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Role of pyruvate dehydrogenase and insulin in the regulation of lipogenesis in the lactating mammary gland of the rat during the starved–refed transition

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Administration of insulin with glucose to starved lactating rats, which activates pyruvate dehydrogenase [M. A. Baxter & H. G. Coore (1978) *Biochem. J.* **174**, 553–561], restored lipogenesis in mammary gland *in vivo* to 50% of the value observed in refed (2.5h) rats. The correlations between pyruvate dehydrogenase activity and the rate of lipogenesis persisted in isolated acini. Activation of pyruvate dehydrogenase *in vitro* with dichloroacetate increased lipogenesis from [6-^{14}C]glucose in acini from starved and refed rats by 250% and 100% respectively. However, in the presence of dichloroacetate, only 70% of the increased flux through pyruvate dehydrogenase was converted into lipid in acini from starved rats, whereas all of the increase could be accounted for as lipid in acini from refed rats. Addition of insulin plus dichloroacetate was required to obtain maximal rates of lipogenesis in acini from starved rats. Similarly, insulin increased the incorporation of [1-^{14}C]acetate into lipid only in acini from starved rats. Although the activity of pyruvate dehydrogenase plays an important role in the control of mammary-gland lipogenesis, the evidence presented suggests a second regulatory site which is insulin-sensitive and is located after the generation of cytosolic acetyl-CoA.

A substantial decrease in lipogenesis in the lactating mammary gland occurs after 24h starvation. This has been shown both *in vivo*, by measurement of ^3H_2O incorporation into lipid (Robinson *et al.*, 1978), and *in vitro*, in acini, by the incorporation of ^{14}C-labelled glucose into lipid (Robinson & Williamson, 1977*a*). Refeeding chow to a starved lactating rat for 2h restores the rates of lipogenesis in the mammary gland, and this inhibition and re-activation of lipogenesis observed on starvation and refeeding correlates with the expected changes in circulating insulin concentrations (Robinson *et al.*, 1978).

It has also been reported that pyruvate dehydrogenase in the lactating mammary gland is inactivated *in vivo* by 24h starvation (Kankel & Reinauer, 1976; Baxter & Coore, 1978) and that this correlates with an increase in the phosphorylation of the pyruvate dehydrogenase complex. This appears to be a result of both an enhanced pyruvate dehydrogenase kinase activity (Baxter & Coore, 1978, 1979*a*) and a decreased pyruvate dehydrogenase phosphatase activity (Baxter & Coore, 1979*b*); consequently the proportion of the enzyme in its inactive form is increased. *In vitro* there is a large increase in lactate and pyruvate production by acini from starved rats compared with acini from fed ones (Robinson & Williamson, 1977*a*), and this confirms the inactivation of pyruvate dehydrogenase in starvation. Administration of insulin *in vivo* reverses the effects of 24h starvation on mammary-gland pyruvate dehydrogenase and re-activates the enzyme (Baxter & Coore, 1978, 1979*b*). However, it has consistently proved impossible to reproduce this effect in acini *in vitro*, where insulin has no effect on the accumulation of pyruvate or lactate, nor does it significantly relieve the inhibition of lipogenesis in acini from starved lactating rats (Robinson & Williamson, 1977*a*). There is thus some rapid short-term response to refeeding which switches on lipogenesis in the mammary gland, and from evidence *in vivo*, the signal for this response would appear to be insulin, although so far this has not been confirmed *in vitro*.

It is likely that pyruvate dehydrogenase inactivation can account for a large proportion of the decreased rate of lipogenesis in mammary gland in starvation, and the activation of the enzyme by

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insulin could well be the short-term response to refeeding that de-inhibits lipogenesis. However, it has been suggested that there might be a second site of insulin action situated within the lipogenic pathway itself. This has been indicated in acini *in vitro* by the ability of insulin to relieve the acetoacetate inhibition of glucose uptake in acini from fed rats (Robinson & Williamson, 1977*b*). A likely candidate for this second site is acetyl-CoA carboxylase, which has low activity in rat mammary gland (Baldwin & Yang, 1974) and which is activated by insulin in other tissues (Halestrap & Denton, 1973, 1974).

