About 5 ml of blood was obtained from 348 volunteers by veni-puncture in the left arm using the syringe and needle after brief questions on age, sex and duration of residence in the area. Samples were placed in Bijou bottles containing anticoagulant (heparin) and labelled specimens were immediately transported to our nearby Vom laboratory for examination.

The Bijou bottles containing blood samples were held between the thumb and forefringer and agitated gently. Samples were examined first by the wet blood film method under light microscope for microfilariae. Positive samples were carefully noted and at least five thick blood films were made from each on new microscope glass slides and allowed to dry at room temperature. Samples thus made were quickly fixed in cold acetone (0–4°C) and again allowed to dry. Smears were stored in deep freezer (–20°C) wrapped in moist proof material until staining.

Staining of microfilariae

A smear from each positive case was stained in Mayer's haematoxylin for morphological study of microfilariae. Measurements were subsequently made using WILD micrometer.

Within two weeks of collection, all other smears were incubated singly in horizontal position for 1 to 2 hours at pH 5.0 and at 37°C for microfilarial acid phosphatase by the method described by Chalifoux and Hunt (1971) using 1-Naphthyl disodium orthophosphate (naphthol AS-TR-phosphate) as substrate at pH 5.0 and at 37°C. Control smears were incubated in the medium without medium. Each smear was subsequently examined for acid phosphatase activity in microfilariae and photomicrographs were made as required. All chemical reagents used to compose the staining solution were manufactured by the British Drug House (B.D.H.), Poole, England.

Results

The morphological features of blood microfilariae were identical to the *D. perstans* and/or *L. loa* microfilariae reported by Kozek *et al.* (1982), and Bell (1967), respectively. Seventeen (4.9%) villagers (11 males and 6 females) were positive for *D. perstans. L. loa* infection was revealed in the blood of 19 (5.5%) villagers (14 males and 5 females). Forty-five percent of these infections were found among Fulanis. The mean length of *D. perstans* microfilariae was 194 μm (range, 192–199), mean width 4.5 μm (range, 4–5) (Fig. 3). For *L. loa* microfilariae, mean length was 275 μm (range, 250–300) and mean width, 7.5 μm (range, 6–8) (Fig. 4). Measurements were undertaken on 30 to 50 microfilariae of each species. The concentration of microfilariae in peripheral blood varied from 3 to 340 mf/ml.

Acid phosphatase activity in microfilariae: Enzyme activity appeared as brick-red spots or light and diffuse staining in microfilariae. Control smears were negative for acid phosphatase activity.

(a) In *D. perstans,* following two enzyme patterns were revealed:
   (I) amphids, excretory pore/vesicle, anal pore/vesicle and phasmids (A+EP+AP+P) (Fig. 5).
   (II) amphids, excretory pore/vesicle, Innenkörper, anal pore/vesicle nad phasmids (A+EP+IB+AP+P) (Fig. 6).

Enzyme staining was more pronounced in the excretory and anal vesicles than in other enzyme-positive structures and a light and diffuse staining was seen in the area of G-cell (Fig. 6). A higher proportion of pattern I (A+

Table 1 Frequency of the two acid phosphatase patterns in *D. perstans* microfilariae (mf) from four villages of the Jos Plateau

Village	No. of persons postive	No. of mf observed	Acid phosphatase patterns* (%)†	
			I	II
Gada	5	273	217 (79.5)	56 (20.5)
Fursum	2	12	10 (83.3)	2 (16.7)
Maigemu	8	185	126 (68.1)	59 (31.9)
Tere	2	76	59 (77.6)	17 (22.4)
Total	17	546	412 (75.5)	134 (24.5)

* I, amphids, excretory pore/vesicle, anal pore/vesicle and phasmids.
II, amphids, excretory pore/vesicle, Innenkörper, anal pore/vesicle and phasmids.
† Percent of the total number of stained specimens.

