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Regulation of Ketogenesis during the Suckling–Weanling Transition in the Rat

STUDIES WITH ISOLATED HEPATOCYTES

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The rates of ketogenesis from endogenous substrates, butyrate or oleate, have been measured in isolated hepatocytes from suckling and weanling rats. Ketogenesis from endogenous substrate and from oleate decreased on weaning, whereas the rate from butyrate remained unchanged. It is concluded that the major site of regulation of ketogenesis during this period of development involves the disposal of long-chain fatty acyl-CoA between the esterification and β-oxidation pathways. Modulators of lipogenesis [dihydroxyacetone and 5-(tetradecyloxy)-2-furoic acid] did not alter the rate of ketogenesis in hepatocytes from suckling rats, and it is suggested that this is due to the low rate of lipogenesis in these cells. Hepatocytes from fed weanling rats have a high rate of lipogenesis and evidence is presented for a reciprocal relationship between ketogenesis and lipogenesis, and ketogenesis and esterification in these cells. Dibutyryl cyclic AMP stimulated ketogenesis from oleate in hepatocytes from fed weanling rats, even in the presence of an inhibitor of lipogenesis [5-(tetradecyloxy)-2-furoic acid], but not in cells from suckling rats. It is suggested that cyclic AMP may act via inhibition of esterification and that in hepatocytes from suckling rats ketogenesis is already maximally stimulated by the high basal concentrations of cyclic AMP [Beaudry, Chiasson & Exton (1977) *Am. J. Physiol.* **233**, E175–E180].

During the suckling period the rat pup has a raised concentration of blood ketone bodies (acetoacetate and D-3-hydroxybutyrate), which decreases when the pups are weaned at 21 days on to the commercial high-carbohydrate diet (Drahota *et al.*, 1964; Page *et al.*, 1971; Lockwood & Bailey, 1971; Dahlquist *et al.*, 1972; Robles-Valdes *et al.*, 1976). The enzymes of ketone-body utilization in peripheral tissues are active at this time, indeed the activities in brain are some 2–3-fold higher than in the adult (Klee & Sokoloff, 1967; Page *et al.*, 1971; Tildon *et al.*, 1971; Dahlquist *et al.*, 1972), and therefore it is generally assumed that the hyperketonaemia of the suckling period is due to increased hepatic production of ketone bodies. The high fat content of rat milk (Dymsza *et al.*, 1964) and the consequent ready availability of fatty acid substrates presumably plays an important role in the control of ketogenesis during the suckling period. Little information is available about possible intrahepatic regulation of ketogenesis during the suckling–weanling transition in the rat. The activities of some of the enzymes concerned in fatty acid metabolism and ketone-body synthesis are higher before weaning and then decline towards adult values (Foster & Bailey, 1976*a*; Shah & Bailey, 1977); particularly, marked changes occur in the activity of carnitine acyltransferase (EC 2.3.1.21; Foster & Bailey, 1976*a*). The developmental pattern of this enzyme correlates well with the rate of palmitate conversion into ketone bodies by liver mitochondria (Foster & Bailey, 1976*b*). Experiments with isolated rat hepatocytes (Sly & Walker, 1978) and with rat liver homogenates (Yeh *et al.*, 1977) have also shown that formation of ketone bodies from palmitate is highest during the suckling period and decreases on weaning.

Current theories on the control of ketogenesis in livers from adult rats suggest that the primary intrahepatic regulation is exerted at the level of the disposal of long-chain acyl-CoA between the pathways of esterification and β-oxidation (for reviews, see McGarry & Foster, 1977; Williamson & Whitelaw, 1978). The present experiments with isolated hepatocytes are concerned with whether this also is the case during the suckling–weanling transition, and, in addition, with examining the effects during this period of development of modulators of ketogenesis, namely dihydroxyacetone (decreases ketogenesis and stimulates lipogenesis; Williamson *et al.*, 1969; Benito & Williamson, 1978), 5-(tetradecyloxy)-2-furoic acid (stimulates ketogenesis and inhibits lipogenesis; Benito & Williamson, 1978) and dibutyryl cyclic AMP (stimulates ketogenesis and

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decreases esterification; Heimberg *et al.*, 1969; Cole & Margolis, 1974; Klausner *et al.*, 1978).

