Adv. Odonatol. 1: 93-104 *December 31, 1982*

LIPID ABSORPTION IN THE MIDGUT OF LARVAL *AESHNA CYANEA*

H. KOMNICK[1], J. BONGERS[2] and W. FISCHER[2]

[1]Institute of Cytology, University of Bonn, 5300 Bonn 1, Ulrich Haberlandstr. 61a
[2]Institute of Applied Zoology, University of Bonn, 5300 Bonn 1, An der Immenburg 1, Federal Republic of Germany.

Lipid absorption through the larval midgut of *Aeshna cyanea* was studied with cytological and biochemical methods. The oral ingestion of triolein leads to the formation of lipid-absorption droplets in the epithelial cells. After fat feeding there is first an increase and finally a decrease in the number and size of droplets per cell. Feeding also causes an initial rise and subsequent reduction of lipolytic activity in the midgut lumen. Orally administered ^{14}C-oleic acid is rapidly taken up by the epithelial cells and esterified into di- and triglycerides. Lipid discharge into the haemolymph occurs in the form of free fatty acids and diglycerides. The results clearly demonstrate that alimentary fat is hydrolyzed in the midgut lumen, the hydrolysates then being absorbed by the epithelial cells and utilized for the resynthesis of triglycerides. Lipid release from the midgut epithelium into the haemolymph occurs in the form of free fatty acid and diglyceride.

INTRODUCTION

Many prey animals of larval dragonflies represent a lipid-rich nutriment, in particular those possessing a more or less developed fat body (e.g. tadpoles and larval insects). Hence, dragonfly larvae should be endowed with a high capability of enteric lipid digestion and absorption. This prediction and the fact that the current knowledge of lipid absorption through the insect midgut (EISNER 1955; TREHERNE 1967; BOLLADE & BOUCROT 1973) is still relatively limited in comparison to lipid uptake through the mammalian small intestine (e.g. PALAY & KARLIN 1959; ASHWORTH et al. 1960; PALAY & REVEL 1964; STRAUSS 1963, 1966; STRAUSS & ITO 1965; CARDELL et al. 1967) have prompted the investigation of enteric lipid uptake in *Aeshna cyanea* larvae.

METHODS

For a time-sequential morphological study with the light and electron microscopes, *Aeshna* larvae starved for up to 5 weeks received a single meal of either commercial margarine or triolein mixed with Aerosil as an endocytosis marker (SiO_2 powder, particle size ca. 5-10 nm: STOCKEM 1967). Midguts were isolated and fixed after different lapses of time (3 hours to 20 days after feeding). Additionally, several biochemical experiments including the oral administration of radioactive-labelled oleic acid were designed for the exploration of whether the endocytosis theory (PALAY & KARLIN 1959; PALAY & REVEL 1964) or the diffusion theory (STRAUSS & ITO 1965; STRAUSS 1966; CARDELL et al. 1967) proposed for the mammalian small intestine can be applied to the midgut of dragonfly larvae.

RESULTS AND CONCLUSIONS

Our structural examination of the midgut epithelium of larval *A. cyanea* confirmed and extended the observations made by ANDRIES (1972, 1976a, b, 1977). The results are diagrammatically summarized in Figure 1 which serves as a brief introduction to the morphology necessary for the understanding of the physiological observations.

The midgut epithelium measures approx. 30 to 100 μm in thickness, depending on whether the gut is empty or filled with food. The epithelium is composed of a single layer of columnar cells intermingled with groups of smaller regenerative cells (Fig. 1a). At the base of these so-called nidi a further type of granulated cells is often observed whose fine structure is reminiscent of endocrine cells in the vertebrate intestine (WELSCH & LOWE 1973).

The columnar cells simultaneously show the fine-structural features of absorptive cells and secretory cells. Indicative of secretory protein synthesis is the elaborate rough endoplasmic reticulum (ER) which extends from the apex to the base of the cell and is heavily concentrated in the supranuclear Golgi zone. The numerous dictyosomes of the Golgi apparatus are closely associated with secretion granules. These are also present in the cell apex where discharge stages of the granular contents into the gut lumen by exocytosis were observed.