EP+AP+P) was consistently observed in the samples examined than pattern II (A+EP+IB+AP+A). The relative frequency of pattern I to pattern II was: Gada, 79.5% and 20.5%; Fursum, 83.3% and 16.7%; Maigemu, 68.1% and 31.9%; and Tere, 77.6% and 22.4% (Table 1). Although infection occurred mostly in older villagers *i.e.* 30 years old and above, the frequency of enzyme patterns could not be ascribed to age or sex of the host.

(b) In *L. loa,* following four enzyme patterns were observed:
  (I) excretory pore/vesicle and anal pore/vesicle (EP+AP) (Fig. 7).
  (II) amphids, excretory pore/vesicle and anal pore/vesicle (A+EP+AP) (Fig. 8).
  (III) amphids, excretory pore/vesicle, anal pore/vesicle and phasmids (A+EP+AP+P) (Fig. 9).
  (IV) amphids, excretory pore/vesicle, Innenköper, anal pore/vesicle and phasmids (A+EP+IB+AP+P). (Fig. 10).

The sheath of *L. loa* microfilariae did not stain for acid phosphatase but its presence was evident. Generally, the enzyme activity was marked in the amphids, excretory and anal vesicles, and phasmids, particularly intense in the former two regions. In pattern IV, diffuse red staining was observed all over microfilaria with brick-brown staining in the excretory and anal vesicles, moderate to intense enzyme activity in the amphids and phasmids, and diffuse staining in the G- and R-cells areas (Fig. 10). Pattern IV was the most frequent (48.3%) in *L. loa* microfilariae (Table 2).

There were no mixed infections in all the blood specimens examined. Due to low numbers we could not subject these variations to statistical analysis.

Discussion

This study demonstrated acid phosphatase variations in the microfilariae of *D. perstans* and *L. loa.* Pattern I (A+EP+AP+P) in *D. perstans* and pattern IV (A+EP+IB+AP+P) in *L. loa* are identical to the staining patterns of parasites from the forest region of neighbouring Cameroon (Omar and Kuhlow, 1977). Pattern I in *D. perstans* of this plateau corresponds as well to the pattern reported in the micro-

Table 2 Frequency of acid phosphatase patterns in *L. loa* microfilariae (mf) from positives in three villages of the Jos plateau

Village	No. of persons positive	No. of mf observed	Acid phosphatase patterns* (%)†			
			I	II	III	IV
Fursum	1	239	19 (7.9)	14 (5.9)	86 (36.0)	120 (50.2)
Tere	3	130	19 (14.6)	13 (10.0)	35 (26.9)	63 (48.5)
Maigemu	15	555	78 (14.1)	40 (7.2)	174 (31.4)	263 (47.4)
Total	19	924	116 (12.6)	67 (7.3)	295 (31.9)	446 (48.3)

* I, excretory pore/vesicle and anal pore/vesicle.
II, amphids, excretory pore/vesicle and anal pore/vesicle.
III, amphids, excretory pore/vesicle anal pore/vesicle and phasmids.
IV, amphids, excretory pore/vesicle, Innenkörper, anal pore/vesicle and phasmids.
† Percent of the total number of stained specimens.

filariae from 2 persons in Zaria area of Niegeria (Schillhorn van Veen and Blotkamp, 1978). However, those from the Jos Plateau differ from above mentioned reports with regard to the presence of pattern II in *D. perstans* and patterns I, II and III in *L. loa* microfilariae. The difference in the number of microflariae tested and/or in the microfilarial sources would be attributable to the discrepancy between these two reports.

In the novel pattern (II, A+EP+IB+AP+P) revealed in *D. perstans,* the areas corresponding to the G and R cells exhibited enzyme activity. The G-cell divides first when microfilaria is ingested by the invertebrate host (Lawrence and Simpson, 1971). Enzyme activity in this structure probably suggests the presence in the population of microfilariae of a group that is metabolically active (Barka, 1962) in contrast to the dying or dead in the ageing process (Omar, 1978). In agreement with this conception, the absence of acid phosphatase staining in some enzyme reactive structures in *L. loa* microfilariae (I, II and III) does not necessarily imply loss of enzyme activity during storage after fixation (Omar and Schulz-Key, 1976; Schillhorn and Blotkamp, 1978), but most likely reflects the age differences in the population of parasites.

