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Influence of starvation and feeding on the hepatopancreas of larval *Hyas araneus* (Decapoda, Majidae)*

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ABSTRACT: Zoea-1 larvae of *Hyas araneus* were kept under different nutritional conditions. Their midgut glands were investigated with a transmission electron microscope. The glandular epithelium consists of the cell types known from adult decapods. It is mainly the R-cell type that undergoes ultrastructural alterations which reflect nutritional conditions. R-cells of fed larvae are characterized by large lipid inclusions; after a certain period of food deprivation (point-of-no-return) the original ultrastructure cannot be reestablished. Refeeding results in large glycogen deposits in these cells.

INTRODUCTION

Food is considered one of the key factors controlling survival in meroplanktonic larvae and an extensive literature exists on the influence of both quality and quantity of nutrition. Effects of starvation periods, however, which must be expected in a patchy environment such as the marine plankton, are far less known. Anger & Dawirs (1981) experimentally investigated impacts of food deprivation on the rate of larval development and survival of the spider crab, *Hyas araneus.* They reviewed our present knowledge about starvation resistance in decapod larvae. Later they suggested that some response patterns in relation to absence of suitable food may be general features of brachyuran larvae (Anger et al., 1981). Although there is already some information on changes in the elemental and biochemical composition of larvae exposed to starvation (Anger & Dawirs, 1982), the physiological and ultrastructural basis of the effects observed has remained unclear. This lack of understanding is particularly obvious in the phenomena called "point-of-no-return" and "point-of-reserve-saturation" (Anger & Dawirs, 1981), both characterized by unknown irreversible processes.

The present study was conducted to depict ultrastructural changes in the midgut glands (hepatopancreas) of zoea-I stage larvae of *H. araneus* both developing regularly,

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and exposed to differential periods of starvation. These changes are compared with those after different schedules of food presence and absence.

MATERIAL AND METHODS

In January 1982, ovigerous females were dredged near the North Sea island of Helgoland. Thereafter they were maintained in a laboratory recirculating system with natural seawater (ca 32 ‰) kept at constant 2 °C, until the first larvae hatched. These were collected at 6 °C as described by Anger & Dawirs (1981) and then transferred individually into vials with 25 ml of filtered seawater (Millipore membrane filter, 0.4 µm pore size).

When fed, the larvae received fresh food (*Artemia* spec. nauplii and *Brachionus plicatilis*) and fresh seawater every second day. Further details on the maintenance and treatment of both fed and starved larvae are given by Anger & Dawirs (1981). All experiments were carried out at 12 °C.

The experimental design is shown in Figure 1. For electron microscopy, larvae were sampled and treated in the following way: to facilitate penetration of the fixative, the abdomen, dorsal spine, and rostrum of each larva were cut off under a stereo microscope. The rest of the larval body was immediately transferred into cold 3.5 % glutaraldehyde in 0.1 M Sörensen's buffer (pH 7.5) for 2 h. After rinsing the material five times for 5 min, respectively, in cold 0.1 M Sörensen's buffer, it was postfixed in 1 % osmium tetroxide solution for 2 h. At all stages of fixation, the solutions were maintained at 2 °C. After

