THE DIGESTION AND ABSORPTION OF TRIPALMITIN IN THE COCKROACH, *PERIPLANETA AMERICANA* L.

By J. E. TREHERNE

A.R.C. Unit of Insect Physiology, Department of Zoology, University of Cambridge

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INTRODUCTION

The digestion and absorption of fats in the cockroach has in the past been the subject of several extensive investigations. Plateau (1876), Sanford (1918), Swingle (1925), Abbott (1926), Wigglesworth (1928) and Schlottke (1937) have all made *in vitro* studies of fat hydrolysis with various species of cockroach. It is only relatively recently that an *in vivo* study has been made of the hydrolysis of some fats in the cockroach crop by Eisner (1955).

There has been considerable controversy as to the site of absorption of fats in these insects. Petrunkevitsch (1900) demonstrated histologically an accumulation of fat droplets in the crop epithelium following a fat meal, and concluded that this organ was of major importance in fat absorption. Subsequently, Sanford (1918) and Abbott (1926) obtained similar results and attempted to dispose of the objections made to this hypothesis by Cuénot (1896), de Sinéty (1901) and Schlüter (1912). More recently, Eisner (1955) has made a detailed study of the factors involved in the appearance of fat droplets in the crop epithelium following fat meals. All these investigations have been qualitative in nature and no quantitative study has been made of fat absorption in the alimentary canal of the cockroach. In the present investigation an attempt has been made to follow the digestion and absorption of a ^{14}C-labelled triglyceride using the technique previously employed to follow the absorption of glucose in *Periplaneta americana* L. Tripalmitin was available suitably labelled with ^{14}C and was chosen for use in this investigation. The glyceryl esters of palmitic acid are among those most commonly occurring in natural fats (Hilditch, 1947).

METHODS

As in a previous investigation (Treherne, 1957*b*), adult cockroaches were starved for 7 days in order to obtain individuals in which the alimentary canal was empty. They were given access to water, except for 24 hr. immediately preceding the experiment. In each experiment the starved individual was allowed to consume 0·10 ml. of an experimental fluid. After an appropriate experimental period the insect was killed by immersion in boiling water. The whole of the alimentary canal was immediately removed and the contents of the various parts squeezed into calibrated centrifuge tubes using the tips of fine pairs of forceps.

The experimental fluid contained 0·2 % ^{14}C-labelled tripalmitin (palmitate-1-C_{14}) which was suspended in a 2·0 % solution of the inert cellulose ester 'Cellofas B' (sodium carboxymethylcellulose). This substance produced a fluid of treacle-like consistency. In some experiments the tripalmitin was dissolved in oleic acid, a 10·0 % suspension being emulsified with 'Cellofas B'. The experimental fluid also contained 0·5 M/l. glucose and 0·008 M/l. of the dye Amaranth (Azo-Rubin S).

The gut contents were extracted in the centrifuge tubes with hot chloroform and, after cooling, the volume was adjusted to 1·0 ml. This solution was then pipetted off for assay of radioactivity and chromatographic analysis. 5·0 ml. of a solution buffered to pH 10·0 was then added to each tube to bring the dye into the solution. This was centrifuged at 4000 r.p.m. and the concentration of the dye in the supernatant was determined at pH 10·0, using a Unicam absorptiometer at an absorption maximum of 510 mμ. The radioactivity of the labelled material was assayed by determining the activity of measured volumes placed on lens papers (Reid, 1947), using a thin-windowed Geiger-Müller tube (G.E.C. CV 2139).

The dye Amaranth was used as a marker in these experiments, for it has been shown that it is not absorbed from the lumen of the cockroach gut (Treherne, 1957*b*). As in previous studies the dye has been used to measure transit in the gut and, by comparing the ratio of dye to radioactive substances in the various parts of the gut, to determine the net percentage absorption of tripalmitin and its derivatives.

