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RESISTANCE OF THE ALIMENTARY CANAL OF Daphnia magna STRAUS TO ENTEROPATHOGENIC NAG VIBRIOS

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At the present time daphnias are being used on an ever-increasing scale as bioindicators of exposure to various exogenous pathogens. In the study of mechanisms of susceptibility and resistance of animals of different classes to bacteria enteropathogenic for man it has been shown that lower crustaceans (daphnias), even after direct contact with enormous numbers of NAG vibrios, do not develop the disease, but utilize the vibrios as food [1]. This fact can be partly explained by the peculiarities of the structure of the alimentary tract of daphnias, as established in the course of evolution.

This paper describes a study of the normal structure of the alimentary tract of daphnias and its response to the action of NAG vibrios.

EXPERIMENTAL METHOD

The investigation was conducted on 30 daphnias (*Daphnia magna* Straus), infected by the scheme described previously [1]. To obtain semithin sections the daphnias were fixed whole in 2.5% glutaraldehyde solution in 0.1M phosphate buffer (pH 7.3) for 1 h. After rinsing in several portions of the same buffer, the daphnias were tested with 1% OsO_4 solution in the same buffer and dehydrated in acetone solutions of increasing concentration. Semithin sections 1 μ thick were cut from blocks embedded in epoxide resins on the LKB-III ultrotome, and these were then stained with methylene blue, azure II, and fuchsine. The daphnias were investigated by a luminescence serologic method at different times after contact with NAG vibrios. For this purpose the daphnias were fixed whole in a 4% solution of neutral formalin, buffered according to Lillie, and embedded in paraffin wax. The resulting serial sections were subjected to parallel histologic investigation by the indirect Coons' method [5]. Specific sera to a culture of NAG vibrios (agglutination titer 1:800) were used to identify the bacteria by this method. Necessary controls for specificity of fluorescence were set up.

EXPERIMENTAL RESULTS

The study of semithin sections shows the precise structure of the epithelial layer of the alimentary tract of the daphnias. In particular, the proximal portion of the intestine was found to be lined with simple cubical epithelium, the cells of which were 11.3 ± 0.56 μ in height (Fig. 1a). The apical surface of the epitheliocytes was covered with a dense fuchsine-positive chitin membrane 2.18 ± 0.31 μ thick. The cell nuclei were large, oval in shape, and contained one or two dark nucleoli. The nuclei as a rule were located in the central part of the cell. The terminal zone was clearly distinguishable and contained tiny vacuoles. Sometimes epitheliocytes with a holocrine type of secretion were observed: their cytoplasm was highly translucent on account of numerous large, optically empty vacuoles (Fig. 1a).

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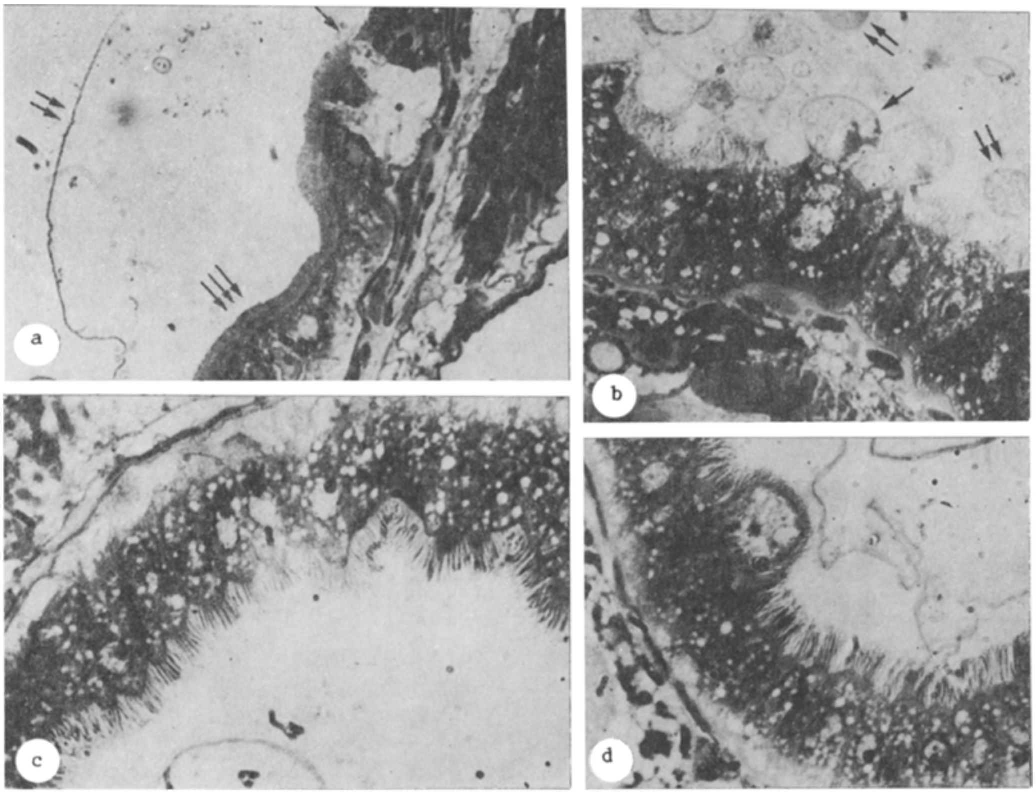

