METHODS

Functional Activity of Isolated Perfused Rat Liver Depends on Medium Composition

A. P. Rupenko, O. V. Kruglik, and I. I. Morgulis

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It is convenient to study liver function and metabolism on the model of isolated perfused organ. The results of the present study indicate that viability and metabolic activity of the organ largely depend on the composition of the medium. Under conditions of isolated perfusion, the known pathway of oxygen transport through capillary filtration is supplemented by oxygen delivery to cells through the organ surface making an important contribution to liver oxygenation.

Key Words: *isolated liver perfusion; oxygen consumption by tissues*

Isolated perfused organ is a unique experimental model for evaluation of its status and function: on the one hand, natural architectonics of tissues and circulation are retained, on the other, there are no regulatory effects from the body. One of these models is isolated perfused liver. Analysis of published data indicates that the use of perfusion media of different composition [4-6] impedes comparison and evaluation of the results.

We compared the effects of perfusion media of different composition on functional activity of isolated liver.

MATERIALS AND METHODS

Experiments were carried out on female Wistar rats (200-260 g). The animals were narcotized with sodium thiopental (100 mg/kg intraperitoneally) and hemostasis was stabilized (heparin, 1000 U/kg intravenously), the portal vein was cannulated and infused with cold solution (50% medium 199 and 50% Krebs—Henseleit saline with 80 g/liter polyglucin); the vena cava was cannulated, after which the organ was isolated and placed into a Homeostat-3M device for culturing of isolated organs of small laboratory animals designed at Krasnoyarsk Research Center [1].

Perfusion of rat liver was carried out under normothermal conditions at constant perfusate flow rate (~3-3.5 ml/min/g).

The specific rate of O_2 consumption through the blood and separately through the organ surface was evaluated by the manometric method [3] (μmol/min/g). In order to characterize liver function, the specific rate of bile flow (ml/min/g) and peripheral vascular resistance (through perfusion pressure, cm H_2O) were measured.

Five series of experiments with perfusion media of different compositions (6 animals per series) were carried out:

— medium 1: 35% medium 199, 35% Krebs—Henseleit bicarbonate buffer with 80 g/liter polyglucin, and 30% homoblood (Ht=10);
— medium 2: 90% Krebs—Henseleit bicarbonate buffer with 40 g/liter polyglucin and 10% homoerythrocytes washed 4 times;

International Center for Research of Critical States, the Board of Krasnoyarsk Research Center, Siberian Division of Russian Academy of Sciences, Russia. ***Address for correspondence:*** olikru@yandex.ru. O. V. Kruglik

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— medium 3: 45% medium 199, 45% Krebs—Henseleit bicarbonate buffer with 60 g/liter BSA, and 10% homoerythrocytes washed 4 times;
— medium 4: 90% Krebs—Henseleit bicarbonate buffer with 20 mM Tris-buffer and 30 g/liter BSA and 10% homoerythrocytes washed 4 times;
— medium 5: 90% Krebs—Henseleit bicarbonate buffer with 20 mM Tris-buffer and 30 g/liter BSA and 10% perfluorotributylamine (PFTBA) and P-268 proxanol emulgator.

Liver function was evaluated by peripheral vascular resistance, bile flow rate, and O_2 consumption rate.

For evaluation of the impact of perfusion medium composition on organ reaction, adrenaline hydrochloride in a final concentration of 7×10^{-7} M was added to the same functional sample in all experimental series for 10 min starting from the 60th minute of perfusion.

The results were statistically processed using Student's *t* test.

RESULTS

The use of media of different composition detects the effects of their components on the vascular tone or microcirculation changes, which is seen from such an integral parameter as vascular resistance. Perfusion rate was constant for all experiments (23 ml/min), specific blood flow was the same in all series (mean value 3.3 ml/min/g), and hence, peripheral vascular resistance can be evaluated by the level of perfusion pressure. The initial pressure was higher than before adrenaline addition in all experiments (Fig. 1). The mean perfusion pressure is 15-25 cm H_2O at blood flow rate of 1-3 ml/min/g. In media 1, 3, 4, and 5 the pressure did not surpass 14 cm H_2O at a rate of 3.3 ml/min/g, this indicating more "physiological" conditions of perfusion than in medium 2, containing no plasma and its components. The pressure increased after addition of adrenaline, most markedly (by 57% of the initial level) in medium 4 and less so with other media (by 48% in medium 5, by 39% in medium 3, by 25% in medium 2, and by 17% of initial level in medium 1). Hence, all media used in the experiment provide more or less normal reaction of the isolated perfused organ to functional test.

