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Correlative biochemical and histochemical observations on the lipids of *Centrorhynchus corvi* (Acanthocephala)

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Summary. Lipids of *Centrorhynchus corvi* (Acanthocephala) have been analyzed using biochemical and histochemical techniques. Males contained more than females. However, no qualitative differences were detected. Of the total lipids, phospholipids constituted 11.1 p. 100 and 13.6 p. 100 in female and male worms, respectively. The rest being neutral fats. Triglycerides formed 52.3 p. 100 and 53.4 p. 100, unsaponifiable lipids 14.1 p. 100 and 19.2 p. 100 and free fatty acids 16.1 p. 100 and 14.3 p. 100 in female and male worms, respectively. Their localization in different parts of the worms has been established by histochemical techniques. The role of the body wall in lipid absorption and excretion is discussed.

Although knowledge about the nature and distribution of lipids in helminths is rapidly increasing, relatively little work has been carried out on the lipids of acanthocephalans. Only two species, *Macracanthorhynchus hirudinaceus* and *Moniliformis dubius* have been studied for their lipids (Beames and Fisher, 1964). The lipid content of helminths is generally high (Von Brand, 1966) ; this seems to have great physiological significance. Since our knowledge about acanthocephalan lipids is fragmentary, the present paper describes the results of correlative biochemical and histochemical studies on the lipids of *Centrorhynchus corvi*.

Material and methods.

The adult worms of *Centrorhynchus corvi* Fukuii, 1928 (Acanthocephala) were obtained from the posterior ileum of the crow *Corvus splendens*. Worms obtained from several specimens of *C. splendens* were pooled. After washing in normal saline, the male and female worms were separately weighed and homogenized in cold methanol. The homogenates were extracted for total lipids by the method of Folch, Lees and Sloane-Stanley (1957), then washed with a solution of NaCl. The aliquots were evaporated to dryness under reduced pressure and weighed.

Lipid saponification was carried out by the methold of Mangold (1965). After evaporating the methanol, the unsaponifiable fraction was extracted from the aqueous

phase in three washes of 1 volume of diethyl ether (Frayha and Fairbairn, 1969). The ether was evaporated to assess the amount of unsaponifiable lipid ; this lipid was then fractionated with TLC using chloroforme : benzene (50 : 50 V/V) as a solvent. The amount of triglycerides was ascertained as glycerol after alkaline hydrolysis (Van Handel and Zilversmit, 1957) ; free fatty acid content was found by the photometric method of Chakrabarty, Bhattacharya and Kundu (1969). Phospholipids were determined as phosphorus using the method of Allen as given in Kates (1972).

Fractionation of lipids was carried out with ascending thin layer chromatography (TLC), using petroleum ether :diethyl ether :acetic acid (80 : 20 : 1, V/V/V) and chloroform :methanol : 7N ammonia (65 : 25 : 4, V/V/V) for nonpolar and polar lipids, respectively (Kates, 1972). The developed chromatoplates were air dried and detection of individual lipid component was carried out with the help of different spray reagents including sulphuric acid, cupric acetatephosphoric acid, ninhydrin, Dragendorff, phospholipid spray reagent and α-naphtol as given in Kates (1972). Rf values of individual lipid components were compared with those of standards.

For the histochemical localization of lipids, complete worms and their pieces were fixed in formol-calcium and postchromed in dichromate-calcium. Frozen gelatin sections were subjected to various histochemical tests for lipids (as cited in Pearse, 1968), which included Sudan Black B in 70 p. 100 ethanol for general lipids, oil red O and Fettrot 7B for neutral lipids, acid haematein for phospholipids and lipoproteins, and Fishler's method for free fatty acids. Material fixed in weak Bouin's fluid after pyridine extraction was used as a control for lipids in general.

Results.

Lipid composition.

