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The Influence of Starvation and Different Diets on the Hindgut of Isopoda *(Mesidotea entomon, Oniscus asellus, Porcellio scaber)*

Z. HRYNIEWIECKA-SZYFTER [1] and V. STORCH [2],*

[1] Institute of Biology, Department of Cytology and Histology, A. Mickiewicz University, Poznań, [2] Zoology Department I (Morphology/Ecology), University of Heidelberg

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Summary

Under conditions of food deprivation, the hindgut epithelium of the experimental animals (*Mesidotea entomon, Oniscus asellus, Porcellio scaber*) undergoes ultrastructural changes. After the application of different diets it was demonstrated that this part of the alimentary canal contains nutrients, though it is lined by a cuticle. Experimental evidence for the formation of glycogen from glucose offered as the only diet comes from autoradiographic experiments. Amino acids, too, were detected in the hindgut cells soon after refeeding. Lipids, on the other hand, which are first absorbed by the large cells of the midgut glands, were not found in the hindgut epithelium. The existence of lipid inclusions in the hindgut epithelium some weeks after refeeding, however, supports the hypothesis that lipids reach the epithelial cells of the hindgut *via* midgut glands and hemolymph.

Keywords: Isopoda; Hindgut; Absorption; Nutrition.

1. Introduction

There exist a number of conflicting views in the literature concerning the origin, function and structure of particular sections of the gut of isopods. Additionally, the terms applied in the description of the same gut sections vary. The reason of the confusion is the fact that the entire gut of isopods, except for the hepatopancreas, is lined by a chitinous cuticle commonly called the intima.

An exhaustive discussion on this subject is presented by HOLDICH (1973) and HOLDICH and MAYES (1975). Following GOODRICH (1939), HOLDICH and MAYES (1975) are of the opinion that the alimentary canal of isopods, extending from the point of insertion of the hepatopancreatic ducts to the anus, is of an ectodermal origin and that the whole of it represents the hindgut. This is an interpretation also accepted by HEROLD *et al.* (1976) and WÄGELE *et al.* (1981).

The section of the hindgut of isopods situated between the insertion point of the ducts of the hepatopancreas and the sphincter is termed the anterior region of the hindgut or simply hindgut, while the remaining part of the gut up to the anus is called the posterior region or rectum (SUTTON 1972, VERNON *et al.* 1974, HOLDICH and MAYES 1975). Conflicting interpretations have been presented as to its syncytial nature, but electron microscopy has clearly proved its cellular composition (HOLDICH and RATCLIFFE 1970, HOLDICH and MAYES 1975).

Furthermore, recent ultrastructural studies have produced additional information on the cell ultrastructure (CORUZZI *et al.* 1982, HRYNIEWIECKA-SZYFTER and TYCZEWSKA 1979), and there is agreement that the hindgut of terrestrial isopods, apart from propelling undigested food material, may act as a site for water storage as an adaptation to the animals' habitat. LINDQUIST and FITZGERALD (1976) demonstrated the buffering effect of water in the gut on osmotic values of the blood during desiccation, and electron microscopy revealed the ultrastructure and cytochemistry typical of osmoregulatory epithelia (HOLDICH and RATCLIFFE 1970, HRYNIEWIECKA-SZYFTER and TYCZEWSKA 1979, CORRUZZI *et al.* 1982).

The presence of the cuticle in the whole gut of isopods

* Correspondence and Reprints: Zoologisches Institut (Morphologie/Ökologie) der Universität Heidelberg, Im Neuenheimer Feld 230, D-6900 Heidelberg, Federal Republic of Germany.

raises the question of whether any part of it is able to absorb nutrients. Although the ultrastructure of the isopod hindgut epithelial cells has been described by several authors there seems to be no proof if this part of the gut is capable of absorption and storage of nutrients or not. According to HOLDICH and RATCLIFFE (1970) in the herbivorous isopod *Dynamene bidentata* "the hindgut appears to take no part in the production of digestive enzymes, food absorption, or glycogen and lipid storage." SUTTON (1972) thinks "that some food absorption almost certainly takes place here". This last mentioned assumption is based on older light microscopic studies (MURLIN 1902, NICHOLLS 1931) which have dealt perforce with a paucity of structural characters. For a further understanding of the gut of isopods this electron microscopic analysis including radioautography has been performed on animals fed with varied diets.

