AN ISOLATED RAT ABDOMINAL PREPARATION

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Two main approaches to the study of the digestive system are known, namely methods *in vivo* and *in vitro*. Each has both its advantages and its disadvantages. There is ever-increasing urgency for the creation of a preparation of "intermediate" type, combining some properties of the *in vivo* and *in vitro* systems. The idea of using functional complexes for investigation of the digestive system was recently partly realized by Kowalewski et al. [7], who perfused a preparation consisting of the stomach, duodenum, pancreas, and liver. With this preparation it was possible to study only the initial stages of digestion, but it proved to be extremely useful for the study of certain effects of physiologically active substances: pentagastrin, secretin, and cholecystokinin. At the same time, it was impossible by means of this preparation to study function of the gastrointestinal tract as a whole and, in particular, the intermediate and final stages of digestion, as well as absorption and utilization of food substances. Interest in this problem has increased in recent times, but authors of many publications have mainly limited their attention to perfusion of blood vessels and (or) of the digestive organs of one or two sections of the gastrointestinal tract [3-6, 8-11]. An isolated alimentary complex of large laboratory animals (dogs) was recently developed for the first time [2]. This complex included all parts of the gastrointestinal tract except the supradiaphragmatic part of the esophagus and the rectum, and the technique provides a new approach to experimental gastroenterology.

For a combined approach to the study of functions of the digestive organs, the writers have used an alimentary complex from a small laboratory animal (rat). The abdominal preparation consists of stomach, duodenum, small and large intestines, and pancreas. The method consists essentially of perfusing the blood vessels of the abdominal preparation of the gastrointestinal tract and (or) portions of it both *in situ* and after isolation.

Method of Obtaining Preparation. For obtaining the abdominal preparation male Wistar rats weighing about 200 g were used. The animals were kept in special chambers with natural illumination and at room temperature (22°C), under laboratory conditions. The work was done on the animals after starvation for 18-20 h, and in some cases, on fed rats. The animals were anesthetized with pentobarbital sodium (3.5 mg/100 g body weight, intraperitoneally), and heparin (100 U/100 g body weight intraperitoneally) was used to prevent blood clotting. After the animal had been anesthetized, laprotomy was performed in the midline. To obtain better access to the abdominal organs, a cushion 20 mm in diameter was placed beneath the rat's spine. The abdomonal organs were shifted to the side and placed on an ice bag, covered with a towel soaked in Ringer's solution. The rectum and esophagus were then divided between two ligatures. The abdominal aorta and vessels leaving it were then dissected. The site of application of the ligatures and of division of the vessels is shown in Fig. 1. Attention is drawn to the variability of the site of origin of the vessel supplying the stomach from the celiac artery, and also its topography relative to that organ.

To perfuse the vessels of the abdominal preparation two versions of introduction of the cannula into the aorta were used: upper and lower. In the first case the cannula for supplying perfusion fluid was introduced into the aorta above the origin of the celiac artery (Fig. 1b), in the second version, below the origin of the caudal mesenteric artery (Fig. 1c). Each of these two versions had its limitations. For instance, if the cannula was introduced above the origin of the celiac artery, the cannula fixed in the proximal portion of the abdominal

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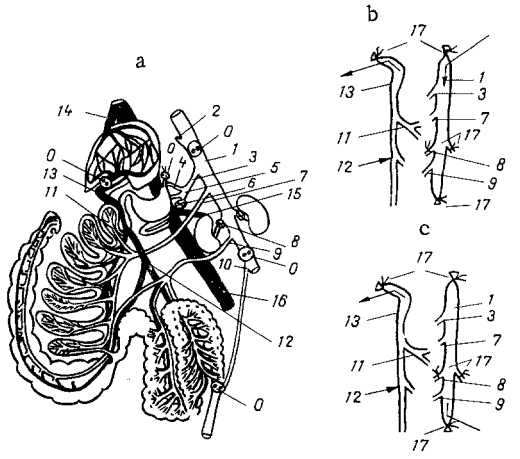

Fig. 1. Scheme of upper (b) and lower (c) versions of perfusion of blood vessels of abdominal preparation (a). Arteries (unshaded) and veins (shaded black) of rat abdomen shown according to Gambaryan and Dukel'skaya [1], with modifications. 0) Site of application of ligature and division of blood vessel, esophagus, or rectum; 1) abdominal aorta; 2) caudal phrenic artery; 3) Celiac artery; 4) gastric artery; 5) hepatic artery; 6) splenic artery; 7) cranial mesenteric artery; 8) renal artery; 9) caudal mesenteric artery; 10) rectal artery; 11) cranial intestinal vein; 12) caudal intestinal vein; 13) portal vein of liver; 14) hepatic vein; 15) renal vein; 16) caudal vena cava; 17) site of ligation of vessel. Arrows indicate site of introduction of cannulas.

aorta can impair or disturb the blood supply to the stomach. If the cannula was inserted below the caudal mesenteric artery, it had to be introduced into a vessel of smaller diameter.

