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Effect of the detergent alkylbenzene sulphonate (ABS) on hepatopancreas of the shrimp *Crangon crangon* L.

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Abstract

Shrimps *Crangon crangon* L., acclimated in the laboratory for 24 h in brackish water (7 $^{0}/_{00}$ salinity), were incubated in solutions of anionic detergent alkylbenzene sulphonate (ABS) in concentrations of 5 – 50 ppm. After 24 h or 96 h of incubation, preparations of hepatopancreas were stained and examined by light or transmission electrone microscopy. The light microscopy examination revealed a flattening of the duct cells, pyknotic nuclei in these cells, fine granular secretions in the ducts of the gland, and cellular infiltration. The transmission electron microscopy examination revealed an impairment and destruction of the lysosomal and mitochondrial membranes of the hepatopancreas cells in the shrimps exposed to alkylbenzene sulphonate.

Introduction

Synthetic detergents are a diverse group of compounds and part of a larger group known as surface active agents or surfactants. The otherwise diverse molecular forms of detergents have in common a hydrophilic and hydrophobic polarity, hence arise their three important general properties:

1. the tendency to concentrate at surfaces,
2. the reduction of surface tension,
3. the formation of aggregates of ions and micelles when present in solutions above a certain critical concentration (ANASTASIU and JELESCU 1973).

Anionic detergents are the best studied group, since they are the most widely used and represent the major source of detergent pollution of waters. The most common anionic detergents are the alkyl-aryl sulphonates such as alkylbenzene sulphonate (ABS) and alkyl sulphate. An ABS with an unbranched hydrocarbon chain is usually referred to as a linear alkylate sulphonate or LAS. Alkyl sulphonates figure prominently among the “soft”, rapidly biodegradable detergents. ABS derived from tetrapropylene, and having a branched hydrocarbon chain, is more resistant to biodegradation, and thus more persistent in the environment (so-called “hard” detergent). At a time of increasing contamination of the seas with detergents, studies of the survival and pathophysiology of marine animals seem to be of considerable importance. This study is an attempt to present a picture of the morphological changes of the hepatopancreas of the shrimp induced by the detergent alkylbenzene sulphonate.

Material and Methods

The experiments were carried out under laboratory conditions. Shrimps *Crangon crangon L.* were collected during summer months in coastal waters of Gdańsk Bay (7 $^{0}/_{00}$ salinity, 12–15° C). After 24 h of acclimation, the shrimps were kept in plastic containers (26 x 20 x

30 cm; 10 l of brackish water; depth of water 20 cm). Mean body weight ranged from 300 to 400 mg, body length varied from 25 to 40 mm.

A stock solution of 1 g of ABS/l was prepared from alkylbenzene sulphonate obtained from the "Pollena" plant in Gdańsk, which contained 80 % of pure ABS detergent. To 10 l of brackish water (7 $^{0}/_{00}$ salinity) in plastic containers, the proper amounts of the stock solution where added, in order to obtain final concentrations of 5, 7.5, 10, 25 and 50 ppm of the ABS detergent. The animals were incubated in these solutions for 24, 48 or 96 h. The solutions of the detergent, as well as the bottom sand in the tanks, were exchanged once daily. The animals were fed during the experiment with chopped shrimps.

For the histological preparations, the hepatopancreas was removed immediately after the shrimp's decapitation. The preparations were stained with hematoxilin-eosin according to ZAWISTOWSKI (1970).

For electron microscope studies, double fixation was employed. The hepatopancreas was fixed in a 3 % solution of glutaraldehyde in 0.1 M phosphate buffer of pH 7.2 at 4° C for 3 h. The preparations were then thoroughly washed in phosphate buffer pH 7.2. Subsequent secondary fixation was for 2 h at 4° C in 1 % osmium tetroxide, buffered to pH 7.2 with 0.1 M phosphate. Following fixation, the specimens were routinely dehydrated and embedded in EPON 812. Ultra-thin sections were cut on a LKB Ultratome using a glass knife. They were then stained with uranyl acetate and lead citrate, and examined in a "Tesla" transmission electron microscope.