The aim of this work was firstly to correlate the re-activation of pyruvate dehydrogenase by insulin administration *in vivo* with any possible effect on lipogenesis in the mammary gland, and secondly, by the activation of pyruvate dehydrogenase *in vitro* with dichloroacetate (Whitehouse & Randle, 1973; Whitehouse *et al.*, 1974), to investigate whether the re-activation of this enzyme can fully account for the increase in lipogenesis observed on short-term refeeding, or whether insulin acts at other sites in the pathway of lipogenesis.

Experimental

Materials

Radioactive compounds. D-[1-^{14}C]Glucose, D-[6-^{14}C]glucose, [1-^{14}C]acetate (sodium salt) and $^{3}H_2O$ were obtained from The Radiochemical Centre, Amersham, Bucks., U.K.

Chemicals. D-Glucose, sodium acetate and sodium dichloroacetate were obtained from BDH Chemicals, Poole, Dorset, U.K.

Biochemicals. All enzymes, including collagenase (grade V), and coenzymes were obtained from Boehringer Corp. (London) Ltd., London W.5, U.K. Highly purified insulin for experiments *in vitro* was a gift from Dr. N. Lazarus, Wellcome Research Laboratories, Beckenham, Kent, U.K. Long-acting insulin (Insulin Leo Retard; Leo Laboratories, Hayes, Middx., U.K.) was used for administration to rats.

Animals. Lactating rats (10–16 days) of the Wistar strain (270–350g) with between 8 and 12 pups were used. The rats were fed *ad libitum* on Oxoid breeding diet (Oxoid, London S.E.1, U.K.) throughout lactation and were starved for 24h with the pups before the experiments.

On the day of the experiment, the rats were divided into four groups. One group received no treatment (starved control group), a second group was refed with the normal chow diet, a third group received an intragastric load of 4ml of 2M-glucose, and the fourth group received the same intragastric load together with a subcutaneous injection of 10 units of long-acting insulin. The litters were kept with mothers throughout the experiment. The feeding or other treatment commenced at 09:00h, and all rats were dissected 2½h later under pentobarbital anaesthesia (60mg/kg body wt.; solution in 0.9% NaCl). Anaesthesia was not used for the intragastric intubation.

Measurements in vivo of lipogenesis

Rats were injected intraperitoneally with 5mCi (0.5ml) of $^{3}H_2O$ at 10:30h. After 60min the animals were killed, and arterial blood was collected from the aorta into a heparinized syringe for the determination of the specific radioactivity of plasma water and blood metabolite concentrations. Duplicate weighed samples of inguinal mammary gland, liver and parametrial white adipose tissue were removed and added to 3ml of 30% (w/v) KOH. Lipid was saponified and extracted by the method of Stansbie *et al.* (1976).

Arterial blood (0.5ml) was added to 2ml of 10% (w/v) $HClO_4$ and then treated as described previously (Robinson & Williamson, 1977*a*).

Experiments in vitro with acini

Acini were prepared from the remainder of the mammary gland of each rat as described by Robinson & Williamson (1977*a*). The acini (about 100mg wet wt.) were incubated in a total volume of 4ml of Krebs–Henseleit (1932) saline containing glucose (5mM) and other additions where indicated. These were sodium acetate (2mM), sodium dichloroacetate (0.5mM) and insulin (30munits/ml). For further details see Robinson & Williamson (1977*a*).

Determination of metabolites

The following metabolites were determined by enzymic methods: glucose (Slein, 1963); L-lactate and pyruvate (Hohorst *et al.*, 1959).

Measurements of radioactivity

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

Expression of results

Incubations *in vitro* were carried out for 20, 40 and 60min, and rates of metabolite accumulation or utilization or incorporation of ^{14}C label that were not linear over this time period were discarded. Initial experiments have shown that less than 5% of the lipid in the acini which had become labelled with ^{3}H during the study *in vivo* was lost during the 60min incubation *in vitro*. Since the rate of incorporation of ^{14}C-labelled substrates was measured between 20 and 60min of the incubation, and the amount of ^{3}H label in the acini remained constant over this period, any contribution of this ^{3}H label to the total radioactivity in the lipid did not affect the measured

rate. The results in the experiments *in vitro* are expressed as μmol/min per 100mg defatted dry wt.