The absorptive function of the columnar cells is suggested by other widespread structures. These are (1) the apical brush border; (2) the apical concentration of smooth ER which locally forms dense membrane stacks that are continuous with membranes of the rough ER; (3) the dense population of mitochondria which are concen-

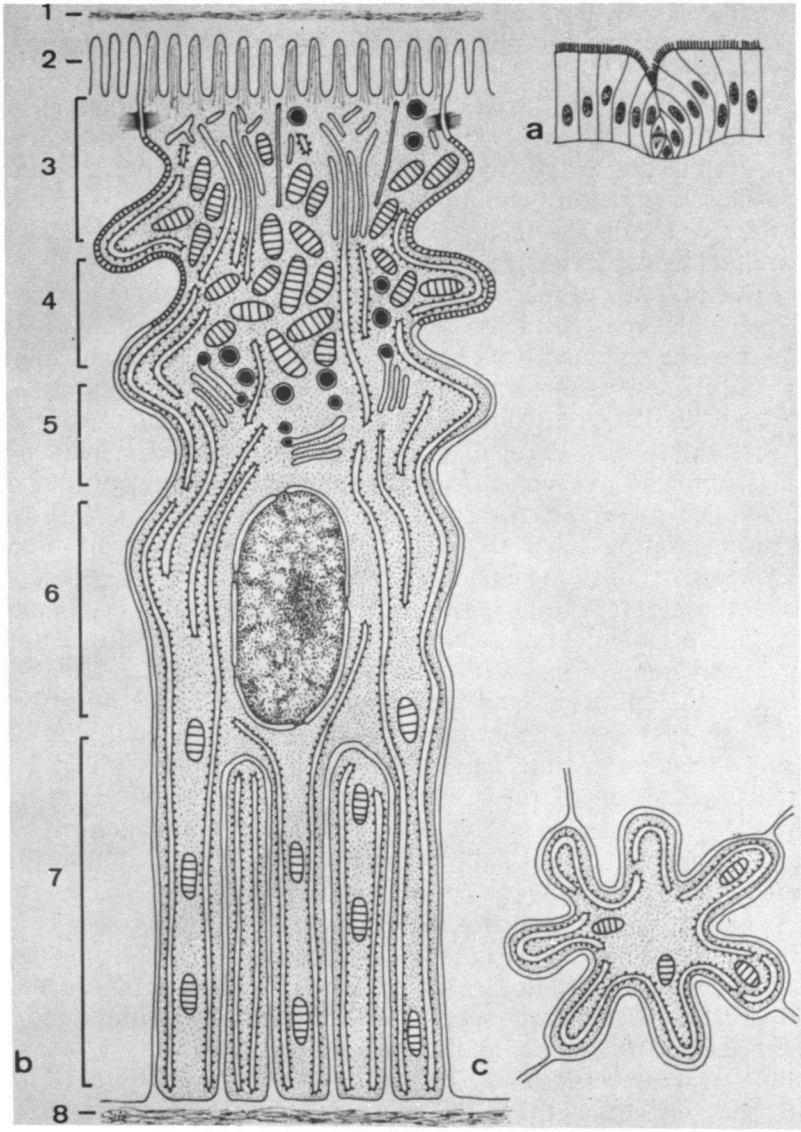

Fig. 1. Diagram of the midgut epithelium of larval *Aeshna cyanea*: (a) Columnar cells and a nidus of regenerative cells at the light-microscopic level; (b) Diagram of a columnar cell at the electron-microscopic level. 1 peritrophic membrane; 2 brush border showing a core of microfilaments in the microvilli; 3 zone of smooth endoplasmic reticulum; 4 zone of mitochondria accumulation; 5 Golgi zone; 6 muclear zone; 7 zone of baso-lateral cell interdigitation; 8 basal lamina; (c) Diagram of a cross section through the basal region of a columnar cell.

trated underneath the zone of smooth ER; and (4) the basal interdigitation of the cells resulting in a basal labyrinth of intercellular spaces (Fig. 1b, c).

