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MICROCALORIMETRIC INVESTIGATIONS OF THE METABOLISM OF AN ISOLATED PERFUSED RAT LIVER*

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ABSTRACT

The combination of the well-known perfusion technique with a Calvet microcalorimeter by means of a specially developed calorimetric vessel is described. The inherent medical, hydrodynamic and thermodynamic problems together with ways of solving them are discussed. The instrumental set-up allows one to investigate the influence of metabolites and drugs on the metabolism of a completely isolated rat liver by measuring the alternation of the heat production.

INTRODUCTION

Until recently, only a few microcalorimetric experiments were performed on complete isolated organs like hearts of frogs and snails[1–3]. They were placed in buffer solutions and could be kept alive for some hours. Therefore, it seemed interesting to look for the metabolism under normal or stress conditions in an organ of a higher animal. Since the livers of guinea pigs and rats have often been studied with perfusion technique[4], these organs offered great advantages for microcalorimetric research.

Whole organs in contrast to mechanically destroyed or trypsin-treated tissue, or to tissue cultures, possess the normal regulation facilities of an intact tissue. Therefore, only under these experimental conditions can the normal interplay between different cells as in the liver or in the kidney parenchyma be observed. Moreover, it is known that in the same tissue not all cells are equally suited for different activities. For instance, there is a division of labour in the small liver lobes along the blood capillaries or sinusoids.

A partial list of the known functions of the liver is as follows: decontamination (conjugation); excretion (bile); synthesis (hormones and clotting factors); storage (glycogen).

To obtain further metabolic information about the various functions of the liver, a totally isolated rat liver is perfused in a specially developed vessel of a microcalorimeter (Fig. 1).

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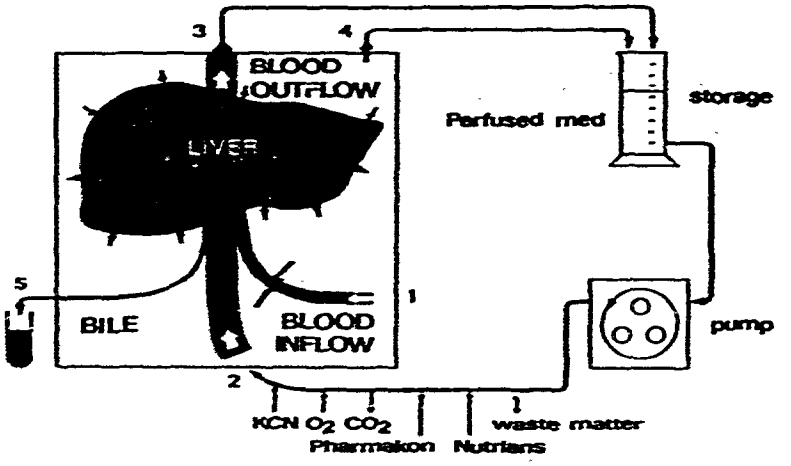


Fig. 1. Perfusion flow system. The liver is connected as follows: Inflow: 1 = Arterial supply (it is ligatured for the isolated liver.); 2 = portal vein. Outflow: 3 = Vena carva inferior; 4 = vessel outflow; 5 = bile outflow. In the inflow-line there may be an exchange of gases, metabolites and waste matter via dialysis membranes. Drugs are added to this line by means of syringes.

The advantage of calorimetry is that it does not interfere with the system under research—perhaps except for the artificial isothermy in the case of the active liver. But it has the same unspecificity as the perfusion technique which does not allow any conclusions concerning the exact place of formation or elimination of substances in the tissue. Therefore, it is necessary to have a basic knowledge of the system, qualitative information about special effects, or a model of the events. Under these circumstances, it may be possible to gain insight into the dynamics of a reaction by means of a simple experimental parameter, the heat of the process.

For both methods, calorimetry and perfusion, one may state that they allow verification of known or assumed reaction pathways.

GENERAL CONSIDERATIONS FOR THE EXPERIMENTS

For the simultaneous performance of calorimetry and perfusion, different and sometimes controversial points of view have to be fitted together[4, 5]:

(1) To perfuse a rat liver without the arterial blood supply, one needs appr. 1 to 2 ml perfusing solution per g liver and per minute. For a standard liver of 10–14 g: $U_{max} = 20$ ml min^{-1}.

