SOME OBSERVATIONS ON
THE ROLE OF THE BODY WALL OF ACANTHOCEPHALUS RANAE IN LIPID UPTAKE

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The acanthocephalan body wall is of interest because it is unique in structure and serves a number of functions (Hammond, 1967). One of its functions is to take up nutrients, acanthocephalans having no gut. Whether the entire body wall, or only part of it, serves this function is not known; nor is it known whether all classes of nutrient enter through the same area of the body surface.

The body of acanthocephalans is divided into two main regions, the trunk and the praesoma (the proboscis apparatus and associated muscles) (see Hammond, 1966, Text-fig. 1). The proboscis is the external part of the praesoma. The larger part of its surface bears hooks, but at its base is a small unarmed 'neck' region. The fine structure of the praesoma and trunk regions of the body wall is now quite well known (Crompton & Lee, 1965; Nicholas & Mercer, 1965; Stranack, Woodhouse, Griffin, 1966; Hammond, 1967). However, these structural studies in themselves have contributed rather little to our understanding of regional functional differentiation in the body wall.

Bullock (1949) suggested that the trunk and praesoma regions of the body wall differ physiologically. He reached this conclusion largely because of the great difference between these areas in the distribution of lipids. He found that the proboscis wall and lemnisci, organs which are in effect outgrowths of the praesoma wall, contain far greater amounts of glyceride and fatty acid than the wall of the trunk. He suggested that the praesoma wall and lemnisci may be concerned either in the absorption or excretion of lipid.

Pflugfelder (1949) suggested that lipid uptake takes place through the praesoma wall and lemnisci. He starved frogs infected with Acanthocephalus ranae for 6 weeks and found the parasites to be 'fat free' after this treatment. If such frogs were then fed with pork fat mixed with the dye Scharlach R a red coloration appeared in their acanthocephalan parasites, first in the neck region of the proboscis and later in the lemnisci. In similar experiments Hammond (1964) dosed toads infected with Acanthocephalus ranae with a solution of Scharlach R in olive oil. Red lipid droplets were detectable in the lemnisci of these worms after 24 hr.

More recent work with the electron microscope on Polymorphus minutus (Crompton & Lee, 1965) and on Acanthocephalus ranae (Hammond, 1967) has also shown that the praesoma wall and lemnisci contain far larger amounts of lipid than the trunk wall. Crompton & Lee suggested that lipids may be absorbed through the surface of the praesoma and pass, through the lemnisci, to the rest of the body. Hammond
(1967) suggested that the reverse process occurs and that lipids are discharged as a waste product at the surface of the proboscis. If the latter theory is correct, lipid uptake may not occur through the surface of the praesoma, but elsewhere. The aim of the present work was to investigate this problem further.

MATERIALS AND METHODS

Experimental animals

Specimens of *Acanthocephalus ranae* (Schrank, 1788; Lühe, 1911) were obtained from the intestines of naturally infected common toads (*Bufo bufo*). Worms from well fed hosts were used in all experiments.

Experiments with dyes

A number of experiments were carried out with the aim of extending and further investigating the findings of Pflugfelder (1949) and Hammond (1964). Toads infected with *Acanthocephalus ranae* received doses of one of three dyes (Scharlach R, Sudan Black, or Lipid Crimson (Edward Gurr)) in saturated solution in olive oil. The doses (1 ml.) were given orally. The toads were killed and the parasites examined 1, 2, or 3 days after dosing. Toads maintained for longer than 1 day were given repeat doses at 24 hr. intervals. Thus toads maintained for 2 days received two doses, and those maintained for 3 days three doses. The worms were first examined microscopically while alive and were later plunged into liquid nitrogen and subsequently sectioned at 10 μ in a cryostat.

Some experiments were carried out in which worms from toads which had received three doses of dye were transferred to fresh hosts. The worms were administered to the new toads from a pipette inserted into the oesophagus, as described by Hammond (1964). Each experimental toad received twenty parasites, each containing a good concentration of dye. The parasites, and the intestines of the toads, were examined after 1, 2, or 3 days.