Putting these factors concerned together, the discrepancies between the results from the present histochemical study and previous ones (Omar and Kuhlow, 1977; Schillhorn and Blotkamp, 1978) indicate that the microfilariae of the two filarial species are not uniform in enzyme staining. Recently, Omar *et al.* (1982) demonstrated variations of this enzyme in the microfilariae of *O. volvulus* in the two known geographical strains from the rain-forest and the Sudan savanna zones of West Africa. The possibility of the existence of geographical strains of *D. perstans* and *L. loa* cannot be completely ruled out.

Summary

The microflariae of *Depetalonema perstans* and *Loa loa* from venous blood of 36 human carriers of the Jos Plateau, Nigeria, were stained histochemically for the distribution of acid phosphatase using 1-Naphthyl disodium orthophosphate (naphthol AS-TR-phosphate) as substrate. Results have shown enzyme variation in the microfilariae of the two species found in

Jos area of Nigeria. Two distinct enzyme patterns were observed in *D. perstans* and four patterns in *L. loa* microfilariae. The distribution of acid phosphatase and its relative frequency in parasites were as follows: *D. perstans*, pattern I (enzymatic activity in the areas of amphids, excretory pore/vesicle and anal pore/vesicle), 75.5%; II (amphids, excretory pore/vesicle and anal pore/vesicles, Innenkörper and phasmids), 24.5%, respectively. In *L. loa* pattern I (excretory and anal vesicles), 12.6%; II (amphids, excretory and anal vesicles), 7.3%; III (amphids, excretory pore/vesicle and anal pore/vesicles and phasmids), 31.9%; and IV (amphids, excretory pore/vesicle and anal pore/vesicles, Innenkörper and phasmids), 48.3%, respectively. These results suggest that *D. perstans* and *L. loa* of man are not uniform in enzyme staining. The question of whether the enzyme variations in their microfilariae from different geographical zones of West Africa are due to differences in geographical strains of the parasites is discussed.

