SURFACE OF MICROFILARIAE AND IMPACT OF CHEMICAL ALTERATIONS *IN VITRO*

V. M. L. SRIVASTAVA, K. MASOOD, P. Y. GURU*, SATYAVAN SHARMA[+] and R. K. CHATTERJEE*

*Departments of Biochemistry, *Parasitology and [+]Chemistry, Central Drug Research Institute, Lucknow 226 001, India.*

ABSTRACT

Amongst various lectins examined, only wheat germ agglutinin reacted with appreciable affinity to the sheath of the microfilariae of *Litomosoides carinii.* The microfilariae of *Dipetalonema viteae*, on the other hand, did not bind any of the lectins used. Trypsin and papain in appropriate concentrations caused rapid exsheathing of *L. carinii* microfilariae. Treatment with trypsin generated new sites on the microfilarial surface for the binding of lectins and also enhanced their capacity to attract the peritoneal exudate cells.

INTRODUCTION

SURFACE alteration, chemical or otherwise, appear to be an important step to make the microfilariae vulnerable to the defence mechanisms of the host[1-5]. Such changes may be brought about by certain exo- or endogenous substances. Nevertheless, a comprehensive idea on biochemical topography of microfilarial surface would be of help for a rational search of such agents. The present communication deals with the effectiveness of various enzymes as an exsheathing agent for the microfilariae of *Litomosoides carinii in vitro* and the influence of this change on cellular adhesion reaction and lectin binding.

MATERIALS AND METHODS

Chemicals

Trypsin and lysozyme were obtained from the Serva Biochemicals, West Germany, and pepsin from the Loba Chemie, Bombay. Hyaluronidase, phospholipase-A and fluoresceinisothiocyanate (FITC) were purchased from the V. P. Chest Institute, New Delhi, while chitinase, wheat germ agglutinin (WGA), N-acetylglucosamine, N-acetyl galactosamine and sialic acid were procured from the Sigma Chemical Company, USA. All other lectins namely, concanavalin-A (Con-A), *Ricinus communis* agglutinin (RCA_1 and RCA_2), soybean agglutinin (SBA) and carcinoscorpin (CSN) were procured from the Hy-Gro Chemicals, Calcutta.

Collection of microfilariae

Microfilariae of *L. carinii* were obtained by filtration of the blood of heavily infected cotton rats (*Sigmodon hispidus*) through 3 μm filters while the blood of infected *Mastomys natalensis* was filtered through 5 μm millipore filters (Maxflow, India) for separation of *Dipetalonema viteae* microfilariae.

Lectin binding

Approximately 50,000 microfilariae were incubated at 37°C with FITC conjugated lectins in 0.5 ml basic filarial medium (BFM)[6] in the dark. After 30 min, 5 ml BFM were added to each tube and the contents were centrifuged at 900 g. The supernatant was carefully removed. The microfilariae were repeatedly washed (generally thrice) with BFM till the supernatant became free of fluorescence. The microfilariae were suspended in BFM. For quantitative measurement of binding, fluorescence of the microfilarial suspension was recorded in a Bowman spectrofluorophotometer with excitation and emission wavelengths of 490 and 520 nm respectively. The microfilariae which were incubated without lectin served as control. The difference between the fluorescent values of experimental and control was used to calculate the amount of the lectin bound to the microfilariae.

Each tube was also examined for a qualitative assessment on a Vickers fluorescence microscope (model M 17) at a final magnification of 1000. An aliquot of the suspension was also measured for microfilarial count.

Binding specificity was checked by preincubating the microfilariae with inhibitory saccharides at a final concentration of 100 mM.

Exsheathing agent(s)

To detect the exsheathing property of different agents, approximately 20,000 microfilariae of *L. carinii* were shaken at 37°C with various enzymes in

BFM. Incubations were carried out in a Techno shaker bath and the final volume adjusted to 0.5 ml. At different time intervals, one drop of the medium was applied on the glass slide, stained with Leishman dye and examined under the microscope for any change in sheath or surface of microfilariae.

Cell adhesion

The effect of exsheathing on cell adhesion was investigated in the presence of both normal and immune sera. Immune serum was obtained from cotton rats showing low microfilarial counts following an intense microfilariaemia. Peritoneal exudate cells were isolated from healthy cotton rats by administering normal saline. Adhesion reaction was carried out in a microtitre plate[5]. After 3 and 12 h of incubation, cell adhesion with microfilarial surface was examined on a glass slide before and after staining with Leishman dye.