2. Materials and Methods

Mesidotea entomon obtained from the Sea Fisheries Institute in Gdynia were kept in the laboratory in Poznań as described previously (HRYNIEWIECKA-SZYFTER and TYCZEWSKA 1979). Only mature, intermoult animals were used.

For experimental purposes specimens were starved for 4 days and then were fed with *Tubifex tubifex* (*Oligochaeta*) which contain hemoglobin in their blood (PROSSER 1973). Animals were taken 4, 10 and 24 hours after the start off feeding as well as after 4 and 7 days of starvation. The anterior regions of the hindguts were dissected and fixed at 4 °C in 2.5% glutaraldehyde in 0,.1 M cacodylate buffer at pH 7.4. After postfixation in 1% OsO_4 (buffered as above) the tissues were stained with uranyl acetate and lead citrate and examined in a JEOLCO JEM-7 A.

Adult specimens of woodlice (*Oniscus asellus, Porcellio scaber*) were taken from their natural habitats near Heidelberg. They were kept singly in plastic vials the bottoms of which were made of gypsum and did not receive any food for 4 to 6 weeks. The holding vessels were inspected daily and the wastes were removed whenever necessary. Following the period of starvation, the experimental animals were fed with pure carbohydrates (glucose, sucrose), foodstuffs with high protein content (curds) and high fat content (butter).

Only one type of the foregoing food was offered to each of the experimental animals. Refeeding lasted 2 hours, 2 and 14 days, respectively.

Sections of the anterior hindgut were fixed in a 3.5% glutaraldehyde phosphate buffer solution (pH 7.5) for 2 hours at a temperature of 4 °C, rinsed several times in phosphate buffer, and postfixed in 2% OsO_4 solution. The samples were dehydrated in ethanol, embedded in araldite, sectioned, and stained with uranyl acetate and lead citrate. The sections were observed in a Zeiss EM 9 S 2 microscope.

For electron microscopic radioautography, D-glucose-^{3}H was added to glucose and sucrose diets, and ^{3}H-labeled amino acids were offered with curds. Thin sections mounted on grids were coated with a thin film of Ilford L-4 emulsion using an expanded wire loop. After being exposed for 4 weeks at 4 °C, the radioautographs were developed following the method described by ZIMMERMANN *et al.* (1976).

3. Results

3.1. Mesidotea entomon

The general organization and histology of the anterior region of the hindgut of *Mesidotoa entomon* has been described in a previous paper (HRYNIEWIECKA-SZYFTER, in press).