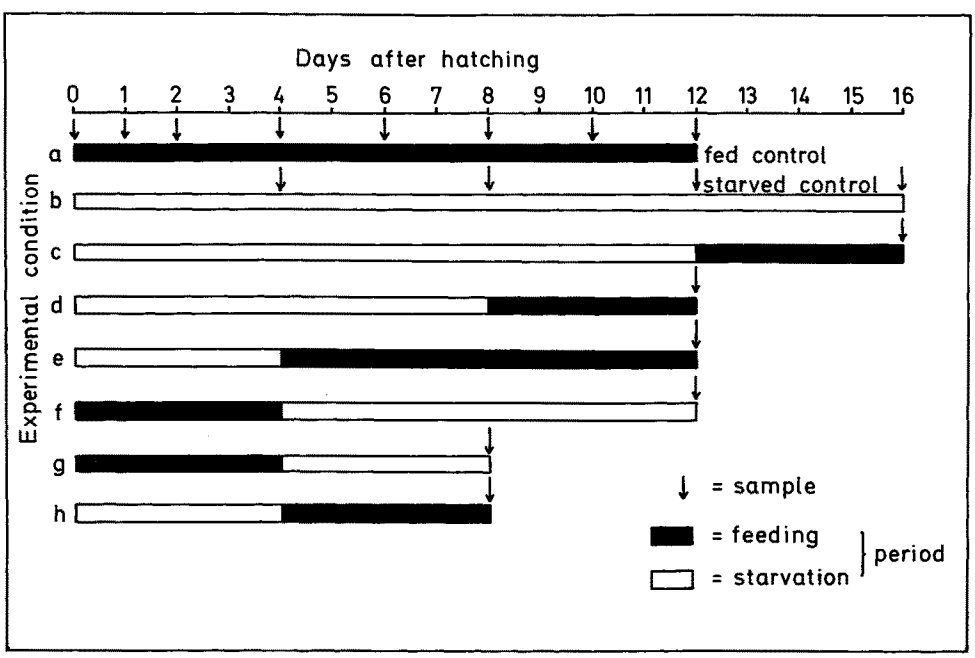

Fig. 1. Experimental design: Schedule of feeding and starving zoea-1 stage larvae of *Hyas araneus*. Arrows: times of sampling larvae for electron microscopy

dehydration through a graded series of ethanol at room temperature, the material was embedded in araldite. Sections were cut on to water with an ultramicrotome (Ultracut Reichert) and picked up on coated copper grids. After staining with uranyl acetate (saturated solution in 70 % methanol) and lead citrate, they were examined with a Zeiss EM 9 S-2 electron microscope.

1 µm sections used for light microscopy were stained with an aqueous solution of 1 % toluidine blue.

RESULTS

As in other decapods so far investigated with the transmission electron microscope the epithelium of the midgut-glands of *Hyas araneus* larvae consists of clearly distinguishable cell types (R-, F-, B-cells) which arise from E-cells located in the blind, distal tip of the hepatopancreas tubules. The question of cell lineage within the organ, however, has not yet been answered definitively (for recent discussion see: Gibson & Barker, 1979; Hopkin & Nott, 1980).

All of these cells bear an apical microvillous border. Basally they are characterized by a tubular labyrinth, also typical of other decapod species, which at least partly is connected to the lateral and basal plasma membrane (Fig. 2a). The glandular tubuli are surrounded by a basal lamina underlain by a meshwork of striated muscle cells and flat extensions of cells containing electron-dense granules probably representing pigment cells (Fig. 2b). Additionally, cells considered excretory or white pigment cells (Green & Neff, 1972) were found (Fig. 2b).

R-cell (Figs 2–4): R-cells or resorptive cells are the most numerous of the above-mentioned cell types. They are mainly concerned with the assimilation and storage of nutrients and thus contain much lipid and/or glycogen when the animals were well fed. Lipid inclusions differ in size according to experimental conditions. Also glycogen can occupy large areas of this cell type. The endoplasmic reticulum is not a prominent feature of R-cells but varies in extent and ultrastructure according to feeding/starvation conditions. It is this cell type that may contain calcium, phosphorus, and magnesium which accumulate in concentrically structured granules (Hopkin & Nott, 1979; Storch et al., 1982). Mitochondria again vary ultrastructurally; the same applies to the number of lysosome-like inclusions.

In newly hatched larvae this cell type is characterized by many lipid inclusions the largest of which may occupy most of the cellular volume (Fig. 3a). The nucleus occurs singly and tends to be somewhat flattened. The rough endoplasmic reticulum occurs as individual cisternae throughout the cytoplasm and may form bulky stacks. Mitochondria tend to be in apical location and do not show peculiarities. The small or intermediate Golgi apparatus is normally located in a supranuclear position; glycogen is lacking.