RESULTS AND DISCUSSION

The effect of various enzymes on the sheath of *L. carinii* microfilariae is presented in table 1. These enzymes were selected by assuming that the sheath is composed of materials like proteins, lipids, mucopolysaccharides and other complex carbohydrates. Hence trypsin, pepsin, papain and collagenase were chosen for proteins; hyaluronidase, lysozyme and chitinase for mucopolysaccharides and complex carbohydrates, and phospholipase-A for phospholipids. The results clearly indicate that trypsin or papain may be successfully employed as the *in vitro* exsheathing agent for *L. carinii* microfilariae (figure 1). It is also evident that a major part of the microfilarial sheath is composed of proteins and polysaccharides which may be degraded by trypsin, papain and chitinase. Chitin is known to occur in the rigid exoskeleton of insects, crustaceans and certain other invertebrates. Recently, the synthesis of chitin has been related to the morphogenesis of the sheath of *Brugia malayi* microfilariae[7]. Hence, the occurrence of chitin in the sheath of microfilariae of *L. carinii* appears quite natural. On the other hand, collagen, phosphoglycerides and other complex polysaccharides do not appear to be present in the sheath. Even if present, they are in very small amounts and are probably shielded from the corresponding enzymes such that these enzymes do not affect the integrity of the sheath covering.

Table 1 *Effect of various enzymes on the sheath of L. carinii microfilariae*

Enzyme	Concentration (mg/ml)	Effect after hour of incubation				
		0.5	1	2	4	10
None	–	+	+	+	+	+
Trypsin	2.0	–	–	–	*	*
Pepsin	2.0	+	+	+	+	+
Papain	2.0	–	–	*	*	*
Collagenase	4.0	+	+	+	+	+
Chitinase	4.0	+	+	+	?	–
Lysozyme	4.0	+	+	+	+	?
Hyaluronidase	4.0	+	+	+	+	+
Phospholipase	0.2 ml	+	+	+	+	+

+ Sheath intact; ? Sheath distorted or damaged; – 95% microfilariae exsheathed; * Most of the microfilariae under digestion.