Results and discussion

Lipogenesis in vivo

Refeeding a starved (24h) lactating rat with chow resulted in a large increase (66-fold) in mammary-gland lipogenesis (Table 1), in agreement with previous work from this laboratory (Robinson *et al.*, 1978). There was no change in the rate of lipogenesis in adipose tissue, but that in liver increased 4-fold. An oral load of glucose given to starved lactating rats caused a smaller increase (8-fold) in mammary-gland lipogenesis and no significant change in the rate in adipose tissue or liver. It has been shown that a similar oral load of [U-^{14}C]-glucose is totally absorbed within 90min, a high proportion of the radioactivity appearing in mammary gland (L. Agius & D. H. Williamson, unpublished work). When insulin (10 units) was administered simultaneously with the glucose load, the rate of lipogenesis in mammary gland was 4 times that with an oral load of glucose alone and 50% of that in the glands of rats refed with chow. This increase in lipogenesis occurred despite marked hypoglycaemia (Table 1). The rate of lipogenesis was also significantly increased in liver and adipose tissue.

These results indicate that an oral load of glucose on its own is unable to restore lipogenesis *in vivo* in the mammary gland of the starved lactating rat to the rate observed in the refed rat. Administration of a large dose of insulin together with the glucose is required to give a stimulation of the rate of lipogenesis approaching that in the refed rat. The fact that insulin administration did not elicit a response of the same magnitude as did refeeding with chow may be due to the hypoglycaemia (Table 1) or other factors (blood flow, changes in the concentrations of amino acids or other signals generated by refeeding with the chow diet).

Glucose metabolism and lipogenesis in acini

To examine whether the alterations in mammary-gland lipogenesis *in vivo* in response to the various treatments were present *in vitro*, the metabolism of [1-^{14}C]glucose was studied in isolated acini (Table 2). Glucose uptake was not significantly altered by any of the three treatments. Compared with the starved control, an oral glucose load did not change the percentage of glucose utilized which accumulated as lactate plus pyruvate (57%), whereas administration of insulin with the glucose load decreased the percentage to a value (37%) approaching that obtained by refeeding with the chow diet (30%). These changes in lactate and pyruvate accumulation indicate a re-activation of

Table 1. *Effects of refeeding, or an oral glucose load and insulin administration on ^3H_2O incorporation into lipid in vivo in mammary gland, liver and adipose tissue of starved lactating rats*

For experimental details see the text. Results are mean values ± S.E.M. for the numbers of rats shown in parentheses. Values that are significantly different from corresponding values for control (24h-starved) lactating rats are shown: *$P < 0.01$; **$P < 0.001$.

Treatment of rats		^3H_2O incorporated (μmol/h per g wet wt. of tissue)			Blood glucose (μmol/ml)
		Mammary gland	Liver	Adipose tissue	
Control (24h starved)	(10)	2.3 ± 0.23	2.73 ± 0.23	1.05 ± 0.08	5.62 ± 0.18
Refed with chow (2.5h)	(10)	152 ± 7.9**	11.8 ± 1.9**	1.09 ± 0.13	5.71 ± 0.25
Oral glucose load (2.5h)	(5)	18.2 ± 2.8**	3.16 ± 0.29	0.76 ± 0.10	5.72 ± 0.16
Oral glucose load (2.5h) + insulin (10 units)	(4)	76.3 ± 4.1**	4.98 ± 0.46**	3.10 ± 1.2*	1.64 ± 0.20**

Table 2. *Glucose metabolism in acini from mammary glands of lactating rats*

For experimental details see the text. The acini were incubated with [1-^{14}C]glucose (5mM). Results are expressed as μmol/min per 100mg defatted dry wt., and are mean values ± S.E.M., with the numbers of rats in each group shown in parentheses. Values that are significantly different from corresponding values for the acini from the control rats are shown: *$P < 0.01$; **$P < 0.001$.

