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CHOLESTEROL ABSORPTION IN THE ROACH, *PERIPLANETA AMERICANA*

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The absorption of cholesterol in the alimentary canal of the roach, *Periplaneta americana*, takes place predominantly in the oesophagus and crop followed by the gizzard and gastric caeca. The cholesterol which was esterified in the gut increased gradually in some tissues with increase in time.

INTRODUCTION

Insects are unable to synthesize sterols which are therefore essential components of their diet (CLAYTON, 1964). The sterol requirement of all insects can be met with dietary cholesterol (DADD, 1973). The insects are apparently able to absorb the dietary sterols from their alimentary canal. Detailed studies on the absorption of sterols in mammals are on record (WISEMAN, 1964), but such studies in insects are, however, greatly inadequate. The absorption of cholesterol and other sterols has been investigated in the roach, *Eurycotis floridana* (CLAYTON *et al.*, 1964) where it occurs predominantly in the crop and to some extent in gastric caeca. In the silkworm larvae, *Philosamia cynthia*, the ingested cholesterol is absorbed from the gut wall (CHINO & GILBERT, 1971). However, according to HOUSE (1974), lipids are generally not absorbed in the crop of insects. As the main site of cholesterol absorption in insects is not very clear, studies were undertaken on the cholesterol absorption in *P. americana*.

MATERIALS AND METHODS

Adult male American cockroaches reared and maintained on rat food were used in these experiments. The insects were used within 4 months after the final moult. The experimental insects were starved for 24 hours prior to the test meal. The test meal consisted of a paste of casein and glucose (1:2). Cholesterol (25 μg) and 0.058 μCi cholesterol-4-^{14}C (sp. act. 42.4 mCi/mM) were added dissolved in diethyl ether to the paste and mixed thoroughly. Ether was evaporated completely and the roaches allowed to feed individually. Almost all the test meal was consumed and the roaches were not given any food thereafter. The quantity of the left over food was 1.5 to 5.0 per cent of the diet. At different time intervals hemolymph was collected by cutting the antennae of the roaches and centrifuging them at a low speed. The quantity of haemolymph collected was measured. The roaches were dissected in cold saline and various parts of the alimentary canal were rinsed with cold saline and the unabsorbed food removed and the quantity determined. As the roaches were not fed with any more food, no faeces were obtained during the experimental period. Fat body was also collected. The tissues were extracted for lipids after homogenizing in chloroform : methanol (2:1, v/v) and the non-lipid contaminants washed by the method of FOLCH *et al.* (1957). The purified extracts were evaporated to dryness *in vacuo* and the residues were dissolved in a known volume of chloroform. Separation of lipid classes was achieved by thin layer chromatography using hexane, diethyl ether and acetic acid (90 : 10 : 1, v/v) as the developing solvent system. The spots were visualised by exposure to iodine vapours. The cholesterol and its esters were identified by using appropriate standards. The spots were scraped off and mixed with 10 ml of scintillation fluid (0.5% PPO and 0.05% POPOP in toluene) and the radioactivity determined in a Packard Liquid Scintillation

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TABLE 1. Cholesterol-4-^{14}C incorporation into the tissues of *P. americana* after feeding 25μg cholesterol and 0.058 μCi cholesterol-4-^{14}C per roach.

Time hrs	Cholesterol absorbed mμgm / mg tissue (wet wt.)								% Cholesterol left unabsorbed in intestine
	Oesophagus	Crop	Gizzard	Gastric caeca	Midgut	Hindgut	Fat body	Hemolymph	
1.0	201.9*	255	36.3	4.33	1.8	—	10.0	1.11	19.7
	3.78**	2.55	0.77	0.36	0.07	—	0.16	0.19	
	3.6+	11.2	1.6	0.23	0.1	—	4.9	0.24	
2.0	732	214	197	10.4	2.6	0.8	9.3	0.5	19.8
	7.0	1.6	0.12	0.21	0.2	—	0.03	0.05	
	10.1	17.3	6.2	2.7	0.1	0.1	0.9	0.19	
4.0	443	321	68.0	16.0	7.0	1	14.2	0.9	38.1
	5.2	2.03	0.83	0.23	0.26	0.16	1.2	0.05	
	6.5	13.2	2.8	0.7	0.14	0.1	3.9	0.28	
6.0	580	418	144.6	32.9	9.0	2.46	15.3	8.6	20.2
	9.8	3.86	4.6	0.05	—	0.03	0.91	0.09	
	3.5	17.5	5.2	1.5	0.16	0.08	4.6	1.6	
12.0	93	130	89.9	32.6	15.6	1.4	1.04	0.46	24.1
	26	1.09	0.19	0.34	0.96	0.1	0.09	0.07	
	0.58	6.01	3.6	2.5	0.2	0.09	0.38	0.17	
24.0	60	138.8	29.8	48.7	30.5	1.95	1.79	7.4	14.07
	18.3	1.25	0.37	0.07	0.15	0.03	0.01	0.22	
	0.72	7.1	1.1	2.5	0.8	0.02	0.9	1.65	

* Cholesterol

** Cholestrol ester

\+ Per cent cholesterol absorbed

Spectrometer (Model 3320). The efficiency of counting of individual samples was determined and the appropriate quenching corrections made.