Results

The light microscopic appearance of the hepatopancreas of control shrimp is shown in Fig. 1. The epithelial cells of the control shrimp's hepatopancreas cells varied in shape and size, but they were higher and their large nuclei were situated basally. The lumen of the glands was narrow and free of granular secretion. Intense proliferation and regeneration of cells was also observed (Fig. 1).

After 96 h exposure to the 5 ppm of the ABS detergent, epithelial cells were strongly flattened, their nuclei pyknotic, no proliferation of cells was visible, and an excress of fine-granular secretion in the ducts was observed (Fig. 3).

After 24 h exposure to the 5 ppm of the ABS detergent, large cells with numerous large vacuoles were ovserved in the sections of hepatopancreas.

Infiltrations with mononuclear cells also occurred (Fig. 4). In transmission electron micrographs (TEM) after 96 h exposure to the 5 ppm of the ABS detergent, lysis of the cell membranes and "overflowing" of the cell content into the gland duct of the hepatopancreas were revealed (Fig. 5). The membranes of the cell organellae were destroyed (Fig. 6), and cell disorganization and destruction appeared (Fig. 7).

Discussion

Detergents penetrate into aquatic animals through the respiratory epithelium, alimentary system and integument, although in crustaceans the integument exerts isolating effects (GOLDRACE 1968, THANG et al. 1980). It is obvious, however, that the thick carapace of crabs protects the body from detergents more effectively than the carapace of shrimp or krill (DREWA et al. 1980, DREWA 1981). Detergents bind to biological membranes, thus leading to their impairment and damage. These mechanisms of detergent action have been described by RAZIN (1972), ANASTASIU and JELESCU (1973), ZUBRZYCKA (1975), HELENIUS and SIMONS (1975) and ZASLAVSKY et al. (1978). The detergent-induced lability of biological membranes causes, among other things, changes in the serum ion

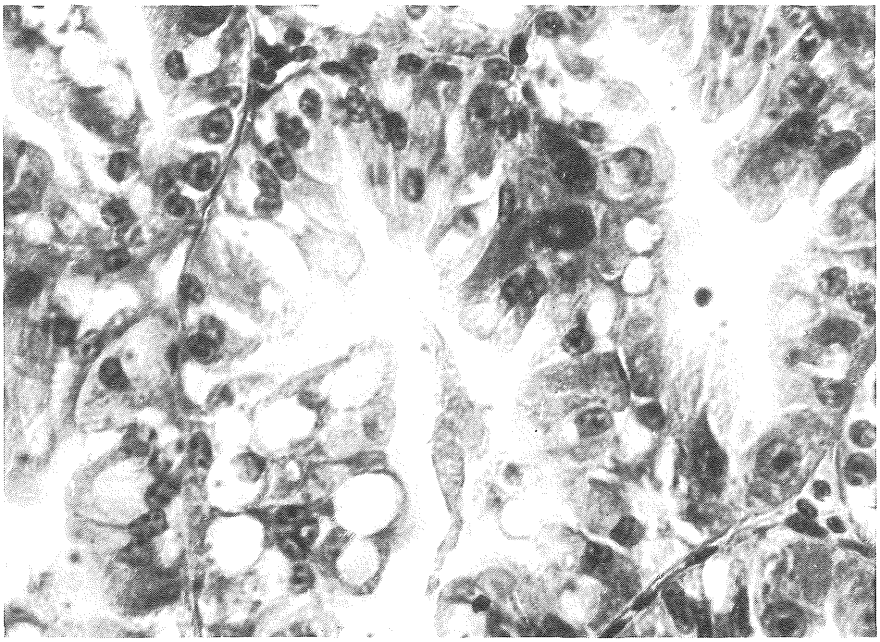

Figure 1

Hepatopancreas of control shrimp, *Crangon crangon.* High epithelial cells. Intense proliferation of the cells. Gland ducts free of granular secretion. HE staining. Magnification: 400X

