FAT ABSORPTION AFTER TEMPORARY ISCHEMIA OF THE SMALL INTESTINE

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UDC 616.341-005.4-036.8-07: 616.34-008.839.15-008.6

KEY WORDS: small intestine; ischemia; fat.

Interest in acute ischemia of the small intestine, one of the most serious pathological states of the abdominal organs, has arisen with the introduction of transplantation of cadaveric organs into clinical practice. The adverse effects of anoxia on intestinal structure and function are not in doubt. However, it is important to know the character of disturbances and the duration of action of the traumatic factor. No reference could be found in the literature to the study of fat absorption after temporary ischemia of the small intestine, and the investigation described below was accordingly carried out to study this problem.

EXPERIMENTAL METHOD

Experiments were carried out on 34 inbred rats weighing 180-200 g. After the animals had been starved for 24 h, under ether anesthesia the mesenteric artery was occluded and the intestine clamped at the junction of the duodenum with the jejunum and of the ileum with the cecum for 40 min (long enough for the formation of vascular anastomoses during transplantation of organs). At intervals of 1, 3, 7, 13, 30, 60, and 90 days after restoration of the circulation, 1.5 ml of sunflower oil was injected into the rats' stomach by means of a tube, and the animals were decapitated 1 h later. The material was taken from the proximal part of the intestine. For histologic and electron-microscopic investigations, 2 or 3 animals were used at each time (18 altogether). Four intact rats, also receiving oil and decapitated 1 h later, served as the control. Activity of succinate (SDH), lactate (LDH), glucose-6-phosphate (G6PHD), glutamate (GDH), and β-hydroxybutyrate (HBDH) dehydrogenases was determined in tissue homogenate from 27 rats by the method in [1]. Parallel tests were carried out on frozen sections. The quantity of fat absorbed was determined by means of balance experiments. Fat was extracted from pelleted food and feces by the method in [5]. The numerical results were subjected to statistical analysis.

EXPERIMENTAL RESULTS

After removal of the ligature from the artery the intestinal lumen was dilated and filled with dark red fluid. Necrosis of the upper third of the villi was discovered histologically. After one day the villi were a little shortened and the vessels congested with blood. By the 3rd day changes in the intestinal wall were no longer visible. Complete reconstruction of the mucosa 72 h after ischemia of the rat intestine for 1 h has been observed by other workers also [2].

Electron-microscopy of the small intestine of the control animals 1 h after fat loading revealed lipid drops in the cavities of the smooth endoplasmic reticulum (SER) and they filled the whole cytoplasm of the enterocytes uniformly (Fig. 1a). Infrequent tubules of the rough endoplasmic reticulum (RER) were detected only above the lamellar complex (LC), whereas in animals not receiving fat it occupied most of the cytoplasm. Vacuoles of LC contained large lipid drops, but the membranes of the cisterns were shortened and reduced in number. Besides lipids, covered with membranes of SER and LC, the cytoplasm of the enterocytes also contained single small so-called matrix lipids, not covered by a membrane. Chylomicrons left the enterocytes at the LC level by reversed pinocytosis, entered the interepithelial space, penetrated

Scientific Group for Organ Transplantation, Department of Operative Surgery and Topographic Anatomy, P. Lumumba Peoples' Friendship University, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR, A. I. Strukov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 97, No. 3, pp. 274-277, March, 1984. Original article submitted March 3, 1983.