R-cells undergo marked ultrastructural alterations in response to food deprivation. Presumably this cell type enables lipid storing crustacea to withstand starvation periods for more than one year (Storch et al., 1982). The alterations increase gradually during the course of starvation and are more or less identical in different specimens. After 16 days (Fig. 3b) their prominent features are: a reduced cell size, lack of stored lipid, reduction of mitochondria, increase of the thickness of the basal lamina which exhibits many irregular infoldings. The basal tubular system is voluminous (Figs 3b, 4a).

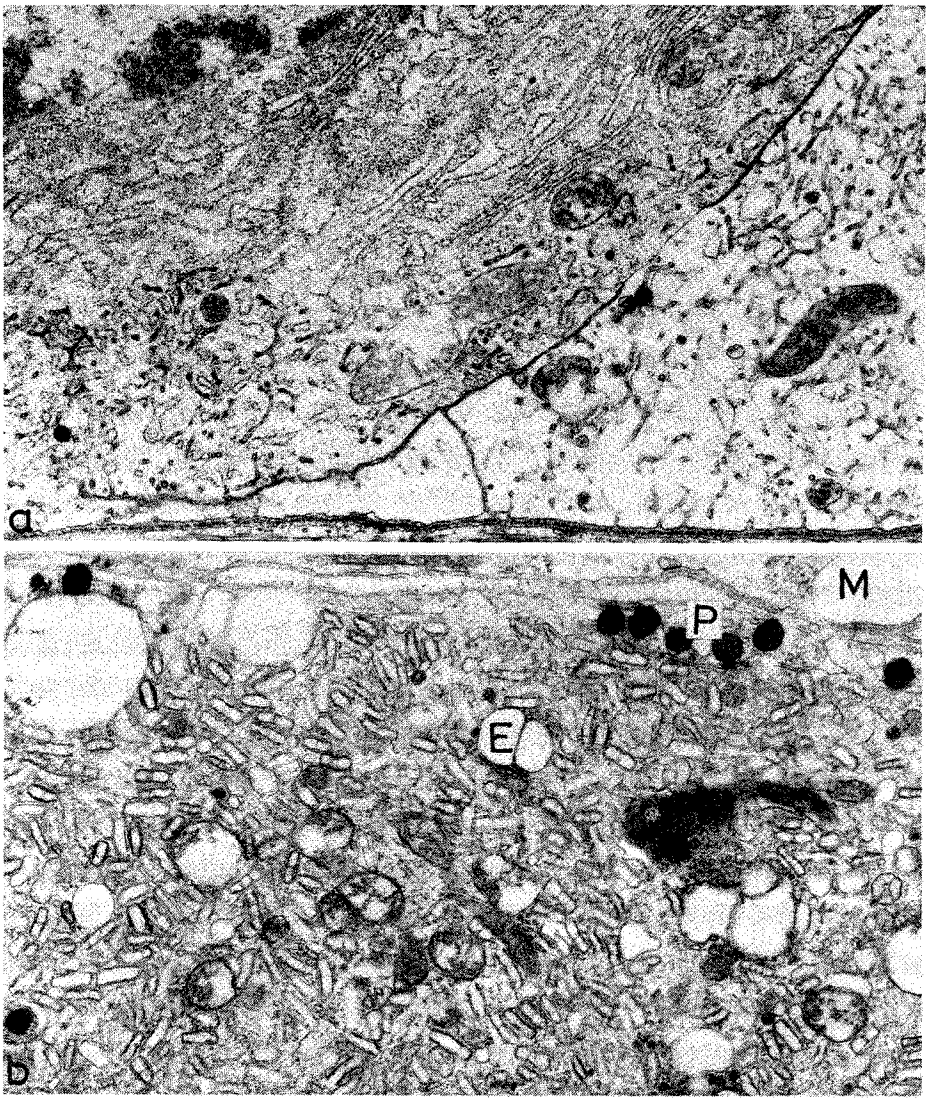

Fig. 2. (a) Basal portion of R-cells (12 000:1). (b) Cells immediately below the glandular cells of the midgut gland (M): pigment cells (P) and excretory or white pigment cells (E) (14 300:1)

A starvation period of 12 days (which causes principally identical changes as one of 16 days) followed by a feeding period of 4 days, results in a completely different ultrastructure (Figs 3c, 4b): Glycogen fields and small lipid droplets are the prominent features. The mitochondria are heavily swollen, ER profiles are rare. Electron-dense granules and different inclusion bodies may occur singly or in groups.

