Effects of Lactation on Ketogenesis from Oleate or Butyrate in Rat Hepatocytes

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1. Rates of ketogenesis from endogenous butyrate or oleate were measured in isolated hepatocytes prepared from fed rats during different reproductive states [virgin, pregnant, early-lactating (2–4 days) and peak-lactating (10–17 days)]. In the peak-lactation group there was a decrease (25%) in the rate of ketogenesis from butyrate, but there were no differences in the rates between the other groups. With oleate, the rate of ketogenesis was increased in the pregnant and in the early-lactation groups compared with the virgin group, whereas the rate was 50% lower in the peak-lactation group. 2. Experiments with [1-¹⁴C]oleate indicated that these differences in rates of ketogenesis were not due to alterations in the rate of oleate utilization, but to changes in the amount of oleoyl-CoA converted into ketone bodies. 3. Although the addition of carnitine increased the rates of ketogenesis from oleate in all groups of rats, it did not abolish the differences between the groups. 4. Measurements of the accumulation of glucose and lactate showed that hepatocytes from rats at peak lactation had a higher rate of glycolytic flux than did hepatocytes from the other groups. After starvation, the rate of ketogenesis from oleate was still lower in the peak-lactation group compared with the control group. This suggests that the alteration in ketogenic capacity in the former group is not merely due to a higher glycolytic flux. 5. It is concluded that livers from rats at peak lactation have a lower capacity to produce ketone bodies from long-chain fatty acids which is due to an alteration in the partitioning of long-chain acyl-CoA esters between the pathways of triacylglycerol synthesis and β -oxidation. The physiological relevance of this finding is discussed.

Lactation in the rat is characterized by an increased demand for metabolic substrates to provide the constituents of milk (e.g. lactose, lipid and protein) and this necessitates a number of metabolic adaptations in various tissues, such as mammary gland, adipose tissue, intestine and liver. For example, reciprocal changes in the activity of lipoprotein lipase in adipose tissue and in mammary gland result in the directing of available triacylglycerols to the gland (Otway & Robinson, 1968; Hamosh *et al.*, 1970). Similarly, alterations in the hepatic activities of key enzymes suggest that glycolysis and lipogenesis are increased during peak lactation (Smith, 1973, 1975).

The concentrations of ketone bodies in the blood are lower in starved lactating rats compared with starved virgin rats. By contrast, the concentration of plasma unesterified fatty acids, the major ketogenic substrate, is slightly higher (Hawkins & Williamson, 1972). One explanation for this finding is that the ketogenic capacity of the liver is decreased during lactation. To investigate this possibility, we have measured rates of ketogenesis in isolated hepatocytes from virgin, pregnant and lactating rats.

Intrahepatic control of ketogenesis from long-

chain fatty acids (e.g. oleate) can occur at the stage of partitioning of acyl-CoA between triacylglycerol synthesis and β -oxidation (Mayes & Felts, 1967; Ontko, 1972; McGarry & Foster, 1972; Williamson, 1973) or later, at the stage of partitioning of acetyl-CoA between citrate synthesis and ketone-body formation (Wieland, 1968). Butyrate cannot be incorporated into triacylglycerol (Fritz, 1961) and is converted into butyryl-CoA in the mitochondrial matrix (Aas & Bremer, 1968). Comparison of the rates of ketogenesis from butyrate with those from oleate in liver cells from rats in different reproductive states should therefore indicate the degree of control occurring at sites before β -oxidation.

Materials and Methods

Rats

Female rats of the Wistar strain were used. Virgin rats weighed between 180 and 250g and lactating rats between 260 and 320g. The litter size was eight to twelve pups. Non-lactating rats refers to rats whose pups had been removed on the day of parturition and were then studied 14 days later. The rats

were fed *ad libitum* on Oxoid breeding diet for rats and mice (Oxoid Ltd., London S.E.1, U.K.). The starved lactating rats had their food removed for 24 h, but were kept with their litters, and suckling was maintained. Virgin and pregnant rats were starved for the same period. Rats were anaesthetized with Nembutal (50 mg/kg body wt.; solution in 0.9% NaCl).

Biochemicals

All enzymes and coenzymes were obtained from the Boehringer Corp. (London) Ltd., London W.5, U.K.

Radioactive compounds

[1-¹⁴C]Oleate was obtained from The Radiochemical Centre, Amersham, Bucks., U.K.

Liver cells

Isolated hepatocytes were prepared essentially by the method of Berry & Friend (1969) as modified by Krebs *et al.* (1974). Routine tests indicated that 90% of the hepatocytes excluded Trypan Blue.