Materials and Methods

Rats of the Wistar strain were used. Suckling rats weighed between 20 and 50g, and weanling rats between 60 and 90g. Suckling animals were weaned on the 21st day after parturition by removing them from the mother. The weanling rats were fed *ad libitum* on Oxoid breeding diet for rats and mice (Oxoid Ltd., London S.E.1, U.K.). Rats were anaesthetized with Nembutal (60mg/kg body wt.; solution in 0.9% NaCl).

All biochemicals, including $N^6O^{2'}$-dibutyryl cyclic AMP, were obtained from Boehringer Corp. (London) Ltd., London W.5, U.K. 5-(Tetradecyloxy)-2-furoic acid was a gift from Dr. A. Richardson (Merrell National Laboratories, Lockland Station, Cincinnati, OH, U.S.A.).

[1-^{14}C]Oleate, [1-^{14}C]acetate and ^3H_2O were obtained from The Radiochemical Centre, Amersham, Bucks., U.K.

Hepatocytes from rats less than 5 weeks old were prepared by a modification of the reverse perfusion technique first described by Trowell (1942). A cannula was inserted into the upper vena cava through the right atrium and an incision was made in the portal vein close to the liver. The result was that the liver was perfused in the reverse direction to normal. The perfusion fluid ran out of the portal vein, over the animal and collected in a plastic beaker, situated below the operating tray, to be recycled. This perfusion was carried out, like the perfusion of adult liver, *in situ*. Owing to the small size of the liver, experiments on suckling and weanling rats usually involved the pooling of hepatocytes from two or more animals. Other details of the preparation of isolated hepatocytes were as described by Krebs *et al.* (1974).

The incubation procedure and measurements of esterification and removal of [1-^{14}C]oleate, and conversion of [1-^{14}C]acetate into lipids were as described by Whitelaw & Williamson (1977). Lipogenesis was measured with ^3H_2O by the method described by Harris (1975). The following metabolites were determined in the neutralized $HClO_4$ extracts by enzymic methods: glucose (Slein, 1963); L-lactate (Hohorst *et al.*, 1959); acetoacetate and D-3-hydroxybutyrate (Williamson *et al.*, 1962). Measurements of radioactivity were carried out as described by Williamson *et al.* (1975).

The rates of ketogenesis, lipogenesis, esterification of [1-^{14}C]oleate and its removal, are expressed as μmol/min per g wet wt. of liver and were calculated from plots of the 20, 40 and 60min values; these were usually linear. The production of glucose and lactate was not linear, especially on addition of dibutyryl cyclic AMP, and therefore the results are expressed as μmol formed/h per g wet wt. of liver.

Results and Discussion

Developmental pattern of ketogenesis in isolated hepatocytes

The rate of ketogenesis from endogenous substrate was as high in hepatocytes from suckling rats as in hepatocytes from starved adult rats (Whitelaw & Williamson, 1977) and did not change significantly over the suckling period examined (Table 1). After weaning, when the change from a high-fat to a high-

Table 1. *Rates of ketogenesis, esterification of* [1-^{14}*C*]*oleate and conversion of* [1-^{14}*C*]*acetate into lipid during the suckling–weanling transition*

For experimental details see the Materials and Methods section. The final concentrations of the added substrates are given in the Table. All results are mean values ± S.E.M. with the numbers of observations in parentheses. Ketogenesis is expressed as μmol of ketone bodies formed/min per g wet wt., esterification as μmol of [1-^{14}C]oleate esterified/min per g wet wt. and conversion of [1-^{14}C]acetate to lipid as μmol of acetate incorporated/min per g wet wt.

