ORIGIN OF BIOLOGICALLY ACTIVE EXCRETORY SECRETORY MATERIALS FROM *SETARIA DIGITATA*

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ABSTRACT

Setaria digitata, a filarial parasite in the peritoneal cavity of cattle, released appreciable quantity of non-dialyzable materials and microfilariae (MF) during *in vitro* incubation at 37°C in Tyrode solution. The number of MF as well as the concentration of non-dialyzable materials gradually decreased. Protease activity was detected in the nòn-dialyzable materials and the latter rose after an initial fall, in line with the release of MF. Sublethal concentrations (0.25 mM) of diethyl carbamazine (DEC), reversibly inhibited the release of MF. Worms so treated for short periods behaved like normal worms when transferred into a drug-free medium, where they released MF with a corresponding raise in non-dialyzable materials including protease. Evidence indicates that the source of ES materials is the hatching fluid.

INTRODUCTION

The importance of excretory secretory (ES) materials from parasites is widely accepted today, both in relation to diagnosis and immunization in diseases such as Chaga's disease[1], Toxocariasis[2], Onchocerciasis[3], and Bancroftian filariasis[4-6]. Recently Kaleysa Raj *et al*[7] showed the presence of protease, lipase, alkaline phosphatase, and large quantities of protein in the ES products of adult *Setaria digitata*, a filarial parasite of cattle used as a model in the study. Rogers[8] suggested that a proteinase may be involved in the hatching of eggs of *Ascaris lumbricoides*, a nematode parasite of the human gastrointestinal tract. However, he could not detect any proteolytic activity in the hatching fluid with dialysed Ox serum as substrate. The release of MF under *in vitro* conditions and the associated raise in the level of non-dialyzable materials including protease activity is the subject matter of this communication. Hatching fluid emerging during the release of MF is suggested as the source of excretory secretory materials in the filarial nematode.

MATERIALS AND METHODS

S. digitata adults were collected from the local slaughter house in Tyrode solution (composition w/v: sodium chloride 0.8%, potassium chloride 0.02%, calcium chloride 0.02%, sodium bicarbonate 0.015%, sodium dihydrogen phosphate 0.05% and glucose 0.5%). The washed worms were incubated in Tyrode solution at 37°C for 4 hr with the change of medium every hour. At the end of the experimental period, the hourly samples were separately centrifuged. The pellets were resuspended in a known volume of Tyrode solution and the number of MF released was determined by counting the total number present in a measured aliquot of the suspension.

Protein was precipitated from the supernatant using solid ammonium sulphate to saturation under ice-cold conditions. The precipitate was collected by centrifugation in a refrigerated centrifuge, washed, dissolved in buffer and dialyzed against distilled water for 24 hr. Protein was estimated from an aliquot of the dialyzed material by the method of Lowry *et al*[9]. The method described by Mycek[10] was followed to estimate protease. The effect of DEC was studied by incubating the worms in Tyrode solution containing 0.25 mM concentration of the drug. As before, the medium was changed every hour for 6 hr. Drug-containing medium was used for the first 3 hr and drug-free medium for the latter 3 hr.

DEC used in the study was a gift from the Kerala Drugs and Pharmaceuticals, Alleppey. Haemoglobin was purchased from Sigma Chemicals. Other reagents and chemicals used were of Analar grade.

RESULTS AND DISCUSSION

The amount of protein released into the incubation medium at hourly intervals, along with the protease activity and MF count is given in table 1. The results

Table 1 *Excretory secretory release during in vitro incubation of Setaria digitata at 37°C*

Time (hr)	Protein ($\mu g \pm SD$)	Enzyme activity*	Micro-filariae
0–1	316 ± 113	1.58 ± 1.13	34,400
1–2	255 ± 118	0.44 ± 0.38	24,500
2–3	383 ± 240	3.46 ± 2.30	62,400
3–4	160 ± 110	2.73 ± 2.30	18,800
0–4	1115	8.21	140,100

Materials released/g wet weight of *S. digitata* during the time
*One unit of enzyme activity is defined as that amount of enzyme which causes the optical density to increase by 1.0 in a 10-min period of incubation.

Table 2 *Excretory secretory release during in vitro incubation of Setaria digitata at 37°C—effect of diethylcarbamazine* (DEC)

Time (hr)	Protein $\mu g \pm SD$	Enzyme activity*	DEC mM	Microfi-lariae
0–1	250 ± 72	1.7 ± 0.8	0.25	Nil
1–2	197 ± 65	1.3 ± 0.9	0.25	Nil
2–3	250 ± 77	1.3 ± 0.8	0.25	Nil
3–4	217 ± 50	0.7 ± 0.2	Nil	Nil
4–5	167 ± 40	1.0 ± 0.7	Nil	Nil
5–6	198 ± 50	1.6 ± 0.2	Nil	49,500

Materials released/g wet weight of *S. digitata* during the time
*One unit of enzyme activity is defined as that amount of enzyme which causes the optical density to increase by 1.000 in a 10-min period of incubation.

clearly showed a raise in the level of non-dialyzable materials including protease, associated with the release of MF. It is true that appreciable quantity of non-dialyzable material is released at the beginning of the incubation. Unpublished observations made in this laboratory indicated that many of these initial proteins are host-derived materials.