Incubation procedure

The cells were incubated with constant shaking for 20, 40 or 60 min in Krebs–Henseleit (1932) saline containing dialysed albumin (fatty acid-poor; final concentration 2.5%, w/v). The gas phase was O₂/CO₂ (19:1), and the temperature was 37.5°C. The total incubation volume, containing 65–95 mg wet wt. of cells and added substrates (1 mM-oleate, 10 mM-butyrate), was 4.0 ml. Incubations were stopped by adding 0.4 ml of 20% (w/v) HClO₄, the mixture was centrifuged to remove denatured protein and the supernatant was neutralized with KOH. The precipitate of KClO₄ was removed by centrifugation.

All incubations with [1-¹⁴C]oleate were performed in duplicate. One of each pair was incubated in a 50 ml Erlenmeyer flask with a centre well and rubber seal. The incubation was stopped by the injection of 0.4 ml of 20% (w/v) HClO₄ with a long-needed syringe. Hyamine hydroxide (0.5 ml) was injected into the centre well and metabolic CO₂ was collected by shaking the flasks for a further 2 h at room temperature. The Hyamine was then removed for measurements of radioactivity and the deproteinized incubation medium was treated as described above.

The reaction in the duplicate flask was stopped by centrifugation for 1 min at 5000g to separate cells from the medium. Chloroform/methanol [2:1 (v/v); 20 vol.] was added to the cell pellet and the lipids were extracted as described by Folch *et al.* (1957). The radioactivity in the chloroform phase was used as a measure of the [1-¹⁴C]oleate converted into esterified fats. The disappearance of radioactivity which is extracted by chloroform from the incubation medium (minus pellet) was used to calculate the

oleate uptake: 0.5 ml of medium plus 1.5 ml of 0.1 M-potassium phosphate buffer, pH 7.0, was extracted with 6.0 ml of chloroform by shaking for 90 s. The chloroform and aqueous phases were separated by centrifugation, the upper phase was removed and the organic phase was washed with 1 ml of potassium phosphate buffer, pH 7.0. The radioactivity in a sample of the chloroform phase was measured. This method relies on the fact that esterified fats are not released in appreciable amounts to the incubation medium. To test this the chloroform extract of the medium after 60 min incubation was analysed by t.l.c. on silica-gel plates. The solvent system used was hexane/diethyl ether/acetic acid (80:15:1, by vol.). Less than 7% of the radioactivity contained in the chloroform extract was present as esterified fats, and over 90% was present as unesterified fatty acids.

The radioactivity in ketone bodies was determined in both the carbonyl and the carboxyl moieties of acetoacetate. Acetoacetate was decarboxylated with aniline citrate (Krebs & Eggleston, 1945) and the ¹⁴CO₂ was collected in Hyamine hydroxide. The carbonyl carbon was collected in hydrazine/lactate (pH 5.0) after decarboxylation with H₂SO₄ at 80°C (Mayes & Felts, 1967). This process consistently gives recoveries of 85–90% with [3-¹⁴C]acetoacetate. Assuming that acetoacetate and 3-hydroxybutyrate are in isotopic equilibrium, the radioactivity of 3-hydroxybutyrate was calculated from the specific radioactivity of acetoacetate and from the ratio of concentrations of 3-hydroxybutyrate and acetoacetate. Acetoacetate and D-3-hydroxybutyrate were determined enzymically as described by Williamson *et al.* (1962). Glycogen was measured as glucosyl units after hydrolysis with amyloglucosidase (EC 3.2.1.3; Keppler & Decker, 1974).

Measurements of radioactivity

These were carried out as described by Williamson *et al.* (1975).

Calculation of rates

The time course of ketone-body formation from endogenous butyrate or oleate was linear between 20 and 60 min in all the situations studied. In the first 20 min of incubation there was a tendency for slightly higher rates of ketogenesis to occur. The rates of ketogenesis presented in the Tables have been calculated for the linear period (20–60 min). For lactate and glucose accumulation in incubations with hepatocytes from fed rats the rates tended to decrease after 40 min; this presumably is partly due to the decreased availability of glycogen. Values for accumulation of glucose and lactate after 60 min incubation are given in the Tables; the same pattern of results in the various experimental groups is obtained if the 20 min or 40 min values are used.