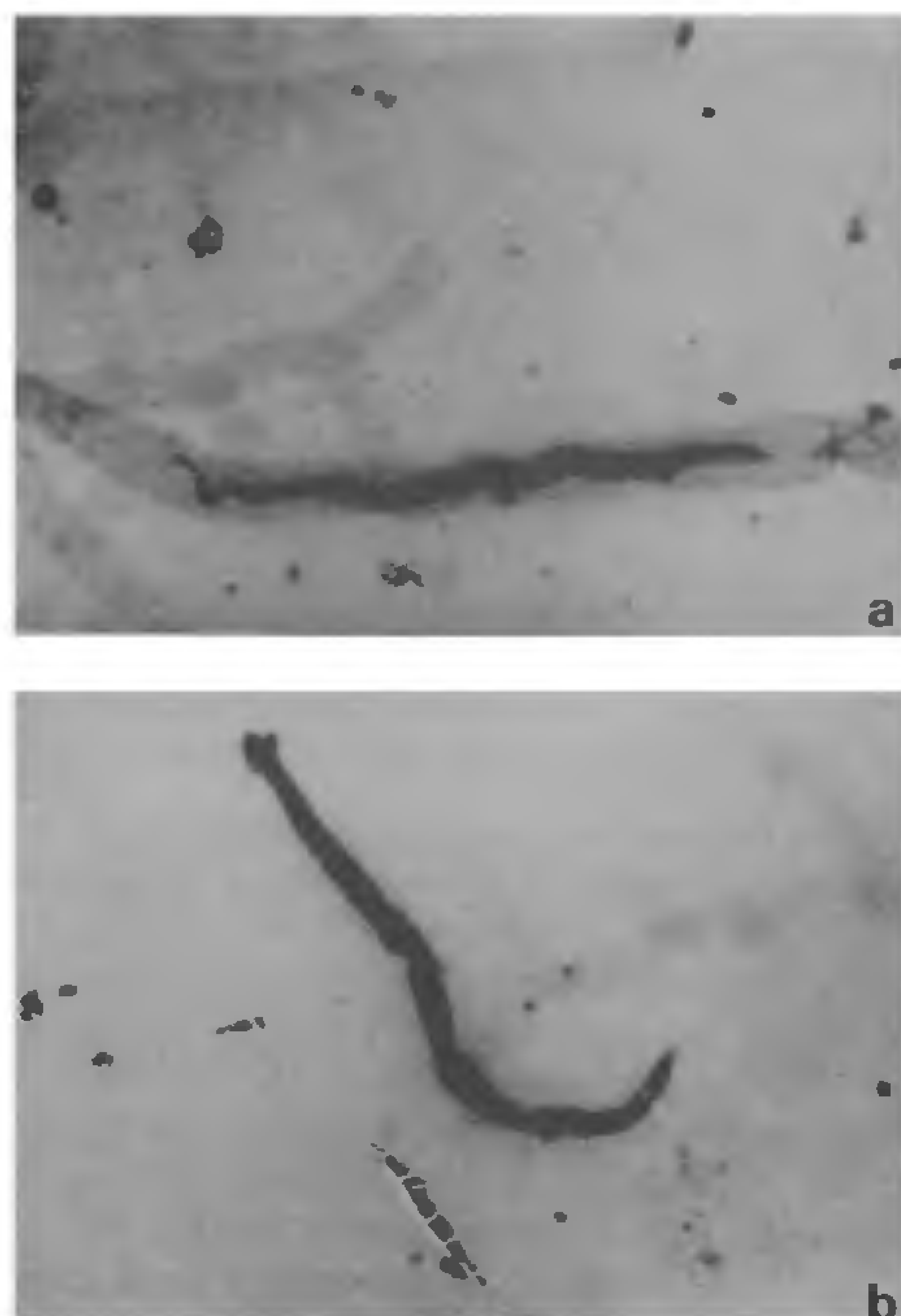

Figure 1a, b. Microscopic appearance of *L. carinii* microfilariae. (a) before (sheathed) and (b) after trypsin treatment (exsheathed).

Amongst six lectins, only WGA was bound to the sheath of *L. carinii* with intense staining (table 2). None of the other lectins showed affinity for the microfilariae of either *L. carinii* or *D. viteae*. The binding of WGA to the microfilariae of *L. carinii* suggests that N-acetyl glucosamine residues occur at

Table 2 *Binding of lectins to the microfilariae of L. carinii and D. viteae*

	Sheath	Cuticle		Anal pore		Excretory pore		Trypsin treated
Lectin	L.C.	L.C.	D.V.	L.C.	D.V.	L.C.	D.V.	L.C.
Con A	–	–	–	+	+	+	+	+
RCA_1	–	–	–	±	–	–	–	+
RCA_2	–	–	–	+	–	+	–	+
SBA	–	–	±	–	–	±	±	+
WGA	+++	–	–	+	+	+	+	+++
CSN	–	–	–	±	±	–	–	–

– No binding; ± Faint reaction; + Moderate binding; ++ Bright; +++ Intense binding; L.C., *L. carinii*; D.V., *D. viteae*.

the surface of this parasite. According to a few reports, the binding of WGA to certain glycoproteins may occur due to its interaction with sialic acid as well as with N-acetyl glucosamine residues[8,9]. However, the absence of CSN binding sites on the microfilarial surface as well as the inhibition of WGA interaction by N-acetyl glucosamine provide strong evidence that WGA specifically binds to the exposed N-acetyl-glucosamine residues of the sheath of *L. carinii* microfilariae. The presence of this saccharide has unequivocally been reported on the microfilarial sheath of *B. pahangi*[10] and *B. malayi*[11].

Quantitative analysis also suggests that only N-acetyl-glucosamine residues are present in significant amount on the microfilariae of *L. carinii* but not on those of *D. viteae* (table 3). The residues responsible for the binding of other lectins have negligible population and their interaction possibly does not produce illumination sufficient for visualizing in a microscope (table 2). The density of the reacting molecules therefore appears to be an important factor for visual detection.

Table 3 *Quantitative binding of lectins to microfilariae**

	L. carinii		
Lectin	Untreated	Trypsin-treated	*D. viteae*
Con A	0.64	2.18	0.31
RCA_1	0.13	1.37	0.06
RCA_2	0.47	1.69	0 23
SBA	0.36	1.80	0.17
WGA	8.07	10.34	0.89
CSN	0.04	0.15	0.05

* f mol/million microfilariae.