The terminal bars between the apices of the columnar cells are composed of 3 types of cellular junction: (1) adhesive junction (zonula adhaerens); (2) communicative junction (gap junction); and (3) occlusive junction (smooth septate junction or zonula continua; NOIROT & NOIROT-TIMOTHÉE 1967). The last probably bars the paracellular route across the epithelium.

After oral ingestion of lipid, the midgut epithelium (Fig. 2b-f) is strikingly different from the midgut epithelium in the unfed state (Fig. 2a). The columnar cells are more or less filled with lipid absorption droplets of various sizes. The largest droplets observed measure ca. 20 μm in diameter. Three hours after lipid ingestion the droplets are first visible with the light microscope. They are very small, measuring approx. 1 μm across. They are commonest in the apical region but are also present in the basal cell processes (Fig. 6c). With time the droplets grow larger (Fig. 2b, c) and extend towards the basal region (Fig. 2c) until the columnar cells appear completely filled over their entire height (Fig. 2d). This state is reached after approximately 1 day and lasts for about 3 days. Thereafter the absorption droplets decrease in number and size (Fig. 2e). However, this phase of decrease of the epithelial lipid load appears quite variable in different individuals and in different cells of the same individual (Fig. 2e). In some larvae numerous small absorption droplets are still present in the basal region of the epithelium 20 days after the lipid meal (Fig. 2f). Examination of the structure at different times gives the overall impression of a tidal growth and decline of lipid absorption droplets across the midgut epithelium (see sequence of Fig. 2a-f).

It is tempting to interpret these light-microscopical observations in terms of the endocytosis theory. However, careful search with the electron microscope failed to reveal any indications of endocytotic lipid uptake. Even in the space between the brush border and the peritrophic membrane no lipid globule was detectable.

Fine-structural and X-ray analysis after feeding of lipid mixed with the inert endocytosis marker Aerosil clearly demonstrates that the SiO_2 particles are present in the gut lumen but absent from the epithelial side of the peritrophic membrane, brush border and apical lipid absorption droplets (Fig. 3). Therefore, only lipid micelles below the size of the smallest SiO_2 particles (approx. 5 nm) could pass through the meshes of the peritrophic membrane and reach the epithelial cells. These observations suggest that endocytosis of lipid