(2) As the metabolic heat of erythrocytes and leucocytes in the blood superpose the liver signal—blood is taken as a special organ in the body—the blood particles must be strongly reduced or eliminated from the perfusion liquid. Therefore, the appropriate supply with oxygen must be secured by other techniques:

(a) the reaction temperature is decreased in such a way, that the rates of oxygen consumption correspond to the oxygen concentration in the perfusing liquid (Van 't Hoff hibernation),

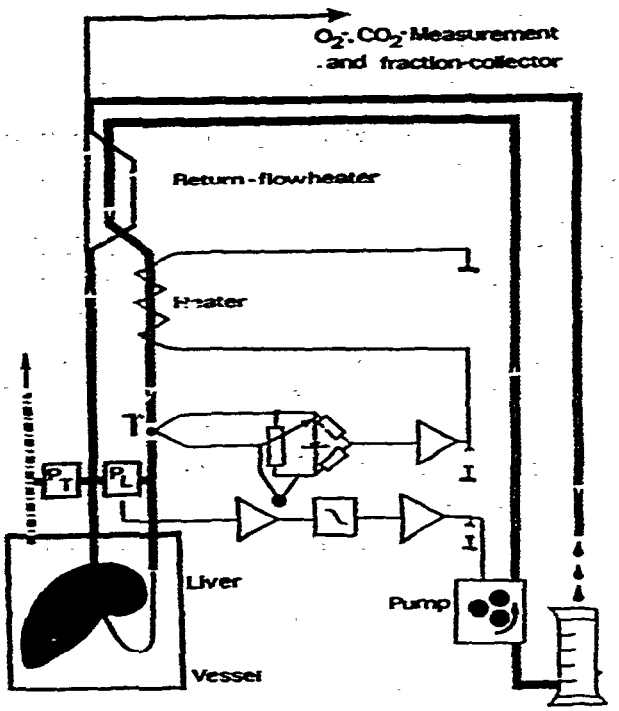


Fig. 2. Control loop for pressure and temperature in the perfusion flow system. The pressure transducer P_L monitors the difference between inflow and outflow; P_T between outflow and reaction vessel. An electronic filter suppresses unwanted oscillation of the pump control. The temperature of the inflowing medium is measured a few centimeters above the entrance to the reaction vessel and compared with that of the heat sink of the Calvet microcalorimeter. The probe T is placed in a bulge of the glass tube leading to the liver. Thus, it is situated in the flow without disturbing it. The heater is a glass helix wrapped with a resistance wire.

(b) an oxygen carrier replaces the storage capacities of the erythrocytes, but is thermally inert in the system (fluorocarbones),

(c) the partial pressure of the oxygen is increased so that the erythrocytes do not take part in the gas transport under physiological conditions. This holds true for pressures higher than 2.5 bar (35 psi).

(3) To obtain a uniform perfusion of the tissue, the deviation from the normal intracorporal conditions must be small. The pressure difference between inflow and outflow may not exceed 30 cm of water column.

(4) To keep the external concentrations of excreted metabolites at a finite level, the perfusion liquid has to circulate in the system with a volume of liquid smaller than 20 ml.

At present no flow calorimeter is commercially available which meets the desired conditions. For a maximum flow of 30 ml h^{-1} in a LKB-flow-calorimeter the volume for the passive heat exchanger amounts to 1 ml in a spiral with 120 cm total length. In the perfusion experiments the flow is nearly forty-fold. For this reason an adaption of the LKB-flow-calorimeter seemed to be unpromising, especially since the liver can not be placed in the calorimetric vessel.

METHODS AND MATERIALS

In the chosen instrumental set-up, the complete liver is situated in a specially developed 100 ml vessel of a Calvet microcalorimeter (Setaram/Lyon)[6]. The organ is perfused via glass tubes from the outside. This perfusion system is divided into several parts:

(a) A waterbath thermostats the perfusate exactly to a few hundredths K. The absolute temperature of the bath is lower than that of the calorimetric block by a few hundredths K.

(b) The perfusate flows in a kind of return flow heater to the reaction vessel.

(c) It passes an active preheater, a glass spiral which is wound with a resistance wire. Here, the remaining 1/1000 K temperature difference between perfusate and calorimetric block is removed. At the beginning of the spiral, a NTC resistor is placed in the liquid flow and a similar resistor at the block. They both form a branch of a Wheatstone bridge. The difference in voltage of the bridge controls the heating current in the resistance wire.