Results and Discussion

Rates of ketogenesis by hepatocytes from fed rats

The rates of ketogenesis by hepatocytes from fed rats at various stages during the process of reproduction are shown in Table 1. The endogenous rate of ketogenesis was low in all groups of rats and there were no significant differences between the groups. The addition of butyrate (10mM) resulted in a large increase in the rate of ketogenesis in all the groups, but in the peak-lactating (10–17 days) group the rate was significantly lower ($P < 0.025$; 25% decrease compared with the virgin group). With oleate (1mM) as substrate, the rates of ketogenesis were lower than with butyrate. In the early-lactating (2–4 days) group there was a 100% increase in the rate from oleate compared with the virgin group, whereas at peak lactation (10–17 days) the rate from oleate was approx. 50% lower than in the virgin group (Table 1). As the metabolism of butyrate does not involve either triacylglycerol formation or the carnitine acyltransferase system (see the introduction), the similar rates of ketogenesis from this substrate in all groups suggest that the greater variation in rates from oleate is due to alterations in regulation before β -oxidation. These changes could be due to an alteration in the rate of oleate uptake into the hepatocytes or to a change in the amount of oleate esterified. Lactating rats whose pups had been removed immediately

after parturition (non-lactating group) and which were then studied after a further 14 days did not show the decreased rate of ketogenesis observed in the peak-lactation group. This implies that the alteration in oleate metabolism is associated with a high rate of lactation.

Metabolism of [1- 14 C]oleate by hepatocytes from fed rats

The rate of uptake of [1- 14 C]oleate decreased with time, presumably as a result of the decreasing concentration of substrate in the medium (Heimberg *et al.*, 1969). There was no significant difference in the rate of oleate uptake (measured at 20 and 60min) between the groups studied (Table 2). This indicates that changes in the rate of formation of oleoyl-CoA were not responsible for the variations in the rate of ketogenesis.

Of the [1- 14 C]oleate taken up by the liver cells during a 60min incubation, 85–90% could be accounted for in three fractions: esterified fats, ketone bodies and 'metabolic' CO_2 . The distribution of the radioactivity in the various fractions is shown as a percentage of the total radioactivity recovered after a 60min incubation (Table 3). There was a significant ($P < 0.025$) decrease in the rate of conversion of [1- 14 C]oleate into esterified fats in the pregnant, early-lactating (2–4 days) and non-lactating groups and a concomitant increase in the rate of formation of

Table 1. *Rates of ketogenesis by hepatocytes from fed rats*

For experimental details see the Materials and Methods section. Rates of ketogenesis are mean values $\pm$ S.D. expressed as μmol of ketone bodies formed/min per g wet wt. (20–60min period). The numbers of rats in each group are shown in parentheses. Values which are significantly different from the virgin group of rats are shown: * $P < 0.025$; ** $P < 0.005$.

Substrate added	State of rats ...	Rate of ketogenesis				
		Virgin (12)	Pregnant (18–21 days) (4)	Non-lactating (3)	Lactating (2–4 days) (4)	Lactating (10–17 days) (12)
None		0.04 ± 0.02	0.01 ± 0.02	0.04 ± 0.02	0.07 ± 0.04	0.03 ± 0.02
Butyrate (10mM)		1.22 ± 0.28	1.14 ± 0.09	1.34 ± 0.24	1.46 ± 0.27	$0.97 \pm 0.23^*$
Oleate (1mM)		0.39 ± 0.15	0.52 ± 0.18	$0.63 \pm 0.16^*$	$0.78 \pm 0.12^{**}$	$0.18 \pm 0.09^{**}$

Table 2. *Rates of [1- 14 C]oleate uptake by hepatocytes from fed rats*

For experimental details, see the Materials and Methods section. Rates of [1- 14 C]oleate uptake are mean values $\pm$ S.D. expressed as μmol of oleate removal/min per g wet wt., with the numbers of rats shown in parentheses. None of the groups was significantly different from the virgin group.

Incubation time (min)	State of rats ...	Rate of [14 C]oleate uptake				
		Virgin (5)	Pregnant (18–21 days) (4)	Non-lactating (3)	Lactating (2–4 days) (4)	Lactating (10–17 days) (5)
20		0.66 ± 0.14	0.63 ± 0.14	0.52 ± 0.05	0.71 ± 0.07	0.61 ± 0.07
60		0.49 ± 0.03	0.44 ± 0.06	0.43 ± 0.04	0.51 ± 0.02	0.47 ± 0.08

Table 3. *Percentage distribution in various products of [1-¹⁴C]oleate taken up by hepatocytes from fed rats*

For experimental details, see the Materials and Methods section. The distribution of ¹⁴C radioactivity is shown as %±s.d. of the total radioactivity recovered in all three fractions: esterified fats, ketone bodies and CO₂. The numbers of rats in each group are shown in parentheses. Values which are significantly different from those of the virgin group of rats are shown: **P*<0.025; ***P*<0.005.