Table 1 gives the lipid composition of male and female specimens of *C. corvi* on wet weight basis of the tissue. The figures in the table are the mean values of three determinations. The males contain relatively more lipids than the females ; however, the nature of the lipids was the same in both sexes. TLC of lipids revealed the free sterols, sterol esters, diglycerides, triglycerides and free fatty acids in the neutral

TABLE 1

Lipid composition of centrorhynchus corvi

	Female *C. corvi* (6.841 g. wet wt.)			Male *C. corvi* (3.136 g. wet wt.)		
	Lipid wt. (mg)	p. 100 wet wt.	p. 100 Total lipids	Lipid wt (mg)	p. 100 wet wt.	p. 100 Total lipids
Total lipids	297	4.34	100	161	5.12	100
Phospholipids	30	0.44	11.1	22	0.70	13.6
Unsaponifiable	42	0.61	14.1	31	0.98	19.2
Triglycerides	156	2.28	52.3	86	2.42	53.4
Free fatty acids	48	0.70	16.1	23	0.89	14.3

fraction and lysophosphatidylcholine, lysophosphatidyl-ethanolamine, phosphatidyl-ethanolamine and glycolipids in the polar fraction. Phospholipids formed 11.13 p. 100 and 13.6 p. 100 of the total lipids in female and male worms, respectively, the remainder being neutral fat. Triglycerides constituted the major lipid component of the worm. The unsaponifiable lipids formed 14.1 p. 100 and 19.2 p. 100 in females and males, respectively. TLC of unsaponifiable lipids revealed the occurrence of three components with different mobilities ; one was cholesterol but the other two could not be identified.

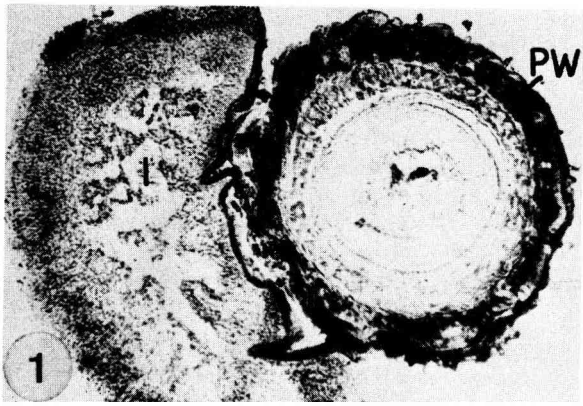
Lipid histochemistry.

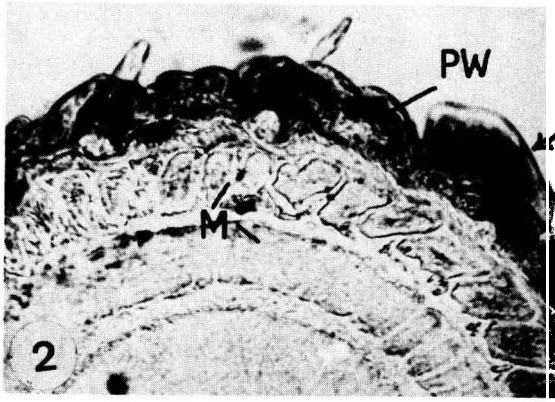
The proboscis wall, lemnisci and radial layer of the metasomal tegument showed heavy accumulation of sudanophilic lipids (fig. 1, 2, 3, 4). Deeply sudanophilic lipid droplets of these structures coalesced to form large lipid deposits. The part of the host intestine attached to the worm proboscis showed relatively less lipid (fig. 1) than the uninfested tissue. The outermost surface covering the proboscis wall showed deep sudanophilia (fig. 2), mainly to phospholipids and lipoproteins. The fibrous network of the epicuticle and cuticle of the metasomal tegument contained sudanophilic material which resisted pyridine extraction and was acid haematein-positive, revealing its lipoprotein nature (fig. 5, 6). The walls of the pore canals in the striped layer stained for lipids (lipropoteins). Some neutral lipid material was observed in the pore canals (fig. 6). Small lipid droplets were present at the base of pore canals ; the number and relative size of these lipid droplets increased in the felt layer and formed a heavy accumulation in the radial layer. The giant nuclei, lacunar canals and connective tissue showed the absence of lipids. In the neck region and anterior metasoma, some coarse neutral lipid droplets reactive for triglycerides were present in the outer part of the tegument ; the radial layer of these sites was devoid of any lipid material (fig. 3).

Relatively less lipid was observed in the proboscis musculature than in that of the body wall, the latter also showing the presence of abundant triglycerides and fatty acids. The lipids of the pseudocoel were also preserved in frozen sections of complete worms which reacted mainly for triglycerides (fig. 4). The oogonial syncytia in the ovarian balls showed heavy deposition of neutral fat (fig. 5) ; the detailed lipid histochemistry of the ovarian ball has already been described (Parshad and Guruya, 1977*a*). The developing acanthors reacted feebly to diffuse lipids and the inner shell envelopes contained lipoproteins. The testis showed diffusely distributed sudanophilia, and the amount of lipid demonstrable with histochemical techniques was apparently less than that of the ovarian balls.