The reverse-phase method of paper chromatography devised by Mangold, Lamp & Schlenk (1955) was used in an attempt to follow any hydrolysis of tripalmitin occurring in the gut lumen. For this purpose, Whatman No. 1 filter-paper was dried for at least 2 hr. at 120° C. and then drawn through a 5·0 % solution of silicone fluid (M.S. 200/10 c.) dissolved in ether. 2·0 μl. samples of radioactive material dissolved in chloroform were applied to the base-line on these papers. The chromatograms were developed by descending chromatography using the solvent systems chloroform/methanol and tetrahydrofuran/water (Mangold *et al.* 1955). Samples of tripalmitin and its derivatives were run as markers on the chromatograms. Palmitic acid was detected by treating the paper with 1·0 % copper acetate followed by 0·1 % Rhodamine B according to the method of Savary (1954). Tri- and dipalmitin were hydrolysed on the paper by spraying with 1·0 % pancreatin and incubating at 37·0° C. (Mangold *et al.* 1955), prior to the copper acetate treatment. Monopalmitin was detected by spraying the papers with a 1·0 % solution of lead tetra-acetate in absolute benzene (Mangold *et al.* 1955). To assay the chromatograms for radioactivity the papers were cut into strips and placed over a 1·0 cm. wide slit in a piece of Perspex, beneath which was a thin-windowed Geiger-Müller tube. Counts were made on successive 1·0 cm. wide areas until the whole of the strip had been assayed.

All the insects were reared and the experiments carried out at a temperature of 28·0 ± 1·0° C.

RESULTS

To investigate the extent of any hydrolysis of tripalmitin cockroaches were fed 0·1 ml. amounts of the experimental fluid containing ^{14}C-labelled tripalmitin. In one set of experiments 0·2% tripalmitin was suspended in the experimental fluid, in another the tripalmitin was dissolved in emulsified 10·0% oleic acid. The extent of the hydrolysis of 0·2% tripalmitin in oleic acid is illustrated in Fig. 1 for chromatograms developed with chloroform/methanol as the solvent system. At 2·0

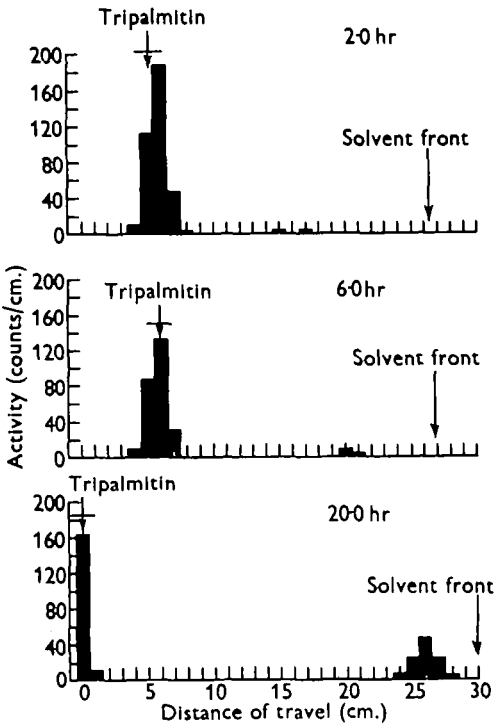

Fig. 1

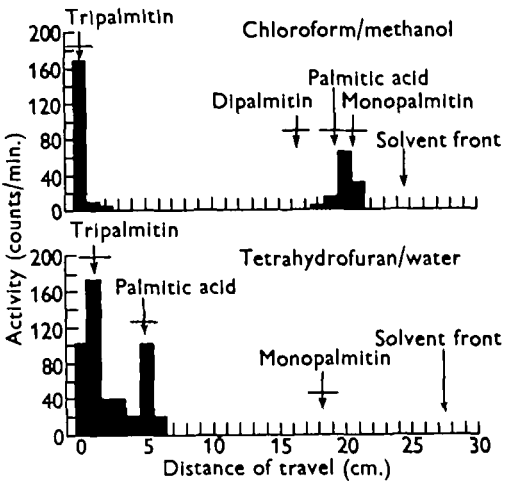

Fig. 2

Fig. 1. The distribution of radioactivity on paper chromatograms of the crop contents at various times after ingestion of ^{14}C-labelled tripalmitin. The solvent system used in these experiments was chloroform/methanol.

Fig. 2. The radioactivity on paper chromatograms of the crop contents, developed in two solvent systems, 20·0 hr. after the ingestion of ^{14}C-labelled tripalmitin. The symbols above the histograms represent the position and extent of control spots run adjacent to the experimental ones.

and 6·0 hr. after feeding most of the radioactivity was associated with the tripalmitin spot, which showed R_F values varying between 0 and 0·22. After 20 hr. a second, smaller, peak of radioactivity was obtained with an R_F value of 0·80–0·90. When control samples of tripalmitin, dipalmitin, monopalmitin and palmitic acid were run on chromatograms this second radioactive peak was associated with the palmitic acid spot in both solvent systems (Fig. 2). Substantially similar results were obtained

with tripalmitin suspended alone in the experimental fluid. The amount of radioactive material which could be recovered from the lumen of the mid-gut was not sufficient for a satisfactory chromatographic analysis.