Feeding immediately after hatching followed by a starvation period of 4 or 8 days is different again. Large crescent and roundish lipid droplets are existent, glycogen is lacking.

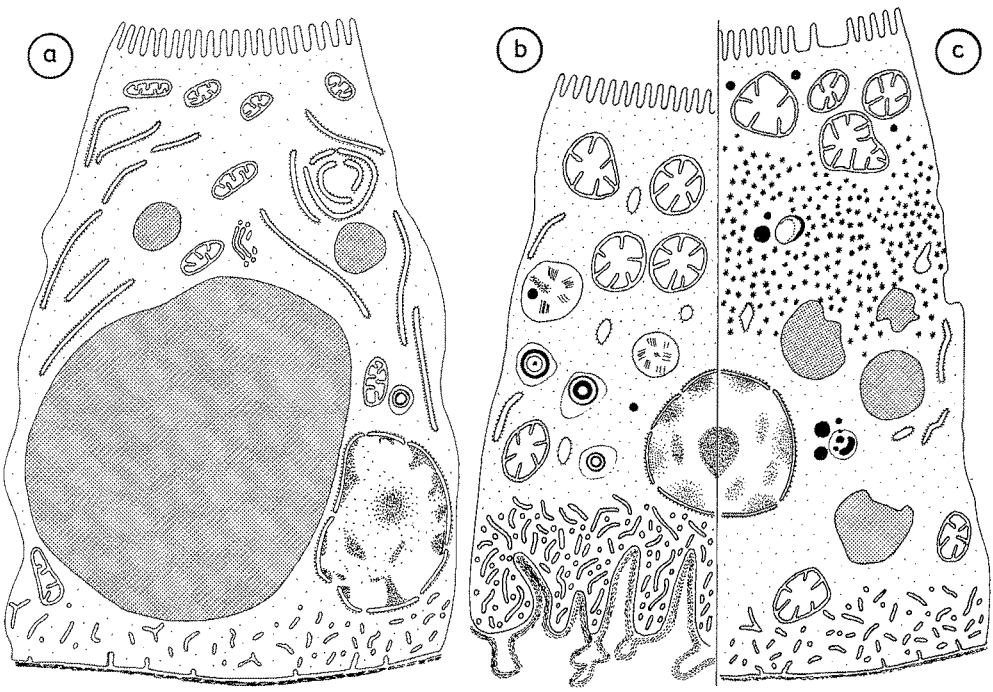

Fig. 3. Diagrams of R-cells under different conditions. (a) fed with *Artemia* and *Brachionus*. (b) starvation period of 16 days. (c) 12 days starvation and subsequent feeding (4 days)

F-cell (Fig. 5a): F-cells or fibrillar cells are highly basophilic, which is characteristic of enzyme-producing cells. The F-cells of *Hyas araneus* zoeae are rather uniform and more or less independent on food deprivation and subsequent feeding procedures. They exhibit extensive ribosome-studded endoplasmic reticulum the profiles of which are more or less parallel to the long axis of the cell. Mitochondria and numerous Golgi bodies are present. As in other cells, starvation results in dilatation of ER cisternae.

B-cell (Fig. 5b): In shape the B-cells correspond more or less to goblet cells of the vertebrate intestine. They bear a single, large vacuole (blisterlike cells) which squeezes the cytoplasm to the periphery, the cell organelles thus being concentrated in a very thin marginal layer of the cytoplasm. Basically and apically there is some more space for organelles left. A single cell nucleus is located at the base, where the cell is reduced to a narrow stem which is tenuously connected to the basal lamina. The apical portion is characterized by numerous membrane invaginations which form channels extending deep into the cell (Fig. 5b). Alterations provoked by feeding or starvation were not found.

