OBSERVATIONS ON A FACTOR DETERMINING THE METABOLIC RATE OF THE LIVER

by

EINAR LUNDSGAARD

Institute of Medical Physiology, University of Copenhagen (Denmark)

In a paper published some years ago[1] brief mention was made of experiments on isolated, artificially perfused livers in which the rate of oxygen uptake in the liver was consistently found to decrease during the first 30–45 minutes after the liver had been isolated. This phenomenon has intrigued me ever since, and although the cause of this drop in metabolic rate in a liver isolated from the "periphery" is not ascertained a short appraisal of the experience gained so far may be presented.

Most of the experiments have been carried out on cat livers. The metabolism of the isolated cat liver is peculiar in that carbohydrates are not metabolized[2]. The respiratory quotient of the isolated cat liver is always very low — generally below 0.7. The blood sugar concentration never decreases. Irrespectively of the blood sugar level a steady increase in blood sugar concentration is observed. This increase must be due to a gluconeogenesis as it is observed also in livers in which the glycogen store has been exhausted by starvation. It appears most likely that the lack of carbohydrate metabolism in the isolated cat liver is not an artefact but a characteristic feature in the liver metabolism of this species. Nevertheless one might claim that a liver which does not metabolize carbohydrate must be in an abnormal state and that the drop in metabolic rate might have some connection with this abnormal state. Contrary to the cat liver the isolated rabbit liver, however, stores glucose as glykogen and oxidizes carbohydrate and although my experience with the rate of oxygen consumption in the isolated rabbit liver is far more limited than my experience with cat livers it can safely be stated that in the isolated rabbit liver also a drop in metabolic rate is encountered immediately after isolation.

It might well be questioned whether any importance can be attached to a drop in metabolic rate in an organ kept alive by artificial perfusion. Such a view appears justified, however, since such a decline in oxygen uptake is observed in experiments on livers only and not in experiments on other organs. In the—unfortunately unsuccessful—endeavour to make preparations of isolated cat intestines function normally with respect to absorption a considerable number of experiments have been carried out in which the oxygen uptake of the isolated cat intestine was determined. The oxygen consumption of such a preparation always remains constant. In perfusing experiments on hind limb preparations the oxygen uptake always increases markedly. This increase generally continues for the entire experimental period of two hours which is the time most often used in my experiments. The marked difference between the changes in oxygen uptake in a typical experiment on a liver preparation as compared with a hind limb preparation

References p. 329.

is shown in Fig. 1. The oxygen uptake has been followed by frequent photoelectric determinations of the oxygen content in the venous blood. The galvanometer readings in each experiment have been standardized by at least 4 determinations of the venous oxygen content by the VAN SLYKE technique. Care has been taken to obtain, as great differences between the oxygen content in the samples used for the standardization as possible. The oxygen content in the arterial blood was determined with the VAN SLYKE technique at the beginning and at the end of the experimental period and in some experiments also in the middle of this period. Though the initial pronounced decrease in oxygen consumption is only observed in experiments with isolated livers it can not of course be ruled out that this decrease might be due to an impairment of the circulation in the liver or some other damage developing during the first period after the isolation of the organ. The question whether it is possible to restore the oxygen uptake after it has attained its low and rather constant level must be of decisive importance for the evaluation of the phenomenon.

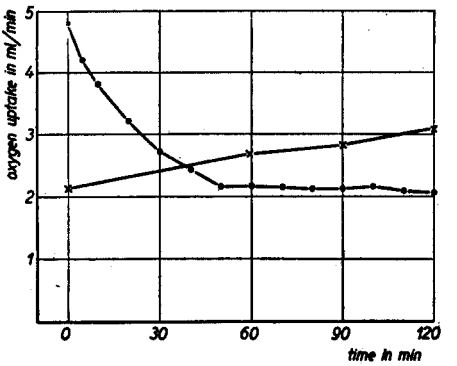

Fig. 1. Spontaneous changes in oxygen consumption during artificial perfusion of a cat liver (•——•) and a hind limb preparation (×——×).