Treatment of rats ...	Control, starved (24h)	Refed with chow (2.5h)	Oral glucose load (2.5h)	Oral glucose load + insulin (2.5h)
Glucose removal	1.14 ± 0.05 (20)	1.19 ± 0.05 (17)	1.22 ± 0.14 (5)	0.98 ± 0.10 (4)
Lactate formation	1.14 ± 0.07 (20)	0.58 ± 0.05 (17)**	1.21 ± 0.12 (5)	0.60 ± 0.10 (4)**
Pyruvate formation	0.16 ± 0.01 (20)	0.11 ± 0.01 (17)**	0.19 ± 0.01 (5)	0.12 ± 0.03 (4)
[1-^{14}C]Glucose → $^{14}CO_2$	0.21 ± 0.03 (13)	0.39 ± 0.04 (12)*	0.22 ± 0.06 (5)	0.25 ± 0.04 (4)
[1-^{14}C]Glucose → lipid	0.14 ± 0.02 (13)	0.30 ± 0.03 (12)**	0.14 ± 0.04 (5)	0.20 ± 0.03 (4)

pyruvate dehydrogenase, which is inactivated in the mammary glands of starved lactating rats (Kankel & Reinauer, 1976; Baxter & Coore, 1978). Baxter & Coore (1978) have shown by direct measurements that administration of glucose and insulin *in vivo* can increase the proportion of active pyruvate dehydrogenase in mammary glands of starved lactating rats to values approaching those for fed lactating rats. In the present work, acini from lactating rats treated with glucose and insulin showed a tendency to an increased rate of lipogenesis from [1-^{14}C]glucose compared with values for starved lactating rats. In acini from rats refed with chow, the increased rate of lipogenesis (100%) was significant ($P < 0.005$; Student's t test). Thus it appears from these results and those of Baxter & Coore (1978) that there is a degree of parallelism between the activation state of pyruvate dehydrogenase and the rates of mammary-gland lipogenesis *in vivo* and *in vitro*.

Effects of activation of pyruvate dehydrogenase in vitro with dichloroacetate

A question posed by the above results is whether activation of pyruvate dehydrogenase *in vitro* can restore rates of lipogenesis from glucose in acini from starved rats. We have therefore studied the effects of dichloroacetate, which increases the proportion of active enzyme by inhibition of pyruvate dehydrogenase kinase (Whitehouse & Randle, 1973; Whitehouse *et al.*, 1974).

Addition of dichloroacetate (0.5mM) to the incubation medium resulted in an appreciable activation of pyruvate dehydrogenase, as indicated by the decreased accumulation of lactate plus pyruvate in acini from both starved lactating rats and lactating rats refed with chow (Table 3). This activation of pyruvate dehydrogenase was accompanied by substantial increases in the incorporation of [6-^{14}C]glucose into lipid (250% in acini from starved rats and 100% in refed rats). These findings confirm the key role of the activation state of pyruvate dehydrogenase in regulating the flux of glucose carbon to lipid in mammary gland. However, in the presence of dichloroacetate the rate of glucose incorporation into lipid in acini from starved rats was still below that found with acini from refed rats. The rate of oxidation of [6-^{14}C]glucose to $^{14}CO_2$ is comparatively low in acini, but it was increased 2.5-fold on addition of dichloroacetate to acini from starved lactating rats (Table 3), indicating increased oxidation of glucose carbon in the tricarboxylic acid cycle. Dichloroacetate did not alter the rate of conversion of [6-^{14}C]glucose into $^{14}CO_2$ in acini from lactating rats refed with chow.

Effects of insulin and dichloroacetate

Although activation of pyruvate dehydrogenase

Table 3. *Effects of dichloroacetate and insulin on the metabolism of glucose in acini from the mammary glands of starved and refed lactating rats*

For experimental details see the text. The acini were incubated with [6-^{14}C]glucose (5mM). Results are expressed as μmol/min per 100mg dry wt. acini and are means ± S.E.M., with the numbers of experiments in parentheses. Values that are significantly different (Student's t test) from their own control values are shown: *$P < 0.05$; **$P < 0.005$; ***$P < 0.0005$. Values for incubations in the presence of dichloroacetate and insulin that are significantly different (Student's t test) from values for incubations in the presence of dichloroacetate alone are shown: †$P < 0.05$; ††$P < 0.025$.