0007-4888/84/9703-0245$08.50

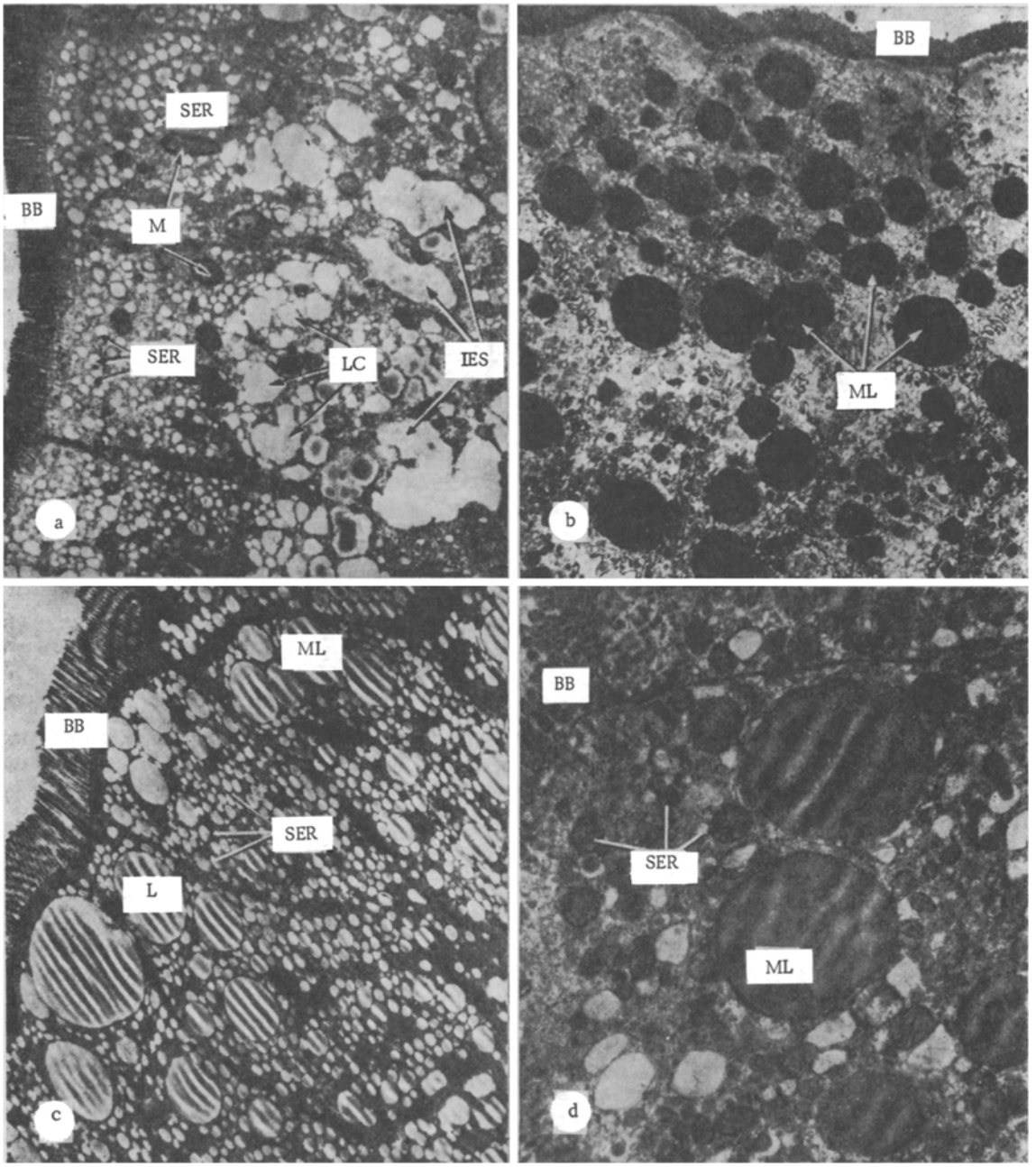

Fig. 1. Ultrastructure of epithelium of villi of normal and ischemic intestine 60 min after fat loading. a) Control: lipid drops fill cavities of SER, which spreads over the whole apical part of cytoplasm of enterocytes; vacuoles of LC are dilated and contain lipids; interepithelial spaces (IES) are dilated and contain chylomicrons. BB) Brush border, M) mitochondria, 5400 ×; b) 1 day after ischemia. Hyaloplasm of enterocytes contains numerous large matrix lipids (ML). 3130 ×; c) 3 days after ischemia. Large quantity of matrix lipids (ML) found in hyaloplasm of enterocytes. 4630 ×; d) 7 days after ischemia: lipids present in enterocytes in two forms: membrane-bound (SER) and matrix (ML); membrane-bound lipids vary in size. 15235 ×.

the basement membrane, and entered the lymphatic vessels from the stroma between the connected endothelial cells. Triglyceride resynthesis is known [3, 4, 14] to begin in SER, which rapidly undergoes hyperplasia and occupies a narrow zone above LC. The latter also is involved in the transport conveyor after the first few minutes, its membrane component contracts, and the vacuoles enlarge.

The transport pathways of the enterocytes 1 day after intestinal ischemia and 1 h after fat loading differed considerably from the control. Large and numerous matrix lipids were

present in the epithelium, and were scattered throughout the cytoplasm (Fig. 1b). The diameter was increased to 3.0 μ, and they were easily distinguished from drops covered with SER membranes, which also were somewhat enlarged. Tubules of the RER were sharply reduced and almost indistinguishable. Structures of LC either could not be detected or their place was occupied by large lipid drops. The character and number of the chylomicrons in the interepithelial spaces, stroma, and lymphatic vessels were indistinguishable from the control.

Two groups of cells were discovered 3 days after the operation and fat loading. In the majority of enterocytes the arrangement and size of the lipids covered with SER membranes and the zone of the lamellar complex and RER were almost indistinguishable from those in the control. In a smaller number of enterocytes changes described for the preceding period were still present; there were many large matrix lipids, LC was disorganized, and reduction of the tubules of RER was much more distinct (Fig. 1c).

