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ABSORPTION OF CARBOHYDRATES AND FATS IN AN ALLOGENEIC GRAFT OF THE SMALL INTESTINE

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The results of a study of the absorptive function of an allogeneic graft of the small intestine, using D-xylose loading and electron-microscopic investigation of neutral fat transport in the intestinal wall, are described. An unusual increase in the absorption of D-xylose was shown to take place in association with structural changes in the wall of the intestine and disturbance of fat transport.

KEY WORDS: *transplantation; absorption; electron microscopy.*

Several attempts have now been made outside the USSR to perform allogeneic transplantation of the small intestine in man [3, 6, 10, 12]. The unsuccessful results of these operations have shown the importance of the study of the functional state of the graft. In particular, the absorptive function of an allogeneic intestinal graft has received very little study and such investigations as have been made have been mainly on an isolated segment of the small intestine [4, 5, 7, 8, 11].

The object of this investigation was to study the absorption of carbohydrates and fats in dogs after total allotransplantation of the small intestine.

EXPERIMENTAL METHOD

Functional and morphological investigations were carried out on 25 mongrel dogs with an allogeneic small intestine. The control consisted of 20 intact animals and 35 dogs with an autologous intestine (the results of these investigations were published previously [1]). The operation of total orthotopic transplantation of the small intestine was carried out by the generally accepted technique [2]. Loading with D-xylose (5 g in 300 ml water) was carried out after starvation for 12 h and emptying of the urinary bladder. In the course of 5 h, urine was collected from the dogs by catheterization of the bladder and the concentration of D-xylose in it was determined [13]. To study lipid transport the dogs were fed with sunflower oil at the rate of 5-6 ml/kg body weight. The animals were killed 1 h after loading and a portion of the graft was removed 5-10 cm distally to the proximal anastomosis for histological (staining with hematoxylin–eosin and Sudan III) and electron-microscopic investigation.

EXPERIMENTAL RESULTS AND DISCUSSION

Control tests showed that the quantity of D-xylose excreted with the urine of the intact

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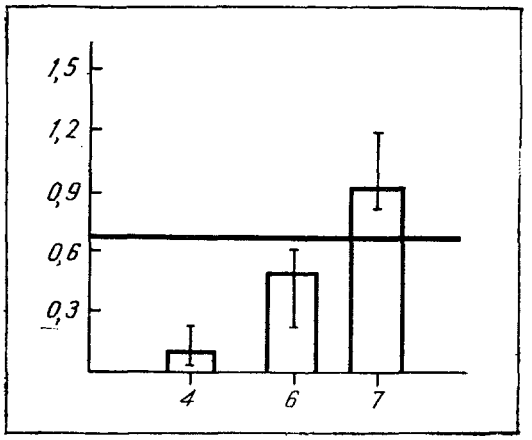

Fig. 1. Excretion of D-xylose with urine of dogs with allogeneic small intestine. Ordinate, quantity of D-xylose excreted with urine (in g/5 h); abscissa, times of observation (in days). Horizontal line marks lower level of excretion of D-xylose in intact dogs. Values of M ± 3 m shown.

