AN APPARATUS FOR PERFUSING THE RAT LIVER IN SITU

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An apparatus for perfusing the rat liver in situ is described. Its advantages are simplicity of construction, the possibility of carrying out two perfusions simultaneously, maintenance of pCO_2 of the perfusion fluid without supplying CO_2, a low level of hemolysis, and the possibility of applying measured doses of necessary substances to the liver continuously.

Key words: perfusion of liver – apparatus.

The first successful types of apparatus for perfusing the rat liver were suggested in 1951 [2, 4].

Further improvements [3, 5-8] brought no essential simplification, and indeed some of them [5-7] were actually more complicated than the first models.

The writer has designed and built a simplified apparatus by means of which two livers can be perfused simultaneously under absolutely identical conditions with separate fluids (of the same or of different types). The scheme of one of the two parallel systems is shown in Fig. 1. It includes the following principal components.

1. Pump. A digital (peristaltic) pump (Peripump, MTK, type 5096; Hungary) was used. The perfusion fluid is propelled after consecutive digital compression of a thick silicone-rubber tube, forcing the liquid to move in one direction. Precise selection of the degree of compression of the walls of the tube is essential in order to reduce hemolysis. Two separate rows of digits, connected with a common mechanical drive system, generate the peristaltic waves. The pump can thus carry out two parallel perfusions simultaneously and with identical throughput. The throughput is controlled automatically by engaging the corresponding pinions. By varying the throughput of the pump, perfusion of the liver can be carried out at a constant rate: 0.8-1 ml perfusion fluid/g liver/min.

The pump is the component common to the two symmetrical systems.

2. Oxygenator (Artificial Lungs). Saturation of venous blood leaving the inferior vena cava with oxygen takes place on the surface of the film that it forms while flowing over the inner surface of a glass tube (diameter 20-25 mm). Moist oxygen is supplied continuously at the rate of 0.3 liter/min. The risk of air embolism [1] is prevented by preliminary treatment of the walls of the reservoir with foam suppressor and collecting the blood from the bottom of the reservoir.

3. Temperature Control System. Water heated to 40°C by a thermostatically controlled electric heater is pumped through the system of heater–reservoir–oxygenator–heat exchanger. Wider glass tubes with an inlet in the lower and outlet in the upper part are connected to these components of the apparatus (Fig. 1: 4, 6, 9). Water circulates between these components along rubber hoses. The temperature of the perfusion fluid is kept constant (37.5°C) in all the components of the apparatus. The temperature is controlled by an electric thermometer, the sensitive element of which (Fig. 1: 10) is surrounded by the flowing perfusion fluid. To prevent loss of heat from the perfused liver to the surrounding tissues the rat is fixed on a table consisting of a hollow metal box filled with circulating hot (45°C) water. The heating system of the table is separate from the heating system of the perfusion fluid, but it supplies both apparatuses. In

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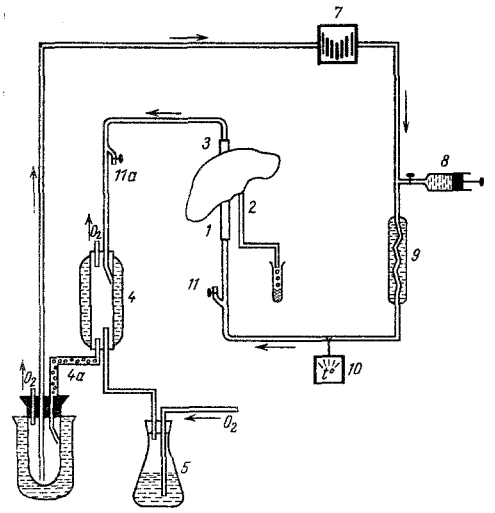

Fig. 1. Diagram of apparatus for perfusing the rat liver: 1) portal vein; 2) bile duct; 3) inferior vena cava; 4 and 4a) oxygenator; 5) oxygen humidifier; 6) storage vessel with heat exchanger; 7) digital pump; 8) microdoser; 9) heat exchanger; 10) electrothermometer; 11 and 11a) points for sampling perfusion fluid.

addition, to limit loss of heat from the liver, the abdomen and chest of the rat are covered by means of a transparent plastic hood.

4. Gas Humidifier. The oxygen passes through a humidifier containing 0.5-0.6% NaCl solution. This concentration was determined experimentally [7].

5. Microdoser. The "apparatus for regional heparinization and lymphography" (manufactured by the Kiev Experimental Medical Equipment Factory) with two syringes and controllable rate of output was used as the microdoser. The syringes were connected with the corresponding main supply lines by means of polyethylene tubes (internal diameter 1.5 mm) and three-way tubes with clips. By means of this instrument, the substance can be injected continuously into the perfusion fluid at a measured rate. The angle of the three-way tube is so designed to ensure complete mixing of the solutions. By changing the syringes (from 1 to 20 ml) and changing the rate of movement of the plungers, the dilution of the injected substance in the perfusion fluid can be varied between 1:2 and 1:400. This facility, coupled with a change in the concentration of the solution, enables the natural gradual inflow of the substances through the portal vein to be reproduced.

6. The Main Supply Lines of Perfusion Fluid and Sampling Points. The apparatus provides for two independent lines – arterial (from the reservoir to the portal vein, length 80 cm) and venous (from the inferior vena cava via the liver to the oxygenator, length 20 cm). As the diagram shows (Fig. 1: 11 and 11a), three-way tubes connected to the arterial and venous lines enable the perfusion fluid to be sampled immediately before and after passage through the liver.

If the technique has been properly mastered, connecting the apparatus to the rat liver requires not more than 3 min, involving hypoxia for not more than 10-15 min (ligatures are placed around the portal vein and inferior vena cava before the vessels are cannulated). Polyethylene cannulas (external diameter 2 mm) are connected with the main supply lines.

In the investigation used, the perfusion fluid was blood diluted with Ringer – Locke solution in the ratio of 4:1 (hematocrit 28-30%). The increase in hemolysis in the course of perfusion was small, namely 0.5-0.6 mg% per hour (using 50 ml perfusion fluid).

In more than 100 perfusions carried out with the aid of this apparatus various indices of liver function of the rat were determined (bile production, pO_2, oxygen demand of the liver, lactate : pyruvate ratio in the outflowing perfusion fluid, etc.). The state of the acid–base balance of the perfusion fluid was assessed from the pH and also from the buffer base shift, the standard bicarbonate, the normal buffer bases, and the pCO_2 value.

The results indicate that the proposed apparatus can maintain active liver function for over 4 h.

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