On the assumption that the decrease in oxygen consumption is due to a disappearance of some substance present in fresh blood but gradually used up by the liver the simplest way to try to restore the oxygen uptake would be to renew the blood after the drop in oxygen uptake has developed. The result of such a simple experiment is shown in Fig. 2. As is seen the addition of fresh blood to the perfusion apparatus causes a marked but transitory increase in the oxygen uptake. A quantitative comparison between the increase obtained by adding fresh blood and the initial drop in oxygen uptake is difficult since it is not possible to renew the blood in the apparatus completely. It is only possible to remove some of the blood and add some fresh blood. In this way not more than about 50% renewal of the blood is obtained. As some change in the cell volume of the perfusion blood resulting from the addition of fresh blood cannot be avoided, and as this alters the standardization of the galvanometer readings care has been taken to draw simultaneously, a sample of arterial and venous blood for VAN SLYKE determinations as near as possible to the "peak" as judged from the galvanometer readings. In this way the magnitude of the increase in the neighbourhood of the maximum is ascertained by the VAN SLYKE technique.

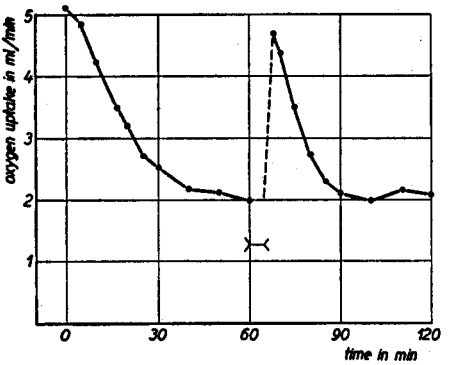

Fig. 2. Oxygen uptake of isolated cat liver, between > and < perfusion blood partly exchanged with fresh blood.

In some experiments blood used for the perfusion of a liver for one to one and a half hours has been used for perfusion of an other freshly prepared liver. In these experiments the oxygen uptake of the second liver was low from the start of the perfusion and remained low.

References p. 329.

From these simple observations it seems safe to conclude that the observed drop in oxygen uptake in an isolated liver is due to changes in the blood and not to changes in the liver tissue as such.

Though as mentioned the most probable assumption is that the decline in oxygen uptake is due to the disappearance of some substance from the blood the possibility remains that it is due to accumulation of some inhibitory substance. Also in that case addition of fresh blood might be expected to cause an increase by dilution of the inhibitory agent. Though the course of the fall in oxygen uptake appears incompatible with such an assumption an attempt has been made to elucidate this possibility experimentally.

Some livers were perfused with washed red, blood corpuscles suspended in an artificial plasma. Dextran, a polysaccharide preparation, was added to the artificial plasma to secure a normal colloid osmotic pressure. Though the result of these experiments was not quite clearcut due to technical difficulties which need not be mentioned here it can safely be stated that only a very slight initial fall in oxygen uptake was observed in these experiments.

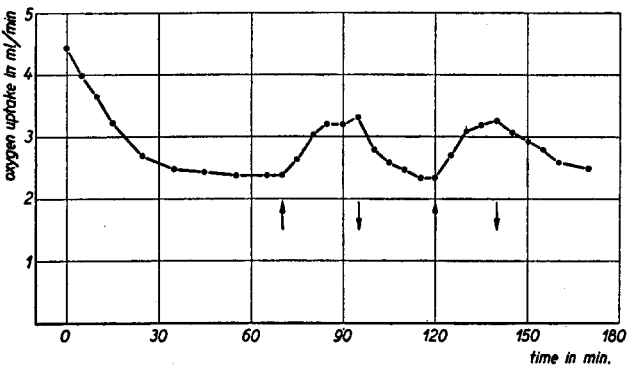

Fig. 3. Oxygen consumption of isolated cat liver. Hind limb preparation shunted in at ↑ and out at ↓.

The observations so far mentioned support the assumption that the liver normally is supplied by the blood with a substance which affects its metabolic rate.

That this hypothetic substance probably is not a specific hormone formed in one of the endocrine glands is indicated by experiments carried out in the following way.