State of rats ...	Distribution of [¹⁴ C]oleate (%)				
	Virgin (5)	Pregnant (18–21 days) (4)	Non-lactating (3)	Lactating (2–4 days) (4)	Lactating (10–17 days) (6)
Products					
Esterified fats	71.7±7.0	57.8±10.6*	57.0±8.4*	53.4±4.5**	85.4±5.6*
Ketone bodies	17.4±6.6	32.6±10.7*	35.1±8.9*	36.2±5.5**	6.3±3.7**
Metabolic CO ₂	10.8±2.0	9.5±1.1	7.9±0.8	10.4±2.1	8.3±2.9

Table 4. *Effect of L-carnitine on rates of ketogenesis from oleate in hepatocytes from fed rats*

For experimental details, see the Materials and Methods section. Rates are mean values±s.d. expressed as μmol/min per g wet wt. with the numbers of rats in each group shown in parentheses. All experimental groups showed a highly significant (*P*<0.005; paired *t* test) increase in the rate of ketogenesis when L-carnitine was present.

State of rats ...	Rate of ketogenesis				
	Virgin (5)	Pregnant (18–21 days) (4)	Non-lactating (3)	Lactating (2–4 days) (4)	Lactating (10–17 days) (5)
1 mM-Oleate	0.45±0.19	0.52±0.18	0.63±0.16	0.78±0.12	0.14±0.05
1 mM-Oleate+1 mM-L-carnitine	0.56±0.19	0.66±0.22	0.76±0.13	0.97±0.21	0.26±0.09

radioactive ketone bodies. In contrast, the peak-lactation group showed a significant increase (*P*<0.025) in the percentage of [1-¹⁴C]oleate converted into esterified fats and a corresponding decrease in the formation of radioactive ketone bodies. There were no significant differences between the groups in the conversion of [1-¹⁴C]oleate into metabolic CO₂. These results, and those presented in the preceding section, suggest that there is an alteration in oleate metabolism in rats lactating for 10–17 days. This results in a higher proportion of the available oleoyl-CoA being converted into esterified products rather than into ketone bodies. They also suggest that there is a tendency for the converse to occur in late pregnancy and in early lactation.

Effects of carnitine on rates of ketogenesis by hepatocytes from fed rats

The transport of long-chain acyl-CoA esters into the mitochondria for oxidation occurs via the carnitine acyltransferase system (EC 2.3.1.21, carnitine palmitoyltransferase) (Kopec & Fritz, 1973; Brosnan *et al.*, 1973) and carnitine is a substrate for carnitine acyltransferase I. Evidence that carnitine can stimulate the rate of hepatic oxidation of fatty acid was originally provided by Fritz (1955). Recent work has shown that the addition of carnitine to livers of

fed rats perfused with oleate (McGarry *et al.*, 1975a) or to isolated hepatocytes from fed rats (Christiansen *et al.*, 1976) increases the rate of ketogenesis.

The addition of L(–)-carnitine (1 mM) to isolated hepatocytes from fed rats had no effect on rates of ketogenesis from endogenous substrates nor on the rates from butyrate (results not shown). However, carnitine caused a significant (*P*<0.005) increase in the rate of ketogenesis from oleate by liver cells from all groups of rats (Table 4). The absolute increase in rate was between 0.12 and 0.19 μmol/min per g wet wt. of tissue. The inclusion of carnitine in the incubation medium did not, however, substantially alter the pattern observed without carnitine, namely that hepatocytes from the rats at peak lactation had the lowest rates of ketogenesis from oleate. Carnitine did not alter the rate of [1-¹⁴C]oleate uptake into the hepatocytes, but caused a 10% decrease in the percentage of oleate recovered as esterified fats and a concomitant increase in the amount of oleate converted into ketone bodies (results not shown). This confirms that carnitine acts at a site before β-oxidation, presumably by enhancing the entry of oleoyl-CoA into the mitochondria. The effects of carnitine on ketogenesis in isolated hepatocytes reported here are in general agreement with those described by Christiansen *et al.* (1976). They concluded that an

increase in the concentration of carnitine could not fully explain the increased diversion of long-chain acyl-CoA esters to β -oxidation in hepatocytes from starved rats. McGarry *et al.* (1975a) have provided evidence that the concentration of hepatic carnitine *in vivo* correlates well with the rate of ketogenesis *in vitro* in a number of situations. However, this relationship does not always hold. The liver of fed lactating rats (1–2 days) has approximately a 2.5-fold higher concentration of carnitine than the liver of virgin rats, yet on perfusion the rates of ketogenesis from added oleate are similar (Robles-Valdes *et al.*, 1976).