Measurement	Age (days) ... 11–14	15–20	22–25	28–32
Ketogenesis from:				
Endogenous substrates	0.52 ± 0.045 (7)	0.44 ± 0.05 (12)	0.15 ± 0.02 (5)	0.085 ± 0.008 (9)
Butyrate (10mM)	1.36 ± 0.12 (5)	1.44 ± 0.055 (4)	1.73 ± 0.31 (3)	1.44 ± 0.08 (5)
Oleate (1mM)	1.85 ± 0.085 (10)	1.55 ± 0.07 (18)	0.89 ± 0.15 (4)	0.63 ± 0.06 (9)
Esterification of [1-^{14}C]oleate	—	0.14 ± 0.015 (18)	0.16 ± 0.07 (7)	0.20 ± 0.02 (9)
Removal of [1-^{14}C]oleate	0.55 ± 0.08 (4)	0.56 ± 0.06 (12)	0.47 ± 0.05 (6)	0.41 ± 0.03 (5)
Conversion of [1-^{14}C]acetate (2mM) to lipid	—	0.029 ± 0.01 (7)	0.089 ± 0.02 (5)	0.145 ± 0.01 (5)

carbohydrate diet took place, the rate of endogenous ketogenesis decreased by 66% (22–25 days of age) and reached values as low as in fed adult rats (Whitelaw & Williamson, 1977; Benito & Williamson, 1978) at 28–32 days of age. The rate of ketogenesis from oleate (1mM) was also similar in hepatocytes from suckling rats (11–14 days) to that in hepatocytes from starved adult rats (Whitelaw & Williamson, 1977) and did not change to any great extent during the rest of the suckling period (Table 1). After the animals were weaned, the rate of ketogenesis from oleate had decreased by 50% at 22–25 days of age and by 63% at 28–32 days compared with those during the second half of the suckling period. The removal of [1-^{14}C]oleate from the incubation medium did not change significantly at any of the ages of the pups studied and therefore the alterations in rates of ketogenesis from this substrate must be due to regulation of the further metabolism of long-chain oleoyl-CoA rather than to its rate of formation.

In contrast, the rate of ketogenesis from butyrate (10mM) did not show any major change during the suckling to weanling transition. Butyrate, unlike long-chain fatty acids such as oleate, is converted into the corresponding CoA derivative within the mitochondrial matrix (Aas & Bremer, 1968), and therefore does not require the carnitine acyltransferase system (Kopec & Fritz, 1973) for entry into mitochondria, nor is it converted into triacylglycerol (Fritz, 1961). Assuming that rate of formation of butyryl-CoA is not limiting when butyrate is the substrate, the alterations in the rate of ketogenesis from endogenous substrates or from oleate during the suckling–weanling transition suggest that the major intrahepatic site of regulation is located at the stage of partitioning of long-chain acyl-CoA between the pathways of esterification and β-oxidation. In relation to the high-fat intake of the suckling period, it is of interest that perfused livers from adult rats fed on a high-fat diet for at least 8 days showed a greater increased rate of ketogenesis from oleate (50%) than from octanoate (10%) (Krebs & Hems, 1970).

The rate of esterification of [1-^{14}C]oleate to complex lipids was appreciable in hepatocytes from suckling rats (15–20 days of age) and increased by 40% on weaning (28–32 days of age). The value for suckling rats contrasts with the lower rate of esterification from [1-^{14}C]oleate (0.085μmol/min per g wet wt.) obtained in hepatocytes from adult rats starved for 48h, which have similar rates of ketogenesis (Whitelaw & Williamson, 1977). Furthermore Yeh *et al.* (1977) found that the rate of esterification of [1-^{14}C]palmitate in liver homogenates of livers from suckling rats (15–20 days old) and adult fed rats was similar, whereas the rate for starved adult rats was some 50% lower. The present findings suggest that it is increased oxidation of long-chain acyl-CoA rather than an impairment in its esterification that is responsible for the higher rates of ketogenesis in hepatocytes from suckling rats. The present experiments do not provide any direct information on the question as to whether the higher activity (2.5-fold) of carnitine acyltransferase (Foster & Bailey, 1976*a*) during the suckling period is a key factor in the increased rate of ketogenesis.