Experiments with radioactively labelled material

The uptake by *Acanthocephalus ranae* of glyceryl trioleate-9,10-3H (obtained from the Radiochemical Centre, Amersham), specific activity 354 mc/mM, was investigated. Where the process was to be followed by light-microscope autoradiography the worms were placed individually in opaque tubes containing 3 μe of glyceryl trioleate in 10 ml. of amphibian Ringer. Fresh toad bile juice was used as an emulsifying agent and the tubes were shaken gently in a flask shaker during the experiment. Control experiments were carried out in which labelled glyceryl trioleate was replaced by non-radioactive material. Worms were removed from their tubes after 5, 30, 60, or 90 min. intervals. They were washed rapidly in clean Ringer then plunged into liquid nitrogen and later sectioned at 10 μ in a cryostat. The sections were mounted on glass microscope slides and fixed in Baker’s formol–calcium. Some sections were extracted for 2 hr. in chloroform–methanol (2:1). All sections were stained by the Fettrot method for neutral fat, as described by Pearse (1960). The sections were coated with Kodak AR 10 stripping film, dried, and stored at 0° C. for an exposure time of 8 days. The material was processed in Kodak D-19b developer (5 min.) and Kodak acid fixer, and after washing was mounted in glycerine jelly.
Lipid uptake in Acanthocephalus ranae

A further series of experiments was carried out as described above, except that the worms were ligatured in various ways before being placed in the medium containing radioactive trioleate. The ligatures, of fine Terylene thread, were applied in the following regions of the worms: (a) at the base of the proboscis to prevent the flow of material between the trunk and proboscis; (b) in the trunk region one-third of the body length from the junction of the trunk and praesoma; (c) at a position two-thirds of the body length from the trunk/praesoma junction; (d) at both points on the trunk. The trunk ligatures were drawn sufficiently tight to act as a barrier to the flow of fluid both in the lacunae of the body wall and in the pseudocoel.

For electron-microscope autoradiography worms were removed from their host and placed in tubes containing 30 μc of glyceryl trioleate, emulsified as described above, in 10 ml of amphibian Ringer. Worms were removed after 5, 30, 60, or 90 min. intervals, rinsed in clean Ringer, then placed in fixative at 4°C in which they were cut into small pieces. The material was fixed for 2 hr. in 6.5% glutaraldehyde (phosphate buffered at pH 7.4), washed in phosphate buffer for 12 hr., then postfixed for 2 hr. in 1% OsO₄ in phosphate buffer (pH 7.4). After dehydration in ethanol the material was transferred to propylene oxide and finally embedded in Araldite (Luft, 1961). Sections were cut on a Huxley ultramicrotome and were mounted on copper grids with carbon support films. The sections were not stained.

The sections were coated with Ilford L.4 Nuclear Research Emulsion. 5 g. of emulsion was dissolved in 10 ml. of distilled water in a water bath at 45°C. This was then cooled to room temperature and a copper wire loop 3 cm. in diameter was dipped into the emulsion and withdrawn. The emulsion held by the loop gelled almost immediately and was applied to the sections by touching it to the surface of the grids supporting them. The sections were stored at 0°C for an exposure time of 8 weeks. The material was developed subsequently in Kodak Microdol-X (5 min.) and fixed in plain hypo. The sections were examined with an AEI EM 6 electron microscope.

THE SURFACE LAYERS OF THE PROBOSCIS AND TRUNK WALL

The fine structure of the proboscis wall, the trunk wall, and the lemnisci of Acanthocephalus ranae has been described recently (Hammond, 1967). The surface of the proboscis is covered by a layer of lipid material up to 1.5 μ in thickness. The trunk has no such covering, its outermost layer consisting of material that is probably acid mucopolysaccharide. Beneath the lipid covering the proboscis is a tenuous layer of material probably corresponding to the acid mucopolysaccharide layer of the trunk. Beneath the acid mucopolysaccharide layer, in both the trunk and proboscis, is a membrane which runs through pores in the matrix of the ‘striped layer’ to form canals which penetrate some way (4 μ in the trunk and 1 μ in the proboscis) toward the inner regions of the body wall (Text-fig. 1). In the proboscis wall these canals are filled with lipid material and beneath them are numerous lipid-filled vesicles and droplets. Far less lipid is present in the wall of the trunk, and the membrane-bounded canals in this region are not filled with such material. The pores in the surface of the proboscis are slightly greater in diameter and more widely spaced than those in the surface of the trunk (Text-fig. 1).
RESULTS

Experiments with dyes

The intestinal mucosa of the toads absorbed Scharlach R, Sudan Black, and Lipid Crimson strongly. The concentration of dye taken up was greatest in the duodenum and fell off towards the rectum. The dyes were detectable in small amounts in the lymphatic system, liver, and blood vessels 24 hr. after administration of the first dose.