Glycogen metabolism and rates of ketogenesis

There is evidence that links high rates of ketogenesis with a low concentration of hepatic glycogen (for a review of earlier literature, see Blixenkrone-Møller, 1938; Mirsky, 1942). During the transition from the fed to the starved state there is a temporal relationship between the depletion of hepatic glycogen *in vivo* and the increased rates of ketogenesis from added oleate in perfused liver (McGarry *et al.*, 1973). Similarly, the injection of anti-insulin serum or of glucagon into fed rats rapidly depletes the liver of glycogen and results in increased rates of ketogenesis from oleate *in vitro* (McGarry *et al.*, 1975b). It was therefore decided to test whether the hepatic glycogen concentration or the rate of glycogenolysis *in vitro* was altered by lactation.

There were no significant differences between the various groups of rats in either the concentration of hepatic glycogen *in vivo* or in the glycogen concentration of isolated hepatocytes before incubation (Table

5). In the absence of oleate, there was no difference between the groups in the amount of lactate accumulating in the medium, but there was a significantly ($P < 0.025$) lower amount of glucose formed in the peak lactation (10–17 days) group (Table 5). In the presence of oleate, there was a decrease in the amount of lactate found in all groups, presumably owing to the inhibition of glycolysis by unesterified fatty acids, either directly (Weber *et al.*, 1967) or indirectly via increased concentration of citrate (Underwood & Newsholme, 1967). The decrease was considerably less in the peak lactation group and concomitant with this was a significantly ($P < 0.025$) lower accumulation of glucose.

By comparing half the lactate formed with the glucose formed it is possible to obtain a measure of the proportion of glucose equivalents from glycogen breakdown which enter the glycolytic pathway as opposed to forming free glucose. This assumes (1) that the rate of oxidation of pyruvate is low in comparison with the glycolytic flux (an assumption which is likely to hold in the presence of oleate because of the decrease in pyruvate dehydrogenase activity) and (2) that glucose carbon which is diverted to glycerol for fatty acid esterification is also low in comparison with glycolytic flux (an assumption which is likely to hold in the absence of oleate). The results presented in Table 5 show that irrespective of the presence or absence of oleate, the peak-lactation group (10–17 days) had the highest apparent glycolytic flux, despite there being no increase in the rate of glycogenolysis (sum of glucose plus half of lactate formed). This finding is in agreement with the changes in activity of key 'opposing' enzymes in the glycolytic-glucose-

Table 5. Glycogen content and formation of glucose and lactate by hepatocytes from fed rats

For experimental details, see the Materials and Methods section. The values for glycogen content of liver *in vivo* and for isolated hepatocytes before incubation are means $\pm$ s.d. expressed as $\mu\text{mol/g}$ fresh wt. of tissue. The values for the formation of glucose and of lactate by isolated hepatocytes during a 60 min period of incubation are means $\pm$ s.d. expressed as $\mu\text{mol/g}$ fresh wt. The presence of oleate (1 mM) in the incubation medium is indicated by +. The numbers of rats in each group are shown in parentheses. Results which are significantly different from those for virgin rats are indicated by: * $P < 0.05$; ** $P < 0.01$.

Measurement	State of rats Oleate in medium	... Virgin (4)	Pregnant (18–21 days) (4)	Non-lactating (3)	Lactating (2–4 days) (4)	Lactating (10–17 days) (5)
Glycogen content in freeze-clamped liver		238 $\pm$ 37	167 $\pm$ 65	—	246 $\pm$ 72	196 $\pm$ 80
Glycogen content in hepatocytes before incubation		218 $\pm$ 73	130 $\pm$ 71	170 $\pm$ 14	135 $\pm$ 56	188 $\pm$ 54
Lactate formed by hepatocytes	—	44.2 $\pm$ 20.1	42.4 $\pm$ 11.2	52.8 $\pm$ 6.5	34.0 $\pm$ 16.7	47.7 $\pm$ 11.9
	+	5.5 $\pm$ 6.6	13.5 $\pm$ 8.7	5.0 $\pm$ 1.0	3.1 $\pm$ 2.5	21.2 $\pm$ 7.0**
Glucose formed by hepatocytes	—	42.1 $\pm$ 2.7	34.6 $\pm$ 9.5	36.8 $\pm$ 8.2	38.1 $\pm$ 6.6	29.0 $\pm$ 7.3**
	+	46.8 $\pm$ 3.6	47.1 $\pm$ 10.8	54.0 $\pm$ 9.3	48.6 $\pm$ 9.5	36.7 $\pm$ 8.3*
$\frac{1}{2}$ (Lactate formed)	—	0.51 $\pm$ 0.21	0.62 $\pm$ 0.17	0.75 $\pm$ 0.24	0.44 $\pm$ 0.22	0.84 $\pm$ 0.16*
(glucose formed)	+	0.06 $\pm$ 0.07	0.14 $\pm$ 0.07	0.04 $\pm$ 0.01	0.03 $\pm$ 0.02	0.30 $\pm$ 0.10**