The cannulas were made from special types of synthetic materials. A ring of slightly larger diameter than the vessel itself and the cannula was first fitted over the cannula to be introduced into the vessel, at a distance of 5-8 mm from its end. Before the canula was introduced into the vessels it was filled with Ringer's solution containing heparin (5000 U heparin to 10 ml solution). The cannula was fixed in the vessel by two ligatures, applied above and below the ring.

During perfusion of the gastrointestinal tract or parts of it, the inlet cannula was introduced into the proximal part of the organ to be studied or of the alimentary complex as a whole, and the outlet cannula into the distal part; the two cannulas were chosen to be of approximate diameter.

During work with the abdominal preparation *in situ*, the abdominal organs were returned to their proper place and the skin wound closed with two or three sutures. During work on the isolated abdominal preparation, it had to be removed from the peritoneal cavity. For this purpose, the soft tissues were divided as closely as possible to the spine. The abdominal preparation, after removal, was transferred quickly to a constant-temperature table (37°C) and covered with a towel soaked in Riger's solution.

Hydraulic dissection can be carried out, when mobilization of the vessels and their division between ligatures must be done after injection of Ringer's solution beneath the serous membranes of the organs and the serosa of the posterior wall of the peritoneal cavity.

To verify isolation of the organs and integrity of the vessels, methylene blue was used. The dye, as a 0.001% solution, was injected into the aorta at the end of the experiment. If the technique of dissecting the preparation was correct, methylene blue stained all the organs of the abdominal complex.

Perfusion of the Abdominal Preparation. Vessels of the abdominal preparation were perfused under constant pressure of the perfusate at the inlet (20-30 mm Hg) and with constant volume of outflowing fluid. The rate of perfusion (0.7-1.0 ml/min) was created by means of various types of pulsating micropumps. The stomach, duodenum, and small and large intestines were perfused by means of such micropumps at a speed of 0.5 ml/min.

During perfusion of the vessels of the alimentary complex, to prevent edema a mixture of Ringer's and polyglucin (1:1), containing monosaccharides, amino acids, or other substances depending on the aim of the experiment, was used. The perfusate was saturated with oxygen for 60 min before perfusion and also throughout the experiment. Addition of polyglucin to the perfusate and its oxygenation enabled the preparation to be maintained in a satisfactory state for 1 h or more. During perfusion of the isolated alimentary complex or of its constituent organs, various food substances in Ringer's solution (pH 7.4) were used. In addi-

dition, in some cases instead of perfusion, nutrient substances were injected once only into different parts of the gastrointestinal tract.

This abdominal preparation, consisting of an alimentary complex including stomach, duodenum, small and large intestines, and pancreas, can thus be used to study many problems in normal and pathological physiology, pharmacology, and in biology as a whole.

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PREPARATION OF LIPOSOMES BY REVERSE-PHASE EVAPORATION AND BY FREEZING AND THAWING

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Preparation of liposomes containing large quantities of drugs is an essential condition for their use as a method of transporting substances into the cells and of achieving a marked

TABLE 1. Dependence of Incorporation of [^{14}C]DTPA into Liposomes on Method of Forming Suspension

Type of liposomes according to method of obtention	Incorporation of [^{14}C]-DTPA, %	Volume of aqueous phase in liposomes, μl/μmole phosphatidylcholine
[^{14}C]-DTPA added to suspension of liposomes	<0,01	—
"Bangham" liposomes	19.3±1,5 (100%)	3,78±0,29
"Bangham" liposomes frozen and thawed 10 times	22.9±1.4 (118%)	4,48±0,27
[^{14}C]-DTPA coprecipitated with lipids before addition of aqueous phase	23.5±4.0 (122%)	4,62±0,78
Liposomes obtained with reverse-phase evaporation and freezing and thawing	54,6±4,4 (281%)	10,6±1,3

Legend. Liposomes formed with phosphatidylcholine and cholesterol each in a concentration of 54 mM.

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