INTERACTION BETWEEN THE CARDIOVASCULAR AND DIGESTIVE SYSTEMS IN THE GENESIS OF EXPERIMENTAL ATHEROSCLEROSIS

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In chronic experiments (lasting up to 2 years) on dogs receiving an atherogenic diet the motor activity of the digestive tract, the cholesterol and phospholipid levels in the blood and intestinal secretion, and the morphology of the small intestine and vascular system were investigated. In the early stages of the experiments (before 4 months) elevation of the blood cholesterol level was accompanied by an increase in the periods of activity of the digestive tract, an increase in the cholesterol secretion by the small intestine, and hypertrophy of its mucosa. In the late stages of the experiments, degenerative and atrophic changes developed in the mucosa of the small intestine, motor activity of the digestive tract was disturbed, and correlation between the cholesterol levels in the blood and intestinal juice was upset. This period was characterized by high hypercholesteremia and by marked atherosclerotic changes in the blood vessels.

KEY WORDS: cardiovascular system; digestive system; experimental atherosclerosis.

One of the main ways of studying the pathogenesis of atherosclerosis is by investigating disturbances of lipid metabolism and seeking methods of their correction [6, 9, 11, 12, 20]. The participation of the digestive system in the regulation of lipid metabolism has been established in a number of investigations [10, 15, 17-19, 23]. Evidence has also been obtained of a disturbance of the functions of the digestive tract in cardiovascular pathology [3, 5, 21].

The object of this investigation was to study the role of the digestive system in adaptation to disturbances of lipid metabolism in experimental hypercholesteremia and atherosclerosis.

EXPERIMENTAL METHOD

Male dogs aged 2-5 years were used and received different atherogenic diets [8]. The 14 dogs of group 1 received cholesterol, 6-methylthiouracil, and vitamin D_2; the four dogs of group 2 received egg yolks and vitamin D_2; the control group consisted of four intact dogs and four dogs receiving 6-methylthiouracil only.

The cholesterol level in the blood and intestinal juice of the animals was determined by the method of Éngel'gardt and Smirnova and phospholipids by the method of Fiske and Subbarow in K. S. Zamychkina's modification. Periodic contractions of the stomach and duodenum were recorded in dogs with a Basow's gastric fistula with the aid of two rubber balloons. The dynamics of the arterial blood pressure (BP) was studied manometrically. The structure of the mucosa of the duodenum and also of the proximal, middle, and distal portions of the small intestine (SI) was investigated at various times (1, 2, 4, 6, 8, 12, 16, and 24 months) in preparations stained as follows: with Sudan black, by the methods of Goldman, Van Gieson,

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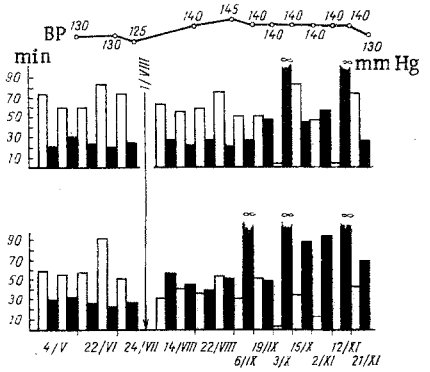

Fig. 1. Dog Druzhok. Increase in periods of contraction of duodenum and stomach after transfer to atherogenic diet (arrow). Abscissa, days and months of experiments; ordinate, duration (in min) of periods of rest (unshaded columns) and contraction (shaded columns). Top row represents stomach, bottom row duodenum.