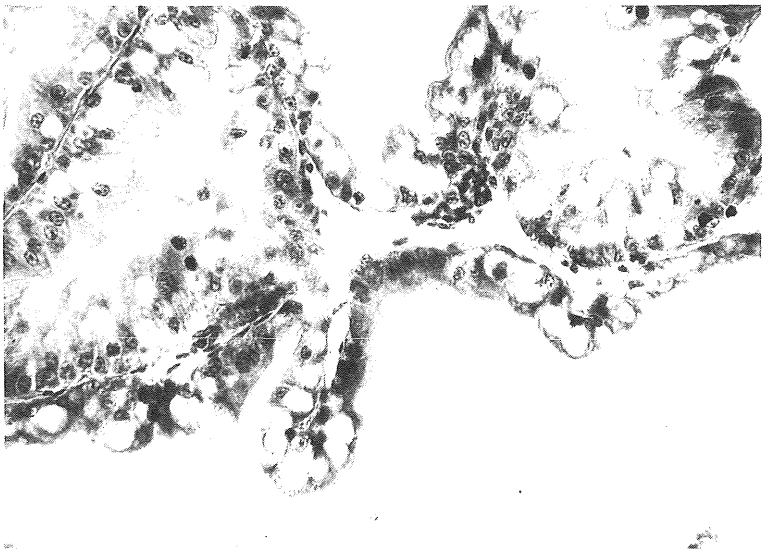

Figure 2

Hepatopancreas of shrimp *C. crangon* after 96 h exposure to 5 ppm of ABS detergent. Epithelial cells strongly flattened. Large quantities of mucus in the gland ducts. No proliferation of cells. HE staining. Magnification: 250X

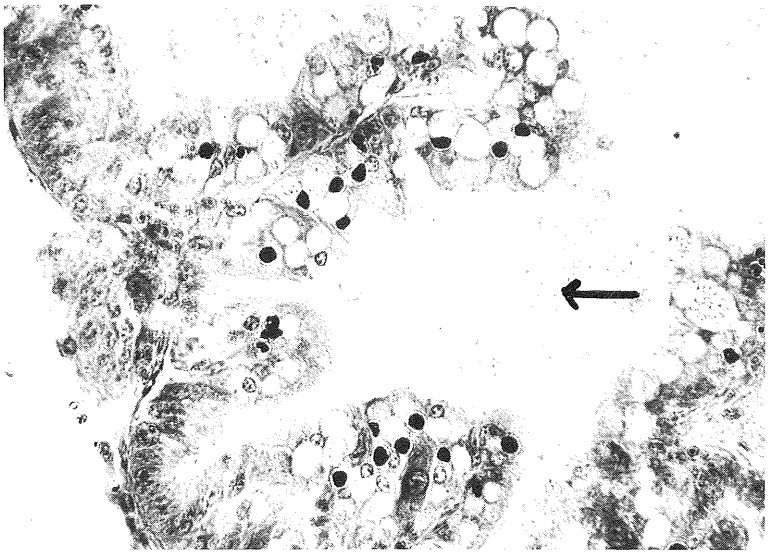

Figure 3

Hepatopancreas of shrimp *C. crangon* after 96 h exposure to 50 ppm of ABS detergent. Pyknotic nuclei. Large quantities of fine-granular secretion in the gland duct. HE staining. Magnification: 250X

