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LIPID, BILE ACID, WATER AND DRY MATTER CONTENT OF THE INTESTINAL TRACT OF DOMESTIC DUCKS WITH REFERENCE TO THE HABITAT OF *POLYMORPHUS MINUTUS* (ACANTHOCEPHALA)

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INTRODUCTION

The data presented in this paper were obtained to define more accurately the environment of *Polymorphus minutus*, an acanthocephalan parasite of ducks. This parasite inhabits a region of the duck's intestine extending from 60% to 80% of the intestinal length (Crompton & Whitfield, 1968).

In the case of *Moniliformis dubius*, an acanthocephalan parasite of rats, Edmonds (1965) found that nutrients are absorbed by the parasite from the host's intestinal lumen rather than the host's intestinal wall with which the parasite makes contact. Thus, nutrients in the rat's intestinal lumen are of prime importance for the growth of *M. dubius*. This conclusion can probably be applied to *P. minutus* and other Acanthocephala since the portion of the body wall of these parasites which is exposed to the intestinal lumen is better adapted for nutrient absorption than that embedded in the intestinal wall (Crompton & Lee, 1965). Consequently, analysis of the substances and conditions in the lumen of the region of the intestine inhabited by *P. minutus* defines the food and other ecological factors in the parasite's environment.

In these investigations the total material, lipid and bile acids in five regions of the duck's intestine have been studied. The results enable comparisons to be made between the environment of *P. minutus* and the other regions of the intestine not favoured by the parasite. Such an approach may provide information about the factors affecting the parasite's selection of its environment. The study of the lipid and bile acid concentrations was also undertaken to obtain evidence about the sites on absorption of the products of lipid digestion, and bile acids. Fats and bile acids are known to be closely associated in the intestine during digestion, but evidence from studies *in vitro* indicates that fatty acids are absorbed more anteriorly in the intestine than bile acids (Wilson, 1962).

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MATERIALS AND METHODS

General procedures

Khaki Campbell ducks, varying in age from 8 to 11 weeks, and of known sex and weight, were allowed to feed *ad libitum* on water and a ration similar in composition to any of those used commercially. Details of the composition of the ration are given by Crompton & Nesheim (1969). Chromic oxide was added to the ration as a marker substance at a level of 0·3 % of the dry weight of the ration. The method described by Nesheim & Carpenter (1967) was used to determine the amount of chromic oxide present in the intestinal samples. The ration was found to be satisfactory for the growth of the ducks and *P. minutus*, which developed as described by Crompton & Whitfield (1968).

The procedure used for collecting samples from the duck's intestine was the same as that given by Crompton & Nesheim (1969). After a duck had been killed, its intestine was stretched out and divided into five sections designated A, B, C, D and E in the text and figures of this paper. Section A extended from 0 to 20 % of the intestinal length, section B from 20 to 40 % of the intestinal length, and so on. The environment of *P. minutus* is located in section D.

Estimations of the total material in the different sections were made by weighing the samples collected from 40 ducks. The water content of each section from 10 of these ducks was determined by weighing the samples before and after they had been subjected to freeze-drying for 24 hr.

The lipid content of each section of the intestine was determined by weighing the lipid extracted from the samples by the method of Renner & Hill (1960) except that a modified Soxhlet apparatus was used. This method involves acidification of the sample prior to solvent extraction so that fatty acids present in intestinal contents as soaps could also be measured. Intestinal samples from each section of 10 ducks were collected, combined, weighed, freeze-dried and re-weighed before the extraction began. The results were expressed as percentage lipid of the dry weight of the total material of the section concerned.

Procedures for bile acids

Bile acid concentrations in the intestinal contents were determined by an isotope dilution procedure involving the use of ^{14}C (carboxyl) cholic acid (New England Nuclear Corp.) and gas–liquid chromatography. The specific activity of the radioactive cholic acid used was 14·5 mC./mM. One ml. of 0·9 % saline, containing 5 μC. of ^{14}C-cholic acid, was injected intraperitoneally into each of five ducks to label their bile acids. The ducks, which were 8½ weeks old, were confined in separate cages and given food and water *ad libitum*. Thirty-six hours later the ducks were killed, their gallbladders were ligatured and removed and samples from the five intestinal sections were collected and weighed.