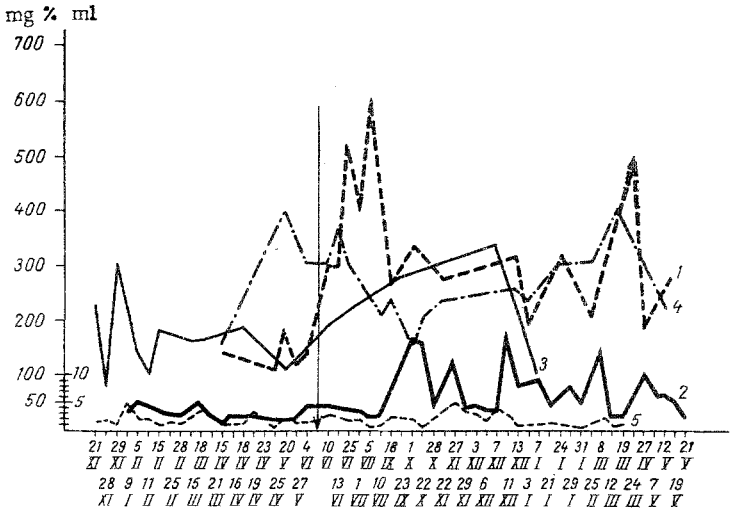

Fig. 2. Dog Rex. Concentrations of cholesterol and phospholipids in blood serum and intestinal secretion after transfer to atherogenic diet (arrow): 1) blood cholesterol (mg%); 2) cholesterol in intestinal juice (mg%); 3) phospholipids in intestinal juice (mg%); 4) blood lecithin (in mg%); 5) volume of juice (in ml). Abscissa, days and months of experiments; ordinate, concentration of substances in blood and intestinal juice (left) and volume of juice (right).

and Weigert–Hart; for acid and neutral mucopolysaccharides (MPS) by Hotchkiss's method, with toluidine blue at pH 1.7-4.0, and with Schiff's reagent in conjunction with alcian blue; for alkaline phosphatase activity by Gomori's method. To obtain a quantitative assessment of the structural changes, drawings of the mucosa made with the RA-6 drawing apparatus (objective 3.5, ocular 4) were subjected to morphometry. In a few cases pieces of mucosa were fixed in osmium tetroxide and embedded in Araldite. Sections were stained with lead citrate and examined in the IEM-100V electron microscope. The various parameters studied were correlated with the stages of experimental atherosclerosis in the cardiovascular system of dogs [2, 4, 7, 16].

EXPERIMENTAL RESULTS

The highest blood cholesterol level was observed in the animals of group 1 (600-1100 mg %, normal value 105-250 mg %); in the dogs of group 2 the hypercholesteremia was less marked (240-400 mg %) and prolonged administration of 6-methylthiouracil with the diet (24 months) likewise gave only a slightly raised blood cholesterol (up to 300 mg %).

In the dogs of group 1 the total cycles of periodic contraction increased after 1-2 weeks in the duodenum and after 1-1.5 months in the stomach also (Fig. 1). The fasting periodic activity of the digestive tract was altered. Regular and peristaltic contractions were unchanged. The BP was raised (to 130-150 mm Hg). In the dogs of group 2 further changes in motor activity did not appear until 7-8 months after transfer to the atherogenic diet.

In most animals of group 1 the changes in the cholesterol level in the blood and intestinal juice were opposite in direction: An increase in the blood cholesterol level (515±72 mg %) was followed by increased cholesterol secretion with the intestinal juice (134±13 mg %), compared with the normal 59±3 mg %), and this was followed by a corresponding decrease in the hypercholesteremia (342±46 mg %).

In the absence of such correlation, the increase in the blood cholesterol level (up to 1100 mg %) and the decrease in the lecithin–cholesterol ratio (to 0.6), and also the atherosclerotic changes in the blood vessels, were more marked. The increase in the periods of activity in these animals occurred only in one organ of the digestive tract and at later stages of the experiment.

In the early stages (1-4 months) of the experiment a varied degree of thickening of the mucosa of SI was observed. In the duodenum, mainly the cryptal portion was enlarged, but elsewhere in SI the height of the villi and depth of the crypts were increased. Evidence of the presence of hypertropy of the mucosa was