Acknowledgments

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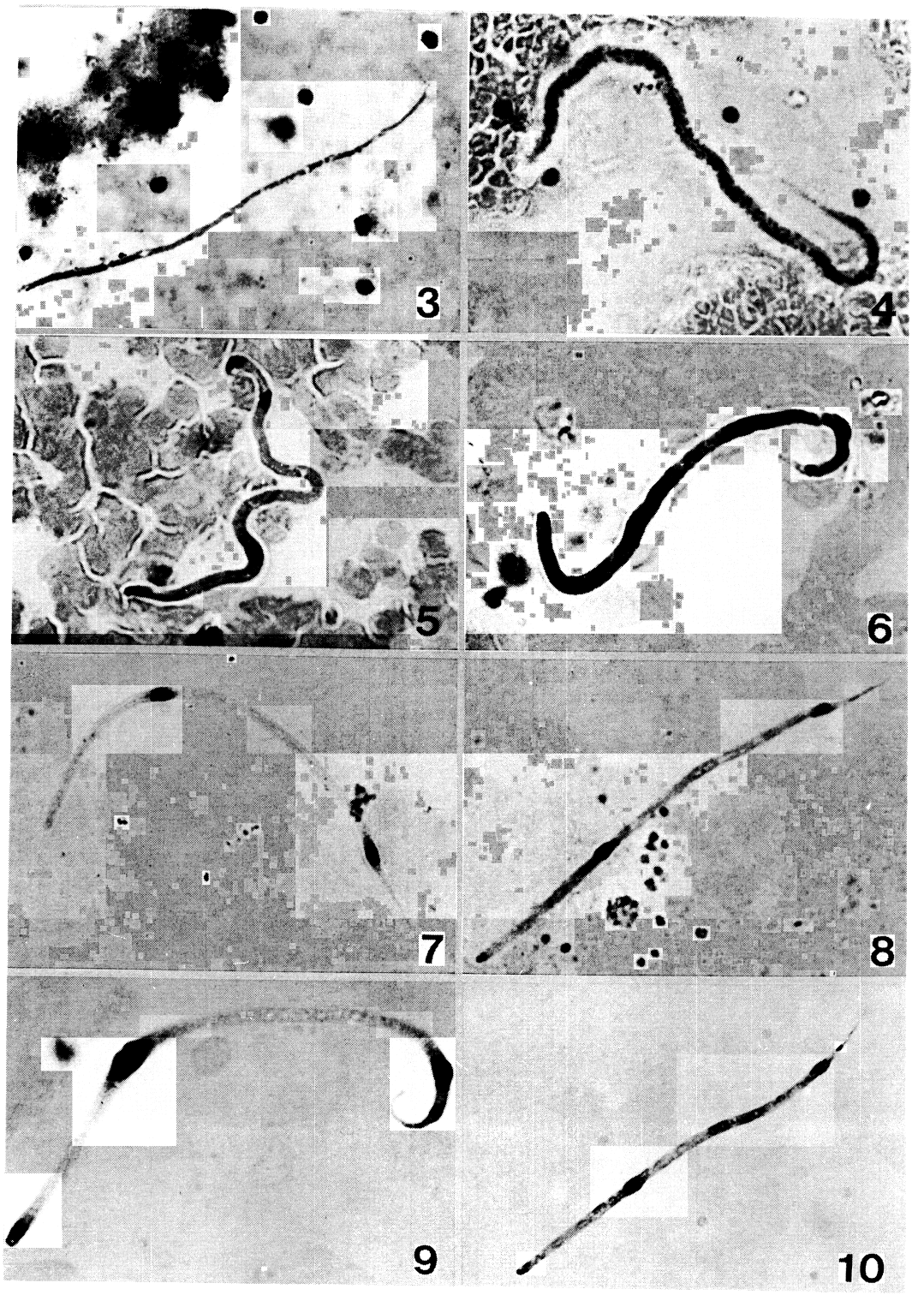
Figs. 3 & 4 Microfilariae of *Dipetalonema perstans* (3) and *Loa loa* (4) in thick blood films; haematoxylin and eosin staining.

Figs. 5 & 6 Acid phosphatase patterns in *Dipetalonema perstans* microfilariae. Fig. 5 shows enzyme activity localized in the regions of the amphid, excretory and anal vesicles and phasmid and Fig. 6, diffuse staining over the body with intense staining in the areas of the amphid, excretory pore/vesicle, Innenkörper, anal pore/vesicle and phasmid.

Figs. 7–10 Acid phosphatase patterns in *Loa loa* microfilaria. Fig. 7 shows enzyme activity localized in the areas of excretory and anal vesicles; Fig. 8, light staining over the whole body and intense staining in the areas of the amphid, excretory and anal pore/vesicles; Fig. 9, enzyme activity confined to the areas of the amphid, excretory and anal pore/vesicles and phasmids; and Fig. 10, enzyme activity all over the body and dense staining in the region of the amphid, excretory pore/vesicle, Innenkörper, anal pore/vesicle and phasmid.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ナイジェリア・ジョス高地の *Dipetalonema perstans* 及び *Loa loa* 仔虫の酸性フォスファターゼ分布の変異性について

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ジョス高地の住民36人の血液から得た *Dipetalonema perstans* と *Loa loa* 仔虫について酸性フォスファターゼ活性を検討した．その結果，両種には酵素活性に変異性があることが明らかとなった．*D. perstans* では2種類の，*L. loa* には4種類のパターンが見られた．その出現頻度はI型（アンフィツド，排泄孔，肛門に活性があるもの）75.5%，II型（I型に加え内体とファスミツドに活性）が24.5%であった．一方，*L. loa* ではI型（排泄孔，肛門に活性）12.6%，II型（I型に加えアンフィツドにも活性）7.2%，III型(II型に加えファスミツドに活性)31.9%，IV型（III型に加え内体に活性）48.3%であった．今回見られた酵素変異性がこれら2種の地理的な株のちがいによるのかという点を考察した．