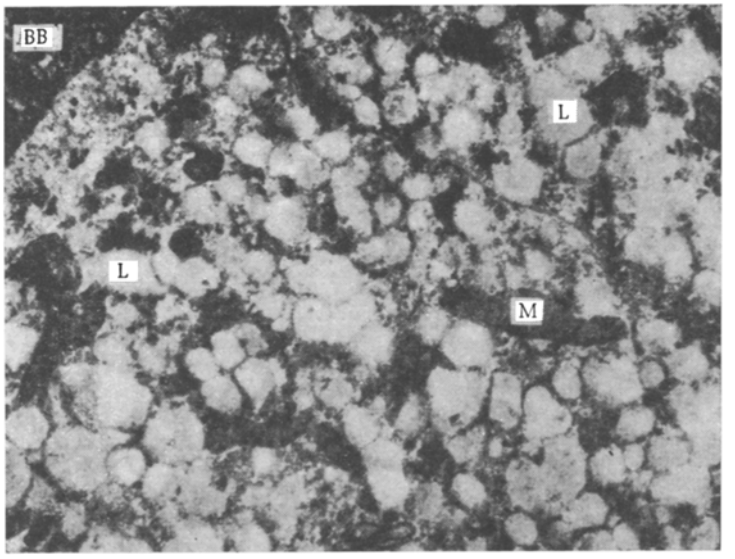

Fig. 2. Enterocytes of intact dog 1 h after loading with neutral fat: BB) brush border; L) lipids covered by membrane of smooth endoplasmic reticulum; M) mitochondria; 10,000 ×.

animals averaged 1.19 g during 5 h. The confidence limits for this mean value (P = 0.05) were 0.69 to 1.89. To assess the absorptive function of the autologous and allogeneic grafts, the excretion of D-xylose was compared with the lower limit of normal (0.69 g in 5 h), so that any decrease in this function or its normalization could be clearly revealed (Fig. 1). Control studies of the absorption of neutral fats were carried out on three intact animals. Laparotomy was performed 1 h after loading. Under these circumstances the dilated milky white lymphatics were visible in the wall of the duodenum and jejunum, and also in the mesentery and under the capsule of the mesenteric lymph node. On histological investigation lipids were seen in the epithelium and stroma of the villi, and in the lymphatic sinuses and vessels of the submucosa and of the muscular and serous coats. On electron-microscopic investigation, small even drops were seen in the dilated cavities of the tubules and smooth endoplasmic reticulum (SER); meanwhile single drops, lying freely in the hyaloplasm and not covered by a membrane (Fig. 2) also were seen. The rough endoplasmic reticulum (RER) occupied only a narrow zone directly above the Golgi complex, by contrast with the starved animals, in which

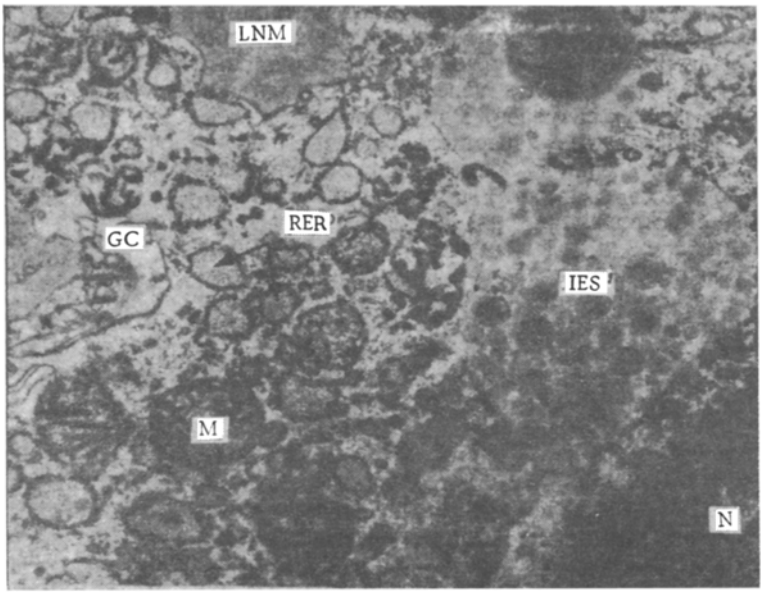

Fig. 3. Enterocytes of dog with allogeneic intestine (6 days) 1 h after loading with neutral fat: LNM) lipids not covered by membrane; RER) rough endoplasmic reticulum; GC) Golgi complex; N) nucleus; IES) lipids in dilated interepithelial space; 14,000 ×.

RER spread over a large part of the hyaloplasm of the enterocytes. The number of membranes of the cisterns of the Golgi complex was somewhat reduced, they were shortened in length, and the vacuoles of the complex were dilated and contained lipid drops. The interepithelial spaces contained a few smooth, round osmiophilic drops.