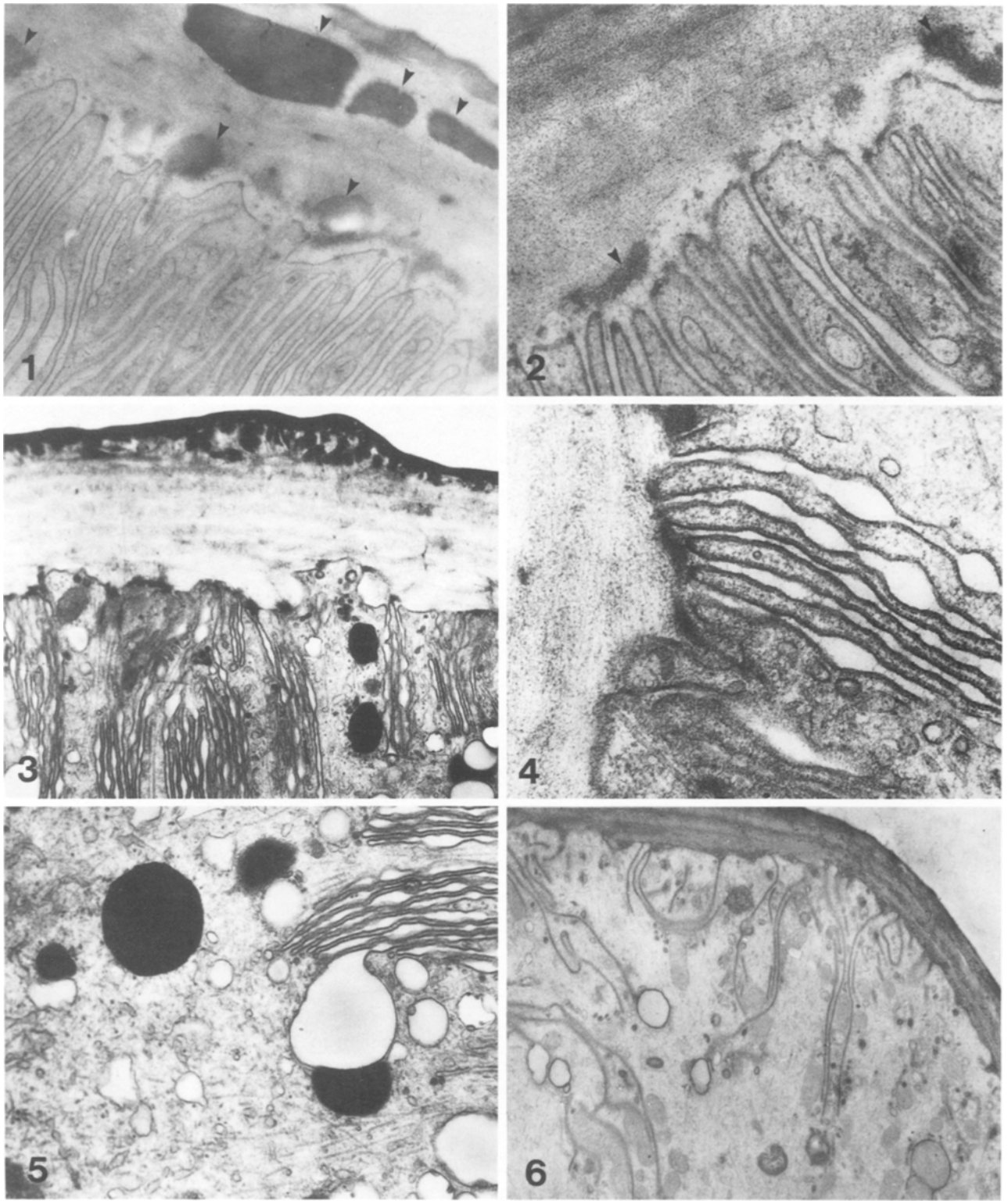
In the present study the ultrastructural description is confined to the apical part of epithelial cells during the postfeeding and starvation period.

The cuticular intima covering the luminal surface of the cells is composed of two layers: an electron dense thin epicuticle and an underlying fine fibrillar much less dense and thicker endocuticle (Figs. 1 and 2). The apical plasma membrane in fed animals forms numerous parallel infoldings penetrating into the apical cytoplasm to a varying depth.

The frequency of infoldings is variable, clearly dependent on the postfeeding period. Four and ten hours after the start of feeding the apical infoldings were extensive and present along the whole cell surface (Figs. 1 and 2). Clearly, during these periods the absorption of food from the lumen of the gut takes place, which is evidenced by the appearance of electron dense material. The absorbed electron dense material of various size and shape was restricted only to the intima and to subcuticular spaces at the top of the infoldings (Figs. 1 and 2). It proves that the passage of the electron dense material through the intima is relatively slow.

Four and ten hours after feeding the infoldings did not widen into vesicles at the base. Also, no free clear vesicles could be observed.

Twenty-four hours after the start of feeding apical infoldings appeared usually at irregular intervals, so that in some regions of cells the apical plasma membrane was relatively smooth (Fig. 3). The apical infoldings were dilated irregularly. In some places the extracellular space between the opposing surface of the plasma membrane of these invaginations was inflated characteristically (Figs. 3 and 4). At the base of numerous infoldings extensive dilations could be observed (Fig. 5). After the twenty-four-hour feeding period the absorbed electron dense material was also present in the intima, though mainly in the subepicuticular layer. In the subcuticular spaces at the top of infoldings the electron dense material appeared in markedly smaller quantities than after 4 and 10 hours after feeding. At the same time, however, a number of dilations of infoldings at the base are filled with electron dense material which probably moved from subcuticular spaces. Fig. 5 clearly shows that the electron dense material lies in the



Figs. 1–6. Hindgut of *Mesidotea entomon*

Fig. 1. Four hours after start of feeding *Tubifex*. Note the electron dense patches in the cuticle (arrows) × 19,000

