NAG INFECTION PRODUCED IN Rana temporaria UNDER HYPOTHERMIA

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Frogs (Rana temporaria), kept under hypothermic conditions close to anabiosis, were infected with NAG vibrios and subjected to clinical, histological, bacteriological, and electron-microscopic investigation. Peroral infection of hypothermic frogs with NAG vibrios was shown to cause the development after 18-24 h of an acute NAG infection resembling the cholera-like syndrome characterized by general toxemia and local enteropathogenic action. After the acute illness the vibrios persisted for a long time in the frogs' gastrointestinal tracts.

KEY WORDS: frog; NAG infection; vibrios; hypothermia.

Among the cholera-like vibrios, the most interesting at the moment are those which are not agglutinated by O-chlorea serum (NAG vibrios), and which are capable of causing infection in man.

Data in the literature show that hydrobionts can be vibrio carriers for a long time [2, 4-7]. However, no investigator has yet established conclusively whether frogs develop the disease when infected with vibrios.

The object of this investigation was to determine whether frogs (Rana temporaria) can develop an experimental NAG infection when kept under various conditions and to attempt to use them as a model with which to study the pathogenesis of this infection.

EXPERIMENTAL METHOD

Altogether 680 grass frogs weighing 25-40 g were used. Before the experiments the animals were kept for 1-3 months in a refrigerator at 4°C, i.e., under conditions of hypothermia close to anabiosis; no food was given to them but they were washed daily with tap water. The animals were infected with two museum strains of NAG vibrios: 4616/p, isolated in 1970 in Tbilisi from the feces of a patient; and 318/g, isolated in 1965 from an open reservoir. The frogs were infected through the mouth by means of a gastric tube. The dose for infection was 500 million bacterial cells in 0.5 ml physiological saline or 0.5 ml Höttinger's broth. Control animals received 0.5 ml of physiological saline or 0.5 ml of Höttinger's broth in the same way. The experimental and control frogs, 15 to 20 at a time, were kept in tall glass jars with 100 ml tap water. All the jars were covered with iron wire gauze.

The experimental and control animals were kept at different temperatures: hypothermia, close to anabiosis (4°C), and 20-24°C.

The experimental frogs were subjected to bacteriological examination, for which purpose they were autopsied in groups of 3 to 5 animals at a time 30 min and 18, 48, and 72 h after infection and then every 2-3 days throughout the period of observation (until 27 days). Blood was seeded from the heart, eggs, bile, and liver, and the contents of the stomach and small and large intestine, on selective and differential diagnostic media (alkaline agar, TCBS, Endo's and Levin's media), and also on Höttinger's broth. The intestine also was

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Fig. 1. Cholera-like syndrome in frog 18 h after infection with broth culture of NAG vibrio 318/g: a) experimental, b) control.

examined histologically and electron microscopically [1]. The reaction of the gastric contents and the antibacterial (against Escherichia coli and against NAG vibrios) and lysozyme activities of the blood serum (by Vasil'ev's method [3]) were tested in 50 frogs in a state of hypothermia and in another 30 frogs kept under natural conditions.

EXPERIMENTAL RESULTS

The antibacterial and lysozyme activity of the blood serum of the frogs kept in a state of hypothermia was zero, and the reaction of the gastric contents was neutral or weakly alkaline (pH 7-7.4). In frogs kept under natural conditions the titer of lysozyme activity was equivalent to a dilution of the serum of 1:64, the antibacterial activity was zero, and the pH of the gastric contents was 4.6-5.0.

At autopsy on the experimental frogs 30 min after infection, vibrios were cultured from the stomach and small and large intestines. After 18-48 h bacteriemia was observed in 12 frogs kept in a state of hypothermia and infected with a broth culture. Vibrios also were seeded from all organs tested. At all other times NAG vibrios were isolated from the stomach and small and large intestines of the experimental frogs; in addition, they were also isolated from the gall bladders and livers of some animals. A well-marked cholera-like effect with a local enteropathogenic action and with evidence of general toxemia, manifested as static congestion of the liver, spleen, and kidneys, was observed in frogs kept in a state of hypothermia 18 h after infection, especially if infected with a 24-h broth culture of NAG vibrio 318/g. The stomach was enlarged. Its vessels and those of the mesentery were dilated. The small intestine was increased in volume, atonic, thin-walled, and filled with serous fluid containing floccules and gas bubbles; the large intestine was distended (Fig. 1). On the second day the cholera-like effect was less severe: The parenchymatous organs were changed and congested, the stomach dilated, the duodenum more often hyperemic, the small intestine contained serous contents and in places it was dilated. On the third to fourth day no clinical changes or changes in the internal organs could be seen in any of the animals. Features of poisoning were ill-defined in the experimental frogs kept at 20-24°C.