The radioactivity in the intestinal contents was recovered by means of a saponification procedure followed by extraction with lipid solvents. The sample from each intestinal segment was placed in a 500 ml. round bottom flask with 2 ml. of 2 N-NaOH and several drops of octanol. The mixture was gently warmed until foaming stopped,

50 ml. of 95 % ethanol were added and the sample was saponified for 3 hr. at 15 lb./in.^{2} in a 16 l. pressure cooker. Next, the sample was acidified with concentrated HCl to pH 2 before being extracted with a solution of chloroform and methanol (2:1, v/v) as described by Grundy, Ahrens & Miettinen (1965). After extraction, the volume of the lipid solvent was adjusted to 25 ml. and the radioactivity of suitable aliquots was determined with a scintillation counter (Nuclear Chicago Model 6804) using the scintillation fluid of Bray (1960). The radioactivity of the bile acids from the ducks' gallbladders was also measured after the bladders had been minced and refluxed with 95 % ethanol for 3 hr.

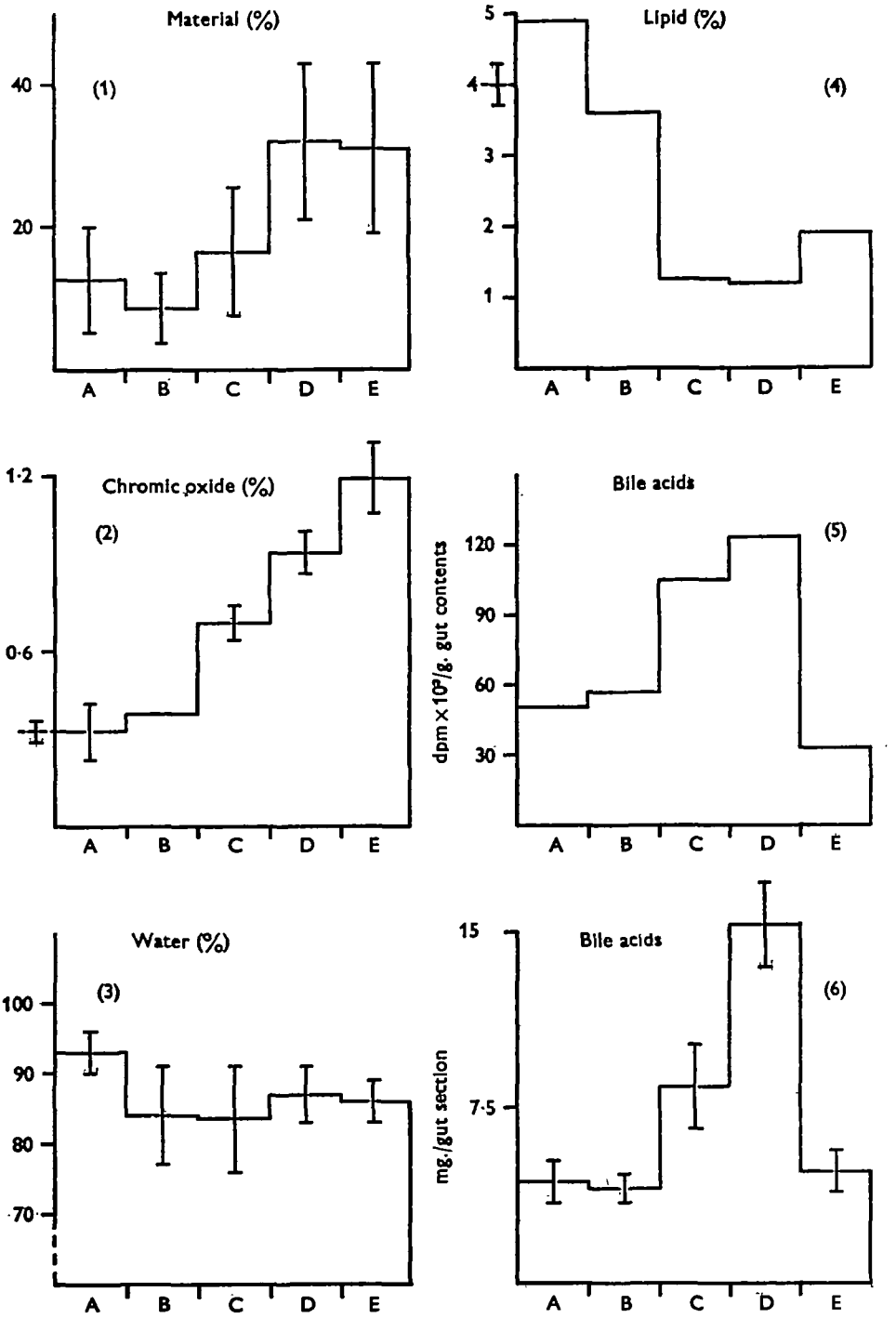
Determination of the amounts of bile acids present in the gallbladder extracts was by means of gas–liquid chromatography of the silyl ethers of the methyl esters of bile acids. The methods for the formation of these compounds have been given by Grundy *et al.* (1965) and modified slightly by Serafin (1968). The conditions for gas–liquid chromatography were the same as those used by Serafin. In order to enable the specific activity of the bile acids to be estimated the radioactivity was measured in samples of the methyl esters of the bile acids before they were converted to silyl ethers.