The conversion of [1-^{14}C]acetate into lipids in hepatocytes increased during the suckling–weanling transition, 3-fold by 22–25 days of age, reaching the highest value at 28–32 days when maximal activities of the enzymes involved in the lipogenic pathway in the liver have previously been reported (Taylor *et al.*, 1967).

Effects of modulators of lipogenesis on the rate of ketogenesis

It has recently been proposed that there is a regulatory link between lipogenesis, esterification and ketogenesis in rat liver, so that inhibition of lipogenesis results in increased ketone-body synthesis and decreased esterification, whereas activation of lipogenesis has the opposite effect (McGarry *et al.*, 1977). These reciprocal changes are considered to be brought about by alterations in the concentration of malonyl-CoA, a key intermediate in lipid synthesis and an inhibitor of carnitine acyltransferase I (McGarry *et al.*, 1977, 1978*a*,*b*) required for entry of long-chain acyl-CoA into the β-oxidation pathway. Experimental support for this postulate has been obtained in hepatocytes from fed virgin and lactating rats (Benito & Williamson, 1978) by using an inhibitor of lipogenesis, 5-(tetradecyloxy)-2-furoic acid (Ribereau-Gayon, 1976; Panek *et al.*, 1977; Kariya & Wille, 1978), and a lipogenic substrate, dihydroxyacetone. Measurements of [malonyl-CoA] and rates of ketogenesis in hepatocytes from meal-fed rats also support the hypothesis (Cook *et al.*, 1978). It seemed of interest therefore to test the effects of the two modulators of lipogenesis, 5-(tetradecyloxy)-2-furoic acid and dihydroxyacetone, in hepatocytes from suckling rats, where lipogenesis is impaired because of the low activity of the key enzymes (Table 1, results with [1-^{14}C]acetate; Taylor *et al.*, 1967), and in hepatocytes from weanling rats, which have higher activities of the enzymes concerned in lipogenesis than do fed adult rats (Taylor *et al.*, 1967).

Dihydroxyacetone (5mM) did not significantly alter the rate of ketogenesis or esterification of [1-^{14}C]oleate in hepatocytes from suckling rats (15–20 days of age) (Table 2). On the other hand, the formation of glucose was increased by 100% in the presence of dihydroxyacetone, indicating no impairment in its metabolism. Similarly, 5-(tetradecyloxy)-2-furoic acid (0.1mM) did not increase the

Table 2. *Effects of dihydroxyacetone and 5-(tetradecyloxy)-2-furoic acid on the metabolism of hepatocytes from suckling and weanling rats*

For experimental details see the Materials and Methods section. The final concentrations of dihydroxyacetone and [1-^{14}C]oleate when added were 5 mM and 1 mM respectively; except for the 'no addition' column the measurements of glucose and lactate production were made in the presence of oleate .The inhibitor of lipogenesis, 5-(tetradecyloxy)-2-furoic acid, was added where indicated to give a final concentration of 0.1 mM. All results are mean values ± S.E.M. with the numbers of observations in parentheses. Ketogenesis is expressed as μmol of ketone bodies formed/min per g wet wt., esterification as μmol of [1-^{14}C]oleate esterified/min per g wet wt., lipogenesis as μmol of ^3H_2O incorporated into lipid/min per g wet wt., and glucose and lactate production as μmol formed/h per g wet wt. Results significantly different by Student's *t* test from the appropriate 'no addition' values are shown: *$P<0.05$; **$P<0.01$; ***$P<0.001$.