neogenic pathway in the livers of rats at peak lactation (Smith, 1975). It is noteworthy that these changes in enzyme activities did not occur in livers of rats that had been lactating for only 2 days (Smith, 1975). This agrees with our observations that there is no change in glycolytic flux during this period.

Rates of ketogenesis by hepatocytes from starved rats

The fact that the decreased rate of ketogenesis from oleate in the peak-lactation group was accompanied by an increased glycolytic flux suggested that the effects of starvation (i.e. glycogen depletion) on ketone-body synthesis should be examined. As expected, starvation of virgin rats resulted in increased rates of ketogenesis from endogenous substrates (approx. 10-fold), butyrate (approx. 2-fold) or oleate (approx. 4-fold) (compare Tables 1 and 6). There was no difference in the endogenous rate of ketogenesis between the three groups (virgin, pregnant and peak lactation). With butyrate there was no difference in the rate in the peak-lactation group, but there was about a 20% lower rate ($P < 0.025$) in the pregnant group (Table 6). When oleate was the substrate, the virgin and pregnant groups had the same rates, whereas the rate of ketogenesis was 28% lower ($P < 0.001$; Table 6) in the peak-lactation group. The finding that a lower rate of ketogenesis from oleate still occurs after starvation in this group of rats

suggests that at least part of the effect is due to factors other than the increased glycolytic flux.

Metabolism of [1-¹⁴C]oleate by hepatocytes from starved rats

The rates of uptake of [1-¹⁴C]oleate by hepatocytes from starved rats were similar to those obtained with liver cells from fed rats (compare Tables 2 and 7) and there were no significant differences between the various groups (Table 7).

Comparison of the percentage distribution of [1-¹⁴C]oleate taken up by the hepatocytes from fed and from starved virgin rats (Tables 3 and 8) indicates that starvation results in a switch in the fate of oleate: a lower percentage is converted into esterified fats and a higher percentage into ketone bodies. This finding confirms the original observation of Mayes & Felts (1967) with perfused rat liver and of Ontko (1972) with isolated hepatocytes. The hepatocytes from the starved lactating rats converted a higher percentage of the [1-¹⁴C]oleate into esterified fats and a lower percentage into ketone bodies than did virgin rats (Table 8), confirming the findings with non-radioactive oleate.

Physiological relevance

The fact that at the time of peak lactation in the rat the liver converts a higher proportion of the long-chain fatty acids presented to it into esterified fats

Table 6. *Rates of ketogenesis by hepatocytes from rats starved for 24 h*

For experimental details, see the Materials and Methods section. Rates are mean values $\pm$ s.d. expressed as μ mol of ketone bodies formed/min per g wet wt. (20–60 min period) with the numbers of rats used in each group in parentheses. Values which are significantly different from those of the virgin group of rats are shown: * $P < 0.025$; ** $P < 0.001$.

State of rats ...	Rate of ketogenesis		
	Virgin	Pregnant	Lactating
Substrate added	(12)	(18–21 days) (4)	(10–17 days) (12)
None	0.56 $\pm$ 0.15	0.48 $\pm$ 0.06	0.48 $\pm$ 0.06
Butyrate (10 mm)	2.31 $\pm$ 0.33	1.87 $\pm$ 0.24*	2.18 $\pm$ 0.45
Oleate (1 mm)	1.58 $\pm$ 0.27	1.36 $\pm$ 0.16	1.14 $\pm$ 0.28**

Table 7. *Rates of [1-¹⁴C]oleate uptake by hepatocytes from rats starved for 24 h*

For experimental details, see the Materials and Methods section. Rates of [1-¹⁴C]oleate uptake are mean values $\pm$ s.d. expressed as μ mol of oleate removed/min per g wet wt. with the numbers of rats shown in parentheses. None of the groups was significantly different from the virgin group.