Serafin (1968) encountered difficulty in determining directly the quantities of bile acids in poultry faeces. Consequently, the concentration of bile acids in an intestinal segment was estimated by dividing the dpm/mg. total bile acid of the gall-bladder of a given bird, into the radioactivity recovered from each intestinal segment of that bird. This procedure is based on the assumption that the specific activity of bile acids in the gallbladder will be the same as that in the intestine and that cholic acid is not absorbed or excreted at different rates from other bile acids present.

RESULTS

Some of the results of the estimations described in this paper are represented diagrammatically in Figs. 1 to 6. The histogram in Fig. 1 shows that material in the intestinal lumen accumulates in the posterior part of the intestine in spite of the fact that nutrients are absorbed more anteriorly (Wilson, 1962). Further evidence for the accumulation of material in the posterior part of the intestine and the absorption of substances in the anterior portion is provided by studying the concentration of chromic oxide in the intestine. The data in Fig. 2 show that the amount of chromic oxide increases in a linear manner along the intestinal length from 0·3 % in section A to approximately 1·2 % in section E. Thus, the chromic oxide content of the total material in the intestine increases by a factor of 4 during the movement of material along the tract while the amount of material itself increases by a factor of less than 3 (Fig. 1). The chromic oxide, therefore, is gradually becoming more concentrated because nutrients are being absorbed. Much of the material in the posterior part of the intestines, including the section inhabited by *P. minutus*, probably consists of indigestible material which will be discharged in the duck's faeces.

The percentage of water in each section of the intestine is shown in Fig. 3. Apart from the result for section A, the water content for the other sections is very similar, with a mean value of 85 % for each section. The mean value of the water content in section A was 93 %, which is significantly different from that of the other sections



Figs. 1–6. Histograms showing the distribution of various substances in the intestines of ducks. (1) percentage total material, (2) percentage chromic oxide, (3) percentage water in total material, (4) percentage lipid of dry weight of total material, (5) distribution of radioactivity associated with bile acids in intestine and (6) quantitative distribution of bile acids in the intestine. On the abscissae of the histograms, A, B, C, D and E are the intestinal sections from which samples were obtained. Each section is a fifth of the intestinal length and section D represents the environment of *Polymorphus minutus*. Dotted lines on Figs. 2 and 4 represent dietary levels.