(d) A second glass spiral, embedded in a copper cylinder, damps the control oscillations like a damping capacitor.

(e) After this glass spiral comes the section in the calorimetric vessel. The liver is supplied with liquid by the portal vein. The perfusate flows through the tissue and accumulates in the liver veins. The medium flowing out of the veins is heated by metabolic processes in the liver.

(f) To obtain an optimal exchange of heat between the perfusate and the heat flow meter, the liquid flows in spirals along the wall of the vessel.

(g) The medium with a temperature approximately equal to that of the block ascends back through the return flow cooler (see b).

(h) After being freed from waste substances and again saturated with oxygen, it circulates back to the liver.

This set-up produces a considerable number of systematic disturbance variables which can falsify the calorimetric signal to a significant extent. Even the consequence of the flow of the perfusate in the glass capillaries, the liver and in the spirals of the calorimetric vessel must be taken into account (Fig. 4).

(1) In the supply capillary system, a decrease in pressure occurs because of the dissipation φ along the line S. This makes it impossible to calculate the pressure differences in the system from simple hydrostatic laws. For instance, if the vertical difference between reservoir and liver is 30 cm, then depending on the rate of the flow, the pressure difference may drop to a few centimeters, as the outflow line from the liver builds up a pressure which acts against the perfusion pressure.

(2) One part of the outflowing line from the liver is placed in the calorimetric vessel itself. As the loss in perfusion pressure is due to the internal friction of the liquid, a heat of friction appears. The frictional power P_f is proportional to the volume flow u and the pressure loss p_{y1y2} along the line S_{x1x2}

$$P_f = p_{y1y2} \cdot u \tag{1}$$

For the flow of a Newtonian liquid in a pipe of constant cross-section, one gets

$$p_{y1y2} = \eta \cdot S_{x1x2} \cdot \Delta v \tag{2}$$

with v velocity of the particles, S_{x1x2} the length of the pipe and η the dynamic viscosity of the liquid.

If the flow stays laminar (Reynolds number smaller than 2.300), one may calculate the friction power in the calorimetric heat exchanger by means of the Poiseuille differential equation (2). The pipe has a total length S_{23} of 130 cm and a half circle cross-section with a radius of appr. 0.1 cm. The solution of the differential equation under these conditions yields

$$P_{23} = \eta \cdot S_{23} \frac{8\pi}{\pi^2 - 8} \cdot \frac{u}{a^4} \tag{3}$$

For a maximum flow of 20 ml min^{-1} the frictional power in the pipe P_f^p amounts to

$$P_f^p \approx 2 \text{ mW}$$

(3) In the liver itself, heat is produced by friction. With the known values of $p_{12} = 30$ cm water column and $u = 20$ ml min^{-1}, one finds a power P_f^l in the liver

$$P_f^l \approx 1 \text{ mW}$$

At maximum metabolic rates, the liver heats up the passing blood by appr. 5°C. This equals a power of metabolism P_m for $u = 20$ ml min^{-1}

$$P_m \approx 7 \text{ W}$$

In preliminary experiments at 25°C (Fig. 3), we obtained values of

$$P_m \approx 0{,}3 \text{ W}.$$

For a standard liver of 20 g, this figure corresponds to 13 cal $h^{-1}g^{-1}$. Following the law of Van 't Hoff, one may calculate a metabolic power

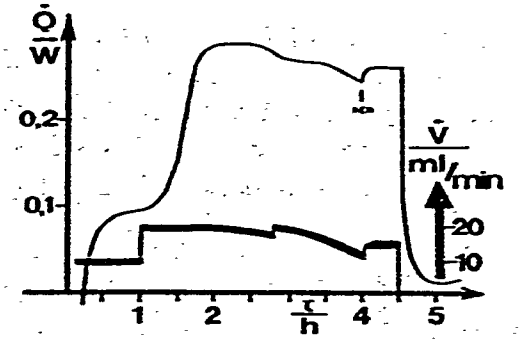


Fig. 3. Thermogram and flow of a perfused liver. The upper curve is a typical thermogram of a perfused liver (at 25°C). The heat flow $\dot{Q}$ corresponds to the lower curve, which describes the flow $\dot{V}$ of the perfusate. The arrow indicates the addition of KCN into the perfusate.