	Suckling rats (15–20 days)		Weanling rats (28–32 days)			
Measurement	No addition	Dihydroxyacetone	No addition	Dihydroxyacetone	Inhibitor	Inhibitor+ dihydroxyacetone
Ketogenesis from:						
Endogenous substrates	—	—	0.085 ± 0.008 (9)	—	0.16 ± 0.02* (9)	—
Oleate (1 mM)	1.55 ± 0.07 (18)	1.38 ± 0.20 (4)	0.63 ± 0.06 (9)	0.35 ± 0.04* (5)	0.64 ± 0.09 (3)	0.59 ± 0.05 (4)
[1-^{14}C]Oleate removal	0.56 ± 0.06 (12)	0.55 ± 0.06 (4)	0.41 ± 0.03 (5)	0.40 ± 0.04 (5)	0.42 ± 0.04 (3)	0.44 ± 0.06 (4)
[1-^{14}C]Oleate esterification	0.14 ± 0.015 (18)	0.14 ± 0.01 (4)	0.19 ± 0.02 (12)	0.27 ± 0.03* (5)	0.15 ± 0.020 (6)	0.18 ± 0.01 (4)
Glucose production	39.9 ± 4 (9)	78.3 ± 8** (4)	44.3 ± 5 (5)	77 ± 3.5*** (5)	40.4 ± 3 (3)	72 ± 4*** (3)
Lactate production	1.85 ± 0.4 (7)	6.4 ± 1.7* (4)	40.4 ± 6 (5)	42 ± 7 (5)	19.6 ± 3* (3)	47 ± 4 (3)
Lipogenesis with ^3H_2O	—	—	0.048 ± 0.008 (8)	0.092 ± 0.010** (5)	0.007 ± 0.001*** (3)	—
Lipogenesis with ^3H_2O when oleate was added	—	—	0.015 ± 0.002 (4)	0.056 ± 0.015* (4)	0.010 ± 0.001* (3)	0.010 ± 0.001* (3)

rate of ketogenesis in hepatocytes from suckling rats (results not shown).

The failure of the two modulators of lipogenesis to alter the rate of ketogenesis from oleate in hepatocytes from suckling rats is consistent with the low rate of lipogenesis in these cells (Table 1) because of the low activities of lipogenic enzymes. Thus changes in [malonyl-CoA] are unlikely to play any significant role in the regulation of ketogenesis during the suckling period. In this connection it is of interest that dihydroxyacetone does not exert any anti-ketogenic effect on ketogenesis from oleate in hepatocytes from starved adult rats (Williamson & Whitelaw, 1978), which also have a low lipogenic rate.

Addition of dihydroxyacetone to hepatocytes from weanling rats increased the rate of lipogenesis (as measured by ^3H_2O incorporation) by 90% in the absence of oleate and by approx. 300% in the presence of oleate (Table 2). As expected, 5-(tetradecyloxy)-2-furoic acid decreased lipogenesis in all cases to low values. In the presence of dihydroxyacetone, ketogenesis from oleate was decreased by 44% and esterification of oleate increased by 35%; 5-(tetradecyloxy)-2-furoic acid completely reversed these changes (Table 2). These results are qualitatively the same as those in hepatocytes from fed virgin and lactating rats (Benito & Williamson, 1978) and suggest that dihydroxyacetone exerts its anti-ketogenic effect by stimulating lipogenesis and presumably increasing [malonyl-CoA]. The fact that 5-(tetradecyloxy)-2-furoic acid increased the rate of ketogenesis from endogenous substrates (Table 2; Benito & Williamson, 1978) is further support for the suggestion that an inverse relationship between lipogenesis and ketogenesis is also present in hepatocytes from weanling rats.

Although there were no significant differences in glucose production from endogenous sources or from added dihydroxyacetone between hepatocytes from suckling (15–20 days) and weanling (28–32 days) rats, accumulation of lactate was 7–20-fold higher respectively in the hepatocytes from weanling rats, indicating the importance of glycolytic flux in these cells as a source of carbon for lipogenesis.