($P < 0.05$). Thus the duodenum and anterior part of a duck's intestine appear to contain more water than the remainder of the tract.

The amount of lipid in the intestine, expressed as a percentage of the dry material in each section, is shown in Fig. 4. The lipid content of the duodenum appears to be slightly greater than that of the diet, an observation which suggests that endogenous lipid, possibly from cellular breakdown, mixes with the diet in the anterior part of the

Table 1. *Details of the bile acids recovered from the intestines of five ducks described in Table 2*

Duck no.		Section of intestine A	B	C	D	E	Totals
1	Sample weight (g.)	1·689	1·280	0·955	2·619	3·266	9·809
	Radioactivity recovered (dpm)	60637	117687	347737	—	149900	675961
	Bile acids recovered (mg.)	3·55	6·89	20·36	—	8·78	39·58
2	Sample weight (g.)	2·455	1·049	1·023	2·451	1·559	8·537
	Radioactivity recovered (dpm)	84487	23137	75081	417970	81012	681687
	Bile acids recovered (mg.)	3·36	0·92	2·98	16·61	3·22	27·09
3	Sample weight (g.)	2·884	2·667	3·898	5·018	5·562	20·029
	Radioactivity recovered (dpm)	89387	96125	167137	151325	169712	673686
	Bile acids recovered (mg.)	4·49	4·83	8·40	7·61	8·53	33·86
4	Sample weight (g.)	0·813	1·209	0·596	1·488	1·112	5·218
	Radioactivity recovered (dpm)	229187	110687	82137	524562	52637	999210
	Bile acids recovered (mg.)	9·72	4·70	3·49	22·26	2·23	42·40
5	Sample weight (g.)	1·703	1·626	1·876	5·594	3·155	13·954
	Radioactivity recovered (dpm)	27500	90325	213950	—	34987	366762
	Bile acids recovered (mg.)	0·93	3·06	7·24	—	1·18	12·41

Table 2. *Details of the gallbladders and associated bile acids*

Duck no., sex and weight	Wet weight (g.)	Radio-activity recovered (dpm)	Total amount of bile acids recovered (mg.)	Specific activity of bile acids (dpm/mg.)	Identity and % composition of bile acids MC	MCC	MLC	Others
1, ♂ 1830 g.	3·822	5596200	327·61	17082	21·5	52·8	18·6	7·1
2, ♂ 1895 g.	2·435	3889600	154·56	25166	18·5	57·0	16·6	7·9
3, ♂ 1825 g.	2·927	5084100	255·66	19886	15·0	54·6	19·2	11·2
4, ♀ 1645 g.	2·236	3297800	139·93	23567	15·4	58·6	14·9	11·1
5, ♀ 1550 g.	1·940	3542900	119·85	29561	11·1	71·6	13·7	3·6
Total	13·360	21410600	997·61	108262	81·5	294·6	83·0	40·9
Mean	2·672	4282120	199·52	21652	16·3	58·9	16·6	8·2
S.D.	0·658	623300	65·53	4530	3·504	6·651	2·124	2·28

MC = methyl cholate; MCC = methyl chenodeoxycholate; MLC = methyl lithocholate

intestine. The fact that the lipid content is lower in section B than in section A and is at a very low value in section C suggests that the major site of lipid absorption is in the anterior half of the duck's intestine.

The distribution of bile acids in the duck's intestine is shown in Figs. 5 and 6 and further data about the identity of the bile acids and their concentrations in the five sections of the intestine are given in Tables 1, 2 and 3. It can be seen from Figs. 5 and 6 that most radioactivity associated with bile acids, and the largest amounts of bile acids, are present in section D of the intestine. The concentration of bile acids falls

sharply in section E, an observation which indicates that bile acids are absorbed in the posterior part of the intestine in a region different from that where the products of lipid digestion are absorbed (Fig. 4). Recently, Dietschy, Salomon & Siperstein (1966) have demonstrated the active uptake of bile acids in the posterior portion of the rat's intestine *in vitro* and *in vivo*.

