CHOLEROGENIC POISONING IN SOME HYDROBIONTS

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Cholerogenic poisoning was simulated in experiments on frogs (*Rana temporaria*). Injection of large doses of cholerogen induces structural and functional changes in the epithelial cells of the frog small intestine similar to those observed in infantile rabbits used as the classical model for the study of mechanisms of cholerogenic poisoning.

KEY WORDS: cholerogenic poisoning; epithelium of frog small intestine.

Recent investigations by Soviet and Western workers have resulted in new ideas regarding the pathogenetic mechanisms of cholera development. The characteristics of the syndrome of rapid intestinal dehydration have been given at the cellular level [1-3, 9-14]. However, many of the mechanisms of development of cholera poisoning still remain unexplained.

Several workers [4-6, 8] have found various members of the genus *Vibrio* in fishes, crustaceans, frogs, and mollusks although no visible signs of the disease could be established in them. Hydrobionts can probably act as potential storehouses for vibrios in nature. No data on the effect of the enterotoxin of the vibrios on frogs could be found in the accessible literature.

In order to study the mechanisms of cholerogenic intoxication an attempt was made in the investigation now described to simulate this condition in hydrobionts.

EXPERIMENTAL METHOD

Experiments were carried out on 165 male grass frogs (*Rana temporaria*) weighing from 30 to 50 g and kept in the laboratory at 6°C in a state close to hibernation.

A cholerogen toxin obtained from the filtrate of a broth culture of *Vibrio cholerae* strain 569B of the "Pakistan" line and series of cholerogens generously provided by Dr. L. F. Zykin were used.

The cholerogen was injected into the duodenum or rectum under ether anesthesia (2% solution of ether in water) in doses of from 0.5 to 1.5 ml (0.013 mg/g) depending on the weight of the frogs and the activity of the cholerogen batch. The equivalent volume of Clark's solution or medium in which the cholerogen was prepared was injected into control animals.

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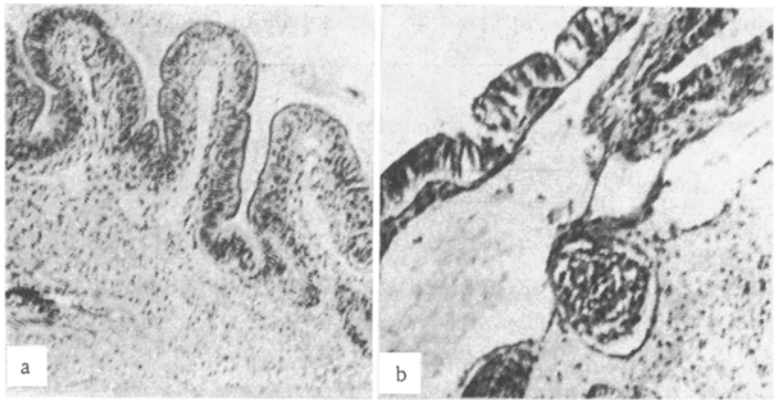

Fig. 1. Mucous membrane of frog small intestine normally and after injection of cholerogen: a) control (75 ×); b) after injection of cholerogen (120 ×). Hematoxylin–eosin.

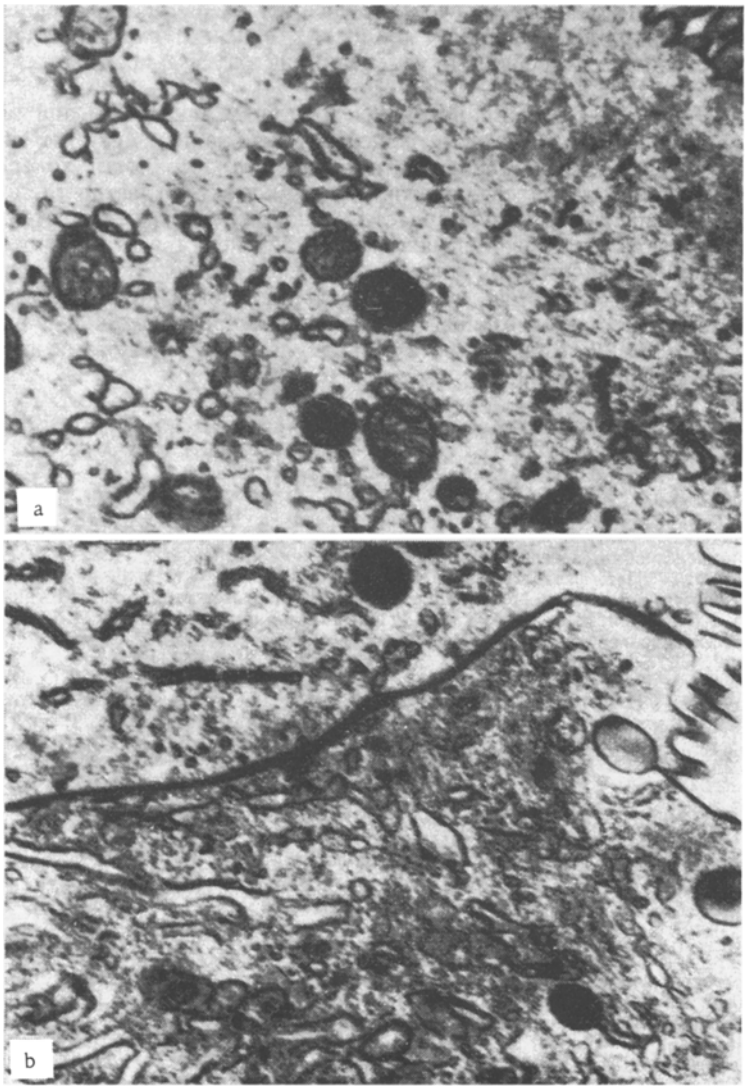

Fig. 2. Epithelial cells of small intestine (20,000 ×): a) control; b) two types of epithelial cells after injection of cholerogen.