Histochemical studies

The roaches were fed 5mg cholesterol each along with casein and glucose as described above. After 24 hours the various parts of the alimentary canal were dissected out and fixed in neutral formalin for one day and then gelatin blocks made according to the method of BARKA & ANDERSON (1963). The sections were cut at a thickness of 10 μm in a cryostat at -20°C. The sections were dehydrated in the usual manner, stained with Sudan Black B and mounted in glycerine jelly. The control roaches were fed on only casein and glucose and the tissues processed as above.

Ligation of the digestive tract

A ligation was attempted between the crop and the gizzard of the roaches. The method used was essentially the same as described by CLAYTON *et al.* (1964), except that the ligation was between the crop and gizzard in *P. americana*. The insects were given food and water *ad libitum* after the operation until they recovered. They were then starved for one week and given a diet of 20 mg ripe banana with 0.36 μCi of ^{3}H-cholesterol (sp. act. 7 Ci/mM). The ligature was tightened soon after the consumption of the test meal. The roaches were dissected after 24 hours and the radioactivity estimated as described above without separating the cholesterol esters from cholesterol. Any roach showing an improper ligature was discarded. The control roaches were treated in the same manner except that no ligature was involved.

RESULTS AND DISCUSSION

The data presented in Table 1 shows the absorption of cholesterol in different parts of the alimentary canal of *P. americana* at various time intervals after feeding. Of the cholesterol present in the diet only about 21% was absorbed in 1 hour which increased to about 38% in 2 hours. Twelve to 24 hours after feeding only about 14% of the cholesterol was detected in the tissues analysed (Table 1). From the data it appears that most of the cholesterol absorption had taken place by 12 hours after feeding.

The oesophagus and crop were the major sites of cholesterol absorption (Table 1). On the basis of per mg tissue, oesophagus had the highest cholesterol content. However, on the basis of total cholesterol content, the crop showed the maximum. In one hour's time the crop accounted for about 11.2 per cent of the cholesterol in the diet whereas oesophagus contained only 3.6 per cent. This is perhaps obvious as the crop is much larger than the oesophagus. The histochemical studies also showed that crop was the major site of cholesterol absorption in *P. americana* (Fig 1). The crop of the cholesterol fed roaches showed the presence of much more Sudan Black B positive material than the controls. The mid gut showed very little of such Sudan Black B positive material. The additional Sudan Black B positive material in the cholesterol fed roaches was apparently due to the cholesterol absorbed as the lipids including cholesterol are stained by Sudan Black B (BARKA & ANDERSON, 1963). The results of the digestive tract ligation experiments further confirm that the crop is a major site of cholesterol absorption (Table 2). It is seen that even in the roaches with ligation between the crop and the gizzard there was sufficient absorption and cholesterol and its distribution in various tissues even though it was slightly

TABLE 2. Per cent ^{3}H-cholesterol incorporation into the tissues of *P. americana*, with a ligature between the crop and the gizzard, 24 hours after feeding.

	% cholesterol absorbed	
	Control (3)	Roaches with ligature (3)
Crop	55.05	84.3
Gizzard	5.3	4.1
Gastric caeca	23.7	6.3
Midgut	5.0	2.3
Fat body	3.9	1.1
Hemolymph	1.7	1.8

less than the controls. In *Eurycotis floridana* crop was found to be the major site of cholesterol absorption. However, in this study apparently the oesophagus was either not considered at all or was taken along with the crop (CLAYTON *et al.*, 1964). In the larvae of the silkworm, *Philosamia cynthia*, the cholesterol absorption was shown to be in the midgut (CHINO & GILBERT, 1971). According to the earlier work, fats were believed to be absorbed in the crop of the roaches, but later work has shown this not to be true (HOUSE, 1974). It is apparently possible that cholesterol is absorbed in the crop of roaches, whereas other lipids are absorbed elsewhere in the gut.

The gizzard also contained a significant amount of cholestrol. The gastric caeca showed a continuous increase in the cholesterol content whereas in oesophagus, crop and gizzard, first there was an increase in the cholesterol content followed by a decline. Cholesterol was also found in the haemolymph and fat body showing that the absorption has indeed taken place (Table 1).