	[6-^{14}C]Glucose → $^{14}CO_2$	[6-^{14}C]Glucose → lipid	Pyruvate formation	Lactate formation	Glucose removal
Acini from starved lactating rats					
Control	0.011 ± 0.002 (6)	0.08 ± 0.02 (6)	0.18 ± 0.02 (11)	1.27 ± 0.10 (11)	1.17 ± 0.07 (11)
+ insulin (30 munits/ml)	0.010 ± 0.001 (6)	0.14 ± 0.03 (6)*	0.17 ± 0.01 (11)	1.36 ± 0.11 (11)	1.27 ± 0.06 (11)
+ dichloroacetate (0.5mM)	0.028 ± 0.003 (6)***	0.29 ± 0.05 (6)**	−0.01 ± 0.01 (11)***	0.35 ± 0.05 (11)***	0.88 ± 0.06 (11)**
+ insulin (30 munits/ml) + dichloroacetate (0.5mM)	0.019 ± 0.002 (6)*,††	0.54 ± 0.09 (6)***,††	0.02 ± 0.01 (11)***	0.47 ± 0.07 (11)***	1.09 ± 0.06 (11)††
Acini from refed lactating rats					
Control	0.016 ± 0.002 (4)	0.31 ± 0.04 (4)	0.12 ± 0.01 (8)	0.69 ± 0.08 (8)	1.07 ± 0.07 (8)
+ insulin (30 munits/ml)	0.014 ± 0.002 (4)	0.38 ± 0.05 (4)	0.13 ± 0.01 (8)	0.84 ± 0.10 (8)	1.14 ± 0.09 (8)
+ dichloroacetate (0.5mM)	0.016 ± 0.002 (4)	0.70 ± 0.07 (4)**	0.03 ± 0.01 (8)***	0.22 ± 0.02 (8)***	1.04 ± 0.07 (8)
+ insulin (30 munits/ml) + dichloroacetate (0.5mM)	0.013 ± 0.002 (4)	0.75 ± 0.08 (4)**	0.05 ± 0.01 (8)***	0.30 ± 0.06 (8)***	1.28 ± 0.09 (8)*,†

partially restored lipogenesis in acini from starved rats, there still appeared to be some limitation of the process. Insulin restores the impaired rate of lipogenesis in acini from cafeteria-fed rats (Agius *et al.*, 1980). We have therefore examined the effects of the hormone in the presence and absence of dichloroacetate (Table 3).

Insulin did not significantly increase glucose uptake by acini from starved rats, but it did cause a significant increase (75%; $P<0.05$) in the conversion of [6-^{14}C]glucose into lipid. There was no apparent activation of pyruvate dehydrogenase, as shown by the unchanged accumulation of lactate and pyruvate (Table 3; Robinson & Williamson, 1977*a*), and therefore the effects of the hormone on the conversion of glucose into lipid must be exerted at some other step in the pathway. The basal rate of incorporation of [6-^{14}C]glucose into lipid was 400% higher in acini from lactating rats refed with chow (2.5 h) than in acini from starved rats, and addition of insulin produced no significant change in the rate (Table 3).

Addition of insulin and dichloroacetate to incubations of acini from starved rats caused a further increase (85%) in the rate of conversion of [6-^{14}C]glucose into lipid compared with that with dichloroacetate alone (Table 3). This stimulation of lipogenesis by insulin in the presence of dichloroacetate was not observed in acini from refed rats. Insulin caused no significant decrease in the amount of lactate plus pyruvate that accumulated in any of the situations studied, and therefore this stimulation of lipogenesis by the hormone presumably occurs at a site of regulation which is after pyruvate dehydrogenase.