Table 1. *The percentage of* ^{14}C *remaining incorporated as tripalmitin in the crop after an experimental period of* 20·0 *hr.*

Experiment	Serial	% tripalmitin	Mean ± S.D.
0·2 % tripalmitin	1	80·0	77·4 ± 10·1
	2	86·1	
	3	62·6	
	4	67·0	
	5	61·8	
	6	83·6	
	7	84·1	
	8	94·4	
0·2 % tripalmitin in oleic acid	1	63·6	77·7 ± 11·7
	2	75·9	
	3	79·2	
	4	92·0	

Table 1 records the proportion of ^{14}C in the crop which remained incorporated as tripalmitin after 20·0 hr. It will be seen that there was considerable variation in the extent of the hydrolysis as between different individuals. These results show that whether administered alone or dissolved in oleic acid the amount of unhydrolysed tripalmitin remaining in the crop after 20·0 hr. averaged approximately 77 % of the total.

The absorption of the ^{14}C-labelled tripalmitin was followed in some experiments in which starved individuals were fed 0·1 ml. amounts of the experimental fluids. The net percentage absorption of the labelled compounds was calculated from the radioactive-material/dye ratio in the various parts of the alimentary canal. The absorption was determined in insects which had been fed either tripalmitin suspended in the experimental fluid or tripalmitin dissolved in emulsified oleic acid (Figs. 3, 4). In both cases little absorption appeared to take place from the crop, but there was a very rapid disappearance from the lumen when the fluid entered the mid-gut region. The results obtained after 2·0 hr. indicate that absorption must be largely confined to the caeca and the anterior part of the ventriculus. Unfortunately it was impossible to separate the parts played by these two regions of the mid-gut using the present technique as the experimental fluid always appeared in them simultaneously.

Table 2 records the net percentage absorption of ^{14}C-labelled tripalmitin and its derivatives from the crop after a period of 20·0 hr. The negative values recorded resulted from experiments in which the ^{14}C recovered from the crop apparently exceeded the amount of dye present, these figures falling within the normal experimental error of the method. The results show that under these experimental conditions any absorption from the crop must be very small, certainly less than about 4 %, which is within the experimental error of the method.

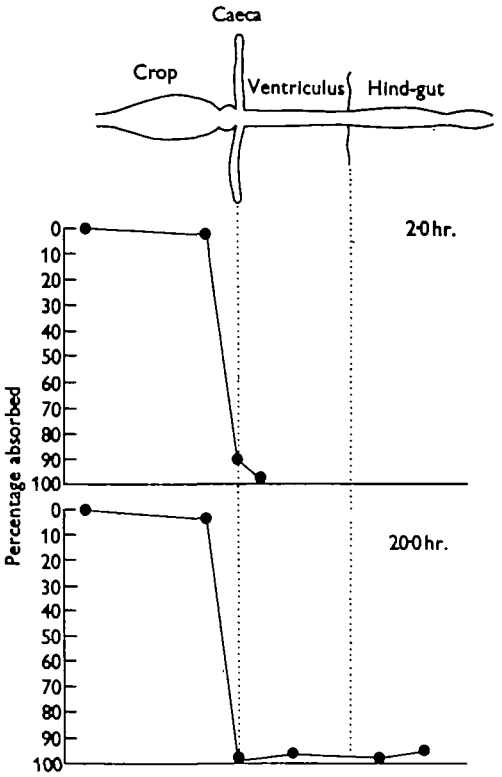

Fig. 3

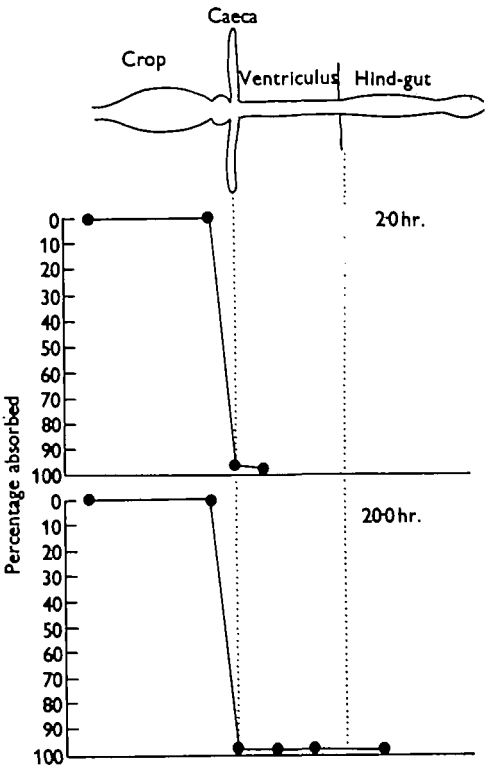

Fig. 4

Fig. 3. The percentage absorption of ^{14}C-labelled tripalmitin and its derivatives 2·0 and 20·0 hr. after ingestion of a suspension of 0·2 % tripalmitin.