Discussion.

As reported for other acanthecophalans, the males of *C. corvi* contain more lipid than the females (Beames and Fisher, 1964) ; no qualitative difference in the lipid composition was recorded. The interspecific difference in the amount of lipids is much less in *C. corvi* than in *Moniliformis dubius* and *Macracanthorhynchus hirudinaceus*. In the latter two species, the males contain almost twice as much lipid as the females (Beames and Fisher, 1964). Even though the reproductive organs of the male *C. corvi* show relatively less lipid demonstrable with histochemical techniques, the


PW
1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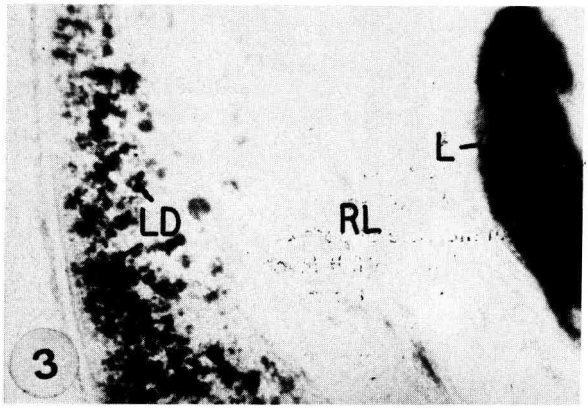
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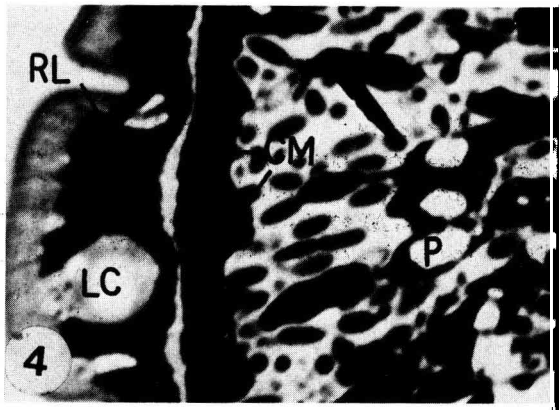
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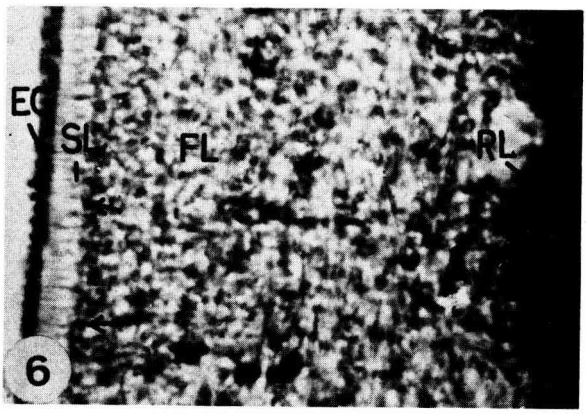
EC
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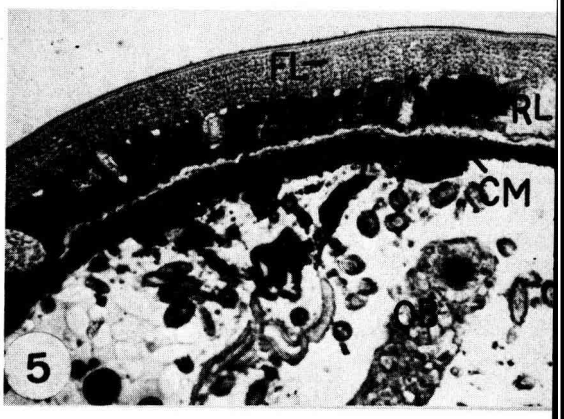
FL
RL
CM
5

males contain more lipids. This divergence in male and female lipids may thus be related to some metabolic differences in the sexes. The high fat content observed in *C. corvi* and other Aschelminthes (Fairbairn, 1969) suggests that lipids are of some special metabolic significance in these organisms. According to Fairbairn (1969), nematodes and acanthocephalans, parasitic in animals, may rely heavily on their hosts for many lipid components either to adaptation to such a constant supply or because their environment may be unsuitable for biosynthesis. More than 50 p. 100 of the total lipids in *C. corvi* were constituted by glycerides accumulating in the proboscis wall, radial layer of the metasoma, tegumentary muscles, lemnisci, pseudocoel and the ovarian balls. Triglycerides represent an important source of animal fuel ; their use in acanthocephalans is not of much significance (Korting and Fairbairn, 1972). In the absence of a distinct protonephridial system in *C. corvi* (Parshad and Guraya, 1977*b*), the occurrence of heavy neutral lipid accumulations may be related to unexcreted waste products, which are relatively less toxic under anaerobic conditions (Von Brand, 1966).