The fact that non-dialyzable materials including protease increases associated with the release of MF is evident from the DEC experiments. DEC reversibly inhibited the release of MF. In homogenate systems DEC partially stimulated protease activity. At a concentration of 0.25 mM DEC concentration, whole worm homogenate showed 30% stimulation of protease activity. Homogenates of the uterus of the worm showed considerably less effect at the same concentration. Worms exposed to 0.25 mM DEC concentration showed only partial paralysis upto 6 hr of incubation. However, irreversible paralysis leading to death was observed when the incubation time or concentration of DEC was increased. In the presence of DEC there was almost 50% reduction in the amount of non-dialyzable materials including the activity of protease and no release of MF. Once they were transferred into a drug-free medium the pattern changed.

Zachariah and Raj[11] recently showed that the metabolism of lipids in *S. digitata* is affected in the presence of DEC. Hatching involves lysis of the egg membranes and this process is reversibly inhibited by sublethal concentrations of DEC and thus hatching is inhibited. The increase in the concentration of non-dialyzable materials including protease, along with the release of MF clearly indicate that hatching does result in the release of these materials and in all probability the materials so released must be very specific to the parasite.

The origin of excretory secretory materials of biological importance is still a matter to be decided. Secretory glands of many nematodes, especially in the intestine have been shown to contain antigenic hydrolytic enzymes such as lipase, protease and acetyl choline esterase[12–14], believed to be originating from the oesophagus of those parasites. However, recent findings in this laboratory indicate that neither the excretory cells and excretory pore nor the oesophagus of *S. digitata* showed appreciable fluorescence when exposed to antisera developed against the ES materials. However, the entire uterine tissue and the space between the embryo and the egg membrane (amniotic fluid) showed strong fluorescence. This indicates that amniotic fluid is antigenic. Hatching fluid is actually the amniotic fluid and hence it has to be the ES material of biological signficance.

The amount of protein, the enzyme activity and the MF, all decreased with increase in incubation time. However, it is interesting to note that the specific activity goes up, though the total activity as well as the non-dialysable materials decreased. The increase in the specific activity may be due to less and less retained host material, while the decrease observed over time may be due to the absence of nutrients other than glucose in the incubation medium. Microscopic examinations showed that there was no difference between the MF released at different time intervals under *in vitro* conditions and those released under *in vivo* conditions.

It can thus be concluded that the ES materials of parasites contain two types of materials—one, host derived and the other, parasite derived. The host specific may be complexes of host materials most of which is already circulating as immune complexes with those of the parasite[15] and the remainder is just adhered to the worm by some means and these are

released during the early hours of *in vitro* incubation. The parasite specific in all probability appears to be associated with the hatching and release of MF as is evidenced by the increased specific activity of protease and a decrease in the non-dialyzable materials. It is speculated that the study of ES materials may prove to be a valuable tool in the detection and ultimate control of filariasis. This is more so because *S. digitata* is very similar to the human filarial parasites *Wuchereria bancrofti* and *Brugia malayi* in its action against DEC[16] and as such the information gathered in the Setaria model system will be related to the human parasite systems.

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1. Tarrant, C. J., Fife, E. H. and Anderson, R. I., *J. Parasitol.*, 1965, **51**, 277.
2. de Savigney, D. H. and Tirand, I. R., *Trans. R. Soc. Trop. Med. Hyg.*, 1977, **71**, 501.
3. Schiller, E. L., D'Antonio, R. and Marroquin, H. F., *Amer. J. Trop. Med. Hyg.*, 1980, **29**, 1215.
4. Kharat, I., Harinath, B. C. and Ghirnikar, S. N., *Indian J. Exp. Biol.*, 1982, **20**, 378.
5. Malhotra, A., Reddy M. V. R., Naidu, J. N., Ghirnikar, S. N. and Harinath, B. C., *J. Biosci.*, 1982, **4**, 507.
6. Reddy, M. V. R., Malhotra, A., Prasad, G. B. K. S. and Harinath, B. C., *J. Biosci.*, 1984, **6**, 165.
7. Kaleysa Raj, R., John Zachariah, T., Suresh Babu, N. P., Thilagavathy, H. P. and Mary Samuel, *Indian J. Med. Res.*, (special Issue), 1985, In press.
8. Rogers, W. P., *Nature* (*London*), 1958, **181**, 1410.
9. Lowry, O. H., Roseborough, N. J., Farr, A. L. and Randall, R. J., *J. Biol. Chem.*, 1951, **193**, 265.
10. Mycek, M. J., In: *Methods Enzymol.*, 1970, **19**, 285.
11. John Zachariah, T. and Kaleysa Raj, R., *Indian J. Med. Res.*, (In press), 1985.
12. Thorson, R. E., *J. Parasitol.*, 1956, **42**, 21.
13. Thorson, R. E., *Am. J. Hyg.*, (Monograph series) 1962, **22**, 63.
14. Ogilvie, B. H., Rothwell, T. L. W., Bremmer, K. C., Schmitzerling, H. J., Nolan, J. and Keith, R. K., *Int. J. Parasitol.*, 1973, **3**, 589.
15. Dissanaike, S., Galahitigava, S. C. and Ismail, M. M., *Bull. W.H.O.*, 1982, **60**, 919.
16. Hawking, F., WHO/ONCHO/78 142, 1978, 1.

ANNOUNCEMENT

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Papers to be presented at the Symposium will be selected on the basis of the extended abstracts to be prepared and submitted as set out below: Three copies of the abstract should be submitted and length may be approximately 1000 words. They should be typed double space; and state clearly the purpose and conclusions of the investigation, and include figures as necessary. Last date for receipt of abstract is *1st June 1986*.

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