State of rats ...	Rate of [¹⁴ C]oleate uptake		
	Virgin	Pregnant	Lactating
Incubation time (min)	(5)	(18–21 days) (2)	(10–17 days) (4)
20	0.55 $\pm$ 0.15	0.62	0.43 $\pm$ 0.08
60	0.46 $\pm$ 0.14	0.49	0.37 $\pm$ 0.06

Table 8. *Percentage distribution in various products of [1-¹⁴C]oleate taken up by hepatocytes from rats starved for 24 h*

For experimental details, see the Materials and Methods section. The distribution of ¹⁴C radioactivity is shown as %±s.d. of the total radioactivity recovered in all three fractions: esterified fats, ketone bodies and CO₂. The numbers of rats in each group are shown in parentheses. Values which are significantly different from those of the virgin group of rats are shown: **P*<0.05.

State of rats ...	Virgin (3)	Pregnant (18–21 days) (2)	Lactating (10–17 days) (4)
Products			
Esterified fats	38.3±7.6	32.8	60.0±15.7*
Ketone bodies	56.7±8.1	63.3	32.5±16.0*
Metabolic CO ₂	4.9±2.4	3.7	7.7±1.3

and a lower proportion into ketone bodies must be seen in relation to the alterations in substrate requirements of the lactating animal. The lactating mammary gland of the rat takes up appreciable amounts of triacylglycerols (Hawkins & Williamson, 1972). Presumably when the rat is fed on the normal laboratory diet which is high in carbohydrate these must be formed by either synthesis *de novo* from glucose or esterification of unesterified fatty acids taken up by the liver. There is evidence from experiments *in vitro* that adipose-tissue lipolysis is faster in the lactating rat (Smith & Walsh, 1976). If this is also true *in vivo*, an ample supply of endogenous unesterified fatty acids would be available for esterification by the liver. The reciprocal alterations in the activity of lipoprotein lipase in adipose tissue and in mammary gland during lactation (Otway & Robinson, 1968; Hamosh *et al.*, 1970) can then 'direct' a high proportion of the liver's output of triacylglycerol to the gland. Other reported changes in hepatic metabolism at peak lactation in the rat, namely the increased activities of key enzymes of lipogenesis (Smith, 1973) and the increased glycolytic capacity (Smith, 1975; this paper), would fit the picture of a liver 'geared' to the production of lipid for utilization by the lactating gland.

The lactating mammary gland of the rat has high activities of the enzymes concerned with the utilization of ketone bodies (Page & Williamson, 1972) and, at least *in vitro*, the ketone bodies taken up by the gland can be converted into lipid (Williamson *et al.*, 1975). This raises the question as to why the liver during lactation 'adapts' so that it has a lower, rather than a higher, ketogenic capacity. *In vitro*, ketone bodies inhibit the utilization of glucose and pyruvate by the gland (Williamson *et al.*, 1975). In the starved lactating rat measurements of arterio-venous differences across the gland have shown that the increased availability of ketone bodies is accompanied by a decreased rate of uptake of glucose and an output of lactate and pyruvate (Hawkins & Williamson, 1972; Robinson & Williamson, 1977).

Thus it is tempting to speculate that acetoacetate might be a 'signal' to the lactating gland of the decreased availability of carbohydrate. If this postulate is valid, then in the fed state the hepatic output of ketone bodies would need to be kept low.

Irrespective of the teleological reasons for the hepatic 'adaptations' in the lactating rats, there presumably must be hormonal or other signal(s) for these changes. A possible mediator during peak lactation is prolactin, whose concentration in the circulation reaches a peak by day 4 of lactation and then falls rapidly after the removal of the young (Amenomori *et al.*, 1970; Simpson *et al.*, 1973). There is a parallel between these changes in the circulating concentration of prolactin and our finding that decreased ketogenic capacity is not present in livers from rats in early lactation (2–4 days) nor in non-lactating rats in which the pups were removed at birth. In this connexion it is noteworthy that the injection of oestrogen into the rat increases the proportion of unesterified fatty acids converted into triacylglycerols by the liver (Heimberg *et al.*, 1977). It also induces an increase in the concentration of plasma prolactin and in the number of hepatic prolactin receptors (Posner *et al.*, 1975).