Hammond (1968) reported lipid excretion in the proboscis wall. The morphological distribution of lipids in the proboscis wall and anterior region of the metasoma suggests lipid absorption at those sites ; the present study does not exclude the possibility of lipid excretion at those sites. The absorption of lipids through the presoma body wall is further supported by the decrease in the amount of lipids in the infested region of the intestine (Varute and Sawant, 1972) and the presence of lipase in the body wall having intense activity in the proboscis (Bullock, 1949). The presence of some lipid material in the pore canals and at their lower ends, suggests the absorption of lipids through the metasomal tegument. This is further confirmed by earlier observations on the absorption of stained fat in *Acanthocephalus renee* (Fairbairn, 1969). The presence of lipase at absorptive sites indicates that triglyceride lipolytic products are absorbed. As also reported for other helminths (Barrett *et al.*, 1970), sterols cons-

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FIG. 1. — *Section through the proboscis and part of the large intestine* (I) *of the host.* Note the presence of heavy lipid accumulation in the proboscis wall (PW). Sudan black B (SBB) × 400.

FIG. 2. — *Higher power view of fig.* 1 *showing sudanophilic covering (arrow) of the proboscis wall* (PW) *and a few scattered lipid droplets in the muscles* (M). SBB × 1000.

FIG. 3. — *Slowing the presence of coarse lipids droplets* (LD) *in the outer part of the tegument of neck region and heavy accumulation of lipids in the lemniscus* (L). Note the absence of lipids in the radial layer (RL). Oil red 0 × 1000.

FIG. 4. — *Showing the presence of lipid deposits in the radial layer* (RL), *circular muscles* (CM) *and the pseudocoel* (P). Note the absence of lipids in the lacunar canal (LC). Oil Red 0 × 100.

FIG. 5. — *Showing the presence of lipids in the radial layer* (RL), *muscles* (CM) *and ovarian balls* (OB). SBB × 100.

FIG. 6. — *Showing the presence of sudanophilic lipid material in the epicuticle* (EC) *and in the pore canals of striped layer* (SL). The lipid droplets which are present at the base of pore canals (arrow) show increased size in the felt layer (FL). SBB × 1000.

tituted the major fraction of unsaponifiable lipids in *C. corvi*. These lipids showed two unidentifiable sterols in addition to cholesterol, while GLC analysis of *M. hirudinaceus* revealed 5 types of sterols (Barrett *et al.*, 1970). These sterols are of exogenous origin since anaerobic worms are unable to synthesize them (Meyer and Meyer, 1972).

The present histochemical study revealed regional differences in lipid distribution at various sites in the body wall ; this can be related to the absorption or excretion of lipids. The lacunar canal system of *C. corvi* is negative for lipids in contrast to other species in which they are commonly present (Fairbairn, 1969). The nature of the neutral lipid fraction in the present species resembles that found in other acanthocephalans (Beames and Fisher, 1964). However, it differs in its phospholipid composition. These differences may be attributed either to species variations or, in some, way, to host metabolism, or to both.

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Résumé. Les lipides de *Centrorhynchus corvi* (Acanthocephale) ont été étudiés par des techniques biochimiques et histochimiques. La teneur est plus élevée chez les mâles que chez les femelles (5, 12 et 4,34 p. 100), mais nous n'avons pas trouvé de différences qualitatives. Les phospholipides des vers mâles et femelles s'élèvent respectivement à 11,1 et 13,6 p. 100 du total des lipides, le reste des lipides étant constitué de graisses neutres. On trouve, respectivement chez les vers mâles et femelles : 52,3 et 53,54 p. 100 de triglycérides, 14,1 et 19,2 p. 100 de lipides insaponifiables et 16,1 et 14,3 p. 100 d'acides gras libres. Nous avons étudié leur localisation par des techniques histochimiques. Le rôle de la paroi du corps dans l'absorption et l'excrétion des lipides est discuté.

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