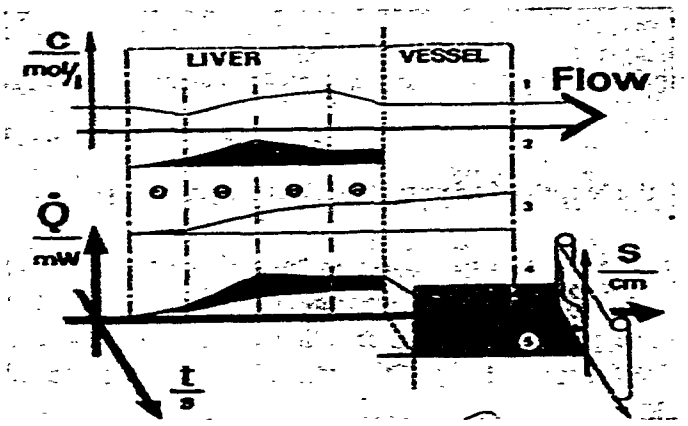


Fig. 4. Hypothesized course of the concentration of a synthesized or metabolized substance C and corresponding heat production $\dot{Q}$ along the liver length S, 1 = Concentration C in the perfusate; 2 = heat production $\dot{Q}$ of the metabolism of substance 1; 3 = heat production due to the friction on the flowing perfusate; 4 = sum of 2 and 3; 5 = recorded signal of 4, transformed by detection equipment. a = Line of self-sufficiency: C ↓ and $\dot{Q}$ ↑; b = line of exothermic synthesis: C ↑ and $\dot{Q}$ ↑; c = line of endothermic synthesis: C ↑ and $\dot{Q}$ ↓; d = line of storage or excretion: C ↓ and $\dot{Q}$ ↓↑. The length S includes the section of the liver as well as the section of the calorimetric vessel.

$P_m \approx 0{,}7$ W

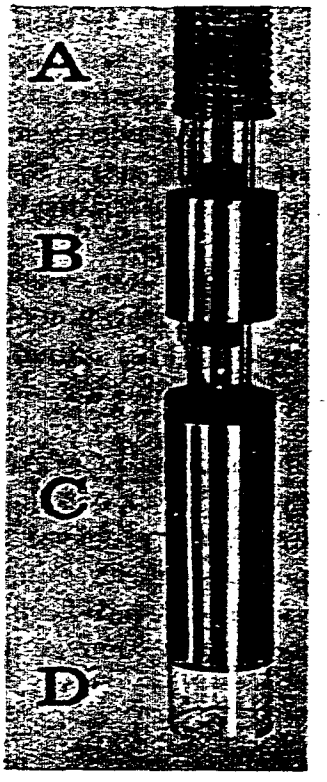
at 40 °C. A ratio of 10 between a mere maintenance metabolism and maximum activity seems to be plausible. Particularly, as in these preliminary experiments, an optimal oxygen supply of the liver was not guaranteed, and the Van 't Hoff law describes only as a first approximation the increase in metabolic heat production in the tissue as a function of maintenance temperature.

For the total heat production of a liver, the terms of frictional heat can be neglected. However, when one is looking for changes in the heat of reaction due to drugs and toxins, the elimination of the systematic error increases the informational value of the thermograms. In these experiments, the changes are in the range of a few per cent of the total heat output. And if the liver changes its parenchymal structure due to a steady abuse of alcohol, the deviations in the flow conditions and the dependent perfusion pressure cannot be neglected

The pressure pick-ups in the calorimetric vessel between inflow and outflow of the liver permit a continuous registration of the circulation value p_{12}. A proportional action control of the peristaltic pump as a function of p_{12} eliminates the problem of the drop in pressure in the supplying line of the perfusion circuit. The dialysis systems for gas and waste exchange do not influence the perfusion pressure between liver entry and exit in the calorimetric vessel (Fig. 4).