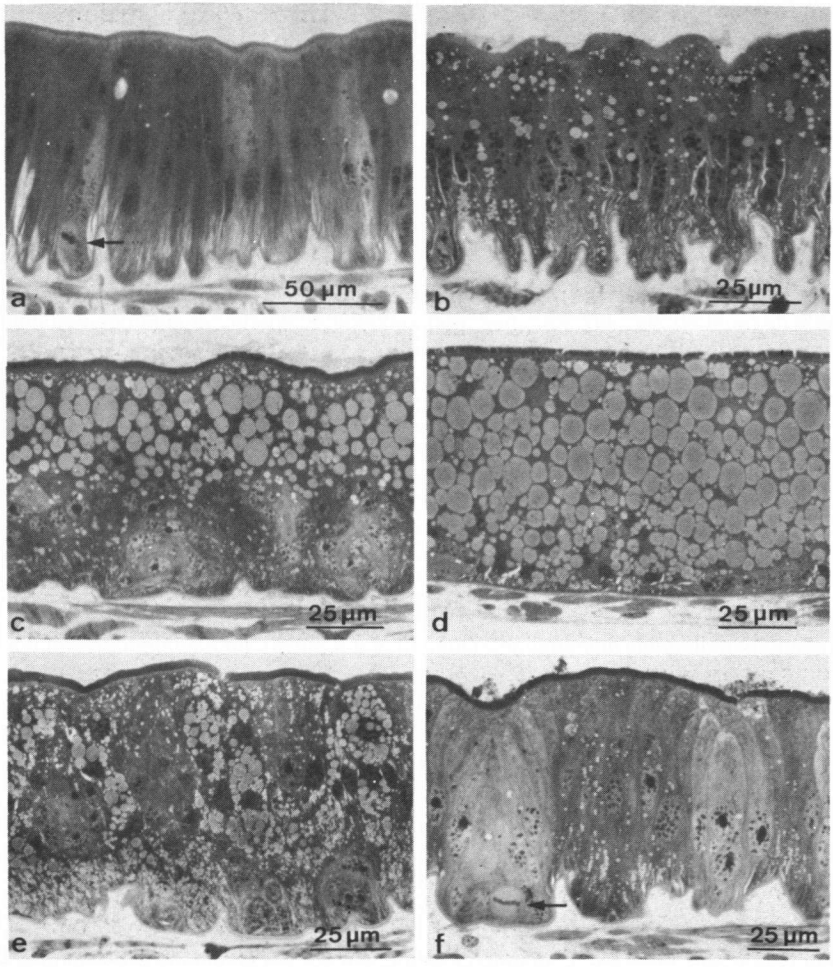

Fig. 2. Midgut epithelium of larval *Aeshna cyanea* fed with lipid after 4 weeks of starvation: (a) Unfed control; (b) 6 hours; (c) 12 hours; (d) 2 days; (e) 6 days; (f) 20 days after feeding. The arrows point to mitotic divisions of regenerative cells. Glutaraldehyde/osmium tetroxide fixation; Araldite embedding; toluidine-blue staining.

plays little or no role.

The diffusion theory implies that lipid is hydrolyzed in the gut lumen and the products then diffuse across the apical plasma membrane into the absorptive cells, where resynthesis of lipid leads to the formation of the lipid absorption droplets.

Morphological indications in favour of this theory are provided by comparing the results of triolein ingestion (Fig. 4a) with the re-