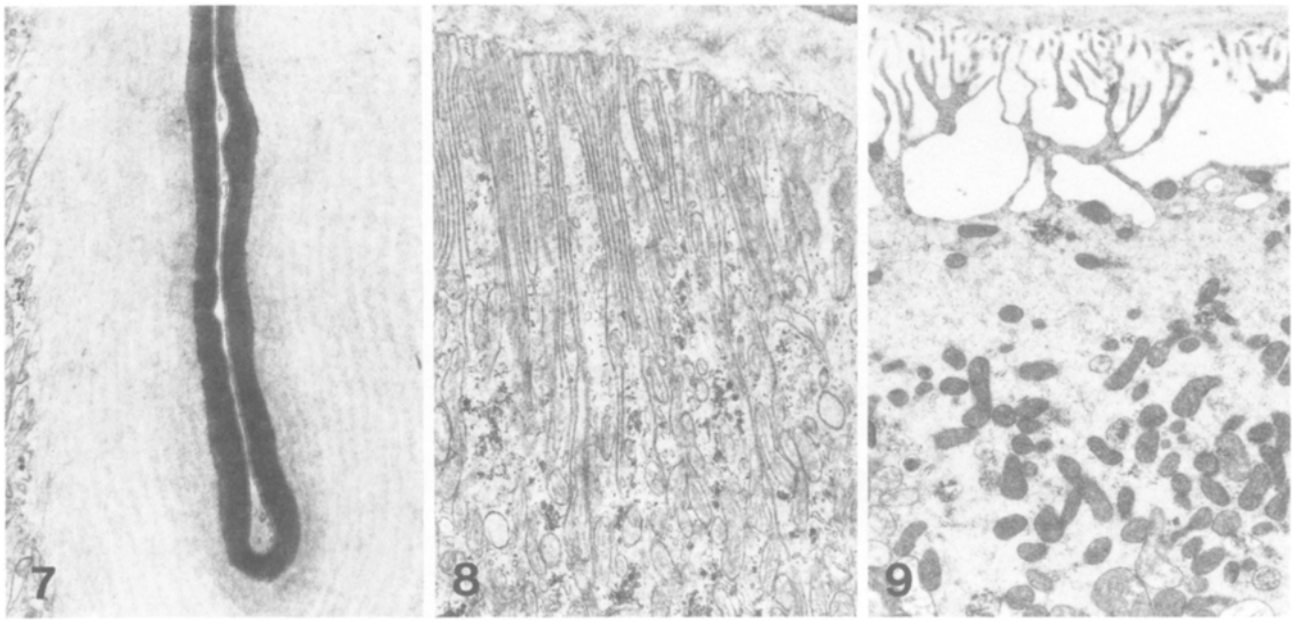
Fig. 2. Ten h s after start of feeding. Note the electron dense patches in the proximal part of cuticle/subcuticular space (arrows). × 26,000

Fig. 3. Twenty four hours after feeding. Note the electron dense inclusions in the cytoplasm and the irregular apical infoldings. × 16,000

Fig. 4. Twenty four hours after feeding. Apical infoldings with dilations. × 42,000

Fig. 5. Twenty four hours after feeding. Basal portion of apical infoldings and electron dense, globular inclusions. × 24,000

Fig. 6. Four days after cessation of feeding. × 14,000



Figs. 7–9. Apical portions of hindgut epithelium of woodlice

Fig. 7. *Porcellio scaber*. Cuticle. × 12,000

Fig. 8. *Oniscus asellus*. Note regularly arranged apical infoldings, which are typical of well-fed animals. × 9,400

Fig. 9. *Porcellio scaber*. Six weeks of starvation. Note irregular apical plasma membrane and space between epithelial cell and cuticle. × 9,400

cytoplasm of a cell, where it accumulates in the form of globular structures of varying size.

In the apical cytoplasm mitochondria were not numerous and were found mainly in the region below infoldings. They were not associated with infoldings, even though sometimes they appeared singly at their base. Single, fairly numerous microtubules extending in various directions were scattered in the cytoplasm almost evenly (Fig. 4), without forming any particular accumulation in the subcuticular region.

After four days of starvation, in the apical part of cells marked changes took place consisting mainly in the disappearance of infoldings. The apical plasma membrane, adhering closely to the cuticular intima, was generally smooth with very few uncharacteristic infoldings whose membranes were very close to each other (Fig. 6). The cytoplasm contained numerous clear vesicles of varying size, fairly numerous mitochondria, and fragments of microtubules.