Effects of dibutyryl cyclic AMP on ketogenesis

It is well established that dibutyryl cyclic AMP stimulates ketogenesis from both endogenous substrates and from added long-chain fatty acids in livers from fed adult rats (Heimberg *et al.*, 1969; Cole & Margolis, 1974; Klausner *et al.*, 1978). Dibutyryl cyclic AMP has also been reported to be an inhibitor of hepatic lipogenesis (Tepperman & Tepperman, 1972; Allred & Roehrig, 1973; Harris, 1975) and therefore it is possible that it exerts its ketogenic effect by modulation of lipogenesis and hence [malonyl-CoA].

Addition of dibutyryl cyclic AMP (10μM) to hepatocytes from suckling rats (15–20 days) did not significantly change the rate of ketogenesis from endogenous substrate or from added oleate, nor did it affect the esterification of [1-^{14}C]oleate (Table 3).

Table 3. *Effects of dibutyryl cyclic AMP on the metabolism of hepatocytes from suckling and weanling rats*

For experimental details see the Materials and Methods section. The final concentration of [1-^{14}C]oleate when added was 1.0mM; except for the 'no addition' column the measurements of glucose and lactate production were made in the presence of oleate. Dibutyryl cyclic AMP (0.01mM) and the inhibitor of lipogenesis, 5-(tetradecyloxy)-2-furoic acid (0.1mM), were added where indicated. All results are mean values ± S.E.M. with the numbers of experiments in parentheses. Ketogenesis is expressed as μmol of ketone bodies formed/min per g wet wt., esterification as μmol of [1-^{14}C]-oleate esterified/min per g wet wt. and glucose and lactate production as μmol formed/h per g wet wt. Results significantly different by Student's *t* test from the appropriate 'no addition' value are shown: *$P<0.05$; **$P<0.01$; *** $P<0.001$.

	Suckling rats (15–20 days)		Weanling rats (28–32 days)		
Measurement	No addition	Dibutyryl cyclic AMP	No addition	Dibutyryl cyclic AMP	Inhibitor+dibutyryl cyclic AMP
Ketogenesis from:					
Endogenous substrates	0.44±0.05 (12)	0.54±0.05 (3)	0.085±0.008 (9)	0.120±0.03 (4)	—
Oleate (1mM)	1.55±0.07 (18)	1.46±0.19 (3)	0.63±0.06 (9)	1.01±0.1** (7)	0.94±0.11* (3)
[1-^{14}C]Oleate removal	0.56±0.06 (12)	0.50±0.05 (3)	0.41±0.03 (5)	0.40±0.04 (4)	0.42±0.003 (3)
[1-^{14}C]Oleate esterification	0.14±0.015 (18)	0.11±0.01 (3)	0.19±0.02 (12)	0.10±0.014*** (7)	0.10±0.027* (3)
Glucose production	39.9±4 (9)	38.0±3 (3)	44.3±5 (5)	114±14*** (7)	135±4*** (3)
Lactate production	1.85±0.4 (7)	−0.56±0.05*** (3)	40.4±6 (5)	−2.64±1*** (4)	−2.8±1*** (3)

Glucose production was also not altered by dibutyryl cyclic AMP, but the low lactate production was completely suppressed. Beaudry *et al.*, (1977) found that glucagon did not increase gluconeogenesis from added lactate in hepatocytes from suckling rats, and that these liver cells had 5-fold higher basal concentrations of cyclic AMP in the absence of the hormone than corresponding hepatocytes from adult rats. Thus the absence of a stimulatory effect of dibutyryl cyclic AMP on ketogenesis in hepatocytes from suckling rats may be due to maximal stimulation of ketone-body synthesis by the high basal concentrations of cyclic AMP or alternatively to the low rate of lipogenesis in these cells and therefore the inability of cyclic AMP to modulate [malonyl-CoA]. Starvation (16–24h) did not increase the rate of ketogenesis from palmitate (Sly & Walker, 1978) or gluconeogenesis from lactate (Beaudry *et al.*, 1977) in hepatocytes from suckling rats, suggesting that both processes are maximally stimulated in the fed state.