A perfusion apparatus with a double pump and two circuits but with a common oxygenator and blood reservoir was used. A liver was isolated and attached to one of the circuits, the other being short circuited. The oxygen uptake of the liver was followed in the usual way and when the oxygen uptake had dropped a hind limb preparation was attached to the previously short circuited circuit. The venous blood returning from the liver and the hind limb preparation in this way is mixed in the oxygenator and the blood reservoir and the liver is supplied with a mixture of blood returning from the liver and the hind limb preparation. As seen from Fig. 3 the oxygen uptake of the liver starts to increase as soon as the hind limb preparation is shunted in. In about 15 minutes it reaches a fairly constant level which is maintained until the hind limb preparation is shunted out. The shunting out of the hind limb preparation is followed by a gradual decline in the oxygen uptake following a course similar to that of the initial fall. The increase is marked though the initial high oxygen uptake is not restored. In the experiment presented in Fig. 3 the hind limb preparation after having been left without circulation for 35 minutes again was shunted in for 20 minutes. The response was practically identical with the first response. The correspondence between the two response must be emphasized inasmuch as it speaks strongly against the possibility that lactic acid may be responsible for the increase in oxygen uptake. This point will be discussed later; it may be only mentioned that the lactic acid concentration in

References p. 329.

the blood at the start of the experiment (oxygen uptake 4.08 ml/min) was 20 mg%, at the maximum of the first response (oxygen uptake 3.30 ml/min) 7 mg% and at the maximum of the second response (oxygen uptake 3.25 ml/min) 26 mg%.

These observations on the rate of oxygen consumption in the isolated liver would probably not have been published if the effect of the periphery on the metabolic rate of the liver had not been revealed in a much more striking manner in some other experiments performed for quite a different purpose.

A cat was hepatectomized by connecting the protal vein with the right renal vein through a cannula of suitable shape and ligating the hepatic vessels. Heparin had been injected to prevent clotting. The blood sugar concentration of the animal was kept as constant as possible by continuous intravenons injection of glucose. In some experiments in which the hepatectomy was not successful the cat was eviscerated. No difference has been observed in the results obtained in experiments on hepatectomized and eviscerated animals. As soon as the operation was finished a cat liver was isolated and run with artificial perfusion for 35 to 50 minutes. After this period of time, the oxygen uptake of the liver has fallen to a constant low level. The glucose concentration in the perfusion blood was followed. From these determinations the glucose output of the isolated liver can be computed with fair accuracy as the blood volume is known. 35 to 50 minutes after the start of the artificial perfusion the oxygen uptake of the liver was determined by means of the VAN SLYKE technique.

The isolated liver was then connected with the hepatectomized cat in the following way. The venous outflow from the liver was connected with the jugular vein of the hepatectomized cat which henceforward shall be denoted the "donor". From the carotic artery of the donor, blood was allowed to run into a 100 ml cylinder containing about 50 ml of blood. Simultaneously the pump was shifted from the blood reservoir connected with the oxygenator to the 100 ml cylinder cutting out the oxygenator and reservoir from the circuit. The blood which flowed from the donor into the cylinder was then taken up by the pump and sent through the liver at a constant rate determined by the pump. From the liver the blood returned to the donor. By means of a clamp on the outflow from the carotic artery of the donor it was fairly easy to manage to keep the blood volume in the cylinder constant, *i.e.*, to secure that the amount of blood leaving equalled the amount of blood entering the donor.

When the liver was connected with the donor the glucose infusion was stopped. At suitable intervals samples were drawn simultaneously from the blood entering and leaving the liver. Oxygen, carbon dioxide, glucose, and lactic acid determinations have been performed on these samples. Oxygen and carbon dioxide were determined with the VAN SLYKE technique, glucose according to HAGEDORN-JENSEN, and lactic acid according to BARKER AND SUMMERSON modified by LEPAGE.

The results related to our problem are presented in Table I. It is seen that within 10 minutes after the connection of the liver with the donor the rate of oxygen consumption in the liver has increased 100% or even more. One hour after the connection the oxygen uptake of the liver in most experiments shows a slight further increase. In other words the connection with a donor of a liver run with artificial perfusion until the oxygen uptake has dropped to a low level increases the rate of oxygen uptake to a rate similar to that observed immediately after isolation of the liver, *i.e.*, presumably to the normal rate. It may be mentioned that this very considerable change in rate of oxidations is not accompanied by any change in the respiratory quotient.

References p. 329.