A functional and morphological study of the allograft of the small intestine was made 4, 6, and 7 days after the operation. On the 4th day of excretion D-xylose with the urine averaged 0.11 g over 5 h (0.05-0.18 for $P = 0.01$). This index was reduced in all the animals studied.

After loading with neutral fat, at the subsequent laparotomy lymphatics were clearly visible in the wall of the duodenum and less clearly in the wall of the graft (in its first loops). On histological investigation of the proximal part of the graft lipids were discovered in all the transport channels. Electron-microscopically, lipids were found in the cavities of SER, which occupied only a narrow zone under the terminal strip. Most of the lipid drops were located in cavities of tubules of the RER, which occupied the usual area of the hyaloplasm. Many large lipid drops not covered by membrane also were found. Similar changes were observed in the autologous small intestine.

On the fifth to sixth day after the operation the mean excretion of D-xylose with the urine was 0.57 g in 5 h (0.21-1.20), i.e., there was a tendency for it to increase. However, this tendency ($P > 0.05$) was due to an increase in the excretion of D-xylose in only one animal. After loading with fat the lymphatics were visible in the wall of the graft, its mesentery, and the mesenteric lymph node. Histologically, lipids were detected in all transport channels. Electron-microscopically, osmiophilic drops were seen mainly in the dilated tubules of the RER, which occupied the greater part of the hyaloplasm. Free drops of different sizes, not covered by a membrane, were situated in different parts of the enterocyte. The sharp dilatation of the interepithelial spaces, where many lipid drops accumulated, will be noted (Fig. 3).

On the seventh to eighth day after the operation the animals were increasingly apathetic and they died with signs of cardiovascular failure. The excretion of D-xylose with the urine at this time averaged 0.96 g in 5 h, which was above the lower limit of normal ($P < 0.05$). This increase was observed in all the animals tested. The lymphatics of the duodenum and grafts could not be seen macroscopically after loading with sunflower oil. Histologically, lipids were found in the epithelium of the villi, stroma, and lymphatics in small amounts. The same picture also was observed on electron-microscopic investigation.

During the first four days after total allotransplantation of the small intestine in dogs the absorption of D-xylose was sharply reduced and lipid transport disturbed. Under these circumstances, transformation of the RER to SER, which plays an important role in the synthesis of triglycerides [14], probably did not take place. For that reason, lipid drops were found mainly in the tubules of the RER and they appeared in the hyaloplasm as large drops, not covered by a membrane. The disturbances of the absorption of carbohydrates and fats at these times of observation were identical to the disturbances of absorption after autografting of the small intestine and, in all probability, were due to the influence of nonspecific transplantation factors (ischemia, denervation, division of lymphatics, operative trauma, anesthesia).

The tendency toward an increase in the excretion of D-xylose with urine on the fifth to sixth day after the operation become specifically significant on the seventh to the eighth day. However, despite the normalization of carbohydrate transport, marked disturbances of lipid transport still remained. Besides intracytoplasmic changes, extracellular changes also were observed in the form of sharp dilatation of the interepithelial spaces, which were packed with drops of fat of various sizes. By this time the process of structural damage in the wall of the graft was increasing in intensity as a result of the manifestation of tissue incompatibility. Plasmatization and damage to the capillaries developed in the stroma of the mucous membrane, whereas monocytic infiltration and perivasculitis were observed in the submucosa and in the muscular coat; desquamation of the epithelium took place. Consequently, the increase in absorption of D-xylose took place against the background of structural disturbances in the wall of the graft. Evidently the transport of dietary monomers under these conditions takes place through passive diffusion through the basement membrane of the epithelium. A similar spurious increase in the absorptive function of the allogeneic graft was observed by other workers previously with respect to the absorption of glucose-^{14}C in an allogeneic segment of small intestine. Meanwhile, at the same time after the operation, in the autologous small intestine, a tendency was observed for the absorption of both carbohydrates and fats to be increased, and this was followed by the normalization of these processes.

After allogeneic grafting of the small intestine, food and therapeutic substances thus either pass through the graft or undergo pathological resorption. It is therefore most rational to feed and treat animals with an allogeneic small intestine parenterally.

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