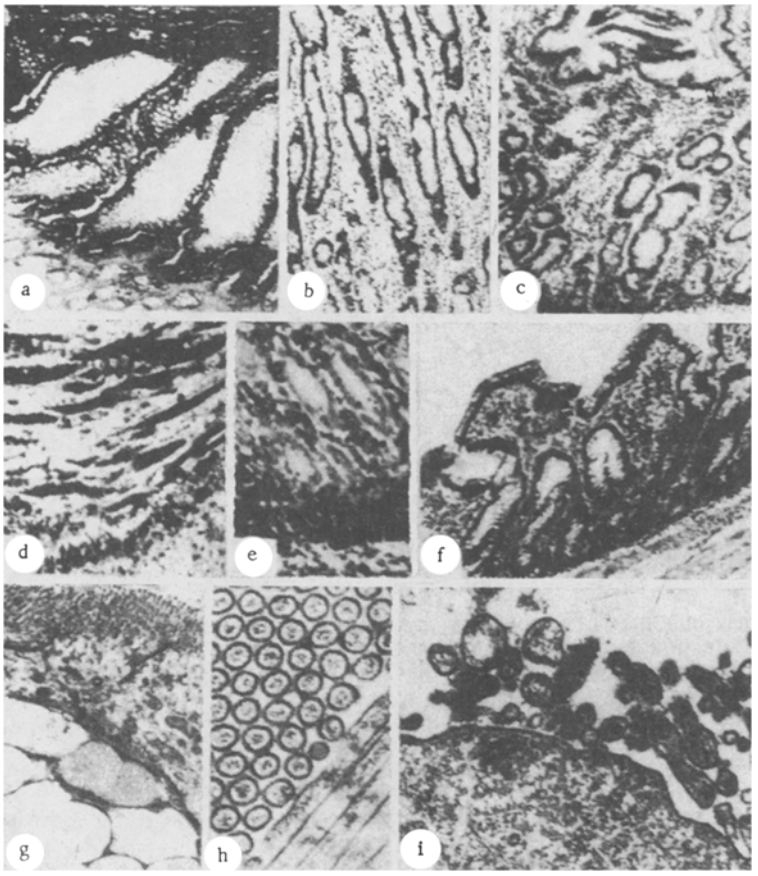

Fig. 3. Structural changes in mucosa of small intestine of dogs with experimental atherosclerosis: a) duodenum: increased alkaline phosphatase activity (Gomori, 63 ×); b) proximal part of jejunum, edema of stroma of mucosa between crypts (Sudan III, 56 ×); c) duodenum: thickening of connective tissue bands between crypts, which are shortened and deformed (Sudan III, 90 ×); d) accumulation of granular material in smooth-muscle cells of circular layer of tunica muscularis externa (Sudan black, 400 ×); e) ileum: stroma of mucosa between crypts and on boundary with tunica propria is infiltrated by macrophages with lipid inclusions in their cytoplasm (Goldman, 140 ×); f) ileum: villi shortened, crypts infrequent and widened (Sudan black, 63 ×); g) prismatic cells (control): compactly arranged microvilli can be seen on apical surface of cells; in lower part of figure there is a goblet cell with typical structure (8000 ×); h) area of brush border under high power (control); microvilli are regular in shape and in arrangement (50,000 ×); i) apical part of prismatic cell: polymorphism, deformation, and decrease in number of microvilli in experimental animals (50,000 ×).

given by an increase in its thickness in the proximal (2716.56±46.2 μ, normally 1972.74±25.41 μ) and distal (1848.0±23.1 μ, normally 1016.4±25.41 μ; $P < 0.05$) portions of SI. Inclusions of fat could be seen in the enterocytes, the lymphatic capillaries of the villi, and the aggregations of macrophages in the tunica propria (Fig. 3e). Alkaline phosphatase activity was increased in the brush border of the enterocytes of the duodenum and proximal portion of the jejunum (Fig. 3a). An increase in the number and size of goblet cells in the epithelium of the villi and crypts was noticable throughout SI, and acid MPS predominated in their secretion. The stroma of the mucosa of SI was edematous (Fig. 3b) and infiltrated with lymphocytes and plasma cells.