Bile flow rate was maximum in medium 1, it decreased from 1.1 to 0.9 μl/min/g over 2.5 h of perfusion and approached the *in vivo* value (Fig. 2). Bile flow rate was minimum in medium containing no BSA or blood plasma. The bile flow was similar

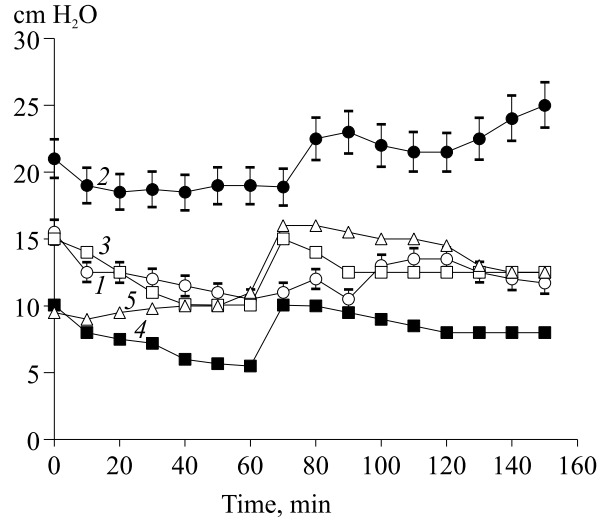

Fig. 1. Changes in the level of perfusion pressure in isolated rat liver perfused with different media. Here and in Fig. 2: *1-5*: medium No.

in all media containing BSA, being lower than in the presence of plasma (homoblood) in the perfusate. Hence, the bile-producing function of cultured organ was most pronounced and best retained in medium 1 containing blood plasma.

The rate of O_2 consumption through the blood during perfusion with albumin-containing media was about 3 μmol/min/g at the start of perfusion and decreased to 2.5 μmol/min/g by the 60th minute (Fig. 3). The rate of O_2 consumption during perfusion with media containing polyglucin (media 1 and 2) was significantly higher than in media 3-5 (3.9 μmol/min/g for medium with washed erythrocytes and 4.8 μmol/min/g for medium with intact erythrocytes) and decreased by the 60th minute.

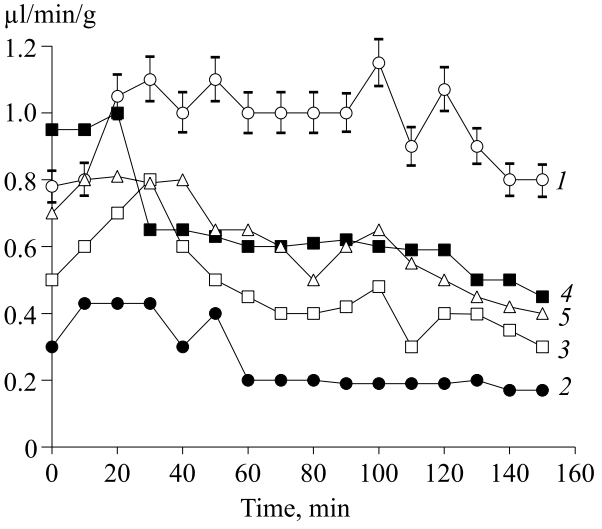

Fig. 2. Changes in bile flow rate in perfusion of isolated rat liver with different media.