Fig. 1. Alimentary canal of a daphnia. Semithin section. a) Anterior portion; single arrow – secreting epitheliocytes (holocrine type of secretion), two arrows – peritrophic membrane, three arrows – chitin membrane. b) Proximal part of middle portion: secreting epitheliocyte. Single arrow – macrolemmocrine type of secretion; two arrows – granules of secretion in peritrophic space; c) distal part of middle portion. Columnar epitheliocytes bearing microvilli, peritrophic space; d) swelling secreting epitheliocyte (macroapocrine type of secretion). Stained with methylene blue, azure II, and fuchsine. Magnification: a) 400, b-d) 1000 ×.

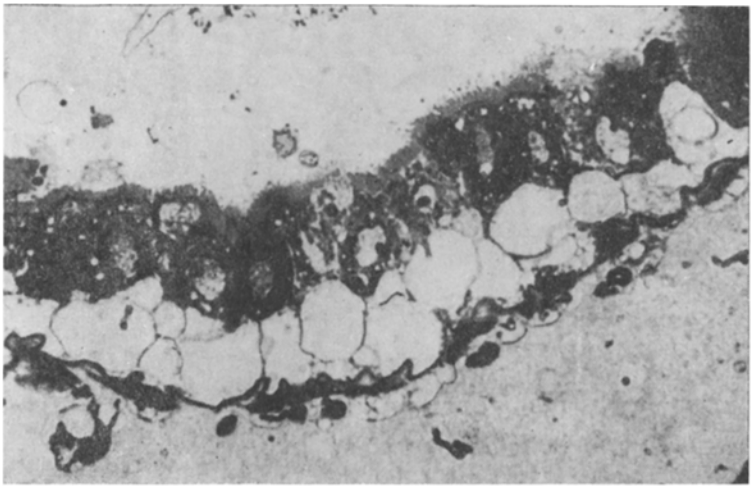

Fig. 2. Posterior portion of digestive tract of a daphnia. Simple cubical epithelium, covered with chitin membrane. Numerous optically empty vacuoles in basal part of cell. Semithin section. Methylene blue, azure II, and fuchsin. 1000 ×.

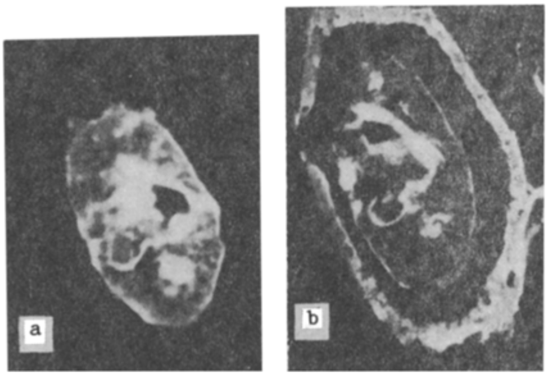

Fig. 3. Middle portion of digestive tract of a daphnia after introduction of a culture of NAG vibrios into the habitat of crustaceans: a) after 24 h: intense fluorescence of peritrophic membrane in proximal part, b) after 48 h: fluorescence of striated border and cytoplasm of cells of epithelial layer. Coons' luminescence-serologic method. 90 ×