In marked contrast, dibutyryl cyclic AMP stimulated both ketogenesis from endogenous substrates (40%) and from added oleate (60%) in hepatocytes from weanling rats (Table 3). The increased rate of ketogenesis was accompanied by a decrease (50%) in the rate of esterification of [1-^{14}C]-oleate, but no change in the rate of removal of the fatty acid. These effects of dibutyryl cyclic AMP are in qualitative agreement with those reported by Heimberg and his colleagues (Klausner *et al.*, 1978) in perfused livers of adult fed rats. As expected, dibutyryl cyclic AMP also increased the production of glucose about 3-fold and completely suppressed the formation of lactate (Table 3; Harris, 1975). The stimulation of ketogenesis in hepatocytes from weanling rats could be due to an inhibition of lipogenesis by cyclic AMP and consequent alteration of [malonyl-CoA], but it must be emphasized that oleate itself is an effective inhibitor of lipogenesis (Table 2; Mayes & Topping, 1974), and therefore cyclic AMP must either potentiate the inhibition of lipogenesis in the presence of oleate or stimulate ketogenesis by an alternative mechanism in these experiments. Some experimental support for the latter proposal is the finding that dibutyryl cyclic AMP still stimulated ketogenesis and decreased esterification (Table 3) in the presence of 5-(tetradecyloxy)-2-furoic acid (another inhibitor of lipogenesis; Table 2) which did not increase ketogenesis from oleate alone (Table 2). Moreover, the inhibitory effect of dibutyryl cyclic AMP on lipogenesis is less than that of oleate (1mM) in hepatocytes from fed lactating rats (M. Benito & D. H. Williamson, unpublished results). Dibutyryl cyclic AMP does not stimulate ketogenesis from a medium-chain fatty acid, octanoate (Raskin *et al.*, 1974), nor from a short-chain fatty acid, butyrate (Williamson & Whitelaw, 1978), suggesting that its site of action must involve the disposition of long-chain acyl-CoA between the pathways of esterification and β-oxidation; Klausner *et al.* (1978) have reached a similar conclusion. In addition, dibutyryl cyclic AMP does not appear to have any direct effect on ketone-body production by isolated mitochondria (Amatruda *et al.*, 1975). It is suggested therefore that cyclic AMP may stimulate ketogenesis by inhibiting esterification of long-chain acyl-CoA (see also Klausner *et al.*, 1978), though effects of cyclic AMP on lipogenesis and consequent stimulation of ketogenesis may occur in other experimental situations, for example, when exogenous long-chain fatty acid is absent, or when the concentration of added fatty acid is too low to exert an appreciable inhibition of lipogenesis.

Relationships between esterification, ketogenesis and lipogenesis

The existence of an inverse relationship between esterification and ketogenesis has been observed in perfused adult rat livers on transition from the fed to the starving state (Mayes & Felts, 1967; McGarry *et al.*, 1973); however, comparatively little information is available on this relationship in livers from fed

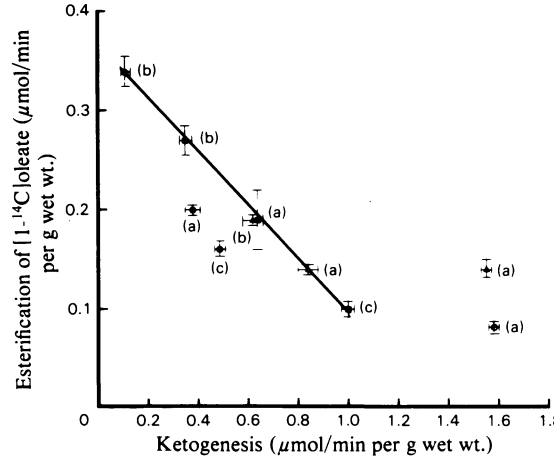

Fig. 1. *Relationship between ketogenesis and esterification of* [1-^{14}C]*oleate in isolated hepatocytes*