TABLE I

OXYGEN CONSUMPTION OF CAT LIVERS BEFORE, 10–15 MINUTES AND 60 MINUTES AFTER CONNECTION WITH A "DONOR" ml/min

Before	10–15 min after	60 min after
1.8	3.8	3.9
2.6	5.0	5.2
2.4	5.1	4.8
2.2	4.0	4.7
1.7	4.6	5.0
2.2	5.4	4.7
2.0	4.4	4.9
Average 2.14	4.61	4.74

As the high rate of oxygen consumption in the liver after connection with the donor is maintained or even increases slightly during the entire experimental period though the liver is still artificially perfused the possibility that the decline in oxygen uptake might be a direct consequence of the artificial perfusion is ruled out. The conditions before and after connection with the donor differ in only one respect. Before the connection when the blood is oxygenated in the oxygenator the oxygen tension is higher in the blood entering the liver than after the connection when the blood is oxygenated in the lungs of the donor. Though it is most improbable that the oxygen tension of the blood entering the liver is of any significance a few experiments have been carried out in which the perfusion blood was oxygenated with alveolar air collected in a DOUGLAS bag instead of the ordinary mixture of oxygen and 4% carbon dioxide. The oxygen uptake of the liver in these experiments showed exactly the same variations as in experiments carried out with the usual technique.

The glucose output from an isolated cat liver averages about 2 mg per minute. The glucose output from a liver after connection with a donor averages about 9 mg per minute. The extra amount of glucose given off by a liver after connection with a donor undoubtedly originates from lactic acid.

In ordinary perfusion experiments on cat livers the lactic acid concentration rapidly falls to very low levels (3 to 5 mg%). In experiments in which the artificially perfused liver is connected with a donor the lactic acid concentration in the blood with which the liver is supplied is as high as 30 to 50 mg%. A definite drop in lactic acid concentration from ingoing to outgoing blood corresponding roughly to the increase in glucose concentration is demonstrable.

As the lactic acid concentration declines during the first period of a liver perfusion experiment during which the oxygen uptake falls off also and as the lactic acid concentration is markedly increased after connection with a donor when the oxygen consumption increases strongly one might think that the concentration of lactic acid in the blood is responsible for the changes in the oxygen uptake of the liver. Observations have previously been mentioned however which do not agree with such an assumption. Furthermore a number of experiments have been carried out in which L (+) lactic acid was added to the perfusion blood. If at the start of the perfusion L (+) lactic acid is added to the blood in amounts increasing the concentration to well above 100 mg% the decline in oxygen uptake proceeds as usual and if lactic acid in varying amounts is

References p. 329.

added to the blood after the oxygen uptake has reached its constant low level only a very slight increase in the oxygen uptake or no increase at all is observed. Consequently the possibility that the lactic acid concentration in the blood is responsible for the changes in oxygen consumption observed in these experiments can be definitely ruled out.

If lactic acid is added to the blood after the oxygen uptake of an isolated liver has been allowed to drop off the rate of disappearance of lactic acid amounts to only one fourth to one third of the rate observed in a liver connected with a donor. Thus not only the rate of oxygen consumption but also the rate of a reaction such as conversion of lactic acid to glucose or glycogen is influenced by the hypothetical substance present in fresh blood. The statement appears justified that this substance influences the "metabolic rate" of the liver.

The nature of the substance influencing the metabolic rate of the liver has not been elucidated; accordingly, this paper can be considered only as a preliminary note. A series of substances however can be ruled out since they have no effect on the rate of oxygen uptake in the liver when added to the blood about one hour after the start of the perfusion. Some of these substances have been added to the blood in a single dose, others have been added continuously at a rate giving concentrations in the blood comparable with the normal concentrations. Without going into details a few of the substances tested so far are listed (Table II).

TABLE II

"Kochsaft" of muscle	Choline
Fresh muscle extract	Methionine
ATP	Tyrosine
Creatine	Tryptophan
Cytochrom C	Arginine
Glutathione	Threonine
Citric acid	Ascorbic acid
Oxalo-acetic acid	Adrenaline
Fumaric acid	nor-Adrenaline
Succinic acid	Desoxycorticosterone glycoside (Ciba)
Pyruvic acid	"Corsunal"*
Lactic acid	Insulin
Acetic acid	Fresh crude extract of anterior pituitary

* Extract of ox-adrenals prepared by *Nordisk Insulin Laboratory* according to GROLLMAN AND FIROR

Among the substances listed in Table II only adrenaline and nor-adrenaline had a definite but quite transitory effect of increasing oxygen uptake. This effect, however, could not be maintained by continuous addition of the substances.