Text-fig. 1. Diagram of the outer layers of the proboscis and trunk of Acanthocephalus ranae (see Hammond 1967). (a) Diagram of whole animal (reproductive organs and musculature omitted); (b) outer layers of proboscis; (c) outer layers of trunk; (d) diameter and spacing of the pores in the surface of the proboscis; (e) diameter and spacing of the pores in the surface of the trunk.

C, membrane-bounded canal; L, lemniscus; LL, lipid layer; LV, lipid-filled vesicle; M, acid mucopolysaccharide layer; OM, outer membrane; P, proboscis; R, receptacle; SL, matrix of 'striped layer'; T, trunk.
Lipid uptake in Acanthocephalus ranae

The three dyes were just detectable within the majority of the acanthocephalans 24 hr. after the initial dosage. In a few individuals no dye was visible. Scharlach R and Sudan Black could be detected in the worms only in lipid droplets within the lemnisci and the lacunae of the proboscis wall. No stain was detectable in any other body region, either in whole animals or in sections.

Lipid Crimson appeared in greatest concentration in lipid droplets within the lemnisci and proboscis wall, but some such droplets within the lacunar system of the trunk wall were slightly coloured also.

Dye could be detected in all worms from toads which had received two or three doses. The distribution of the dye remained as described above, but the concentrations in the worms were higher. A thin layer of coloured material was present on the outer surface of the proboscis of about 40% of the worms examined. In his experiments Pflugfelder (1949) noted that an intense colour appeared first in the neck region of the proboscis. No particularly intense coloration was noted in this region during the present experiments. Indeed it seemed impossible for this to occur. Coloured lipid droplets flowed back and forth between the lemnisci and the lacunae of the proboscis wall as a result of the activity of the proboscis apparatus (see Hammond, 1966) and were thus kept evenly distributed throughout the system.

Of the worms transferred to fresh hosts 40-70% established themselves successfully. The amount of detectable dye in the transplanted worms diminished with time. All these worms retained small but detectable quantities of dye after 3 days however. In the case of worms containing Lipid Crimson the dye could no longer be detected within the trunk wall after 24 hr. in a fresh host, although it remained visible in the lemnisci and proboscis wall after 3 days.

Three days after the introduction of worms containing coloured lipid droplets the intestinal mucosa of the toads concerned had become slightly coloured. Coloration was more difficult to detect where the worms administered contained Sudan Black than when the worms contained a red dye. The coloration was more marked in the general region of the intestine in which the worms had become established than elsewhere. A thin layer of coloured material could be seen on the surface of the proboscis of the majority of worms.

Experiments with radioactive material

The worms removed from the maintenance medium after 5 min. contained insufficient radioactive material to produce satisfactory autoradiographs. Good autoradiographs were obtained from animals maintained in vitro for 30 min. or longer. No radioactive material was detected in the proboscis or lemnisci of any worm, even after a 90 min. exposure in vitro to labelled glyceryl trioleate (Pl. 1 A).

The distribution of radioactivity in the worms is shown in Table 1. In all worms examined radioactivity was confined to the wall of the trunk (Pl. 1 A, B). Little difference was noted in the distribution of radioactivity between worms maintained in vitro for 30, 60 and 90 min. The highest level of activity occurred in the ‘radial fibrillar layer’, the maximum level being reached after 60 min. This is the thickest layer of the trunk wall, and that in which the lacunar channels are situated. The lacunar channels themselves contained a moderate amount of radioactive material.

In each case activity was low in the cuticle, ‘striped layer’ and ‘canal layer’, and
moderate in the ‘felt layer’. No radioactivity was detected further within the worms than the muscular layers of the trunk wall.