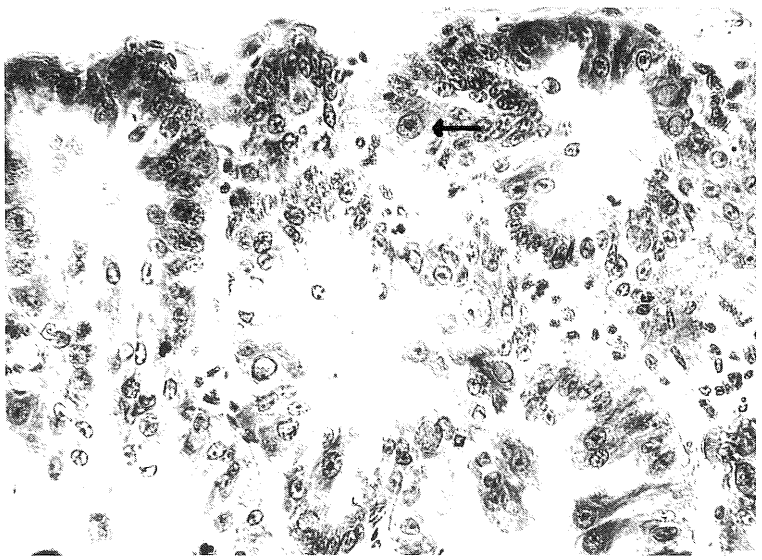

Figure 4

Hepatopancreas of shrimp *C. crangon* after 24 h exposure to 5 ppm of ABS detergent. Visible are single, large cells with numerous large vacuoles (arrow). Mononuclear cell infiltration. HE staining. Magnification: 250X