It must be mentioned that pyruvic acid and the aminoacids glycine and alanine in large doses (300 mg) have a marked effect on the oxygen uptake in the isolated liver[1]. As continuous addition of pyruvic acid at a rate of 2 mg per minute (blood flow 50 to 60 ml/min) has no effect on the oxygen uptake and as the amino acid content in blood perfused through a liver does not decrease as does the oxygen uptake during the first period of the experiment it appears that pyruvic acid and amino acids can safely be ruled out as factors responsible for the changes in oxygen uptake in the liver observed in these experiments.

The problem to which attention is directed in the present paper undoubtedly is

References p. 329.

related to the observation made by many investigators[3, 4, 5, 6, 7] that the respiration of tissue slices is higher and more stable in serum than in Ringer solution. Though this observation is not absolutely identical with those of the writer, it appears most probable that the substance (or substances) in serum which enchances tissue respiration is the same as the substance (or substances) which is gradually removed from the blood by an isolated liver causing a decline in the rate of oxidations. The question of the nature of the serum constituents which enchance tissue respiration has been delt with in a rather explicit manner by WARREN in two publications. In the first of these[8] it has been demonstrated that the stimulating effect of serum on tissue respiration partly can be attributed to its bicarbonate content. According to WARREN the maximal effect of adding bicarbonate to a Ringer-phosphate medium is obtained at a concentration of only 3 mM per liter. Variations in the bicarbonate concentration at higher levels are without any influence on the rate of oxidations. Since whole blood under constant and fairly high carbon dioxide pressure has been used in the experiments described one can certainly rule out changes in bicarbonate content as being responsible for the observed changes in oxygen uptake in the isolated liver.

In accordance with CANZANELLI *et al.*[3], WARREN finds substances capable of enchancing the respiration of tissue slices in the ultrafiltrate of serum. Only about 50% of the effect can be attributed to bicarbonate. In his second paper WARREN[9] reports attempts to fractionate serum with respect to its action in enchancing tissue respiration. From his elaborate experiments WARREN concludes that lactic acid and amino acids are not involved in the stimulating effect of serum on tissue respiration. I draw the same conclusion from my observations. WARREN further suggests that the active substance is a dicarboxylic acid, but he has not put this assumption on a direct trial by adding dicarboxylic acids to the Ringer-phosphate medium used in his experiments. In my experiments I have tested different organic acids assumed to be formed as intermediates in tissue metabolism. However, no effect on the low oxygen uptake of the isolated liver was observed.

SUMMARY

Observations are presented indicating that the normal metabolic rate of the liver is dependent on a substance (or substances) formed in the extrahepatic tissues and carried to the liver through the blood. This still unidentified substance is used or destroyed in the liver tissue.

RÉSUMÉ

L'auteur présente des observations indiquant que la vitesse normale du métabolisme du foie dépend d'une substance (ou de substances) formée dans les tissus extrahépatiques et qui est amenée au foie par le sang. Cette substance non encore identifiée est utilisée ou détruite dans le tissu hépatique.

ZUSAMMENFASSUNG

Beobachtungen werden beschrieben die darauf hinweisen, dass die Normalgeschwindigkeit des Lebermetabolismus von einer Substanz (oder von Substanzen) abhängt, die in ausserhalb der Leber gelegenen Geweben gebildet und durch das Blut der Leber zugeführt wird. Diese noch nicht identifizierte Substanz wird im Lebergewebe verbraucht oder zerstört.

References p. 329.

REFERENCES

1 E. LUNDSGAARD, *Acta Physiol. Scand.*, 4 (1942) 330.
2 E. LUNDSGAARD, NIELS A. NIELSEN, AND S. L. ØRSKOV, *Skand. Arch. Physiol.*, 76 (1936) 296.
3 A. CANZANELLI, G. ROGERS, C. DWYER, AND D. RAPPORT, *Am. J. Physiol.*, 135 (1942) 316.
4 D. FRIEND AND A. B. HASTINGS, *Proc. Soc. Exptl Biol. Med.*, 45 (1940) 137.
5 H. LASER, *Nature*, 136 (1936) 184.
6 M. SCHAFFER, T. CHANG, AND R. GERARD, *Am. J. Physiol.*, 111 (1935) 697.
7 B. WALTHARD, *Z. ges. exptl Med.*, 94 (1934) 45.
8 C. O. WARREN, *J. Biol. Chem.*, 156 (1944) 559.
9 C. O. WARREN, *J. Biol. Chem.*, 167 (1947) 543.

Received March 25th, 1949