One problem in the interpretation of the results presented in Table 3 is that dichloroacetate depresses glucose utilization in acini from starved rats and insulin relieves this effect. To exclude the possibility that the apparent stimulation of lipogenesis by insulin was due to variations in the rate of glucose utilization by the acini, the experimental data presented in Table 3 have been expressed in Table 4 as a percentage of the rate of glucose utilization. It is clear that in acini from starved lactating rats insulin stimulated the percentage of glucose converted into lipid in both the presence and absence of dichloroacetate, and this effect was not accompanied by any change in the percentage of glucose that accumulated as lactate plus pyruvate. The hormone did not alter the percentage of glucose converted into lipid in acini from refed rats. The finding that the percentage of glucose converted into $^{14}CO_2$ is increased 4-fold when pyruvate dehydrogenase is activated with dichloroacetate in acini from starved rats suggests that there is a step in the conversion of acetyl-CoA into lipid which is limiting, and therefore increased flux through pyruvate dehydrogenase is accompanied by increased oxidation of acetyl-CoA in the tricarboxylic acid cycle. The decreased percentage of [6-^{14}C]glucose oxidized to $^{14}CO_2$ on addition of insulin, with a concomitant increase in the percentage of glucose converted into lipid, is further support for the postulate that insulin acts at a site which is after pyruvate dehydrogenase in the pathway of lipogenesis. Thus it appears that addition of dichloroacetate and insulin can restore the percentage of glucose converted into lipid in acini from starved rats to values approaching those for acini from refed rats.

In adipose tissue, dichloroacetate inhibits the

Table 4. *Effects of dichloroacetate and insulin on the percentage of glucose converted into lipid, CO_2 and the pyruvate and lactate pool by acini from starved and refed lactating rats*

The results are taken from the data given in Table 3 and are expressed as a percentage of the rate of glucose utilization. It is assumed that glucose gives rise to two C_3 units. The percentages are means ± S.E.M. with the numbers of experiments shown in parentheses. Values that are significantly different from their own control values (Student's *t* test) are shown: $^{*}P<0.05$; $^{**}P<0.005$; $^{***}P<0.0005$. Values for incubations in the presence of dichloroacetate and insulin that are significantly different (Student's paired *t* test) from values for incubations in the presence of dichloroacetate alone are shown: $\dagger P<0.05$; $\dagger\dagger P<0.005$; $\dagger\dagger\dagger P<0.0005$.

	[6-^{14}C]Glucose → $^{14}CO_2$	[6-^{14}C]Glucose → lipid	Glucose → pyruvate + lactate
Acini from starved lactating rat			
Control	0.88 ± 0.11 (6)	6.39 ± 1.26 (6)	62.7 ± 3.6 (11)
+ insulin (30 munits/ml)	0.81 ± 0.15 (6)	11.0 ± 2.7 (6)*	60.5 ± 4.1 (11)
+ dichloroacetate (0.5 mM)	3.36 ± 0.47 (6)**	33.7 ± 5.8 (6)**	21.5 ± 4.0 (11)***
+ insulin (30 munits/ml) + dichloroacetate (0.5 mM)	1.67 ± 0.18 (6)***,††	48.8 ± 7.7 (6)***,†	23.2 ± 3.1 (11)***
Acini from refed lactating rat			
Control	1.46 ± 0.33 (4)	27.1 ± 3.2 (4)	38.1 ± 3.2 (8)
+ insulin (30 munits/ml)	1.12 ± 0.17 (4)	31.2 ± 4.9 (4)	42.7 ± 4.3 (8)
+ dichloroacetate (0.5 mM)	1.31 ± 0.11 (4)	58.8 ± 3.4 (4)**	12.4 ± 1.0 (8)***
+ insulin (30 munits/ml) + dichloroacetate (0.5 mM)	0.92 ± 0.11 (4)*,†††	55.7 ± 4.1 (4)***	14.1 ± 2.7 (8)***

Table 5. *Effect of insulin on the incorporation of [1-^{14}C]acetate into lipid by acini from starved and refed lactating rats*
For experimental details see the text. The acini were incubated with glucose (5mM) and [1-^{14}C]acetate (2mM). Results are expressed as μmol/min per 100mg of dry wt. acini and are means ± S.E.M. with the numbers of experiments in parentheses. Values that are significantly different from the starved control value (Student's *t* test) are shown: *$P < 0.05$; **$P < 0.005$.