After injection of the preparation the experimental and control frogs were kept at 18-20°C in an atmosphere of increased humidity. The results were read 24 h after the experiment began.

The frog's intestine was studied by histological, histochemical, and electron-microscopic methods.

For histological investigation the material was fixed in Carnoy's fluid and paraffin sections were stained with hematoxylin–eosin. RNA was determined by Brachet's method, neutral mucopolysaccharides by McManus's method with the usual enzyme control, and acid mucopolysaccharides were detected with alcian blue. The mucous membranes of the frogs' gastrointestinal tracts were investigated.

Ten pieces each were taken from the small intestine of six control animals and 12 experimental animals receiving the cholerogen, for electron-microscopic study. The pieces of tissue, immediately after removal from the immobilized frogs, were fixed with 1% osmium tetroxide in veronal–acetate buffer, pH 7.4, at 4°C for 1 h. The material was embedded in Epon with Araldite. Ultrathin sections were examined with the JEM-100B electron microscope, with an accelerating voltage of 80 kV.

EXPERIMENTAL RESULTS

Under the influence of the cholerogen signs of generalized poisoning with lesions of the parenchymatous organs (liver, spleen, kidneys) and a local enteropathogenic action, were observed in 90% of the frogs.

In the light microscope the severest changes after injection of the cholerogen were observed in the jejunum (hyperemia and edema of the tissues; Fig. 1). The villi of the mucous membrane were thickened and shortened. Edema fluid seeped into the lumen of the intestine, as shown by the widened intercellular spaces and displacement of the nuclei from the basal to the apical part, and it also penetrated into the musuclar coat of the intestine, separated the smooth-muscle cells, and thus gave the whole of the muscular layer a reticular structure (Fig. 1b). Some of the changes in the epithelium could be detected only histochemically; the content of neutral and acid mucopolysaccharides was reduced. There was a simultaneous increase in the RNA content.

The electron-microscopic investigation showed the following changes in the epithelial cells of the small intestine after injection of cholerogen compared with the control (Fig. 2). 1) The pale edematous epithelial cells contained large vesicles of agranular endoplasmic reticulum, confluent and easily forming vacuoles, as in the epithelium of the rabbit small intestine after injection of cholerogen [2]. The cytoplasm of these cells contained free polysomes, monosomes, and polymorphic hyperosmiophilic mitochondria with lamellar and tubular cristae; the nuclei were enlarged and translucent. 2) In epithelial cells of another type, distinguished by the higher electron density of the cytoplasm, there were unusually large numbers of round or oval mitochondria with a translucent matrix. The granular endoplasmic reticulum was widened and its membranes were degranulated in places (Fig. 2b). Free monosomes and polysomes also were found in the matrix of the cytoplasm of these cells. Considerable vesiculation was found aroung the Golgi complex. Small and large vesicles in these cells joined together to form vacuoles. Smoothing out of the microvilli and the formation of vesicles from the apical plasmolemma (potocytosis) occurred in the cells of both types.

These structural changes reflect the action of large doses of cholerogen on the plasmalemma and organelles of epithelial cells in different stages of function.

The integrity of the epithelial layer was not disturbed, it must be noted, where the lesions of the small intestine occurred. The intercellular spaces were widened only in the basal part and, to some extent, in the apical part of the cell.

Pathological changes caused by the injection of cholerogen evidently start with disturbances of vascular and capillary tone in the form of hyperemia and congestion, edema of the mucous membrane of the gastrointestinal tract, and disorganization of its structure and cellular and membrane levels with considerable seepage of water into the lumen of the small intestine.

The loss of water by the body in frogs receiving large doses of cholerogens can take place, it may be supposed, not only through the gastrointestinal tract, but also through the skin with a simultaneous decrease in the water content in the muscles. This view was confirmed in some of the frogs by the marked dryness of the skin, the change in its color, and the bent and dry digits of the upper and lower limbs characteristic of cholerogenic poisoning with changes in the tone and color of the skeletal muscles.

Cholerogenic poisoning in frogs (*Rana temporaria*) is thus characterized by signs of general poisoning and by a local enteropathogenic action. Depending on the dose and activity of the cholerogen, the digestive tract was selectively affected, specifically by a paretic dilatation and edema of its walls in surplus parts, notably the caudal segments of the intestine. These changes were often accompanied by hyperemia and congestion of the liver, spleen, and kidneys.

The action of the cholerogen on the epithelial cells led to intraepithelial edema, focal disappearance of the microvilli, and the formation of large vesicles and vacuoles from the agranular endoplasmic reticulum, opening into the lumen of the small intestine.

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