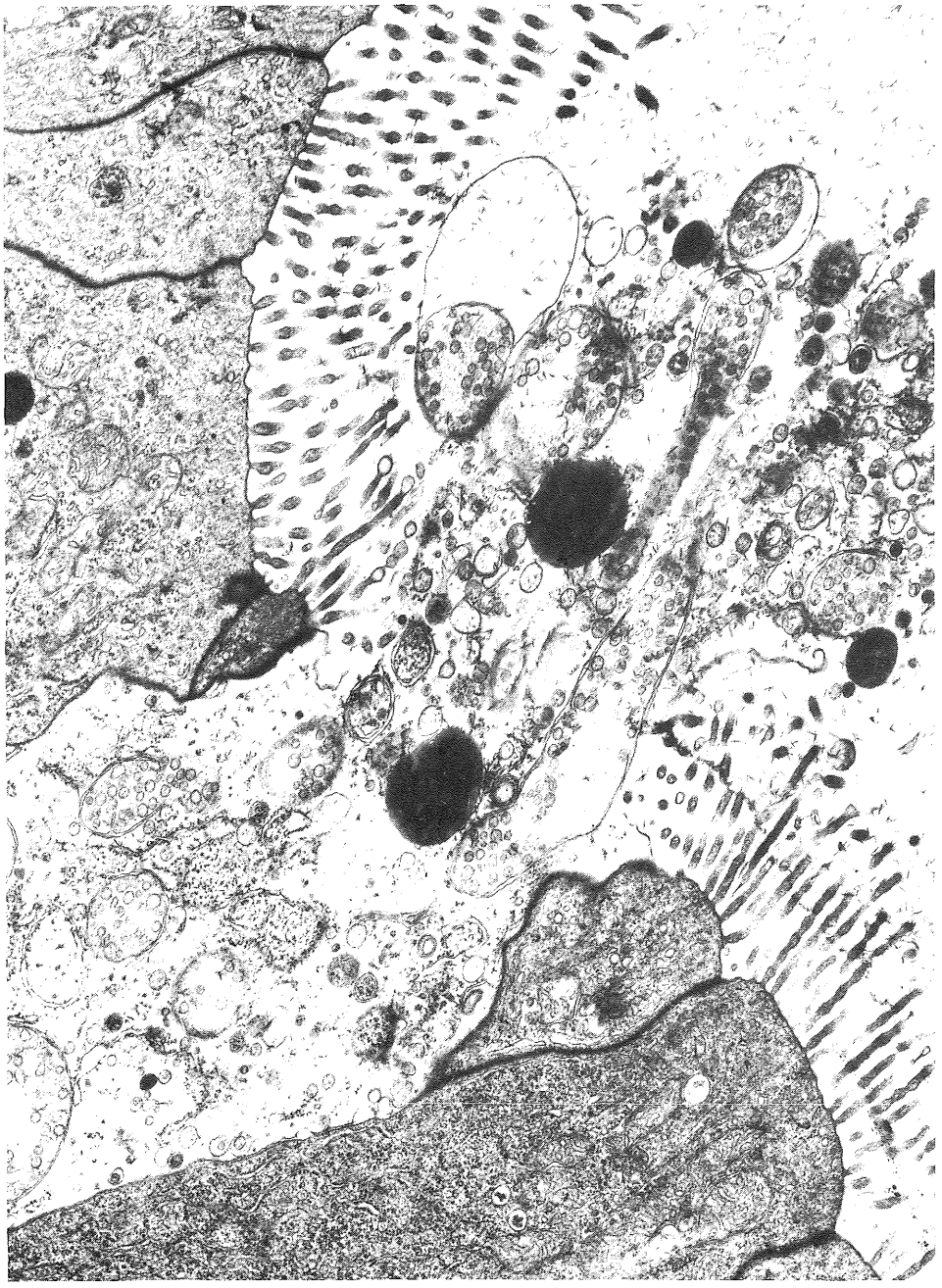

Figure 5

Transmission Electron Micrograph (TEM). Hepatopancreas of shrimp *C. crangon* after 96 h exposure to 5 ppm of ABS detergent. Cytoplasmic membrane damaged, content of the cell "overflows" into the gland duct. Magnification: 4,000X