The histological investigations showed that the relief of the mucous membrane of the small intestine was smoothed out after 24 h in the frogs kept in a state of hypothermia: The invaginations and villus-like projections which were clearly visible in the intact animals had disappeared (Fig. 2a, b). In solitary enterocytes or groups of them karyorrhexis was observed (Fig. 2c). The mitotic index (MI) of the epithelial cells 24 h

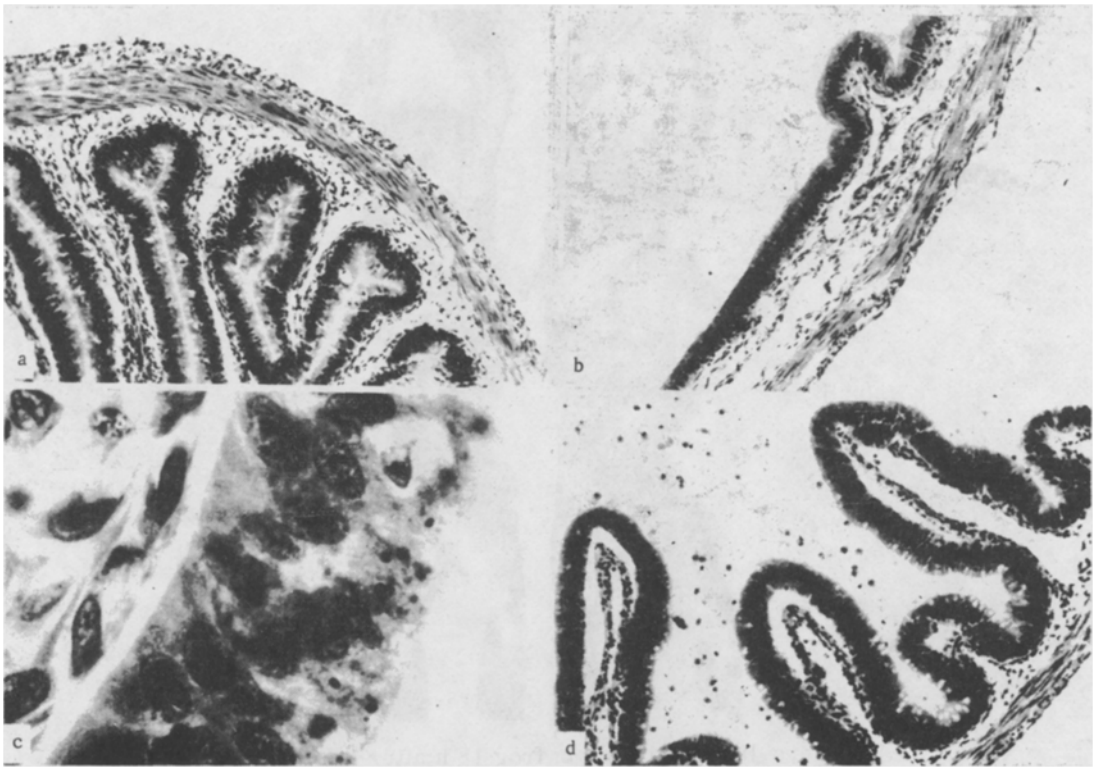

Fig. 2. NAG infection in Rana temporaria kept under hypothermia: a) small intestine of intact frog; invaginations and villus-like projections are clearly visible. Hematoxylin–eosin, 120×; b) small intestine of frog 24 h after infection with NAG vibrios; relief of mucous membrane sharply altered: Invaginations and villus-like projections have disappeared. Hematoxylin–eosin, 120×; c) karyorrhexis in group of epithelial cells of frog small intestine 24 h after infection with NAG vibrios. Hematoxylin–eosin, 1100×; d) restoration of normal structure of mucous membrane of frog small intestine 48 h after infection with NAG vibrios; many desquamated cells visible in lumen. Hematoxylin–eosin, 120×.

after infection was 9‰ (about 2‰ in intact frogs in a state of hypothermia). Invaginations of the epithelium and villus-like formations were again observed 48 h after infection in the small intestine (Fig. 2d). Cells with pycnotic nuclei were arranged mainly at the apices of the villus-like projections or in their upper third. The number of these cells reached 1.3% (normally about 0.2%). Many desquamated cells were present in the lumen of the intestine. The number of mitoses in the epithelium of the invaginations was increased (7‰). Pathological mitoses were found.