Only individual enterocytes 7, 14, and 30 days after the operation contained large matrix lipids (Fig. 1d), but by the 90th day the usual structure of the transport pathways of the epithelial cells during fat absorption was observed.

Activity of enzymes of the various metabolic pathways in the ischemic intestine after fat loading changed variously (Fig. 2). After 1 day, activity of SDH, G6PDH, and GDH in the tissue homogenates was sharply reduced. Enzyme-histochemical investigations showed that this decrease was due to a decrease in intensity of the reactions in the epithelium of the villi and, in particular, of the crypts. Enzyme activity was back to normal after 3 days and showed a tendency to increase further. By the 14th day SDH and HBDH activity was significantly higher than in the control, whereas LDH, G6PDH, and GDH activity was the same as in the control. The results of the enzyme-histochemical investigation showed that increased enzyme activity was due to the epithelium of the crypts.

After one month enzyme activity in the intestinal cells was somewhat reduced, and after 3 months it was back to normal.

Loss of fat with the faeces increased significantly only 24 h after the operation. The very small decrease in fat absorption in the experimental animals compared with the controls at other times was not statistically significant: normal 88 ± 3.8% (n = 4), 1 day after ischemia 73 ± 3.9% (n = 6), after 1 week 80 ± 2.3% (n = 9), after 2 weeks 84 ± 1.9% (n = 5), after 4 weeks 81 ± 2.8% (n = 4) after 8 weeks 80 ± 3.1% (n = 3), and after 12 weeks 85 ± 1.6% (n = 3).

Thus during the first few days the resynthesis of fat and its liberation from the enterocytes were disturbed, causing accumulation of lipids in the cytoplasm and increased fat excretion with the faeces. A clear sign of disturbance of absorption is the appearance of large and numerous matrix lipids in the epithelium. According to data in the literature [10], matrix lipids may consist of newly esterified triglycerides, which are released from solution into the hyaloplasm, when cavities of tubules of SER become filled to overflowing with lipids. In fact the lipid drops located in the cavities of SER were larger in the experimental animals than in the controls. This may be due either to accelerated synthesis or to delayed transport of triglycerides along the tubules. After migrating along the tubules of SER the triglycerides then must pass through LC, where carbohydrates are added to them [8, 9]. After ischemia LC is disorganized, its membranes are almost indistinguishable, and individual vacuoles become enlarged. This is evidence that synthesis of the membranes of the complex is delayed and its function disturbed. If it is recalled that the velocity of processes taking place in an organoid is much less than the absorption of fat on the luminal surface [7], saturation of the enterocytes with lipids, evidently as the result of slower transport both through tubules of SER and through LC will be understandable.

Normal absorption of fat is accompanied by reduction of RER membranes, which are transported into membranes of the SER, undergoing hyperplasia, and membranes of LC [3, 4, 6]. After ischemia reduction of the RER membranes is more marked than in the control. It can therefore be tentatively suggested that synthesis of the protein and enzymes required for renewal of the utilized SER and LC membranes is delayed. On the first days after ischemia there is probably a relative protein-synthesizing deficiency of the enterocytes, brought to light by fat loading. This hypothesis is confirmed by experiments in which protein synthesis was specially blocked by puromycin [6] or cyclohexamide [15]. These substances block synthesis of β-lipoproteins required for chylomicron transport, and under these circumstances lipids accumulate in the cytoplasm. The protein-synthesizing deficiency of RER after ischemia may

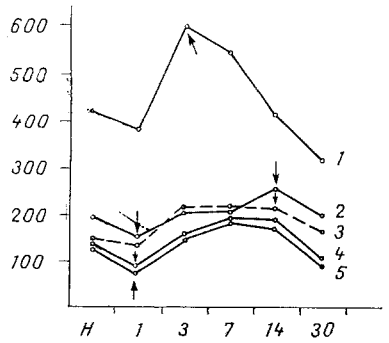

Fig. 2. Activity of oxidation-reduction enzymes in normal and ischemic intestine 60 min after fat loading. 1) LDH, 2) SDH, 3) HBDH, 4) G6PDH, 5) GDH. Abscissa, time (in days) after ischemia (3 rats studied after 30 days, 6 rats in each other case); ordinate — enzyme activity, in μg formazan formed by 1 g wet weight of tissue in 1 min. Arrows indicate times when differences from normal are significant.

be connected with depressed enzyme activity and reduced generalization of high-energy compounds. The enzyme-histochemical investigation revealed a decrease in activity of enzymes of glycolysis, the Krebs' cycle, the pentose pathway, and of GDH, the key enzyme of amino-acid metabolism, in the epithelium of the villi 24 h after ischemia. This reduction was partly due to the presence of dystrophic changes in the enterocytes and, as the additional electron-microscopic study of the epithelium of the villi in the animals undergoing operation and not receiving fat, showed, to incomplete differentiation: small mitochondria, short tubules of RER with only few attached ribosomes, translucent hyaloplasm. In the writers' view, energy expenditure in the ischemic intestine is required for differentiation of the epithelium.