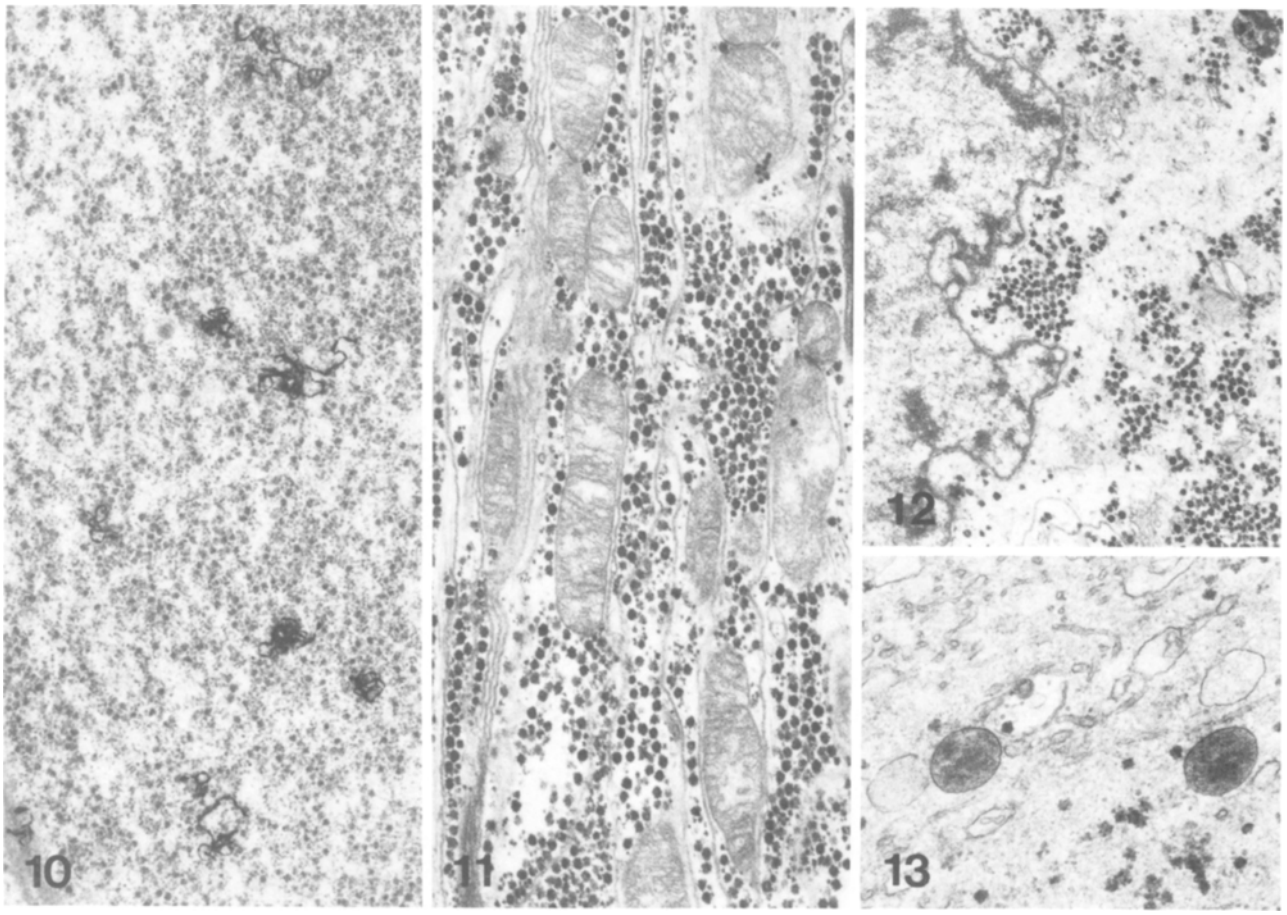
After a week of starvation the changes became drastic. Infoldings disappeared completely, the apical membrane was smooth along the whole surface. In the cytoplasm there were still some mitochondria and a few microtubules.

3.2. Oniscus asellus, Porcellio scaber

Several authors, *e.g.*, ALIKHAN (1972), CORUZZI *et al.* (1982), SMITH *et al.* (1975), VERNON *et al.* (1974), have described the ultrastructure of woodlouse hindgut cells "under normal conditions" including their cuticle (Fig. 7) and the extensive infoldings of the apical plasma membrane (Fig. 8). As will be shown below, a "normal state" can hardly be defined with certainty. Species differences of *Oniscus asellus* and *Porcellio scaber* were not found.