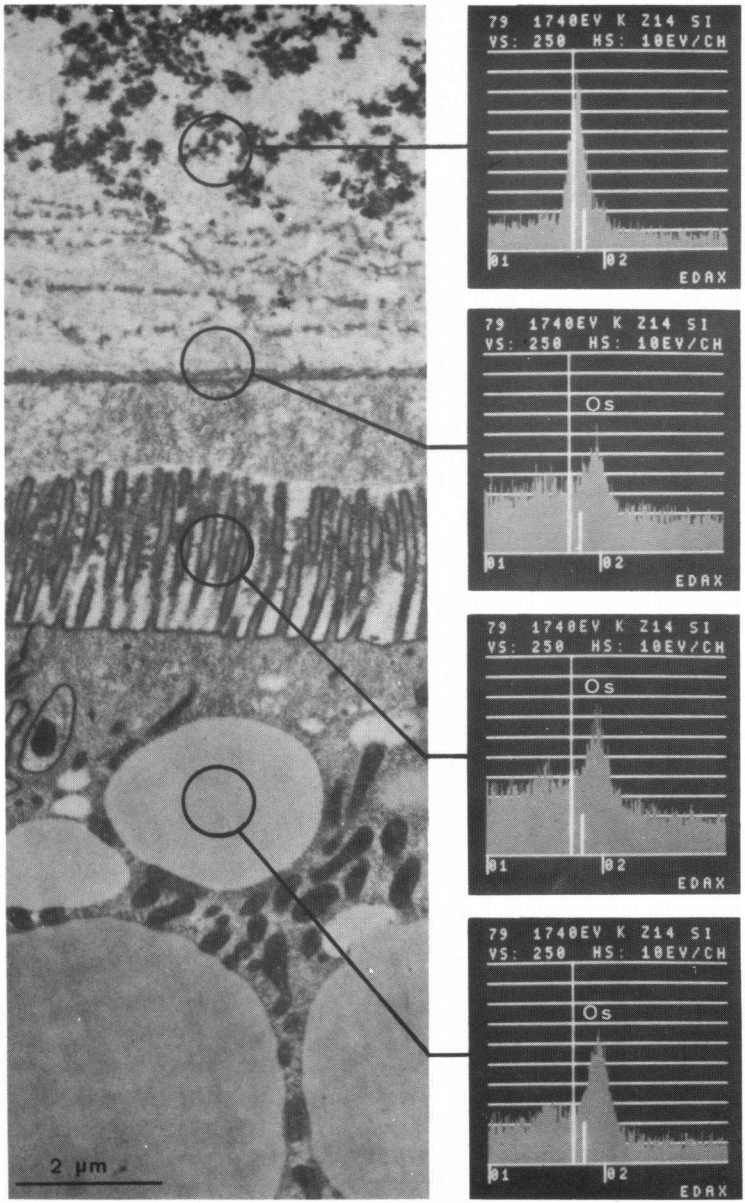

Fig. 3. Cross section of *Aeshna* midgut fixed at 24 hours after feeding triolein containing 10% SiO_2 powder. Electron micrograph. The energy-dispersive X-ray diagrams were obtained from the analysis of the encircled areas. The marker indicates Si which is found exclusively in the gut lumen over the peritrophic membrane.

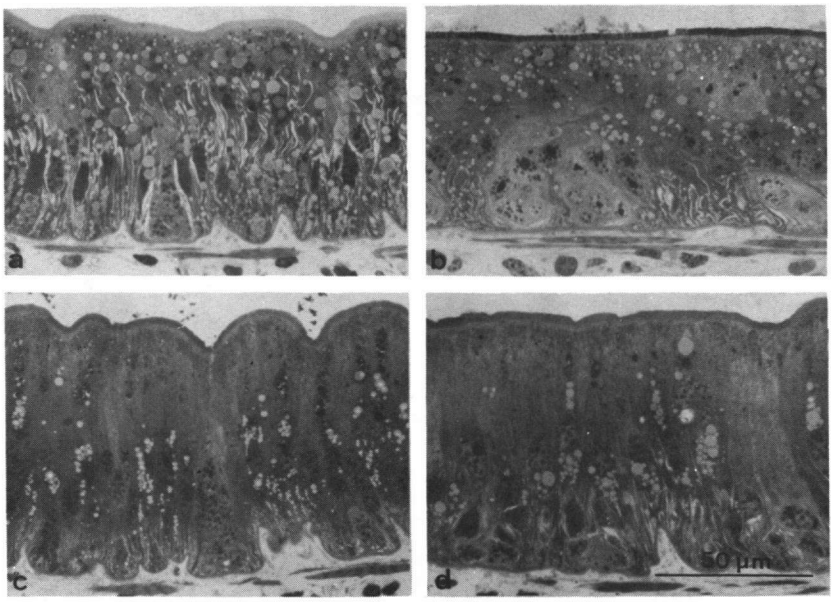

Fig. 4. Midgut epithelium of larval *Aeshna cyanea* starved for 4 weeks and subsequently fed with (a) triolein, (b) oleic acid and glycerol in the molar proportion of 3:1, (c) oleic acid, and (d) glycerol (30% aqueous solution). Fixation in all cases 9 hours after feeding. Same techniques as Fig. 2.

sults of feeding oleic acid and glycerol alone or as a mixture (Fig. 4b-d). In all cases, the epithelial cells contain lipid absorption droplets. Since glycerol is completely soluble and lost during aqueous fixation and alcoholic dehydration, the presence of lipid absorption droplets can result only from lipid synthesis.

The diffusion theory requires that the intestinal lumen is capable of lipid digestion. Therefore, the midgut lumen, midgut epithelium and haemolymph were comparatively assayed for lipolytic activity. At no time in the experiments was lipolytic activity measurable in the haemolymph or in the midgut epithelium, whereas the midgut lumen showed an interesting fluctuation of lipolytic activity. As seen from Figure 5, there is a basal level of lipolytic activity in the unfed state. Feeding causes a sharp rise of activity followed by a relatively fast drop to zero (not measurable) and a slow restoration of the basal level. These data indicate that lipolytic enzymes are released into the midgut lumen upon food uptake, where they become active. The time period of the lowest lipolytic activity in the lumen, i.e., the lowest secretory activity of the midgut epithelium, is approximately