DISCUSSION

The present study demonstrates that the epithelium of the hepatopancreas of larval *Hyas araneus* which is composed of the cell-types well known from adult decapods (Gibson & Barker, 1979; Hopkin & Nott, 1979, 1980; Loizzi, 1971; Storch & Welsch, 1977) undergoes marked ultrastructural changes dependent on the nutritional status. It is

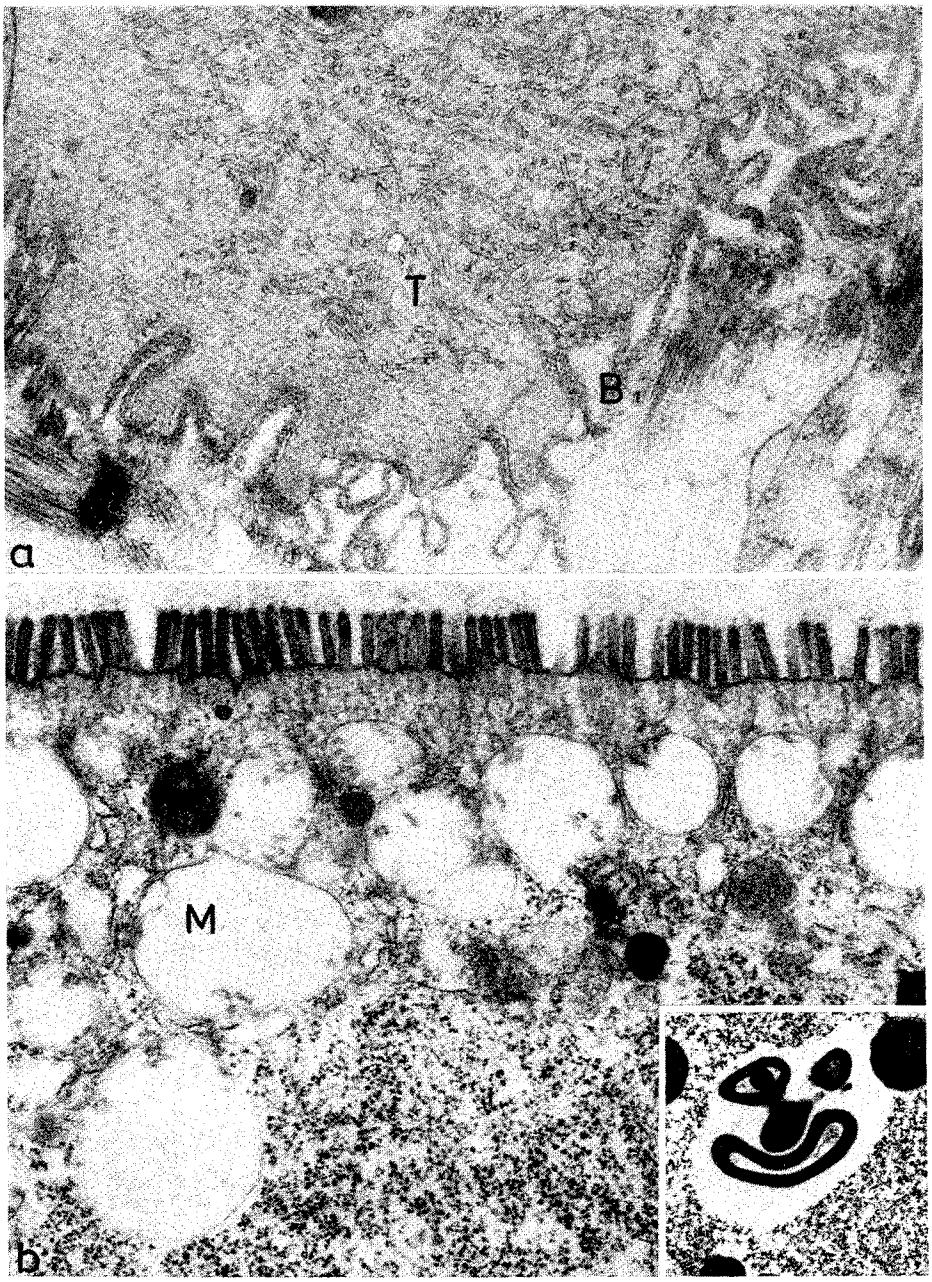

Fig. 4. (a) Basal portion of R-cell after 16 days without food with tubular system (T) and folded basal lamina (B). (b) Apical portion of R-cell (12 days starvation, 4 days refeeding) with irregular microvillous border, swollen mitochondria (M), and large amounts of glycogen (14 300:1). Inset: inclusion body (3800:1)

