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Effect of Haematocrit Value and pO_2 on the Redox State and Metabolism of the Perfused Liver

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1. The haematocrit value and pO_2 of blood perfusing the isolated liver were varied. Provided O_2 content of the blood was not rate-limiting, O_2 consumption was related to haematocrit value rather than O_2 saturation or pO_2. 2. Hypoxia caused the blood-glucose concentration and ketogenesis to increase and the output of very-low-density (d <1.006) lipoproteins to decrease. 3. A decrease in pO_2 caused an increase in both the [lactate]/[pyruvate] and [3-hydroxybutyrate]/[acetoacetate] and a decrease in [ATP]/[ADP] ratios, independently of O_2 consumption. 4. The more reduced redox state was associated with a shift in the balance between the oxidation and esterification of free fatty acids in favour of oxidation. 5. Acetoacetate may be an important hydrogen acceptor during hypoxia of the liver.

The metabolism of the liver under conditions of mild hypoxia (as opposed to anoxia) has not been studied in detail. The liver, which is supplied with blood from two sources, is exposed to a splanchnic supply which may vary widely in pO_2 and rates of flow. Brauer *et al.* (1963) showed that the perfused liver would not control K^+ leakage or regulate the blood glucose at physiological concentrations unless it was perfused with fully saturated blood. These results have been confirmed by others (e.g. Burton & Ishida, 1965). Unlike the majority of other investigators (see Ross, 1972), who perfused with diluted blood at unphysiologically high pO_2 and rates of flow, our standard conditions allow higher rates of O_2 consumption by using whole blood perfused at a lower, but physiological, pO_2 and rate of flow. Our results demonstrate that changes in the rate of O_2 consumption or in pO_2 not only affect glucose regulation, but also have marked effects on lipid metabolism.

Materials and Methods

Livers from fed male Long Evans rats (340–360g) were perfused as described by Mayes & Felts (1966). A membrane-type gas-exchanger was used (Felts & Whayne, 1973). The perfusate of defibrinated whole rat blood was dialysed as described by Mayes & Felts (1966). After dialysis the haematocrit value was approx. 37%. When required, a higher value was obtained by removal of an appropriate volume of serum. The pO_2 of the blood entering the liver was regulated by varying the composition of the gases passing through the gas-exchanger. The pO_2 was minitored continuously by using a flow-through cell containing a pO_2 electrode (Radiometer, Copenhagen, Denmark). The O_2 content of samples of the blood entering and leaving the liver was also measured polarographically by a method similar to that described by Solymar *et al.* (1971) and used to calculate the O_2 consumption of the liver. A constant flow of the perfusate was maintained with the aid of a peristaltic pump at 12ml/min throughout the experimental period of 90min. Free fatty acids in the form of [1-^{14}C]oleate complexed to bovine serum albumin were infused for 90 min to maintain constant perfusate specific radioactivity. ATP and ADP were determined in freeze-clamped livers by the methods of Lamprecht & Trautschold (1974) and Jaworek *et al.* (1974) respectively. Details of other methods have been published (Topping & Mayes, 1972).

Four groups of perfusions were carried out in which the haematocrit value and pO_2 of the perfusate were varied.

Results and Discussion

The infusion of [1-^{14}C]oleate resulted in a constant concentration of free fatty acids in the perfusate throughout the experimental period. There was no

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Table 1. *Effect of haematocrit value and blood pO_2 on O_2 consumption and metabolism of perfused liver*

Livers from fed rats were infused with free fatty acid (oleate) to maintain a mean concentration of 0.82 μmol/ml of serum. The haematocrit value and pO_2 of the blood were varied as described in the text. Each value is the mean ± S.E.M. of three perfusions. The value of *P* refers to the significance of the difference between means which was <0.05 when tested by the analysis of variance. With the exception of the glycogen concentration, which was a terminal value, the results were calculated from the mean of determinations made at 30, 60 and 90 min from the start of infusion of the fatty acid. Over this period the quantities measured had all reached steady values.