The rates of ketogenesis and esterification are taken from Table 2 of the present paper, and from Table 1 of Benito & Williamson (1978), except results for fed lactating rats plus dibutyryl cyclic AMP and starved virgin rats, which are unpublished (M. Benito & D. H. Williamson). For experimental conditions see the Materials and Methods section. Hepatocytes from fed lactating (●), fed virgin (▲), fed weanling (■), suckling (△) and starved virgin rats (○); oleate alone (a), oleate plus dihydroxyacetone (b) and oleate plus dibutyryl cyclic AMP (c). The lines with bars indicate the S.E.M.

rats in other physiological states or in livers subjected to various metabolic manipulations. The relationship between esterification of [1-^{14}C]oleate and ketogenesis in hepatocytes from fed virgin and lactating rats (Benito & Williamson, 1978), fed weanling and suckling rats (present paper) and starved virgin rats (M. Benito & D. H. Williamson, unpublished results) is shown in Fig. 1. There is a highly significant inverse correlation ($r = -0.99$; $P < 0.001$) between esterification and ketogenesis in hepatocytes from fed virgin and weanling rats over the range of experimental situations studied. The relationship appears to be displaced to the left in the case of hepatocytes from lactating rats, suggesting that the factors governing the interrelationship between the two processes are 'set' differently in lactating rats (see Whitelaw & Williamson, 1977). In the case of hepatocytes from suckling rats and starved virgin rats the values are displaced to the right, indicating inappropriately high rates of ketogenesis for the rates of esterification. In hepatocytes from fed virgin, lactating and weanling rats there is significant negative correlation ($r = -0.88$; $P < 0.01$) between ketogenesis and lipogenesis (Fig. 2) and this can be interpreted as further support for the role of malonyl-CoA as an inhibitor of carnitine acyl-CoA transferase I (McGarry *et al.*, 1977, 1978*a*,*b*) and thus a regulator of ketogenesis in rats fed on a high-carbohydrate diet. Nevertheless, as discussed above this relation ship is unlikely to hold in hepatocytes from suckling rats, and therefore it is concluded that the high rate of ketogenesis in these cells, like that in hepatocytes from starved rats, must be due to additional factors.

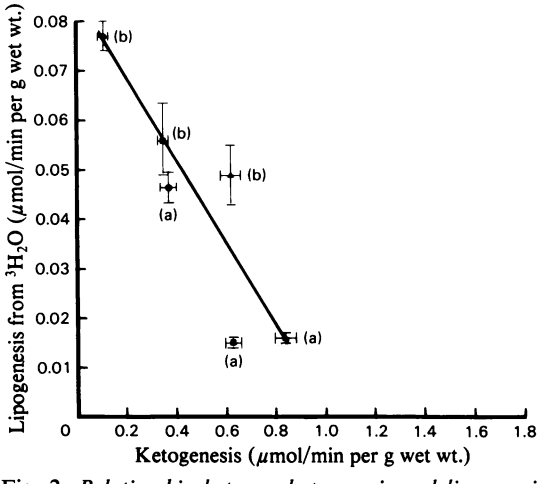

Fig. 2. *Relationship between ketogenesis and lipogenesis measured with ^3H_2O in isolated hepatocytes*

The rates of ketogenesis and lipogenesis are taken from Table 2 of the present paper and from Table 1 of Benito & Williamson (1978). For experimental conditions see the Materials and Methods section. Hepatocytes from fed lactating (●), fed virgin (▲) and fed weanling rats (■); oleate alone (a) and oleate plus dihydroxyacetone (b). The lines with bars indicate the S.E.M.

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