3.2.1. Starvation

Under conditions of food deprivation the hindgut epithelial cells exhibited features similar to those described for the hepatopancreatic cells (STORCH 1984). Their size was reduced. The apical cell parts showed branched microvilli and voluminous subcuticular spaces (Fig. 9). The mitochondria were found more or less evenly distributed all over the cell. They could be subdivided into two populations, the first being swollen with an electron-lucent matrix, the second being elongated with a darker matrix (Fig. 9). The cell nuclei often had an irregular outline. Their chromatin some-



Figs. 10–13. Central portion of hindgut epithelial cells of woodlice

Fig. 10. *Porcellio scaber*. Radioautograph. Accumulation of silver grains over glycogen deposit. 6 weeks starvation, 2 days refeeding with glucose. × 14,000

Fig. 11. *Oniscus asellus*. 6 weeks starvation, 2 days refeeding with sucrose. × 14,000

Fig. 12. *Oniscus asellus*. 6 weeks starvation, 2 days refeeding with sucrose. Note irregular periphery of cell nucleus. × 9,400

Fig. 13. *Oniscus asellus*. 6 weeks starvations, 2 days refeeding with curds. × 19,000

times showed a netlike formation indicating disintegration. The cytoplasm was electron-lucent and homogeneous containing only some scattered RER profiles. Dictyosomes were rarely observed. The predominant features were autolysomes of various outlines. Portions of the basal lamina were found to be considerably reinforced and multilayered. Some parts projected very deeply into the epithelium.

3.2.2. Carbohydrate Diet

Feeding with glucose and sucrose resulted in an extensive concentration of glycogen in the hindgut epithelial cells (α-particels) and in the surrounding muscle cells (β-particles) after 2 days (Fig. 10). After 2 hours small amounts of glycogen were already present in the epithelial cells. Autolyosomes occurred in large numbers. The mitochondria were elongated (Fig. 11). The cell nuclei possessed many minute protrusions (Fig. 12). Even after feeding sucrose for fourteen days this cell ultrastructure was maintained. In contrast to the hindgut epithelial cells of starved animals subcuticular spaces were very small. The apical plasma membrane formed numerous and irregularly arranged projections under which lay elongated mitochondria. The basal plasma membrane was in general found to be less complicated. Single infoldings of the basement lamina again penetrated the cell body.

3.2.3. Protein-oriented Diet

Glycogen was again found to be a predominant feature of the epithelial and muscle cells but the amount was lower than after feeding with sucrose alone.

After two days, the mitochondria were swollen, their matrix being light. Golgi fields were encountered frequently, sometimes occurring in small groups. Stacks of RER were found. In the neighbourhood of endoplasmic reticulum and dictyosomes membrane-bounded inclusion bodies (peroxisomes?) occurred (Fig. 13) which were also found in the absorptive cells of isopod midgut glands (STORCH 1984). They were even found after two hours. Autolysosomes, on the other hand, were rare. The predominant feature of the hindgut cells under these conditions was their comparatively large amount of cell organelles and deposits. Labeled material was found after two hours. Again, there were no large subcuticular spaces.

3.2.4. Lipid-oriented Diet

This diet, too, resulted in glycogen deposits but only in medium amounts. Lipid inclusions were lacking after 2 days feeding, but after fourteen days feeding *ad libitum*, electron-lucent lipid droplets were found to be arranged in small groups in the hindgut epithelial cells. The cytoplasm appeared more or less homogeneous after two days. The cell nucleus was round. Elongated mitochondria were concentrated near the apical plasma membrane which corresponded to categories 2 and 3. Dictyosomes were rarely found, autolysosomes still existed.

4. Discussion

It is generally accepted that in isopods the products of digestion are absorbed through the hepatopancreas (JONES *et al.* 1969, CLIFFORD and WITKUS 1971, VERNON *et al.* 1974, SMITH *et al.* 1975, HRYNIEWIECKA-SZYFTER and TYCZEWSKA 1978), the cells of which clearly reflect the nutrients metabolized (STORCH 1984, ŠTRUS *et al.* 1985).

In spite of numerous studies describing the morphology and ultrastructure of the hindgut of isopods (SMITH *et al.* 1969, HOLDICH and RATCLIFFE 1970, VERNON *et al.* 1974, HEROLD *et al.* 1976, HRYNIEWIECKA-SZYFTER and TYCZEWSKA 1979), few authors have dealt with its participation in the process of food absorption.