Haematocrit value (%)	Blood pO_2 (kPa)	Blood pO_2 (mmHg)	O_2 consumption (μmol of O_2/min per g)	Glycogen (% of wet wt.)	[Blood glucose] (mg/100 ml)	[Blood lactate] (μmol/ml)	[Lactate]/[Pyruvate] (blood)	[3-Hydroxybutyrate]/[Acetoacetate] (blood)	Total ketone-body production (μmol/h per g)	Very-low-density-lipoprotein production (μmol of triglyceride fatty acid/h per g)
A. 44.5 ± 0.3	13.3	100	5.3 ± 0.4	1.9 ± 0.5	147 ± 6	3.1 ± 0.2	8.4 ± 0.7	3.5 ± 0.3	3.8 ± 0.2	7.2 ± 0.6
B. 36.2 ± 0.2	13.3	100	4.0 ± 0.0	2.0 ± 0.6	168 ± 18	3.2 ± 0.3	8.1 ± 0.6	3.1 ± 0.2	3.4 ± 0.2	6.1 ± 1.3
C. 36.8 ± 0.3	9.3–9.9	70–75	4.1 ± 0.2	1.4 ± 0.4	175 ± 12	4.2 ± 0.2	12.1 ± 2.2	4.9 ± 0.9	4.4 ± 0.6	4.4 ± 0.4
D. 38.6 ± 0.3	5.3	40	2.9 ± 0.1	1.5 ± 0.5	211 ± 17	7.9 ± 0.6	28.0 ± 2.9	14.1 ± 1.8	14.4 ± 1.7	3.5 ± 0.2
P values ...			<0.05 (A versus B) <0.05 (C versus D) <0.01 (A versus D)		<0.05 (A versus D)	<0.01 (A + B versus C) <0.001 (A + B versus D)	<0.05 (A + B versus C) <0.05 (C versus D) <0.001 (A + B versus D)	<0.05 (A + B versus C) <0.001 (A + B versus D)	<0.001 (A + B versus D)	<0.01 (A versus D)

significant difference between the four groups of perfusions, the range being 0.81–0.84 μmol/ml of serum. There was no significant difference between the groups in the mean rate of flow of blood through the liver, which was 1.04 ml/min per g. Thus the fractional uptake of free fatty acids was not affected by changes in oxygenation (Table 1), confirming the results of Soler-Argilaga *et al.* (1974).

Oxygen consumption

In livers perfused at a pO_2 of 13.3 kPa (100 mmHg) and a haematocrit value of 44.5, the mean O_2 consumption was 5.3 μmol/min per g (Table 1). When the pO_2 was kept constant, but the haematocrit value was decreased to 36.2, oxygen consumption was decreased to 4.0 μmol/min per g. In both groups of perfusions the pO_2 in the hepatic-vein outflow was approx. 5.3 kPa (40 mmHg). When the haematocrit value was held at 36.8 and the pO_2 was decreased to 9.3–9.9 kPa (70–75 mmHg), thereby decreasing O_2 saturation, the pO_2 of the outflow fell to approx. 4 kPa (30 mmHg), but the O_2 consumption was not diminished. Thus, at the upper-normal range of blood pO_2, when factors such as flow rate and temperature are held constant, O_2 consumption is related to the number of erythrocytes passing through the liver rather than to the O_2 saturation of the blood or to its pO_2. This conclusion is at variance with that of Brauer *et al.* (1963), who considered hepatic O_2 consumption to be uniquely related to O_2 saturation. However, the results support these authors in confirming that the respiration of the liver is normally O_2-limited (i.e. in state 5) and that the rate-limiting step seems to be in O_2 transfer from erythrocyte to parenchyma.

When the pO_2 of the perfusate was decreased to 5.3 kPa (40 mmHg), the pO_2 in the hepatic vein approached zero and O_2 consumption fell to 2.9 μmol/min per g.

pO_2 and redox state

The intracellular redox state as represented by the free [NADH]/[NAD^+] ratio in the cytosol or mitochondria is proportional to the intracellular [lactate]/[pyruvate] ratio and the [3-hydroxybutyrate]/[acetoacetate] ratio respectively, and these metabolites are in equilibrium with the blood or the perfusate of isolated livers (Williamson *et al.*, 1967; Krebs *et al.*, 1969). When the haematocrit value was decreased, and the pO_2 was maintained at 13.3 kPa (100 mmHg) with consequent decrease in O_2 consumption, there was no change in the blood [lactate]/[pyruvate] and the [3-hydroxybutyrate]/[acetoacetate] ratios. They were similar to the corresponding ratios found in the rat liver

in vivo (Williamson *et al.*, 1967). However, when the haematocrit value was held constant, but the pO_2 of the inflowing blood was decreased to 9.3–9.9kPa (70–75mmHg), O_2 consumption did not change but there was a significant increase in both the [lactate]/[pyruvate] and [3-hydroxybutyrate]/[acetoacetate] ratios. Thus changes in pO_2 of the blood entering the liver, which are likely to occur under physiological conditions, can change the intracellular redox state without altering the O_2 consumption. If this effect is mediated via the respiratory chain, it is implied that there is a change in pO_2 at the cytochrome oxidase site, which affects the ratio of oxidized/reduced cytochrome a_3. Owing to the high affinity of cytochrome a_3 for O_2, it is generally considered that cytochrome a_3 is completely oxidized. However, reports (e.g. Jobsis, 1972) have indicated that, under some steady-state conditions in tissues, there may be appreciable reduction of cytochrome a_3 in the presence of an adequate oxygen supply.