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References

- Aas, M. & Bremer, J. (1968) *Biochim. Biophys. Acta* **164**, 157–166
- Amenomori, Y., Chen, C. L. & Meites, J. (1970) *Endocrinology* **86**, 506–510
- Berry, M. N. & Friend, D. S. (1969) *J. Cell Biol.* **43**, 506–520
- Blixenkrone-Møller, N. (1938) *Hoppe-Seyler's Z. Physiol. Chem.* **252**, 117–136

- Brosnan, J. T., Kopec, B. & Fritz, I. B. (1973) *J. Biol. Chem.* **248**, 4075–4082
- Christiansen, R., Borrebaek, B. & Bremer, J. (1976) *FEBS Lett.* **62**, 313–317
- Folch, J., Lees, M. & Sloane Stanley, G. H. (1957) *J. Biol. Chem.* **266**, 497–509
- Fritz, I. B. (1955) *Acta Physiol. Scand.* **34**, 367–385
- Fritz, I. B. (1961) *Physiol. Rev.* **41**, 52–129
- Hamosh, M., Clary, T. R., Chernick, S. S. & Scow, R. O. (1970) *Biochim. Biophys. Acta* **210**, 473–482
- Hawkins, R. A. & Williamson, D. H. (1972) *Biochem. J.* **129**, 1171–1173
- Heimberg, M., Weinstein, I. & Kohout, M. (1969) *J. Biol. Chem.* **244**, 5131–5139
- Heimberg, M., Soler-Argilaga, C., Weinstein, I. & Russell, R. L. (1977) in *Biochemical and Clinical Aspects of Ketone Body Metabolism* (Söling, H.-D. & Seufert, C. D., eds.), Georg Thieme Verlag, in the press
- Keppler, D. & Decker, K. (1974) in *Methods of Enzymatic Analysis* (Bergmeyer, H. U., ed.), vol. 3, pp. 1127–1131, Academic Press, New York and London
- Kopec, B. & Fritz, I. B. (1973) *J. Biol. Chem.* **248**, 4069–4074
- Krebs, H. A. & Eggleston, L. V. (1945) *Biochem. J.* **39**, 408–419
- Krebs, H. A. & Henseleit, K. (1932) *Hoppe-Seyler's Z. Physiol. Chem.* **210**, 33–66
- Krebs, H. A., Cornell, N. W., Lund, P. & Hems, R. (1974) in *Alfred Benzon Symp. 6th* (Lundquist, F. & Tygstrup, N., eds.), pp. 718–743, Munksgaard, Copenhagen
- Mayes, P. A. & Felts, J. M. (1967) *Nature (London)* **215**, 716–718
- McGarry, J. D. & Foster, D. W. (1972) *Metab. Clin. Exp.* **21**, 471–489
- McGarry, J. D., Meier, J. M. & Foster, D. W. (1973) *J. Biol. Chem.* **248**, 270–278
- McGarry, J. D., Robles-Valdes, C. & Foster, D. W. (1975a) *Proc. Natl. Acad. Sci. U.S.A.* **72**, 4385–4388
- McGarry, J. D., Wright, P. H. & Foster, D. W. (1975b) *J. Clin. Invest.* **55**, 1202–1209
- Mirsky, I. A. (1942) *J. Am. Med. Assoc.* **118**, 690–694
- Ontko, J. R. (1972) *J. Biol. Chem.* **247**, 1788–1800
- Otway, S. & Robinson, D. S. (1968) *Biochem. J.* **106**, 677–682
- Page, M. A. & Williamson, D. H. (1972) *Biochem. J.* **128**, 459–460
- Posner, B. I., Kelly, P. A. & Friesen, H. G. (1975) *Science* **188**, 57–59
- Robinson, A. M. & Williamson, D. H. (1977) *Biochem. J.* **164**, 153–159
- Robles-Valdes, C., McGarry, J. D. & Foster, D. W. (1976) *J. Biol. Chem.* **251**, 6007–6012
- Simpson, A. A., Simpson, M. H. W., Sinha, Y. N. & Schmidt, G. H. (1973) *J. Endocrinol.* **58**, 675–676
- Smith, R. W. (1973) *J. Dairy Res.* **40**, 339–351
- Smith, R. W. (1975) *Biochim. Biophys. Acta* **411**, 22–29
- Smith, R. W. & Walsh, A. (1976) *Lipids* **11**, 418–420
- Underwood, A. H. & Newsholme, E. A. (1967) *Biochem. J.* **104**, 300–305
- Weber, G., Lea, M. A., Convery, H. J. & Stamm, N. B. (1967) *Adv. Enzyme Regul.* **5**, 257–298
- Wieland, O. H. (1968) *Adv. Metab. Disord.* **3**, 1–47
- Williamson, D. H. (1973) in *Proc. Congr. Int. Diabetes Fed. 8th* (Malaisse, W. J. & Pirart, J., eds.), Excerpta Medica, Amsterdam
- Williamson, D. H., Mellanby, J. & Krebs, H. A. (1962) *Biochem. J.* **82**, 90–98
- Williamson, D. H., McKeown, S. R. & Ilic, V. (1975) *Biochem. J.* **150**, 145–152