		[1-^{14}C]-Acetate→lipid	[1-^{14}C]-Acetate→CO_2	Pyruvate formation	Lactate formation	Glucose removal
Acini from starved lactating rats						
Control	(7)	0.40 ± 0.04	0.026 ± 0.006	0.23 ± 0.03	0.84 ± 0.08	1.19 ± 0.08
+ insulin (30 munits/ml)	(5)	0.58 ± 0.07*	0.017 ± 0.005	0.21 ± 0.04	1.01 ± 0.13	1.67 ± 0.18*
Acini from refed lactating rats						
Control	(7)	0.59 ± 0.04**	0.022 ± 0.005	0.12 ± 0.02**	0.38 ± 0.06**	1.62 ± 0.17*
+ insulin (30 munits/ml)	(5)	0.69 ± 0.05**	0.010 ± 0.003	0.12 ± 0.01**	0.42 ± 0.05**	1.65 ± 0.14*

conversion of glucose into lipid in the presence of insulin (Whitehouse *et al.*, 1974), which is in contrast with the present findings.

Effects of insulin on conversion of [1-^{14}C]acetate into lipid

Acetyl-CoA synthetase (EC 6.2.1.1), which catalyses the conversion of acetate into acetyl-CoA, is mainly located (90%) in the cytosol of rat mammary gland (Buckley, 1974). Thus when acetate is the substrate it will provide a supply of acetyl-CoA in the cytoplasm for lipogenesis and by-pass those steps involved in the transport of acetyl-CoA generated in the mitochondria from glucose, i.e. citrate synthase (EC 4.1.3.7), citrate transport out of the mitochondria and ATP citrate lyase (EC 4.1.3.8). We have therefore examined the effects of insulin on the conversion of [1-^{14}C]acetate into lipid to obtain further information on the site of action of the hormone.

The incorporation of [1-^{14}C]acetate into lipid in acini from refed rats was significantly greater (50%; $P < 0.005$) than in acini from starved rats (Table 5). Addition of insulin increased the incorporation in acini from starved rats to a value similar to that for acini from refed rats. There was, however, no significant change in the rate of incorporation of [1-^{14}C]acetate into lipid in acini from refed rats on addition of insulin (Table 5). This finding is further evidence for a site of insulin action on lipogenesis which is after pyruvate dehydrogenase and is likely to be located after the generation of cytosolic acetyl-CoA.

There are, however, two criticisms of this interpretation: one is that the activity of acetyl-CoA synthetase may limit the utilization of acetate, and the present experiments do not exclude a stimulatory effect of insulin on the enzyme. Second, the formation of acetyl-CoA from acetate in the cytosol does not generate reducing equivalents (NADPH) via the pyruvate–malate cycle. Thus lipogenesis from acetate is dependent on the pentose phosphate pathway for provision of reducing equivalents. In acini from starved rats the rate of glucose utilization in the presence of acetate was 29% lower than in acini from refed rats (Table 5). Addition of insulin removed this difference, and therefore it might be argued that insulin stimulates membrane transport of glucose in acini from starved rats, and thus increases the availability of NADPH and the conversion of acetate into lipid. Against this argument is the fact that insulin did not significantly increase glucose utilization in acini from starved rats when it was the sole substrate (Table 3).

Conclusions

These experiments provide further evidence that a major factor responsible for the decreased rate of lipogenesis in rat mammary gland during starvation is the inactivation of pyruvate dehydrogenase. The activity of the enzyme can be restored *in vivo* with insulin (Baxter & Coore, 1978) and *in vitro* with dichloroacetate but not with insulin. In both situations there is a parallel increase in the rate of lipogenesis. However, the finding that when pyruvate dehydrogenase is activated in acini from starved rats the addition of insulin produces a further stimulation of the rate of lipogenesis suggests that the hormone acts at a site in the pathway of lipogenesis which is after pyruvate dehydrogenase. By analogy to studies on the regulation of lipogenesis by insulin in adipose tissue (Halestrap & Denton, 1973, 1974; Stansbie *et al.*, 1976), a likely site for the action of the hormone is acetyl-CoA carboxylase.

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