patent. This situation confirms suggestions in the literature [3, 5, 6, 10] that narrowing of the anastomosis is not the only cause of the onset of structural and functional changes in the organs adjacent to the anastomosis.

This investigation thus showed that choledochoduodenostomy by the present writers' method can preserve an anastomosis of statistically satisfactory size in the postoperative period; the creation of the valve and the location of the anastomosis on its efferent side prevents regurgitation of the intestinal contents into the biliary tract. This may explain the fewer number of complications and the less severe pathomorphological changes in the bile duct and liver of the animals of this group.

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ULTRASTRUCTURE OF THE MUCOUS MEMBRANE OF THE SMALL INTESTINE IN GERMFREE RATS BECOMING CARRIERS OF *Vibrio cholerae*

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KEY WORDS: *Vibrio cholerae* carrier state; germfree rats; small intestine; ultrastructure.

Despite the great importance of the cholera carrier state its pathogenesis has been very inadequately investigated [3, 5, 8, 10]. One promising trend in this situation is the formation of a model of a chronic *Vibrio cholerae* carrier state in germfree rats [2].

In this paper we examine the ultrastructure of the mucous membrane of the small intestine of germfree rats in which a carrier state is produced with respect to *Vibrio el-tor* and *V. cholaerae*.

EXPERIMENTAL METHOD

Germfree rats of the OFA breed and the Fisher F-344 strain, aged 2-12 months, were infected either with *V. el-tor* (Ogawa serotype, not secreting an exotoxin) in a dose of 2×10^9

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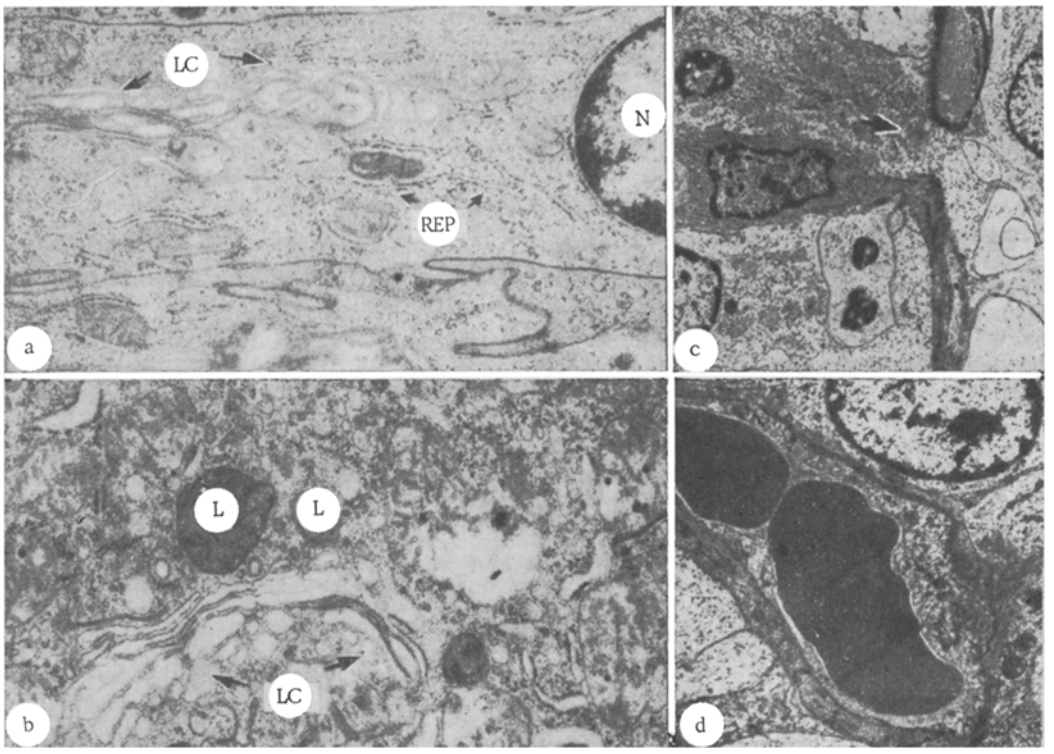