Fig. 4. The percentage absorption of ^{14}C-labelled tripalmitin and its derivatives after the ingestion of 0·2 % tripalmitin dissolved in 10·0 % emulsified oleic acid.

Table 2. *The percentage absorption of* ^{14}C*-labelled material from the crop after an experimental period of* 20·0 *hr.*

Experiment	Serial	% absorbed	Mean ± S.D.
0·2 % tripalmitin	1	1·6	0·6 ± 3·25
	2	5·0	
	3	−3·9	
	4	3·3	
	5	−1·6	
	6	0·2	
0·2 % tripalmitin in oleic acid.	1	2·7	0·5 ± 1·84
	2	−2·3	
	3	1·1	
	4	0·1	
	5	1·0	

It has been shown that the absorption of the radioactive material was largely confined to the anterior part of the mid-gut where the uptake was relatively rapid. This effect suggested that the rate at which the fluid was allowed to leave the crop was likely to be of importance in determining the total absorption of tripalmitin and its derivatives. Some experiments were therefore carried out in which the degree of crop emptying was compared with the total amount of radioactive material absorbed from the gut during the experimental period. The extent of the crop emptying was determined from the amount of dye present in the crop, while the absorption was calculated from the total amount of radioactive material recovered from the crop and the remainder of the alimentary canal. Fig. 5 illustrates the close relation which was obtained between crop emptying and the absorption of tripalmitin in the cockroach.

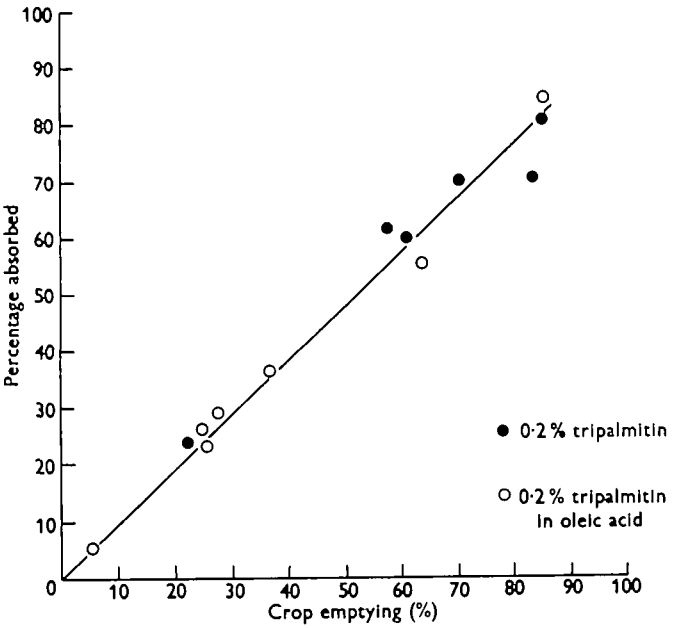

Fig. 5. The relation of crop emptying to the absorption of ^{14}C-labelled tripalmitin.

DISCUSSION

It is evident that under these experimental conditions there was a partial hydrolysis of tripalmitin in the crop. After 20·0 hr., when the crop had nearly emptied, about three-quarters of the triglyceride remained unhydrolysed. These observations are in agreement with those of Eisner (1955) who showed that the hydrolysis of triolein in *Periplaneta americana* did not proceed to completion. He argued that the completion of hydrolysis was prevented by the accumulation of the fatty acid within the partly hydrolysed fat. It was concluded that, as was shown by Frazer (1948), the enzyme was displaced by the end-products which accumulated at the oil/water interface on which the lipase acts. The degree of hydrolysis found for tripalmitin

in the present investigation is of the same order as that obtained by Frazer (1948) who found that hydrolysis stopped when about 30% of the triglyceride had been digested.

During the first few hours after the experimental meal there was no significant hydrolysis of tripalmitin in the crop and it passed into the mid-gut in a largely unhydrolysed condition. Now crop emptying, which is an exponential function of time in this insect (Treherne, 1957*b*), would tend to proceed most rapidly during this period immediately following the meal. These facts cannot, however, be taken as evidence for the absorption of triglycerides in the unhydrolysed condition, for it is possible that there was some further relatively rapid hydrolysis of the tripalmitin in the mid-gut where the enzyme/substrate ratio is likely to be much higher than in the crop lumen. It should be borne in mind in this respect that the main source of lipase in the cockroach gut is the epithelium of the mid-gut (Abbott, 1926).