The distribution of neutral fats revealed by Fettrot staining in non-extracted sections was closely similar to that found in a number of other acanthocephalans by Bullock (1949). The lemnisci and proboscis wall contain large amounts of neutral fat and the wall of the trunk much less. A layer of material up to 1 μ thick stained by Fettrot was noted on the outer surface of the proboscis but not on that of the trunk. It is believed that this material corresponds with the layer shown by the electron microscope to cover the surface of the proboscis (Hammond, 1967). The largest amount of neutral fat found in the wall of the trunk occurred in the radial fibrillar layer.

Table 1. Distribution of radioactivity in Acanthocephalus ranae after in vitro maintenance in a medium containing labelled glyceryl trioleate (for detailed information on the layers of the body wall see Hammond, 1967)

<table>
<thead>
<tr>
<th>Time after dosing the host (min.)</th>
<th>Trunk</th>
<th>Radial fibrillar layer</th>
<th>Basement membrane and muscular layers of trunk wall</th>
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<td></td>
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<td>Proboscis and lemnisci</td>
<td>Cuticle striped layer, canal layer</td>
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<td>5</td>
<td>o (0)</td>
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<td>90</td>
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<td>i (0)</td>
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0, activity not above background level; 1, very low activity; 2 low activity; 3 moderate activity; 4, moderately high activity; 5 high activity.
The figures in brackets are for sections extracted with chloroform–methanol.

Sections which had been extracted in chloroform–methanol contained no material that was stained by Fettrot. The amount of radioactive material contained in such sections was considerably less than in untreated sections (Table 1). Radioactivity in extracted sections was confined to the radial fibrillar layer of the trunk wall.

Ligaturing of worms in the ways described had no effect on their uptake of radioactive material, nor on its subsequent distribution within the body. Autoradiographs indicated that ligatured worms contained similar amounts of radioactive material, similarly distributed, to non-ligatured individuals.

The results of electron-microscope autoradiography (Pl. 2A, B) were in agreement with those obtained with the light microscope. In the felt layer of the trunk wall (Pl. 2B) radioactivity was associated primarily with electron dense ‘droplets’ which it has been suggested (Hammond, 1967) are lipid in nature. In more outer layers (the striped layer and the canal layer) activity was associated with the canals entering the body wall, although apparently not specifically with ‘droplets’ (Pl. 2A). In sections of the radial fibrillar layer roughly 50% of the silver grains developed in the overlying emulsion were apparently associated with ‘lipid droplets’, while the rest were not correlated with objects that were distinctly recognizable in these unstained sections.
DISCUSSION

There seems little doubt that labelled glyceryl trioleate was absorbed by the worms solely through the wall of the trunk. If the proboscis and lemnisci had played any part in absorption it is likely that radioactivity would have been detected in them, but in no experiment was this the case. The fact that the worms' uptake of radioactive material was unimpaired when the proboscis was ligatured also indicates strongly that the proboscis normally plays no part in the uptake of glyceryl trioleate. Similarly worms with ligatures tied around the trunk absorbed as much radioactive material as non-ligatured individuals. This suggests that glyceryl trioleate was absorbed through all parts of the surface of the trunk.

The results of these experiments suggest that the large amounts of lipid associated with the proboscis wall and lemnisci are not evidence of lipid uptake by these organs. Such results, however, would not conflict with the suggestion (Hammond, 1967) that the proboscis and lemnisci are concerned with the discharge of lipid to the exterior.

The rather small amounts of radioactivity found in the striped layer and canal layer of the trunk wall indicate that material did not accumulate in these regions during uptake. In electron-microscope autoradiographs radioactivity, as might be expected, was associated with the canals of these regions. Material presumably enters the animals through the pores in the surface, passes in the canals through the striped and canal layers, and leaves the canals to enter the felt layer, perhaps by a process similar to pinocytosis (Edmonds & Dixon, 1966; Hammond, 1967). It is likely that metabolism of glyceryl trioleate occurs in the trunk wall. However, the great reduction in the amount of radioactive material in sections extracted with chloroform–methanol (Table 1) indicates that even 90 min. after absorption the radioactive label remained associated with lipid. Radioactive material remained only in the radial fibrillar layer in extracted sections. This suggests that in this layer metabolism of the absorbed material proceeded most rapidly. This is supported by the observation that in electron-microscope autoradiographs only about half of the radioactivity present in the radial fibrillar layer appeared to be associated with 'lipid droplets', while in the felt layer there was a much higher correlation between lipid droplets and radioactivity (Pl. 2 B).