The fact that nutrients are absorbed more anteriorly along the intestine will result in the bile acids becoming more concentrated in the posterior sections in a similar manner to that discussed above for chromic oxide (Fig. 2). Inspection of the data in Table 3 shows that the amount of bile acid per g. of intestinal material increases from section A to section D and eliminates the possibility that most bile acid is present in section D because most material is contained in that section (Fig. 1).

Table 3. *Estimated distribution of bile acids in the intestines of domestic ducks*

	Mean amounts of bile acids		Mean wet weight of section contents	Amount of bile acids/section							
				Cholic		Chenodeoxycholic		Lithocholic		Others	
	Mg.	Mg./g.	(g.)	Mg.	Mg./g.	Mg.	Mg./g.	Mg.	Mg./g.	Mg.	Mg./g.
Section A	5·86	3·42	1·714	0·94	0·55	3·45	2·06	1·0	0·58	0·47	0·27
Section B	3·48	2·12	1·641	0·56	0·34	2·06	1·25	0·59	0·36	0·27	0·16
Section C	5·62	3·06	1·838	0·90	0·49	3·32	1·81	0·95	0·51	0·45	0·24
Section D	15·49	5·18	2·986	2·48	0·83	9·14	3·06	2·63	0·87	1·24	0·42
Section E	4·66	1·70	2·744	0·74	0·27	2·75	1·00	0·79	0·29	0·38	0·14

The values in this table are calculated from data applying to ducks 2, 3 and 4 (Table 2) on the assumption that the percentages of cholic acid, chenodeoxycholic acid, lithocholic acid and other bile acids are about 16, 59, 17 and 8 of the total (Table 1).

Nineteen determinations were made with gas–liquid chromatography to determine the concentration of bile acids from the gallbladder extracts of the five ducks. Three major peaks appeared on the gas chromatograph; these peaks correspond to the retention times of the silyl ethers of the methyl esters of cholic acid, chenodeoxycholic acid and lithocholic acid, the mean percentage of each of these acids in the bile acid fraction of duck bile being given in Table 2. Cholic acid, chenodeoxycholic acid and litho-cholic acid form about 16, 59 and 17%, respectively, while some other components, which have not yet been identified, form about 8% of the bile acid fraction. The data for cholic acid, however, may be less accurate than the data for the other bile acids since the cholic acid peak did not appear to be homogenous and was often composed of two partially superimposed peaks.

DISCUSSION

The results reported in this paper apply to young ducks which were allowed to feed *ad libitum* on a diet similar to that produced commercially. The results may not apply directly to wild ducks which will probably feed at set times of day and be expected, therefore, to have intestinal conditions like those depicted in Figs. 1 and 6 for a relatively short time while food is being digested and absorbed.

The fact that material accumulates in the posterior part of the intestine in spite of the fact that nutrients are being absorbed supports the view that peristalsis is weaker

posteriorly than anteriorly (Bell, Davidson & Scarborough, 1959). The opening and closing of the cloacal sphincter, which controls defecation, will also contribute to the accumulation of roughage in the posterior part of the intestine. Many parasitic helminths are adapted for attachment to their host's intestinal wall and the literature implies that peristalsis is a major hazard for them. Parasites in the posterior part of the intestine, like *P. minutus*, may be in more danger from damage from all the abrasive roughage collecting in the environment than from being swept away by peristalsis. Abrasive material in the environment of *P. minutus* is likely to be a serious hazard when the parasite is living in wild ducks, including those species like Eider, *Somateria mollissima*, which eat molluscs and crunch shells.

The identification of chenodeoxycholic acid as the main bile acid of these ducks is in agreement with the report that this is the major bile acid in all the birds examined (Haselwood, 1964). It may be assumed that the duck's bile acids are present in the intestinal lumen as sodium salts of conjugated bile acids. In chicks, most ^{14}C-cholic acid has been shown to be conjugated shortly after injection (Serafin, 1968). No attempt was made to identify the conjugate present during the present study on ducks, but literature reviewed by Haselwood suggests that conjugation with taurine is more likely than with glycine.