Fig. 1. Ultrastructure of enterocytes and blood capillary in small intestine before and after infection with cholera vibrios. a) Structure of supranuclear region of enterocytes from villi of small intestine of germfree rats. LC) Lamellar complex, N) nucleus, RER) rough endoplasmic reticulum, 15, 000 ×; b) lamellar complex of enterocytes from villi of small intestine of germfree rats 20 h after infection with cholera vibrios. L) Lysosome-like structures, 27,000 ×; c) projection of base of enterocytes (arrow) of villi through basement membrane into stroma, 4500 ×; d) blood capillary in stroma of villi of small intestine 20 h after infection with cholera vibrios, 10, 500 ×.

bacterial cells, or *V. cholerae* (Ogawa serotype, strain 154) in a dose of 4×10^9 bacterial cells with the drinking water. The animals became clinically healthy carriers and excreted vibrios constantly at the rate of 1×10^7 cells/g feces. The rats were killed with carbon dioxide 20 h and 18-20 days after infection. Pieces of the ileum of the germfree and infected rats for investigation were fixed in 3% glutaraldehyde and 1% OsO_4 solutions. After dehydration the material was embedded in Araldite. Ultrathin sections were exminaed in the JEM-100B electron microscope.

EXPERIMENTAL RESULTS

The villi of the mucous membrane of the small intestine 20 h after infection with *V. el-tor* or *V. cholerae* were long and finger-shaped, and the crypts were cylindrical with a narrow lumen. The linear parameters of the crypt–villus system were unchanged.

In the electron microscope an increase in the number of electron-dense lysosome-like structures was observed in the cytoplasm of enterocytes from the villi of the infected rats compared with the germfree animals, together with hypertrophy of the lamellar complex, which consisted mainly of vacuoles (Fig. 1a, b). More often than in the germfree animals the base of the enterocytes projected through the basement membrane into the stroma of the villi, where it made contact with connective tissue cells. This part of the enterocytes contained only ribosomes (Fig. 1c). The intercellular spaces were infrequently widened. After infection infiltration of the epithelial layer with connective-tissue cells, especially lymphocytes

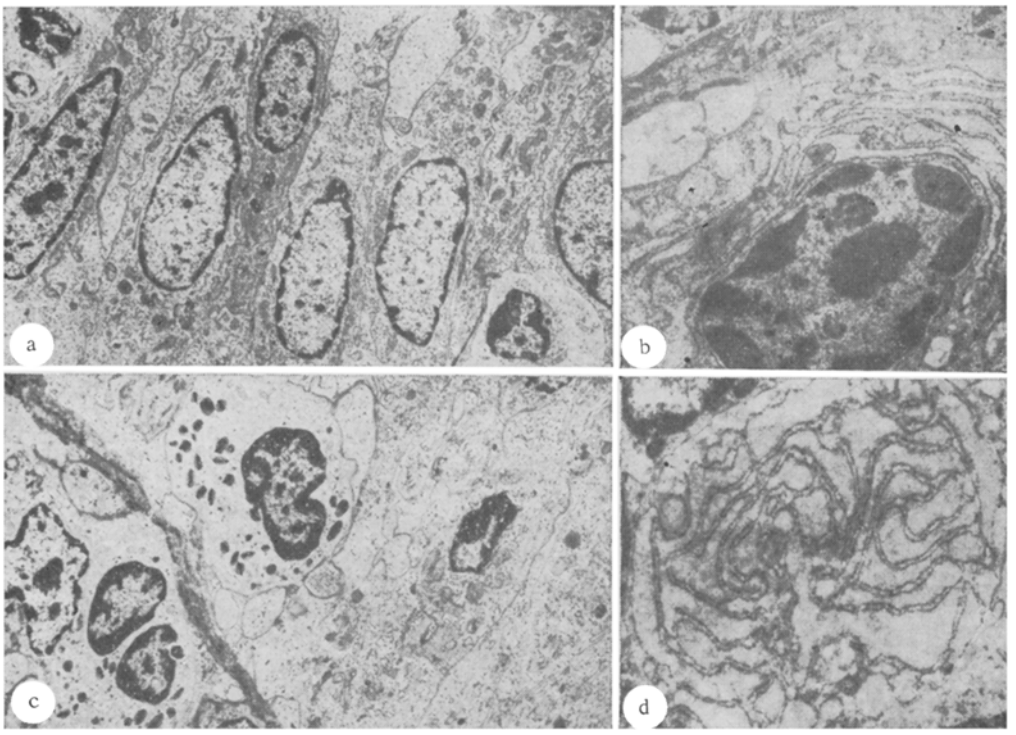

Fig. 2. Ultrastructure of villi of small intestine of germfree rats 18-20 days after infection with cholera vibrios. a) Lymphocytes visible among enterocytes, 7500 ×; b) eosinophil, 12,000 ×; c) structure of plasma cells of stroma of villi of small intestine of germfree rats, 12,000 ×; d) activation of plasma cells from stroma of villi of small intestine 18-20 h after infection with cholera vibrios, 15,000 ×.