Electron-microscopic investigation revealed focal lesions of the epithelial cells of the small intestine. On the first day after infection considerable vesiculation of the cytoplasm of the enterocytes, dilatation of the cisterns of the granular and agranular endoplasmic reticulum, edema and homogenization of the matrix of the mitochondria, and local edema of the cytoplasm were observed (Fig. 3a, b). On the following days an increased number of lysosomes was observed visually in individual enterocytes, evidently in connection with intracellular destructive processes developing during NAG infection. The normal submicroscopic picture of the epithelium of the small intestine was restored on about the 14th day after infection.

It can be concluded from these results that frogs kept under hypothermic conditions can develop a NAG infection accompanied by a marked cholera-like syndrome. The following factors evidently facilitate the development of the infection: a reduction of microbial competition in the gastrointestinal tract, a change in pH of the gastric contents, and weakening of the immunobiological reactivity of the animals. One index of the last of these factors is lysozyme, which participates in combined nonspecific immune responses of the body and, in particular, in the regulation of permeability of the tissue barriers and in local immunity of the mucous membrane. The neutral or weakly alkaline reaction of the gastric contents in frogs kept under hypothermia cannot be regarded as a barrier preventing vibrios from penetrating into the small intestine.

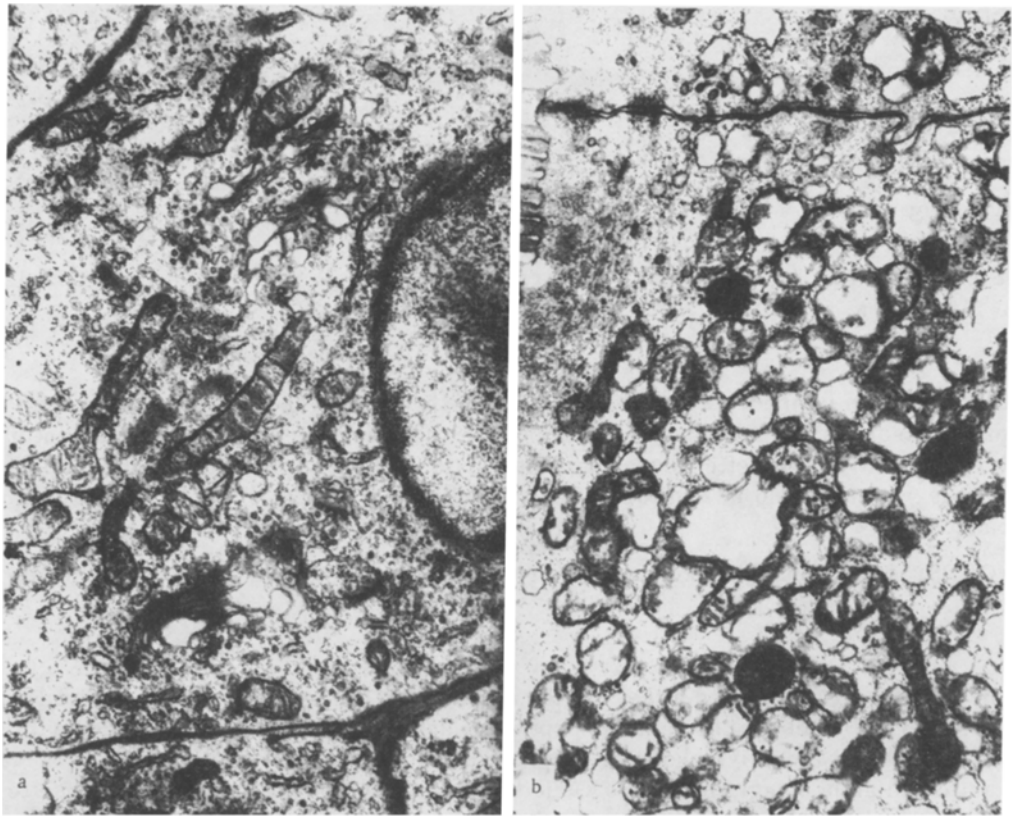

Fig. 3. Epithelial cell of small intestine of control frog (a) and after infection with NAG vibrios (b); 12,000×.

The results of the clinical, bacteriological, histological, and electron-microscopic investigations indicate that frogs kept under hypothermic conditions close to those of anabiosis can develop and recover from an acute NAG infection during the first 2 days after infection. Vibrios persist for a long time thereafter in their bodies.

The suggested model of NAG infection can be used to study the dynamics of the pathogenesis of the infectious process in the acute period, going on to a prolonged vibrio carrier state.

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