HOLDICH and RATCLIFFE (1970) conclude from the ultrastructural evidence that the hindgut of *Dynamene bidentata* does not take part in the absorption of the food material. By contrast, from the histochemical evidence of ALIKHAN (1972) it follows that the anterior region of the hindgut (called midgut by the author) of *Porcellio laevis* plays a role in the absorption of fat. More recently, WÄGELE *et al.* (1981) admitted on the basis of the activity of some enzymes, a possibility of food absorption through the "thin and loosely arranged cuticle" of the hindgut of *Cyathura carinata*.

The results of our investigations suggest that the anterior region of the hindgut of *Mesidotea entomon, Oniscus asellus* and *Porcellio scaber* absorbs food material from the gut lumen and ascertain that it represents a major site of carbohydrate (glycogen) storage, at least in the terrestrial forms, *Oniscus asellus* and *Porcellio scaber*. Under extreme conditions (continuous feeding on butter) it can store lipids, as well, since the large cells of the midgut glands absorb lipids after feeding (STORCH 1984). It is suggested that later hepatopancreatic stores are transported to the hindgut. Moreover, the experiments presented in this paper have shown for the first time that the cuticular intima of the hindgut may be permeable to macromolecules. The chemical nature of these macromolecules detected in *Mesidotea entomon* is not known; it can only by suggested, on the basis of electron density, that they are hemoglobin coming from *Tubifex tubifex* fed to the animals.

The permeability of the hindgut intima to a variety of small organic molecules has been shown by MADRELL and GARDINER (1980) in several species of insects. The absorption of labeled glucose, glycine and palmitic acid by the hindgut of crickets was found by THOMAS and NATION (1984).

The electron dense material absorbed by the intima of the hindgut of *M. entomon* passes to the dilated extracellular spaces. Hence it can be assumed that invaginations of the apical cell membrane play a significant role in the passage of this material from the subcuticular space to the interior of the cell.

In general, apical infoldings in the cells of the anterior region of the hindgut of *M. entomon* are similar to those found in the hindgut of other isopods, which is involved in ionic and osmotic regulation (SMITH *et al.* 1969, WITKUS *et al.* 1969, HOLDICH and RATCLIFFE 1970, VERNON *et al.* 1974, HOLDICH and MAYES 1975). Unlike in osmotic-regulating epithelia, the apical infoldings of the anterior region of the hindgut of *M. entomon* are not associated with mitochondria, and microtubules do not appear in bundles.

The apical infoldings of the anterior region of the hindgut of *M. entomon* also differ from infoldings described in the posterior region of the hindgut (HRYNIEWIECKA-SZYFTER and TYCZEWSKA 1979). In the latter the apical infoldings are tightly closed, whereas in the anterior region they are widely dilated. Since the fixation conditions were identical for both the anterior

and the posterior regions of the hindgut, these differences could not be an artefact.

The results of this study show that the apical infoldings are labile structures whose organization is clearly correlated with the nutritional status. During starvation their gradual reduction can be observed. Hence they could be compared to microvilli, whose reduction in the hepatopancreas of isopods caused by starvation was described by STORCH and LEHNERT-MORITZ (1980).

References

ALIKHAN MA (1972) The fine structure of the midgut epithelium in the woodlouse, *Porcellio laevis* Latreille (Isopoda, Porcelionidae). Crustaceana Sup 3: 101–109

CLIFFORD B, WITKUS ER (1971) The fine structure of the hepatopancreas of the woodlouse *Oniscus asellus*. J Morphol 135: 335–349

CORUZZI L, WITKUS R, VERNON GM (1982) Function-related structural characters and their modifications in the hindgut epithelium of two terrestrial isopods, *Armadillidium vulgare* and *Oniscus asellus*. Exp Cell Biol 50: 229–240

GOODRICH AL (1939) The origin and fate of the entoderm elements in the embryogeny of *Porcellio laevis* Latr. and *Armadillidium nasutum* B. L. (Isopoda). J Morphol 64: 401–429

HEROLD L, GOGGINS JA, WITKUS ER, VERNON GM (1976) Annulate lamellae in hindgut epithelial cells of Isopoda. Protoplasma 87: 291–296