and eosinophils, increased. The lymphocytes contained few organelles but often had single granules of average electron density, surrounded by a membrane.

In the crypts the cytoplasm of the Paneth cells contained far fewer secretory granules, evidently because of increased secretion in response to administration of the vibrios.

Infiltration by lymphocytes and eosinophils was observed in the stroma of the villi and crypts, and fewer fibroblasts and collagen fibers could be seen. The lymphocytes lay in groups of three or four cells in contact with macrophages, in whose cytoplasm the profiles of the rough endoplasmic reticulum were widened and the number of polymorphic lysosomes increased. There was no difference between the wall of the blood vessels before and after infection with vibrios (Fig. 1d). Some dilatation and congestion of the blood capillaries and dilatation of lymphatics were observed.

On the 18th-20th day of the carrier state by comparison with the previous period there were more enterocytes extruded from the villi, more secondary lysosomes in the cytoplasm of the enterocytes, a greater degree of infiltration of the epithelial layer by lymphocytes and eosinophils, and less frequently by other stromal cells (Fig. 2a, b).

Groups of plasma cells with dilated cavities of the rough endoplasmic reticulum of the lamellar complex appeared in the stroma of the villi and crypts (Fig. 2c, d). There were relatively more lymphocytes; eosinophils were found more frequently although there were few secretory granules in their cytoplasm. Mast cells were numerous and in a state of degranulation. With time no visible change took place in the structure of the macrophages.

Unlike *V. el-tor*, *V. cholerae* induces intensive extrusion of enterocytes and considerable infiltration by lymphocytes with granules in the small intesting of germfree rats. Otherwise the changes in the small intestine are identical in type.

According to some workers, cholera in man and in experimental animals causes edema of the basememt membrane of the blood vessels and enterocytes, pallor of the matrix of the

cytoplasm of the enterocytes, and swelling and desquamation of individual microvilli [1, 4, 6, 7]. No such changes could be found in germfree rats with a chronic vibrio carrier state. Intensification of the functional activity of the enterocytes (hypertrophy of the lamellar complex, an increase in the number of lysosomes in the cytoplasm) probably took place because of absorption of some soluble metabolic products of the vibrios. Projection of the base of the enterocytes through the basement membrane must evidently be regarded as potentiation of epitheliomesenchymal interaction [9].

The pattern of activation of immunocompetent cells in response to administration of *cholera vibrios* described above, in the absence of any pathological changes in the mucous membrane of the small intestine, must be regarded as evidence of the basically healthy state of germfree rats with a chronic vibrio carrier state.

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INTENSITY OF RNA SYNTHESIS AND DNA CONTENT IN THE NEONATAL RAT MYOCARDIUM DURING ADAPTATION TO HIGH ALTITUDE HYPOXIA

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In the genesis of heart muscle differentiation of myocytes is not accompanied immediately by their quitting the mitotic cycle. According to data obtained by various workers, proliferative activity of rat cardiomyocytes is considered to continue for a long period after birth [2, 4, 7, 9]. Meanwhile doubts have been expressed even about the data of light microscopy and autoradiography on DNA synthesis and mitotic activity of cardiomyocytes during early postnatal development [10, 12]. Some particularly heated discussions have taken place on the question of the behavior of DNA in muscle cell nuclei during hyperfunction and hypertrophy of the myocardium both in adults and during the period of its early ontogeny [1, 6].

It was accordingly decided to use the method of light autoradiography to study RNA synthesis and DNA content in nuclei of muscle and nonmuscle cells of the neonatal rat heart during gradual adaptation to high-altitude hypoxia, throwing a measurable functional load on the myocardium, and also during a single exposure to high-altitude hypoxia under different conditions.

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