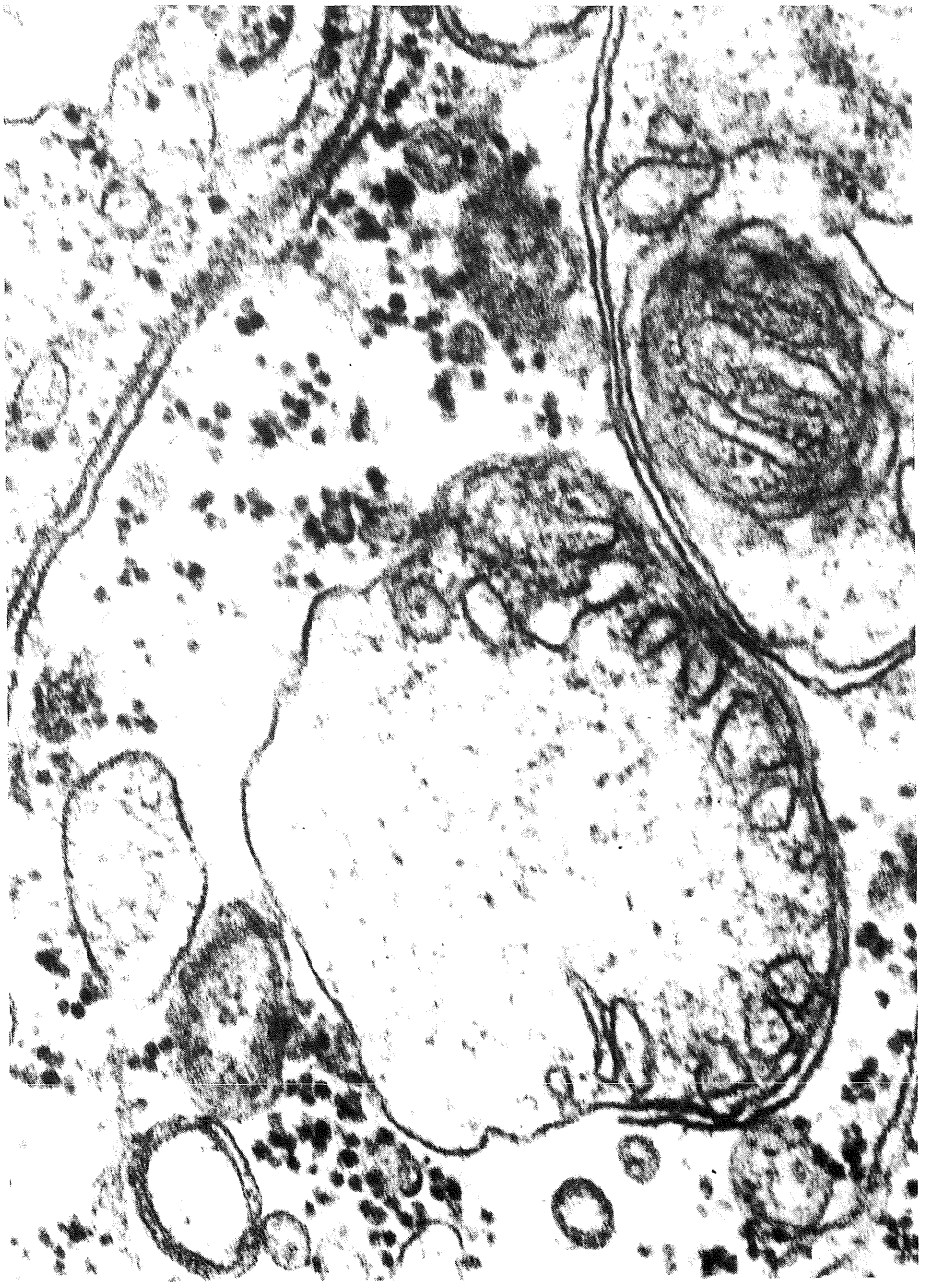

Figure 6

TEM. Hepatopancreas of shrimp *C. crangon* after 96 h exposure to 50 ppm of ABS detergent. Damaged external membrane of mitochondrium. Magnification: 20,000X