The predominant form of cholesterol absorbed was unesterified (Table 1). However, with the increase in time after feeding the cholesterol ester increased gradually at least in some tissues. The proportion of free cholesterol to the ester in *P. americana* was about 71:29 (VROMAN *et al.*, 1964). However, in the present studies the free cholesterol was much more than reported in the earlier study. The enzyme, sterol ester hydrolase is very active in the digestive tract of higher animals and is considered to be essential during the absorption of sterols (VAHOUNY & TREADWELL, 1968). In case of insects such enzyme studies have been carried out in the roach, *P. americana* where the cholesterol esterases were found in the homogenates of the various parts of the alimentary canal and which were active in both esterification and hydrolysis of cholesterol and its esters (CASIDA *et al.*, 1957). The cholesterol esterase also occurs in *Galleria mellonella* (CLEMENT & FRISCH, 1946). Recently AGARWAL & NAIR (1976) have studied cholesterol ester hydrolase in the larvae of *Trogoderma granarium* and found that the enzyme specificity is directly correlated with the utilization of various cholesterol esters by this insect. Further, enzyme inhibitors like cholesteryl methyl ether and cholesteryl chloride also inhibit the utilization of various cholesterol esters by this insect. However, the role if any, of these enzymes is not known in cholesterol absorption in insects. Besides, it will be of interest to know what is the major site of cholesterol absorption in different groups of insects.

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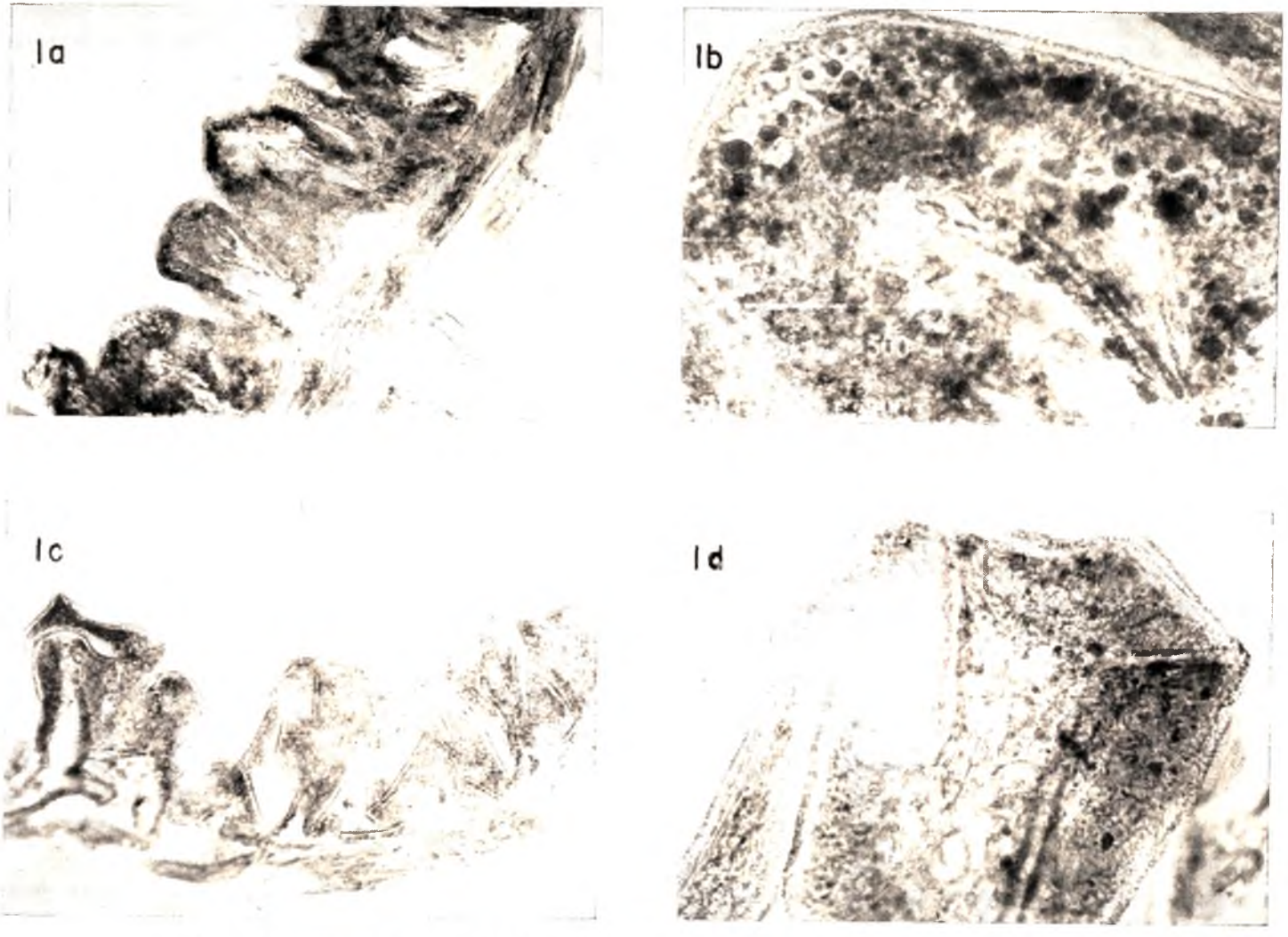

Fig. 1. Photomicrographs showing a part of the cross section of crop of *P. americana* stained with Sudan Black B. (a) Showing Sudan Black B positive material in the crop of roaches fed on cholesterol diet X 100. (b) Same as (a) X 400. (c) Showing Sudan Black B positive material in the crop of control roaches X 100. (d) Same as (c) X 400.