A comparison of the fine structure of the wall of the proboscis with that of the trunk (Hammond, 1967) indicates no basic structural reason for the failure of the proboscis to absorb glyceryl trioleate. Although the pores in the surface of the proboscis are slightly larger in diameter and rather more widely spaced than those in the trunk, the pores of both regions are highly similar. The functional differences between the wall of the trunk and that of the proboscis appear to have a primarily physiological basis (Bullock, 1949; Hammond, 1967). Uptake of materials by the proboscis would be prevented by a continuous outward passage of lipid along the canals running to its surface. It has been found similarly (Hammond, unpublished work) that D-glucose and L-methionine are absorbed by the trunk of Acanthocephalus ranae but not by the proboscis.

The results of the experiments with dyes carried out in the present work are in general agreement with those of Pflugfelder (1949). The results of the experiments with radioactive material, however, suggest that Pflugfelder’s conclusion that the
proboscis and lemnisci are concerned with lipid uptake is incorrect. It is suggested that in the present experiments the olive oil and dye may have been absorbed by the trunk of the worms from the intestine of the host. The proboscis need not have been involved. The dye itself may have become concentrated in the lipid droplets of the lemnisci and proboscis wall later. Dye was not detectable in these organs until 24 hr. after administration of the dye to the host. Accumulation of dye within the lemnisci might suggest that these organs have an excretory function. The fact that in a number of worms, including those transferred to new hosts, a layer of material coloured by the dye was present on the outer surface of the proboscis might be an indication that lipid has been discharged from the proboscis. The failure to detect dyes, apart from Lipid Crimson, in the trunk wall may have been due to the fact that they did not accumulate there, and so did not reach a detectable concentration. Whether Lipid Crimson was visible in the wall of the trunk because it reached higher concentrations there than other dyes, or because it has a more intense colour, is not known. In worms transferred to fresh hosts Lipid Crimson disappeared from the trunk wall after 24 hr., while remaining in the lemnisci and proboscis. While it is possible that the dye passed to the outside directly from the trunk, this observation could be consistent with the passage of dye from the wall of the trunk to the lemnisci before discharge from the proboscis.

**SUMMARY**

1. *Acanthocephalus ranae* has been found to take up glyceryl tri[oleate-9,10-3H] solely through the surface of the trunk. The proboscis and lemnisci play no part in the uptake of this material. The large amounts of lipid present in the latter organs may be evidence of their involvement in lipid excretion.

2. Fat-soluble dyes are taken up by the animal and accumulate in lipid droplets in the lemnisci and proboscis wall. It is suggested that such dyes do not enter the animal through the surface of the proboscis, as has been suggested previously, but through the surface of the trunk.

3. The structure of the acanthocephalan body wall is discussed in relation to the uptake of nutrients.

I wish to thank Dr D. A. Erasmus for helpful discussions during the course of this work, and Mr T. Davies for valuable technical assistance.

**REFERENCES**


**Lipid uptake in Acanthocephalus ranae**


**EXPLANATION OF PLATES**

**PLATE 1. Acanthocephalus ranae**

A. Autoradiograph of longitudinal section of a worm which had been maintained in the medium containing labelled glycercyl trioleate for 60 min. The wall of the trunk contains a relatively high concentration of radioactive material, but the proboscis and lemnisci contain none.

B. Autoradiograph of longitudinal section of the trunk wall. The worm had been maintained in the medium containing labelled glycercyl trioleate for 60 min. The largest concentration of radioactive material is present in the radial fibrillar layer.

**PLATE 2. Acanthocephalus ranae**

A. Electron-microscope autoradiograph of an unstained longitudinal section of the striped layer of the trunk wall. The animal had been maintained in the medium containing labelled glycercyl trioleate for 30 min. Radioactivity is associated with the canals running from the body surface.

B. Electron-microscope autoradiograph showing radioactivity associated with a group of lipid droplets in the felt layer of the trunk wall. The animal had been maintained in the medium containing labelled glycercyl trioleate for 30 min. (Unstained section.)

F.L., felt layer; L., lemniscus; L.C., lacunar channel; L.D., lipid droplet; M.L., muscular layers; M.S., matrix of striped layer; P., proboscis; PO, pore; PS, pseudocoel; R.L., radial fibrillar layer; T.W., trunk wall.