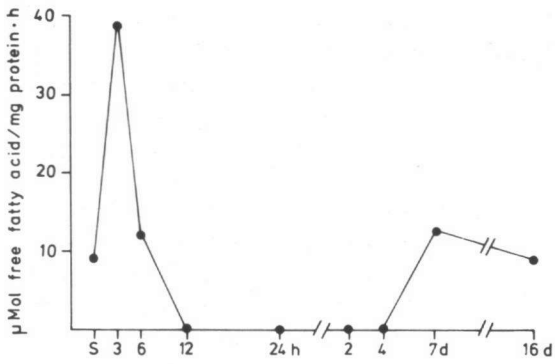

Fig. 5. Lipolytic activity of midgut lumen after oral administration of triolein. Prior to lipid ingestion the larvae had been kept unfed for 3 weeks. S = starvation, i.e. 16 days after triolein ingestion.

identical with the time period when the epithelial cells show the heaviest load of lipid absorption droplets.

Three hours after ingestion of triolein, free fatty acids are present in both the midgut lumen (36 µmoles/mg protein) and the midgut epithelium (48 µmoles/mg protein). These figures clearly demonstrate that hydrolysis of lipid actually takes place in the lumen and the products of lipolysis – at least fatty acids – are taken up by the epithelial cells.

The latter conclusion is confirmed by the transmigration of radioactivity after oral ingestion of ^{14}C-oleic acid. Three hours after ingestion most of the label has already left the midgut lumen and is present in the midgut epithelium. Some label has already been released into the haemolymph and taken up by the fat-body cells of

Table I

Redistribution of ^{14}C-lipid 3 and 6 hours after oral administration of ^{14}C-oleic acid in percent of cpm of total lipid per mg protein

	3 hours	6 hours
midgut contents	1.4 ±0.7	0.6 ±0.3
midgut epithelium	93.8 ±1.6	91.8 ±4.3
haemolymph	4.2 ±0.3	7.6 ±1.5
rectum (fat body)	1.8 ±0.7	2.6 ±0.4

the rectum. At 6 hours the figures have scarcely changed, label still remaining accumulated in the epithelium (Table 1).

The analysis of ^{14}C-incorporation into different lipid fractions provides direct evidence for the uptake of labelled fatty acid by the epithelial cells and its subsequent utilisation for the synthesis of mono-, di- and finally triglycerides (Table 2).

Except for a minor triglyceride fraction, which presumably is indicative of some resynthesis in haemocytes, the lipid of the haemolymph occurs exclusively in the form of diglyceride and free fatty acid (Table 2). This indicates that lipid discharge from the epithelial cells into the haemolymph also takes place in the form of free fatty acid and diglyceride, which in turn are taken up by the fat-body cells and used for the esterification into triglycerides (Table 2).

The sites of lipid resynthesis in the absorptive cells and the mode of discharge into haemolymph remain to be clarified. The first lipid absorption droplets detectable with the electron microscope are located in close association with the apical endoplasmic recticulum. They are often found enclosed in the stacks of smooth ER (Fig. 6 a, b), which appears to be the site of lipid resynthesis in the absorp-

Table II

Distribution of label within the triglyceride (TG), diglyceride (DG), monoglyceride (MG) and free-fatty-acid (FA) fractions of midgut epithelium haemolymph and rectum, 3 and 6 hours after oral administration of ^{14}C-oleic acid in percent of total lipid per mg protein

		3 hours	6 hours
epithelium:	TG	33.7 ± 11.2	65.9 ± 5.9
	DG	41.7 ± 14.3	22.4 ± 4.5
	MG	1.7 ± 0.3	13.9 ± 0
	FA	35.6 ± 4.7	7.1 ± 2.6
haemolymph	TG	2.5 ± 0.8	2.4 ± 0.5
	DG	50.5 ± 5.6	97.6 ± 1.5
	MG	0	0
	FA	45.4 ± 5.5	21.6
rectum (fat-body)	TG	17.1 ± 0.6	22.7 ± 1.8
	DG	72.3 ± 13.6	47.1 ± 1.8
	MG	0	0
	FA	11.2 ± 5.4	29.2 ± 1.1

tive cells of the mammalian small intestine (CARDELL et al. 1967). Growth of the lipid absorptive droplets probably results from the increase in the number of synthesized molecules as well as from the confluence of smaller droplets (Fig. 6b).