In the late stages of the experiment (8-24 months) structural changes in the mucosa of SI were observed, with shortening and deformation of the villi and crypts (Fig. 3c, f), flattening of the enterocytes, and the appearance of degenerative changes in the epithelium of the villi, Brunner's glands, and the crypts; alkaline phosphatase activity fell. At these periods the thickness of the mucosa in the foci of atrophy (the

height of the villi, especially in the proximal part of the jejunum) was reduced (337.26 ±48.51 μ, normally 896.95 ±2.31 μ; $P < 0.05$). A characteristic feature of this period was a decrease in the number of goblet cells in the epithelium of the villi and crypts of SI. Thickening of the bundles of connective-tissue fibers was observed in the stroma of the mucosa with replacement of the Brunner's glands and crypts and marked infiltration by lymphocytes and plasma cells. The arteries and veins of the submucosa were dilated and contained lipemic plasma, but their walls were intact. Distinctive changes included the accumulation of Schiff-positive granules, staining with Sudan black, in the smooth-muscle cells of the inner layer of the tunica muscularis externa (Fig. 3d). Electron-microscopic examination of the brush border of the enterocytes in the animals of group 1 revealed considerable polymorphism and deformation of the microvilli and a marked decrease in their number (Fig. 3i). This period was characterized by marked atherosclerotic changes in the various visceral and main arteries.

The presence of degenerative and atrophic changes in the mucosa of SI in the late stages of the experiment must have affected contact digestion [14], for processes of hydrolysis and absorption depend on the structure of the mucosa [13, 22]. Atrophy of the villi evidently corresponds to the inhibition of absorption of lipids observable in patients with atherosclerosis [1, 20].

Structural and functional changes in the digestive tract in the early stages of the experiment were evidently adaptive responses aimed at maintaining lipid homeostatis. In this period there were in fact no considerable changes in the blood biochemical indices or atherosclerotic changes in the vessels.

Prolonged alimentary cholesterol loading led to the development of degenerative and atrophic changes in the mucosa of SI. The changes in motor activity and cholesterol excretion in SI point to a disturbance of the mechanisms regulating these functions. The results of these changes were a high and persistent hypercholesteremia, a sharp decrease in the lecithin–cholesterol ratio, and marked atherosclerotic changes in the vascular system.

In the case of prolonged exposure of the body to a known risk factor – alimentary cholesterol loading – an important role in the mechanisms of compensation of the disturbances of lipid metabolism is thus played by the morphological and functional state of the digestive system.

LITERATURE CITED

1. M. N. Abramzon and R. Ya. Malkiel', Kardiologiya, No. 10, 90 (1973).
2. A. P. Avtsyn, S. M. Shibaeva, et al., Dokl. Akad. Nauk SSSR, 139, 717 (1961).
3. V. Kh. Vasilenko and M. Yu. Melikova, Klin. Med., No. 2, 35 (1959).
4. V. N. Vinogradov, N. L. Yastrebtsova, and K. A. Gornak, Vestn. Akad. Med. Nauk SSSR, No. 2, 43 (1961).
5. E. G. Grigor'yan, Ter. Arkh., No. 12, 8 (1969).
6. V. S. Zhdanov and A. M. Vikhert, Kardiologiya, No. 12, 42 (1973).
7. E. D. Klimenko, A. I. Evdokimov, and E. V. Mal'ko, Byull. Éksperim. Biol. Med., No. 3, 112 (1971).
8. E. D. Klimenko and L. A. Shekun, in: Proceedings of a Scientific Conference on the Physiology and Pathology of Corticovisceral Relations and Functional Systems of the Organism [in Russian], Vol. 1, Ivanovo (1965), p. 476.
9. A. N. Klimov and N. G. Nikul'cheva, Ter. Arkh., No. 6, 29 (1973).
10. S. M. Leites, Biokhimiya, No. 5-6, 283 (1943).
11. A. A. Pokrovskii, Vestn. Akad. Med. Nauk SSSR, No. 8, 31 (1974).
12. A. S. Smetnev and I. V. Neverov, Sov. Med., No. 1, 3 (1974).
13. A. M. Ugolev, Physiology and Pathology of Contact Digestion [in Russian], Leningrad (1967).
14. A. M. Ugolev, Ter. Arkh., No. 4, 35 (1973).
15. A. N. Fedoseev, Byull. Éksperim. Biol. Med., No. 5, 41 (1960).
16. S. I. Filippovich, Vestn. Akad. Med. Nauk SSSR, No. 12, 3 (1968).
17. G. K. Shlygin, in: Physiology and Pathology of the Digestive System [in Russian], Moscow (1963), p. 157.