The results for the degree of hydrolysis of tripalmitin in the crop were characterized by considerable individual variation. This perhaps results from the mode of production of lipase in the crop, for it is secreted in the mid-gut and passes forward to mix with the crop contents (Abbott, 1926). It seems likely with such a system that the appearance of the enzyme in the crop may be determined by several factors which together might result in a certain degree of variability in the hydrolysis of ingested triglycerides.

It has already been mentioned that some controversy has existed in the past about the part played by the crop in fat absorption in the cockroach. The hypothesis that the crop was of importance in fat absorption was based on the histological observations of Petrunkevitsch (1900), Sanford (1918) and Abbott (1926) that fat droplets appeared in the epithelium of the crop following a meal containing fat. More recently, Eisner (1955) examined this process in some detail and showed qualitatively that the appearance of fat droplets in the epithelium depended on the viscosity of the fat. The accumulation of fatty acids in the partly hydrolysed long-chain triglycerides lowered the viscosity and hastened the appearance of fat droplets in the crop epithelium. Eisner found that heavy mineral oils could be made to appear quite rapidly when mixed with oleic acid. Fat droplets appeared in the epithelium when olive oil was fed to the insects, alone or as an aqueous emulsion.

The results obtained in the present investigation do not support the hypothesis that the crop is an important organ in fat absorption. The experiments carried out on the absorption of tripalmitin and its derivatives demonstrated that under these experimental conditions there was no significant uptake from the crop. These substances were, however, absorbed extremely rapidly from the lumen of the caeca and the anterior part of the ventriculus. It may be that the absorption of tripalmitin in the crop may differ from that of a substance such as olive oil used by these authors. However, from the observations of Eisner (1955) it would certainly be expected that tripalmitin dissolved in oleic acid would be absorbed in the crop, for he showed that the absorption of oil mixtures seemed to depend largely on their viscosity. The fact that it was not absorbed in significant amounts in the present investigation suggests that the droplets of fat observed in the epithelium represented

only a very small proportion of the total fat, the greater part of which was absorbed in the mid-gut region. Furthermore, it should be borne in mind that in these experiments the tripalmitin was administered to the insect suspended in 0·5 M/l. glucose in order to slow down crop emptying (Treherne, 1957*b*) and any absorption from the crop would thus be exaggerated. Wigglesworth (1942) has shown that droplets of oil can be made to appear in the cuticular epithelium of *Rhodnius* when oleic acid or olive oil is placed on the abdominal surface. There is therefore no reason to suppose that the crop wall of the cockroach is any more permeable to fats than the rest of the body surface.

This account does not supply any information about the absorption of the glycerol released on the hydrolysis of the tripalmitin, for this portion of the molecule was not labelled with ^{14}C. It seems unlikely, however, that appreciable amounts of glycerol would be absorbed in the crop. The crop is lined with a cuticular wax layer which is relatively impermeable to water (Eidmann, 1922) and, by analogy with other water-soluble compounds (Treherne, 1957*a*), is likely to provide a relatively impermeable barrier to the diffusion of glycerol.

The total amount of ^{14}C-labelled material absorbed showed a linear relation with crop emptying. Thus, as with glucose (Treherne, 1957*b*), the limiting process in absorption was not the transfer of material across the gut wall, but the rate at which it was allowed to leave the crop. This system effectively masks the processes at the site of absorption and the present investigation does not, therefore, throw any light on the mechanism of uptake in the mid-gut. It is possible that these processes may be better studied in a preparation in which the mid-gut can be filled with a fluid of known concentration, as has been described in some previous investigations (Treherne, 1958*a*, *b*).

SUMMARY

1. A partial hydrolysis of ^{14}C-labelled tripalmitin has been demonstrated in the crop of *Periplaneta americana* L.

2. No significant absorption of tripalmitin and its derivatives could be demonstrated in the crop, whether the tripalmitin was suspended in an experimental fluid or dissolved in emulsified oleic acid.

3. Absorption took place in the mid-gut and appeared to be largely confined to the caeca and the anterior part of the ventriculus.

4. The total absorption of tripalmitin showed a linear relation with crop emptying, suggesting that the rate at which the material was allowed to leave the crop, rather than the uptake in the mid-gut, was the limiting factor in absorption.

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