HOLDICH DM (1973) The midgut/hindgut controversy in isopods. Crustaceana 24: 21–27

— RATCLIFFE NA (1970) A light and electron microscope study of the hindgut of the herbivorous isopod, *Dynamene bidentata* (*Crustacea: Peracarida*). Z Zellforsch 111: 209–227

— MAYES KR (1975) A fine-structural reexamination of the so-called "midgut" of the isopod *Porcellio*. Crustaceana 29: 186–192

HRYNIEWIECKA-SZYFTER Z, TYCZEWSKA J (1978) The fine structure of the hepatopancreas of *Mesidotea entomon* L. (Crustacea, Isopoda). Bull Soc Amis Sci Lettr Poznań, sér D 18: 70–79

— — (1979) Fine structure and localization of alkaline phosphatase in the hindgut of *Mesidotea entomon* L. (Crustacea, Isopoda). Bull Soc Amis Sci Lettr Poznań, sér D 19: 57–64

(in press) Changes in the epithelium of the anterior region of the hindgut of *Mesidotea entomon* (Crustacea, Isopoda) correlated with haematopoiesis. Bull Soc Amis Sci Lettr Poznań, sér D, 26

JONES DA, BABBAGE PC, KING PE (1969) Studies on digestion and the fine structure of digestive caeca in *Eurydice pulchra* (Crustacea: Isopoda). Mar Biol, Berlin (West) 2: 311–320

LINDQUIST OV, FITZGERALD G (1976) Osmotic relationship between blood and gut fluids in the isopod *Porcellio scaber* Latr (Crustacea). Comp Biochem Physiol [A] 53: 57–69

MADRELL SHP, GARDINER BOC (1980) The permeability of the cuticular lining of the insect alimentary canal. J Exp Biol 85: 227–237

MURLIN JR (1902) Absorption and secretion in the digestive system of land isopods. Proc Acad Nat Sci Phi 54: 284–359

NICHOLLIS AG (1931) Studies on *Ligia oceanica*. II. J mar biol Ass UK 17: 675–707

PROSSER CL (1973) Respiratory function of blood. In: PLOSSER CL (ed) Comparative animal physiology. W. B. Saunders Co, Philadelphia

SMITH JM, NADAKAVUKAREN MJ, HETZEL HR (1975) Light and electron microscopy of the hepatopancreas of the isopod *Asellus intermedius*. Cell Tiss Res 163: 403–410

SMITH WJ, WITKUS ER, GRILLO RS (1969) Structural adaptation for ion and water transport in the hindgut of the woodlouse *Oniscus ascellus*. J Cell Biol 43: 135–136

STEEVES HR (1963) The effects of starvation on glycogen and lipid metabolism in the isopod *Lirceus brachyurus* (Harger). J Exp Zool 154: 21–37

STORCH V (1984) The influence of nutritional stress on the ultrastructure of the hepatopancreas of terrestrial isopods. Symp Zool Soc London 53: 167–184

— LEHNERT-MORITZ K (1980) The effects of starvation on the hepatopancreas of the isopod *Ligia oceanica*. Zool Anz 204: 137–146

ŠTRUS J, BURKHARDT P, STORCH V (1985) The ultrastructure of the midgut glands in *Ligia italica* (Isopoda) under different nutritional conditions. Helgoländer Meeresunters 39: 367–374

SUTTON SL (1972) Woodlice. Pergamon Press, pp 144

THOMAS KK, NATION YL (1984) Absorption of glucose, glycine and palmitic acid by isolated midgut and hindgut from crickets. Comp Biochem Physiol 79 A: 289–295

VERNON GM, HEROLD L, WITKUS ER (1974) Fine structure in the digestive tract epithelium in the terrestrial isopod *Armadillidium vulgare*. J Morphol 144: 337–360

WÄGELE JW, WELSCH U, MÜLLER W (1981) Fine structure and function of the digestive tract of *Cyathura carinata* (Krøyer) (Crustacea, Isopoda). Zoomorphology 98: 69–88

WITKUS ER, GRILLO R, SMITH WJ (1969) Microtubule bundles in the hindgut epithelium of the woodlouse *Oniscus asellus*. J Ultrastruct Res 29: 182–190

ZIMMERMANN H-P, GRANZOW V, GRANZOW C (1976) Nuclear glycogen synthesis in Ehrlich ascites cells. J Ultrastruct Res 54: 115–123