Pre-exposure to trypsin markedly increased the affinity of *L. carinii* microfilariae for peritoneal exudate cells (table 4). Thus, in the presence of immune serum following 12 h incubation, the untreated microfilariae showed poor adhesion while the treated ones registered high affinity. The enzyme-treated microfilariae, even in the presence of normal serum, exhibited appreciably high adhesion rate whereas the untreated ones failed to attract the cells. Similar observations have been recorded for the microfilariae of *B. pahangi*[12]. Exposure to the exsheathing enzyme, trypsin, therefore appears to generate new sites for cell attachment. This hypothesis is well-supported by the observations pertaining to the significant increase in the reactivity of microfilariae with various lectins by pre-exposure to trypsin (tables 2, 3). The appearance of new lectin binding sites by treatment with exsheathing agents has also been reported for the microfilariae of *B. pahangi*[10]. This is possibly due to the increase in density of N-acetyl-glucosamine residues which enhances the cell adhering capacity of microfilariae. It is pertinent in this context to mention that the reduction in acidic groups (silaic acid residues) by

Table 4 *Effect of pre exposure to trypsin on the adhering capacity of L. carinii microfilariae*

		Adhesion of peritoneal exudate cells after	
Microfilariae treated with	Type of serum	3 h	12 h
None	Normal	Nil	Nil
None	Immune	Nil	5% (1–2 cells)
Trypsin	Normal	Doubtful	60% (1–4 cells)
Trypsin	Immune	Insignificant	70% (1–8 cells)

neuraminidase treatment is known to enhance the adhesion reaction[13].

Thus it is summarized that the microfilariae of *L. carinii* but not of *D. viteae* possess a large number of WGA binding sites. The binding of other lectins is poor in comparison and appears to be predominantly non-specific. N-acetyl glucosamine residues are primarily contributed by chitin which by the enzymatic action appear to be the prominent polysaccharide of the sheath of *L. carinii* microfilariae. Trypsin may be used to exsheath this species of microfilariae *in vitro*. Treatment of the microfilariae with the enzyme exposes a large number of new lectin binding sites which increases the vulnerability of the parasite to cellular adhesion.

29 April 1987; Revised 5 January 1988

1. Hawking, F., Sewell, P. and Thurston, J. P., *Br. J. Pharmacol. Chemother.*, 1950, **5**, 217.
2. Schardein, J. L., Lucas, J. A. and Dickerson, C. W., *J. Parasitol.*, 1968, **54**, 351.
3. Gibson, D. W., Connor, D. H., Brown, H. L., Fuglsang, H., Anderson, J., Duke, B. O. L. and Buck, A. A., *Am. J. Trop. Med. Hyg.*, 1976, **25**, 76.
4. Hawking, F., *Adv. Pharmacol. Chemother.*, 1979, **16**, 129.
5. Piessens, W. F. and Beldekes, M., *Nature (London)*., 1979, **282**, 845.
6. Bueding, E., *J. Exp. Med.*, 1949, **89**, 107.
7. Furman, J. A. and Piessens, W. F., *Mol. Biochem. Parasitol.*, 1985, **17**, 93.
8. Bhavnandan, V. P. and Katlic, A. W., *J. Biol. Chem.*, 1979, **254**, 4000.
9. Pereira, M. E. A., Loures, M. A., Villalta, F. and Andrade, A. F. B., *J. Exp. Med.*, 1980, **152**, 1375.
10. Devaney, E., *Trop. Med. Parasitol.*, 1985, **36**, 25.
11. Kaushal, N. A., Simpson, A. J. G., Hussain, R. and Ottesen, E. A., *Exp. Parasitol.*, 1984, **58**, 182.
12. Chandrashekar, R., Rao, U. R. and Subrahmanyam, D., *Trop. Med. Parasitol.*, 1984, **35**, 177.
13. Hudson, R. and Kitts, W., *J. Parasitol.*, 1971, **57**, 808.

NEWS

"WHO COLLABORATING CENTRE ON DRUG INFORMATION" AT CDRI LUCKNOW

The World Health Organization has established a "WHO Collaborating Centre on Drug Information" at the Central Drug Research Institute, Lucknow. The National Information Centre for Drugs and Pharmaceuticals (NICDAP), a sectoral discipline-oriented information centre under the NISSAT Plan of the DSIR, Ministry of Science and Technology, Govt. of India, operating at CDRI since October 1977, has been chosen for this purpose. This collaborating centre is the first of its kind established by WHO on drug information and the collaboration with WHO has become effective from February 1988 for a period of four years. The centre will act as a focal point for information activities in the area of drugs and pharmaceuticals for the 11 member-countries of the South East Asian region which include, besides India, Bangladesh, Bhutan, Burma, Indonesia, Democratic Republic of Korea, Maldives, Mongolia, Nepal, Sri Lanka and Thailand.

For further particulars please contact: Dr S. S. Iyer, Scientist-in-Charge, NICDAP, Central Drug Research Institute, Lucknow 226 001.