Bile is known to be involved in the following aspects of host-parasite relationships, (1) excystation of coccidian parasites, (2) hatching of cestode eggs, (3) evagination of cestode scolices, (4) carbohydrate metabolism of cestodes, (5) excystment of the metacercaria of digenean trematodes, (6) growth, and (7) lysis of parasites (Smyth & Haselwood, 1963). Some of these effects may be direct in which the bile acts on the parasite itself; and some may be indirect, in which the parasite responds to conditions produced by the influence of the bile on the host's digestive processes. Since bile is a mixture of substances, bile acids cannot yet be assumed to be involved in all the effects listed above. The work of Smyth (1962) and Smyth & Haselwood (1963) on protoscolices of *Echinococcus granulosus*, however, has shown that this helminth does not become established in many mammals because the bile salts of the refractory hosts lyse the protoscolices. The normal hosts of *E. granulosus* secrete bile in which the salts are either ineffective against the protoscolices or below the threshold level for lysis. Smyth (1962) suggested that bile acids could be responsible for determining the host specificity of a given parasite.

Smyth's hypothesis may apply to *P. minutus*, although this parasite has been reported from more than 80 avian hosts. The concentration and nature of the bile acids in the parasite's environment, however, may be very important in determining the range of organisms which can exist there and, therefore, in regulating the potential competitors of the parasite. The mean concentration of a duck's bile acids in the environment of *P. minutus* is 5·18 mg./g. wet weight of gut contents (Table 3). This concentration probably represents about a twenty-fold dilution of the concentration in duck bile, assuming that concentration of bile acid in duck bile is similar to that in human bile (Smyth & Haselwood, 1963). When living *P. minutus* are placed in freshly collected duck bile *in vitro*, movement stops and the parasite's body wall appears to dissolve within 1 to 2 hr. Specimens of *P. minutus* in duck bile diluted twenty-fold with Hanks's saline show no adverse effects after 8 hr. *in vitro* (D. W. T. Crompton, unpublished observations). It would appear, therefore, that any parasite which is a

potential competitor of *P. minutus* must be either unaffected by duck bile acids or able to withstand concentrations of 5 mg./g. of intestinal contents.

Recently, Graff & Kitzman (1965) have found that bile is essential for the establishment of the acanthocephalan *M. dubius* in the intestine of rats, and that bile salts are necessary for the activation of cystacanths *in vitro*. The obvious conclusion from this work is that the bile acids of the rat bile are vital for the establishment of the parasite, since rats become infected by eating cystacanths. A similar investigation of the establishment of infections of *P. minutus* in ducks could now be undertaken, particularly since the host's bile acids have been identified and estimated from ducks kept under conditions which can support the parasite.

SUMMARY

1. The distributions of total material, water, lipid and bile salts in the intestine of domestic ducks feeding *ad libitum* have been determined.

2. Most material accumulates in the posterior part of the intestine and the duodenal region appears to be wetter than the rest of the intestine.

3. When ducks feed on a diet with a lipid content of 4% of the dry weight, the highest concentration of lipid detected in the intestine was 5% of the dry weight of the ntestinal material. Lipid appears to be absorbed in the anterior half of the intestine.

4. Bile acids accumulate in the posterior part of the intestine where concentrations as high as 5·18 mg./g. of intestinal contents were detected. The evidence indicates that bile acids are absorbed in the posterior part of the intestine.

5. The bile acids of bile from the gallbladders of ducks have been identified by means of gas–liquid chromatography. Cholic acid, chenodeoxycholic acid, lithocholic acid and some unidentified substances were found to form 16, 59, 17 and 8%, respectively, of the bile acids.

6. The results are discussed with reference to *Polymorphus minutus*, an acanthocephalan parasite of ducks.

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