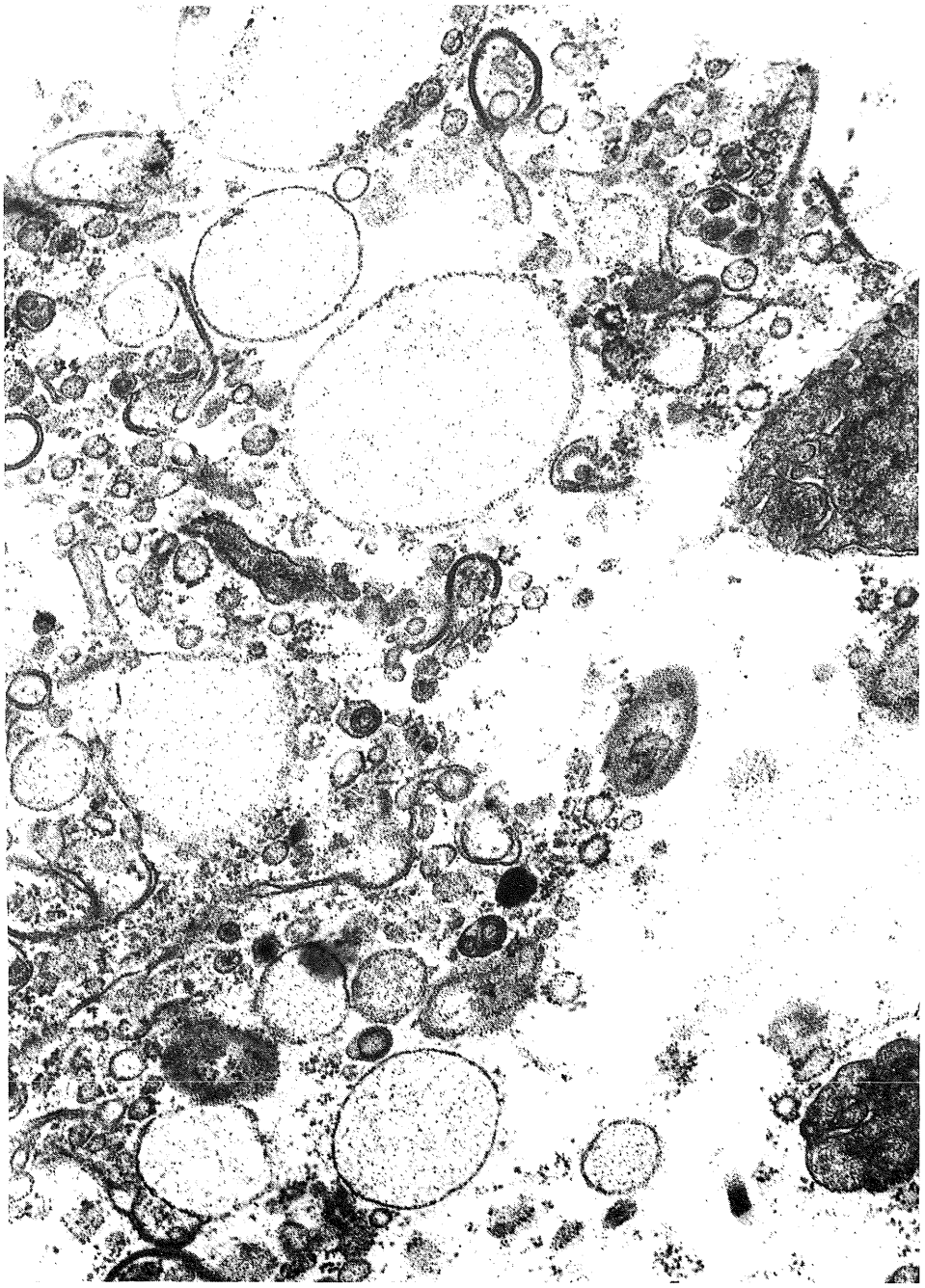

Figure 7

TEM. Hepatopancreas of shrimp *C. crangon* after 96 h exposure to 50 ppm of ABS detergent. Disorganization inside the cell. Damaged cell organellae. Magnification: 44,000X

composition of the shrimps (ZBYTNIEWSKI et al. 1978). By causing membrane lability, detergents induce not only changes in the permeability of cell membranes (cytoplasmic membranes) but also of membranes of cell organelles, e.g. lysosomes and mitochondria. According to the present results, lysosomes damaged by the detergent release acid hydrolases into the cytoplasm (a fine granular secretion occurs in the ducts of hepatopancreas). The detergent causes damage to the structures of the hepatopancreas. Many focal infiltrations and dissolution of membranes were observed. After 96 h exposure, shedding of the duct epithelia takes place. Fine-granular secretion penetrates the gland ducts through the demaged cell membranes (Fig. 3). Electron micrographs (Fig. 5) show destroyed cell membranes and "overflowing" of cell content into the gland ducts as well as destroyed cell organelles (Fig. 7). Internal and external membranes of the mitochondria undergo lysis in the presence of the detergent (Fig. 6).

The morphological picture of the hepatopancreas of the shrimp exposed to the detergent action suggests cell autolysis. Changes resembling those found in the present studies in the shrimp hepatopancreas have been described by LANG (1967), VERNBERG et al. (1979), and MOORE (1979) for gills of fishes exposed to other pollutants. The long-term action of the detergent in aquatic animals inhibits tissue regeneration. Hepatopancreas cells of the shrimp exposed to the detergent exhibit not mitotic activity. It can be assumed that similar disturbances occur also in other tissues.

WALCZAK et al. (1983) have found an increase of mean corpuscular volume (MCV), a decrease of the red blood cells (RBC), of hemoglobin concentration, of white blood cells (WBC), and of total protein concentration in fishes *Cyprinus carpio* exposed to a 5 ppm solution of ABS detergent from 7 to 125 days. Histolocically, the congestion of the vascular system, hypertrophy of epithelial cells of the gills and enlarging of the hepatopancreas of experimental fishes were also observed. The changes were permament and did not disappear after the fishes were kept in pure water without detergent for six months.

In summary, the detergent ABS induces an increase in the number of lysosomes in hepatopancreas cells and brings about changes in the activity of lysosomal enzymes, causes liability of cytoplasmic membranes and membranes of cell organelles, and inhibits mitosis and cell regeneration.

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