CONCLUSIONS

If the sample survives in the calorimeter for a longer time under proper conditions, the influence of drugs and toxins on the liver metabolism can be found. As yet,



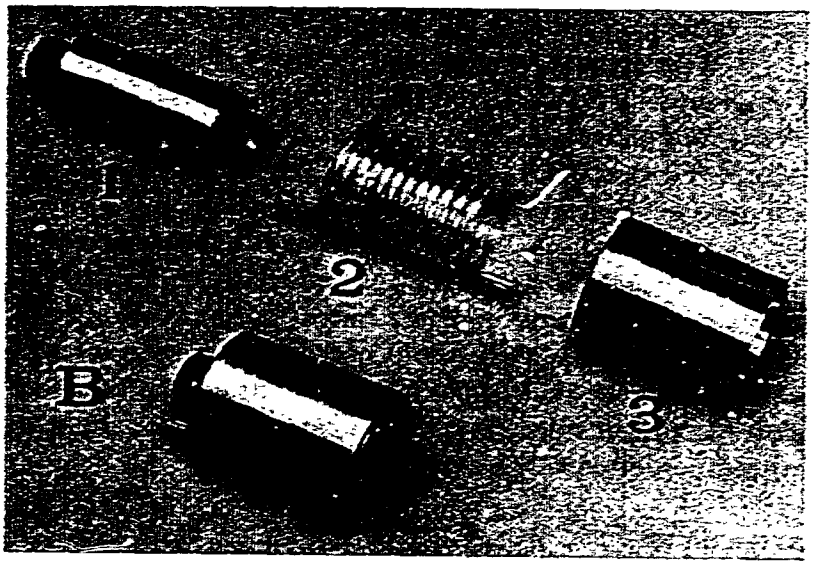


Fig. 5. Special developed measure system. A = Active preheater; B = passive compensating heater; C = special vessel of the microcalorimeter; D = vessel for the liver.

Fig. 6. Passive compensating heater. 1 = Copper cylinder inside; 2 = glass spiral (the joints filled with siliconrubber); 3 = copper cylinder outside.

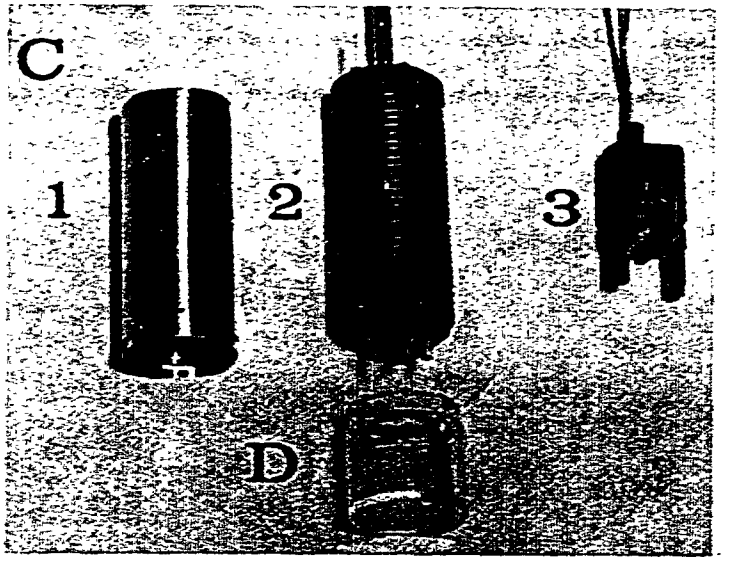


Fig. 7. Special experimental vessel of the microcalorimeter. 1 = Steelpod; 2 = inside parts of synthetic with a milled double helix and glassperformance; 3 = double pressure transformer.

we have only observed maintenance metabolism for a few hours in vitro. In these experiments, glucose was catabolized and its concentration in the perfusate decreased. In vivo the liver may synthesize this substance if necessary, and thus, control the glucose level in the blood. The gluconeogenesis in the liver has an exothermic effect. At the same time, endothermic synthesis processes may take place at other sites of the liver. Moreover, the storage of substances (e.g., glycogen) proceeds in a quantitatively detectable manner with a small reaction heat.

With inflammatorial liver diseases, the change in the enzyme pattern is accompanied by an alteration of the reaction heat. In the same way, the application of drugs stimulates special performances of the liver and changes the heat output. Moreover, deficiency diseases and genetic diseases alter the structure and lead to changed efficiencies for the same synthetic process.

As the bile is drained of and collected separately, the metabolism of active substances and the necessary amount of energy can be investigated. Thus, a conventional microcalorimeter of the batch type may be adapted to perfusion problems[7]. It proves to be a suitable tool for the investigation of complete organs under varying conditions and for metabolic tests of drugs and medical therapies. It would seem possible to transform the instrumental set-up for other purposes and to combine it with different perfusable organs (Figs. 5–7).

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