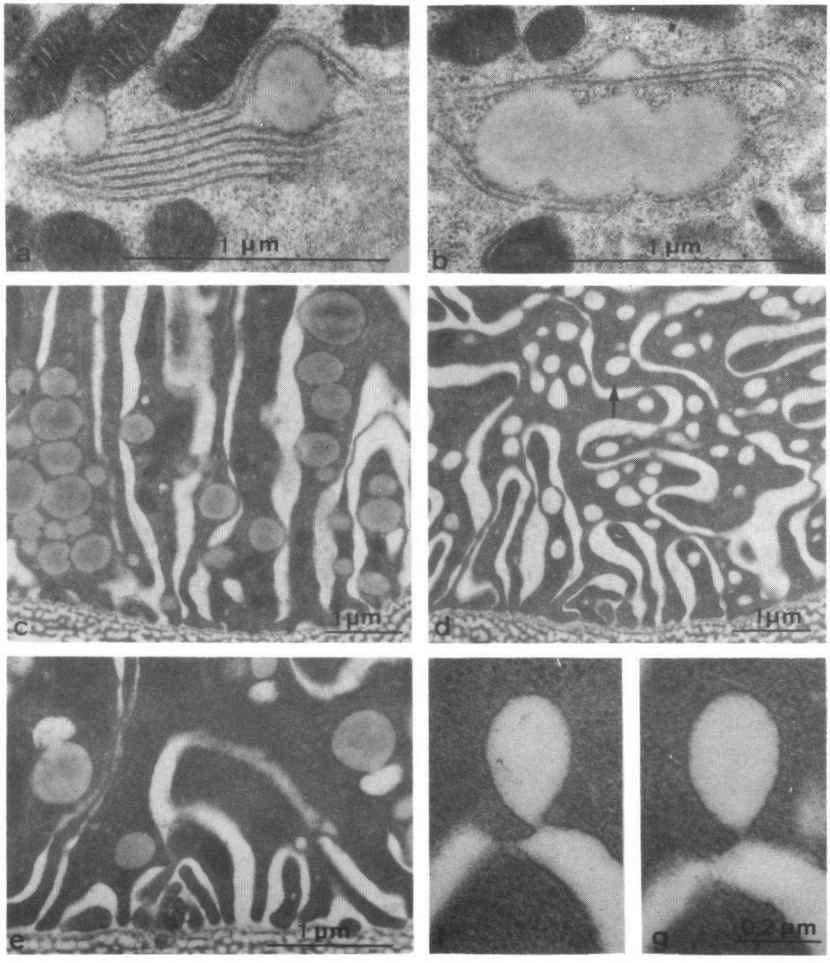

Fig. 6. Electron-microscopical details of the midgut epithelium of larval *Aeshna cyanea* which had been starved for 3 weeks, then fed with triolein and fixed 3 hours after lipid ingestion: (a-b) Lipid absorption droplets within the apical stacks of smooth endoplasmic reticulum; (c-f) Basal regions of the epithelium with interdigitating cell processes showing (c) electron dense and (d) electron translucent droplets, (e) connexions of both and (f -g) droplets opening into the intercellular space. (f) and (g) are serial sections of the same droplet labelled with arrow in (d).

Since the slender baso-lateral cell processes normally contain much smaller lipid droplets than the midregion of the cell, the large droplets again appear to fractionate before discharge. Images interpretable as droplet partition were often observed in the infranuclear region.