When the pO_2 of the inflowing blood was decreased to 5.3kPa (40mmHg), there was a more pronounced increase in the [lactate]/[pyruvate] and [3-hydroxybutyrate]/[acetoacetate] ratios, but, in addition, this was accompanied by a most significant increase in the rate of ketogenesis. Thus hepatic hypoxia appears to be a potent ketogenic factor, and acetoacetate may be an important hydrogen acceptor under these conditions, being comparable with pyruvate in skeletal muscle.

In another series of perfusions the [ATP]/[ADP] ratio in freeze-clamped perfused livers was 3.14 ± 0.14 (mean $\pm$ S.E.M.) and 2.39 ± 0.36 under conditions comparable with B and C respectively (Table 1). The ratio in fed stunned rats was 2.58 ± 0.10. It is to be expected that an increased redox state would lead to a decreased extramitochondrial [ATP]/[ADP] ratio (Krebs, 1974). However, it was confirmed that O_2 consumption did not decrease, in spite of the change in redox and phosphorylation state, indicating that it was unlikely that there was any change in the rate of ATP production.

Blood glucose

The mean blood glucose concentration at zero time for all groups of livers was 142mg/100ml. It stabilized at progressively higher concentrations as O_2 consumption decreased, confirming the results of Brauer *et al.* (1963).

Secretion of very-low-density ($d < 1.006$) lipoproteins

Both a high haematocrit value and a high pO_2 favoured the formation of very-low-density lipoproteins. It follows that anaemic patients might be

Table 2. *Effect of haematocrit value and blood pO_2 on the metabolism of ^{14}C-labelled free fatty acid by perfused livers*

The experiments are the same as those referred to in Table 1. [1-^{14}C]Oleate (5μCi) was added to the fatty acid infused and the incorporation of ^{14}C into the various metabolic products measured over 90min. Each value is the mean $\pm$ S.E.M. from three perfusions. *P* has been calculated in a manner similar to Table 1.

	Haematocrit value (%)	Blood pO_2 (kPa)	Blood pO_2 (mmHg)	[^{14}C]Oleate (%) in: Total liver lipids	Liver triglycerides	Liver phospholipids	Very-low-density-lipoprotein triglyceride	CO_2	Total ketone bodies	Total: Oxidized products	Total: Esterified products
A.	44.5 ± 0.3	13.3	100	47.5 ± 10.0	39.3 ± 9.9	6.0 ± 0.2	17.7 ± 2.4	7.4 ± 1.8	1.4 ± 0.5	8.8 ± 2.2	65.2 ± 12.4
B.	36.2 ± 0.2	13.3	100	39.0 ± 3.3	32.5 ± 2.9	6.4 ± 0.4	23.7 ± 0.5	5.8 ± 0.6	1.5 ± 0.3	7.3 ± 0.9	62.7 ± 3.4
C.	36.8 ± 0.3	9.3–9.9	70–75	30.3 ± 6.2	22.9 ± 4.9	6.5 ± 1.2	8.8 ± 1.1	12.8 ± 2.2	4.0 ± 1.3	16.8 ± 3.4	39.1 ± 7.2
D.	38.6 ± 0.3	5.3	40	36.6 ± 3.4	24.0 ± 2.3	10.6 ± 1.5	7.7 ± 1.5	8.9 ± 0.3	9.7 ± 1.1	18.6 ± 1.3	44.3 ± 3.5
	P values ...				<0.05 (A + B versus C + D)	<0.01 (A + B + C versus D)	<0.001 (A + B versus C + D)	<0.05 (B versus C)	<0.05 (A + B versus C) <0.05 (C versus D) <0.01 (A versus D)	<0.001 (A + B versus C + D)	<0.01 (A + B versus C + D)

expected to show subnormal rates of hepatic very-low-density lipoprotein secretion.