The longest part of the alimentary tract of crustaceans is the middle portion, which has a local expansion in the proximal part, and gradually becomes narrower toward the posterior portion. A peritrophic membrane, characteristic of certain classes of invertebrates [2], consisting of a dense homogeneous fuchsinophilic lamina up to 1 μ in thickness, can be seen in the lumen of the intestine. Between the peritrophic membrane and the epithelial layer lies the peritrophic space — a cavity line by the peritrophic membrane and the apical surface of the epitheliocytes of the alimentary tract of the daphnia, in which contact digestion takes place (Fig. 1c). A special feature of the peritrophic space is its inconstant volume along its length. This is due primarily to the functional state of the particular portion of the digestive tract and to the digestive process. Frequently many spherical formations, bounded by a membrane, with granular contents and staining palely with methylene blue, are found in this space (Fig. 1b). The epithelial layer in this portion has no chitin cover. The cells lining the middle portion in its proximal part are columnar in shape (Fig. 1b), but in the distal portions they become cubical in shape. Most cells have a slightly prominent apical surface, covered with high pyroninophilic microvilli; the average height of the cell is 17.1 ± 1.0 μ, and of the microvilli 5.5 ± 0.48 μ. The predominance of cells with high microvilli indicates that it is in the middle part of the digestive tract of daphnias that absorption takes place. The basal plasmalemma of these cells frequently forms numerous outgrowths and folds. The nucleus of the epitheliocytes is large, round or oval in shape, and contains large, dark nucleoli, as a rule located eccentrically. Many pale vacuoles can be observed in the cytoplasm (Fig. 1b, d). Sometimes club-shaped evaginations of the apical surface of the cells, covered with short microvilli, project into the peritrophic space. Many azurophilic and eosinophilic round inclusions can be seen in their cytoplasm, mainly in its apical part. The external appearance of these cells corresponds to cells with different types of secretion: macroapocrine and macrolemmocrine [4].

The posterior portion of the alimentary canal is shorter than the two preceding portions and lined with simple cubical epithelium 13.0 ± 1.29 μ high; a thick, likewise fuchsinophilic, chitin membrane 5.24 ± 0.7 μ thick can be seen on its apical surface. The cell nuclei are round, with one or two compact nucleoli and their mean diameter is 4.4 ± 0.41 μ. The cytoplasm of the cells contains numerous vacuoles, which fuse together in the basal part of the cell to form larger cistern-like cavities. This creates the impression that the whole cell is apparently resting on an "air cushion." Cells with a holocrine type of secretion, their cytoplasm entirely filled with large vacuoles, can be seen in the epithelial layer (Fig. 2).

The epithelial layer of all portions of the alimentary canal of daphnias rests on a compact, winding basement membrane. All parts of the digestive tract are surrounded by a muscular coat with longitudinal and transverse bundles of muscle cells.

Daphnias did not become ill after direct contact with NAG vibrios, but utilized them as food, and digested and assimilated this food with the aid of digestive enzymes. A study of histologic preparations showed that the morphologic pattern of the alimentary canal as a whole corresponded to that described above for intact animals, and the epithelial cells were not damaged. Changes observed were evidence only of activation of digestive processes. To confirm this observation, we used a luminescence-serologic method with antiserum to NAG vibrios. The investigation showed that antigenic material of the bacteria in each case and the bacterial cells themselves were revealed by their luminescence in the lumen of the alimentary canal of the daphnias 3 h after the experiment began. Intense fluorescence of the peritrophic membrane of the middle portion of the alimentary canal also was observed, where the introduced microorganisms were being digested (Fig. 3a). Fluorescence was found mainly in the peritrophic membrane of the posterior portion 48 h after introduction of the bacteria into the habitat of the crustaceans, whereas in the middle portion fluorescence of the striated border and cytoplasm of the epithelial cells also was observed (Fig. 3b). On the 3rd day of the experiment specific fluorescence could no longer be observed in any portion of the digestive tract of the daphnias.

The methods of investigation which we used showed that introduction of enteropathogenic microorganisms into the habitat of these crustaceans gives rise to no pathological changes in the alimentary tract of daphnias. The absence of phenomena of phagocytosis in daphnias, observed and described by Mechnikov [3] in his classical research, in our experiments can be explained on the grounds that their intestinal epithelium is sufficiently well protected from mechanical injury by a peritrophic membrane, a peritrophic state, where food is digested, and a chitin membrane. The method of peroral infection which we used did not enable the leukocytes of the daphnias to come into contact with the pathogenic vibrios. In addition the epithelial cells of the alimentary canal of crustaceans evidently do not possess receptors for toxins produced by bacteria of the enteric group, and these animals accordingly do not react to them.

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EFFECT OF α-TOCOPHEROL (VITAMIN E ACETATE) ON METABOLISM OF GASTRIC SECRETORY CELLS OF HYPERTHYROID RATS

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KEY WORDS: hyperthyroidism; gastic secretary cells; α-tocopherol.

The leading factor in the complex chain of adaptive reactions of the stomach to an excessive intake of thyroid hormones is modification of the permeability of the biological membranes [3, 4, 10]. It has been shown that thyroid hormones activate lipid peroxidation (LPO) in the liver mitochondria [2]. The writer previously [5, 6] described structural disturbances in mitochondria and lysosomes in the gastric parietal cells of hyperthyroid rats. At this stage it is very important to look for possible ways of influencing metabolism in

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