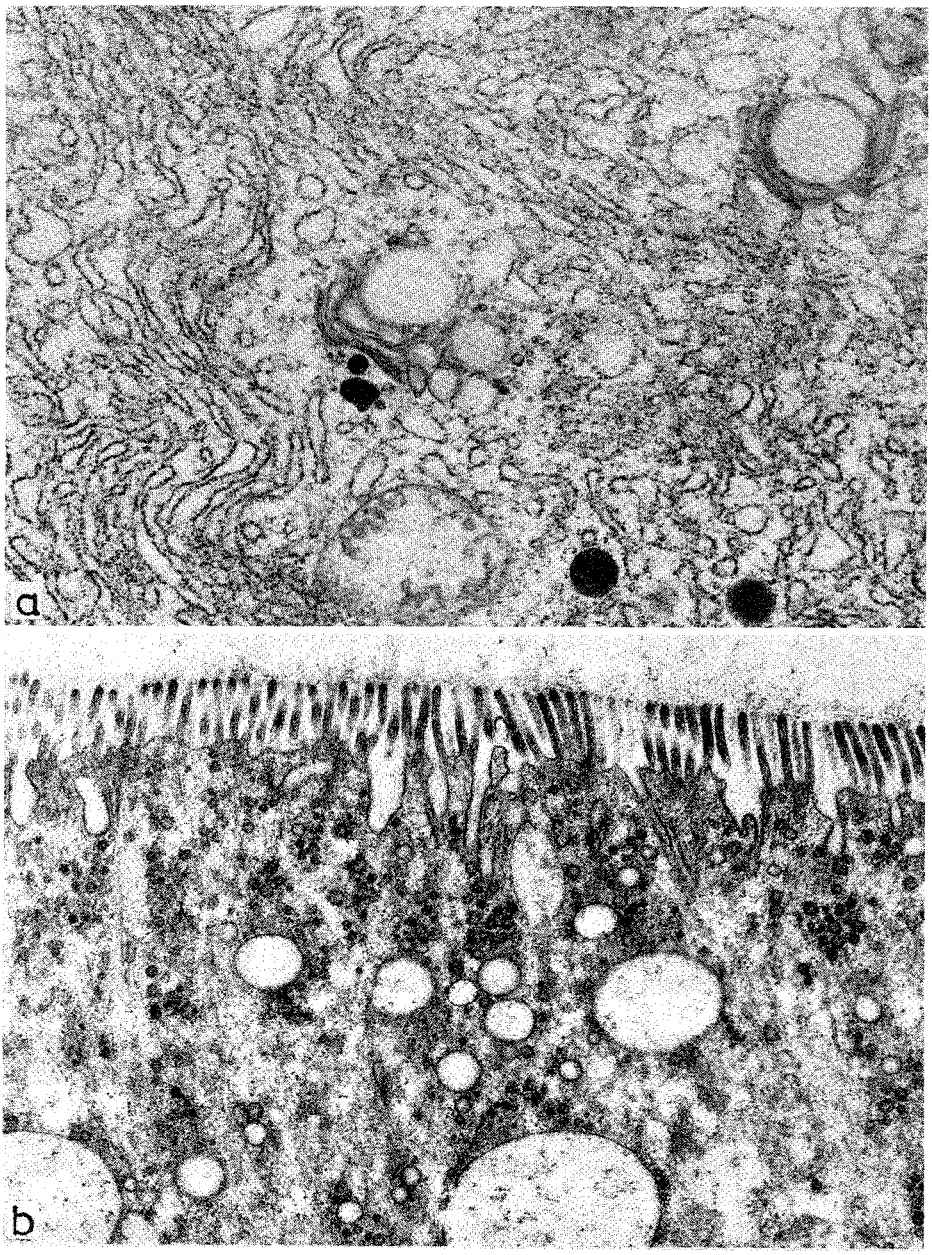

Fig. 5. (a) Central part of F-cell (fed). (b) Apical part of B-cell (fed) (14 300:1)