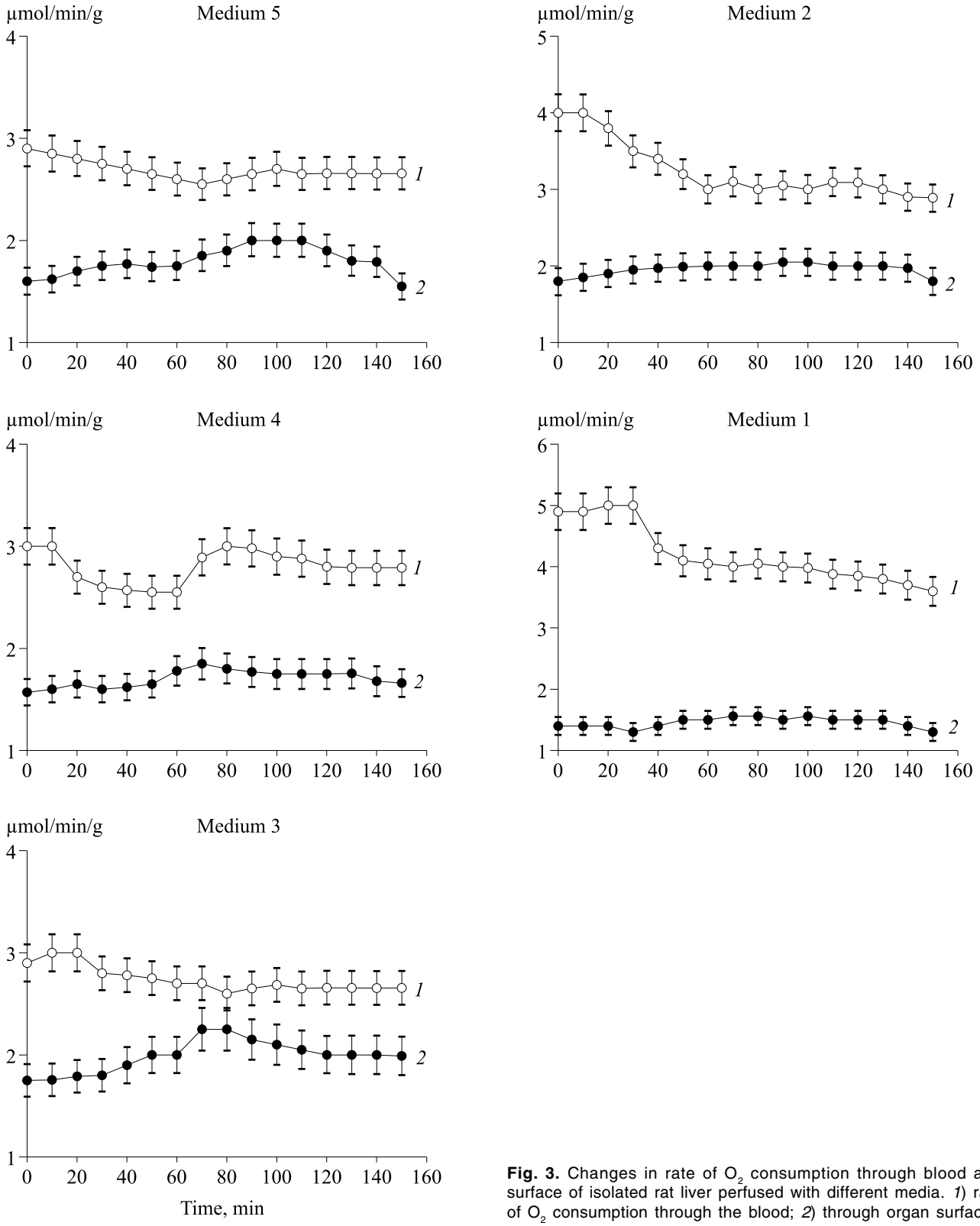

Fig. 3. Changes in rate of O_2 consumption through blood and surface of isolated rat liver perfused with different media. *1*) rate of O_2 consumption through the blood; *2*) through organ surface.

Adrenaline treatment stabilized the rate of O_2 consumption to the end of experiment.

In addition to this universal method of oxygenation, we previously revealed [2] the phenomenon of respiration through the organ surface. The maximum (under conditions of our experiment) O_2 consumption through the organ surface was observed in medium 5

containing PFTBA without blood components (Fig. 3).

The percentage of O_2 consumed through the surface reaches 75% during perfusion with PFTBA-containing medium. Respiration through the surface was the least pronounced during perfusion with plasma-containing medium (medium 1): the rate of O_2 consumption through the organ surface was about 30% of O_2 consumption through the blood. The percentage of O_2 consumption through the organ surface increased during perfusion, this increase was maximum in medium 2.

Adrenaline treatment leads to the appearance of a trend to an increase of O_2 consumption through the surface with all perfusion media, the increase being significant only with albumin-containing media.

Comparison of media for perfusion of isolated rat liver, differing mainly by components used for creation of oncotic pressure (BSA, polyglucin, plasma proteins) and O_2 carriers (homologous and intact erythrocytes, PFTBA) showed that viability and metabolic activity of the organ depended largely on the composition of the perfusion medium. Therefore, identical media should be used for observations and comparison of the results of organ treatment. Oxygen delivery to cells under conditions of isolated perfusion is realized, in addition to the known way of oxygen transport by capillary filtration, by another way, through the organ surface; this route typical of isolated organ in a culture makes an important contribution into liver oxygenation.

Modern studies on isolated organs can be classified into 2 types. Type 1 are studies with maximum approximation of the organ vital activity to the *in vivo* conditions, so that the organ functions were reproduced as much close as possible to those in an intact body. Media with erythrocytes and plasma proteins (in our study it was medium containing intact erythrocytes, medium 1) are preferable for these studies. Similarity to *in vivo* situation limits the use of this method for obtaining precise quantitative characteristics of metabolism. In type 2 studies, the model of isolated perfused organ is approximated to a standard preparation for regulation of a biochemical experiment. Erythrocyte-free media (medium 5) or washed erythrocytes (media 2-4) are preferable for these studies. The behavior of the isolated organ under extreme conditions can be best studied in maximally "depleted" media.

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