Free fatty acid metabolism

The incorporation of [1-^{14}C]oleate into the various products of free fatty acid metabolism was compared (Table 2). Overall there was a highly significant ($P<0.001$) inverse relationship between the label entering esterified products of free fatty acid metabolism and the label entering products of their oxidation, in confirmation of previous results (Mayes & Felts, 1967). When the pO_2 was held at 13.3kPa (100mmHg) and the haematocrit value was decreased from 45 to 36 there were no significant changes in the distribution of label, even though the O_2 consumption was decreased. However, when the haematocrit value was held constant, but the pO_2 of the inflowing blood was decreased from 13.3 to 9.3–9.9kPa (100 to 70–75mmHg), there was a most profound effect on the fate of the labelled oleate. Esterification was markedly decreased, but oxidation was doubled. Thus, although a decrease in pO_2 of the blood was correlated with a change to a more reduced intracellular redox state, there was an increase in aerobic oxidation of fatty acids to both CO_2 and ketone bodies. As O_2 consumption remained constant, it is implied that there must have been a compensatory decrease in the oxidation of some other major respiratory substrate, most likely carbohydrate. The decreased esterification was reflected in a highly significant decrease in incorporation of ^{14}C-labelled free fatty acid into very-low-density-lipoprotein triglycerides.

When the pO_2 was decreased to 5.3kPa (40mmHg), there was no further significant change in the total ^{14}C recoveries in oxidized and esterified products, although there was a marked fall in O_2 consumption. However, the partition of ^{14}C between CO_2 and ketone bodies changed, with a decrease in $^{14}CO_2$ production and a corresponding increase in production of ^{14}C-labelled ketone bodies. This effect was similar to that observed in previous work (Mayes & Felts, 1967), where oxygenation of the blood was held constant but the load of free fatty acid taken up by the liver was raised progressively. $^{14}CO_2$ production decreased as ^{14}C-labelled ketone-body production increased. These former results were interpreted as indicating that ATP production resulting from the oxidation of fatty acids could be regulated by control of the partition in oxidation of fatty acids between the citric acid cycle and ketogenesis. Similarly, it is suggested that in the present experiments, the decrease in ATP formation, which would result from the decreased O_2 consumption, was achieved at least in part by decreasing oxidation to CO_2 while the carbon flow through the pathway of β-oxidation was maintained by increasing the production of ketone bodies, with acetoacetate serving as a final hydrogen acceptor.

Regulation of oxidation and esterification of fatty acids by the redox state

The present experiments demonstrate that a change in intracellular redox state is associated with, and could well be a cause of, the shift in the balance between the oxidation and esterification of long-chain fatty acids. Bremer *et al.* (1974) reported that pyruvate oxidation in liver mitochondria is extremely sensitive to the inhibitory action of an increase in the [NADH]/[NAD$^+$] ratio, whereas β-oxidation of fatty acids is relatively insensitive. This would explain how the oxidation of fatty acids could be increased at the expense of carbohydrate when there was an increase in [NADH]/[NAD$^+$] due to a decrease in pO_2. As oxidation and esterification of fatty acids are reciprocally related, there would be a corresponding decrease in esterification. It is also possible that the change in redox state would inhibit carbohydrate oxidation at the glyceraldehyde 3-phosphate dehydrogenase step in glycolysis (Williamson *et al.*, 1967). A decrease in cytosolic [ATP]/[ADP] ratio or an increase in [AMP] is unlikely to be a controlling factor, since it would lead to a stimulus of glycolysis [at the phosphofructokinase step (Newsholme & Start, 1973)] rather than an inhibition as called for by the results. On the other hand, the increased [NADH]/[NAD$^+$] ratio in mitochondria would lead to an increased mitochondrial [ATP]/[ADP] ratio (Krebs, 1974) with consequent conversion of active into inactive pyruvate dehydrogenase (Wieland *et al.*, 1974). This effect would reinforce any direct effect of a change in [NADH]/[NAD$^+$] and lead to increased fatty acid oxidation and decreased esterification.

The present results taken with those of other authors supply an explanation of our previous investigations (Mayes & Felts, 1967). These showed that there is a shift in the balance between oxidation and esterification of fatty acids in favour of oxidation in livers from starved rats compared with livers from fed rats. This may well be due to the increased [NADH]/[NAD$^+$] ratio found in both the cytosol and mitochondria of starved as compared with fed livers (Williamson *et al.*, 1967). In addition, the increased [NADH]/[NAD$^+$] ratio that results from oxidation of fatty acids (Bremer *et al.*, 1974) may facilitate the switch from esterification towards oxidation, which occurs when increasing quantities of free fatty acids are infused into livers from fed animals (Mayes & Felts, 1967).

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