mainly the R-cell that is affected. R-cells of freshly hatched zoeae are stuffed with lipid inclusions representing yolk reserves. They resemble the R-cells of adult land-dwelling hermit crabs (Storch et al., 1982; Lawrence, 1970) which are well adapted to tolerate severe starvation periods even for more than one year. As has been shown by Anger

& Dawirs (1981), this reserve in zoea-1 larvae of *H. araneus* is not sufficient to reach the next instar when the larva suffers from continual lack of food. In contrast to all other decapods so far investigated electron microscopically, glycogen was never found in conspicuous amounts in R-cells of regularly fed individuals. This is reminiscent of the liver of teleost fish species the metabolism of which can be predominantly more lipid- or more carbohydrate-oriented.

During starvation the amount of lipid decreases steadily. After more than 4 days R-cells appear small due to depletion of lipid. As in other crustacea and in other animal phyla the decrease of lipid is accompanied by ultrastructural alterations that begin to appear soon after the cessation of feeding. As has been found by Anger & Dawirs (1981) 16 days is close to the maximal survival time of zoea-1 larvae at 12 °C. At this stage of starvation the basal lamina shows an increase in thickness as has been reported by earlier authors in bivalves, crustaceans, insects, and teleosts subjected to starvation (for references see Storch et al., 1982).

As in a variety of cells of different phyla affected by starvation, the residual bodies indicating autolysis increase in number. These findings correspond to results obtained e. g. by Schmekel (1972) for molluscs, Janssen & Möller (1981) for cnidarians, and Storch et al. (1982) for crustaceans.

The endoplasmic reticulum, which is regularly developed in well-fed larvae, can be found broken up into rounded vesicles or short and widened cisternae. Again, similar observations have been made in different phyla (for references see David, 1967; Schmekel, 1972; Storch et al., 1982). These findings are in accordance with the observations of Hallberg & Hirche (1980) that in marine calanoid copepods discontinuation of feeding during overwintering is associated with low enzyme activities.

Mitochondria become swollen even after a relatively short period of starvation. This is paralleled by findings, for example, in teleost hepatocytes (Storch & Juario, 1983) where mitochondria can surpass the cell nucleus in size after cessation of feeding.

Refeeding after 8 and 12 days, respectively, does not result in a complete recovery of the affected R-cells. As is shown in Fig. 4b the mitochondria are still swollen and occur mainly in an apical position. They are underlain by considerable quantities of glycogen-particles and lipid droplets which are smaller in size than in well fed larvae. Glycogen is a wide-spread carbohydrate reserve material. When required, for example to supply the energy for muscular contraction, the glycogen is broken down and transported to the muscle as glucose. Only the latter is found in the blood, and the glucose of the blood (or haemolymph) of several species is maintained at a steady level during adverse conditions, such as starvation (Veldhuijzen, 1975). Thus, the glycogen deposit may help to explain why larvae which have starved for some time can survive and move for rather long periods. Anger & Dawirs (1981) found that after a 12 days starvation period still below the lethal threshold at 12 °C no larva recovered and reached the second stage, but some of them lived for up to 29 days before they died. Their assumption that these zoeae had not completely lost their ability to take up food is thus corroborated by our electron microscopic findings.

Additionally, the data presented here indicate that a reestablishment of the R-cell ultrastructure seems to be impossible after a certain period of initial starvation immediately after hatching. When larvae were fed immediately after hatching (and prior to starvation, experiments f and g in Fig. 1), the R-cells were found to be ultrastructurally

different from those treated in the opposite way (experiments d, e, h in Fig. 1). Four days of initial feeding are even sufficient to build up a new cuticle. This, again, corroborates the findings of Anger & Dawirs (1981).

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