Because of the lack of information it is difficult at present to compare the rate of recovery of the transport function of the ischemic intestine so far as proteins, fats, and carbohydrates are concerned. After ischemia of a loop of intestine for 30 min in rats, phenylalanine assimilation was sharply reduced for the first 3 days, and in some animals, up to 8 days [11]. In dogs, after ischemia of a loop of intestine for 1 h, the same workers [13] found that phenylalanine transport is completely restored to normal after only 24 h, and glucose transport rather later. In the same experiments, phenylalanine assimilation after ischemia for 2 h was restored after 3 days, but glucose assimilation only after 7 days [12]. According to data in the literature [2], ischemia of the intestine for 1 h in rats leads to loss of body weight, which is restored in the course of 3-5 weeks. Fat absorption at the lower limit of normal, which we observed for 8 weeks, and also disturbance of absorption of amino acids and carbohydrates can, in our opinion, explain this loss of body weight.

Temporary ischemia of the small intestine thus leads to marked structural disturbances of lipid transport through enterocytes, a reduction in enzyme activity of the cells, in absorption function, and to loss of fat with the faeces. Normalization of these parameters takes place at different times and is complete after one month.

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INSULIN CONTENT IN PANCREAS AND BLOOD PLASMA OF DECAPITATED ANE ENCEPHALECTOMIZED RAT FETUSES

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UDC 616.379-008:6:577.175.722]-09:616.831.41-07:616.154.37:577.175.722]-092.9

KEY WORDS: decapitation; encephalectomy; hypothalamus; pituitary; fetus.

Previous observations on adult animals subjected to stimulation or destruction of certain hypothalamic zones suggested that this brain region is concerned with the regulation of pancreatic function [15]. These effects of the hypothalamus are considered to be mediated through the autonomic nervous system [7]. Meanwhile the insulin-stimulating and inhibitory activity of incubation media and homogenates of certain regions of the hypothalamus has been demonstrated and is evidence of the possible existence of a humoral pathway from hypothalamus to pancreas [5, 11].

Investigations into the connection between the pituitary and pancreatic function have shown that the plasma insulin level and glucose sensitivity of adult hypophysectomized animals are reduced and that proinsulin synthesis is inhibited [12]. However, there is as yet no clear understanding of the nature of the changes observed in the pancreas during disturbances in the hypothalamus–pituitary system.

The writer showed previously that decapitation and encephalectomy of fetuses in utero leads to loss of sensitivity of the pancreatic B cells to glucose. Subsequent replacement injection of hypothalamic homogenate, preincubation with the adenohypophysis, or injection of certain adenohypophyseal hormones (STH, ACTH) abolish the effects of the operations, and B-cell reactivity was restored after 30 min [10]. The writer postulated that this rapid recovery of reactivity may be due to the fact that removal of the hypothalamus and pituitary as a result of the operations inhibits development of structures of the B cell that are responsible for perception of glucose as an insulinotropic signal and does not affect fundamental processes of hormone biosynthesis and secretion.

The aim of this investigation was to study the role of the hypothalamus and pituitary in the development of insulin biosynthesis and its secretion into the blood stream in rat fetuses.

EXPERIMENTAL METHOD

Wistar albino rat fetuses were used. To remove the hypothalamus, the fetuses were encephalectomized in utero [1], and decapitation of the fetuses in utero was used as the experimental model of hypophysectomy [6]. Operations on the fetuses were performed after 17.5 days of pregnancy. After 21.5 days of pregnancy, the mother was anesthetized with pentobarbital and the fetuses removed; blood was taken quickly from the fetuses (by pipet from an incision in the heart) and the pancreas was removed. Blood from fetuses belonging to the same litter was pooled. The isolated glands from each fetus were weighed separately and placed in a plastic test tube with a drop of physiological saline on dry ice, where they were kept until the time of determination of immunoreactive insulin (IRI). The blood was collected in heparinized tubes and the plasma was separated by centrifugation and kept in plastic tubes in dry ice.

Laboratory of Hormonal Regulations, N. K. Kol'tsov Institute of Developmental Biology, Academy of Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. P. Avtsyn.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 97, No. 3, pp. 278-280, March, 1984. Original article submitted May 20, 1983.

0007-4888/84/9703-0249$08.50