There are two types of droplets in the basal cell processes. One type is clearly identifiable as lipid droplets by the osmiophilic contents (Fig. 6c). The second type is usually smaller, electron-translucent and occasionally shows fuzzy or lamellar contents (Fig. 6d). Both types are also found intermingled in the same cell. Direct connexions of both types point to the possibility of fusion or segregation (Fig. 6e). In contrast to the osmiophilic droplets, the translucent droplets are frequently observed in the state of fusion with the baso-lateral plasma membranes (Fig. 6e, f). It is tempting to speculate that the translucent droplets arise from the electron-dense droplets by segregation of triglycerides and diglycerides, the latter then being released by exocytosis into the baso-lateral intercellular spaces. Release of fatty acid may also occur with these droplets or by diffusion directly through the plasma membrane.

ACKNOWLEDGEMENTS

The authors are grateful to Mrs. I. BAAS for technical assistance, to Dr. M. SCHMITZ for the X-ray diagrams, and to Dr. R. STIEMERLING for the drawings.

REFERENCES

ANDRIÈS, J.-C. 1972. Genèse intraépithéliale des microvillosités de l'épithélium mésentérique de la larve d'Aeschna cyanea. J. Microscopie 15: 181-204.

ANDRIES, J.-C. 1976. Variations ultrastructurales au sein des cellules épithéliales mésentériques d'Aeshna cyanea (Insectes, Odonates) en fonction de la prise de nourriture. Cytobiol. 13: 451-468.

ANDRIES, J.-C. 1976. Présence de deux types cellulaires endocrines et d'un type exocrine au sein du mésentéron de la larve d'Aeshna cyanea Müller (Odonata: Aeshnidae). Int. J. Insect Morphol. Embryol. 5: 393-407.

ANDRIES, J.-C. 1977. Specialization of the endoplasmic reticulum in the apex of the midgut cells of Aeshna cyanea (Insecta, Odonata). Cell Tiss. Res. 178: 97-101.

ASHWORTH, C.T., V.A. STEMBRIDGE & E. SANDERS 1960. Lipid absorption, transport and hepatic assimilation studied with electron microscopy. Am.J. Physiol. 198: 1326-1328.

BOLLADE, D. & P. BOUCROT 1973. Incorporation d'acides gras radioactifs alimentaires dans les lipides chez Periplaneta americana. Insect Biochem. 3: 123-137.

CARDELL. R.R., S. BADENHAUSEN & K.R. PORTER 1967. Intestinal triglyceride absorption in the rat. An electron microscopical study. J. Cell Biol. 34: 123-155.

EISNER, T. 1955. The digestion and absorption of fats in the foregut of the cockroach, Periplaneta americana (L). J. exp. Zool. 130: 159-181.

NOIROT, Ch. & C. NOIROT-TIMOTHEE 1967. Un nouveau type de jonction intercellulaire (Zonula continua) dans l'intestin moyen des Insectes. C. r. Acad. Sc. 264: 2796-2798.

PALAY, S.L. & L.J. KARLIN 1959. An electron microscopic study of the intestinal villus. II. The pathway of fat absorption. J. biophys. biochem. Cytol. 5: 373-384.

PALAY, S.L. & P.J. REVEL 1964. The morphology of fat absorption. In: H.C. MENG (ed): Lipid transport p. 1-11. C. Thomas, Springfield.

STOCKEM, W. 1967. Die Eignung von Aerosil fur die Untersuchung endocytotischer (pinocytotischer) Vorgange. Mikroskopie 22: 143-147.

STRAUSS, E.W. 1963. The absorption of fat by intestine of golden hamster in vitro. J. Cell Biol. 17: 597-607.

STRAUSS, E.W. 1966. Electron microscopic study of intestinal fat absorption in vitro, from mixed micelles containing linolenic acid, monoolein, and bile salt. J. Lipid Res. 7: 307-323.

STRAUSS, E. & S. ITO 1965. Autoradiographic and biochemical study of linolenic acid-C^{14} absorption by hamster intestine from mixed micelles in vitro. J. Cell Biol. 27: 101A.

TREHERNE, J.E. 1967. Gut absorption. Ann. Rev. Ent. 12: 43-58.

WELSCH, U. & A. LOWE 1973. Elektronenmikroskopische Beobachtungen an endokrinen Zellen des Magen-Darmkanals vom Grasfrosch (Rana temporaria). Z. wiss. Zool. 186: 79-88.