Evidence that the stimulation of lipogenesis in the mammary glands of starved lactating rats re-fed with a chow diet is dependent on continued hepatic gluconeogenesis during the absorptive period

Effects of a gluconeogenic inhibitor, mercaptopicolinic acid, *in vivo*

Dermot H. WILLIAMSON, Vera ILIC and Richard G. JONES*

Metabolic Research Laboratory, Nuffield Department of Clinical Medicine, Radcliffe Infirmary,
Woodstock Road, Oxford OX2 6HE, U.K.

(Received 30 January 1985/5 March 1985; accepted 12 March 1985)

1. The rapid stimulation of lipogenesis in mammary gland that occurs on re-feeding starved lactating rats with a chow diet was decreased (60%) by injection of mercaptopicolinic acid, an inhibitor of hepatic gluconeogenesis at the phosphoenolpyruvate carboxykinase step. Mercaptopicolinate had no effect on lipogenesis in mammary glands of fed lactating rats. 2. The inhibition of lipogenesis persisted *in vitro* when acini from mammary glands of re-fed rats treated with mercaptopicolinate were incubated with [$1\text{-}^{14}\text{C}$]glucose. Mercaptopicolinate added *in vitro* had no significant effect on lipogenesis in acini from starved–re-fed lactating rats. 3. Mercaptopicolinate prevented the deposition of glycogen and increased the rate of lipogenesis in livers of starved–re-fed lactating rats, whereas it had no significant effect on livers of fed lactating rats. 4. Administration of intraperitoneal glucose restored the rate of mammary-gland lipogenesis in re-fed rats treated with mercaptopicolinate to the values for re-fed rats. Hepatic glycogen deposition was also restored, and the rate of hepatic lipogenesis was stimulated 5-fold. 5. It is concluded that stimulation of mammary-gland lipogenesis on re-feeding with a chow diet after a period of starvation is in part dependent on continued hepatic gluconeogenesis during the absorptive period. Possible sources of the glucose precursors are discussed.

Two major biosynthetic pathways in the lactating mammary gland of the rat, namely lipogenesis and lactose synthesis, require the continual supply of glucose to the gland and are very sensitive to the amount of dietary carbohydrate consumed. Removal of food for a period as short as 6 h decreases by 80–90% the rate of lipogenesis (Williamson *et al.*, 1983) and lactose synthesis (Carrick & Kuhn, 1978) in the gland. On re-feeding with chow diet, the rates of both processes rapidly (in 2 h) return to those observed in the fed state (Wilde & Kuhn, 1979; Williamson *et al.*, 1983). Similar findings have been reported after longer periods (24 h) of starvation (Robinson *et al.*, 1978; Munday & Williamson, 1981; Bussmann *et al.*, 1984). In contrast, the rate of lipogenesis in livers of lactat-

ing rats does not return to normal on short-term re-feeding (Robinson *et al.*, 1978; Munday & Williamson, 1981), nor is it responsive to infusion of physiological amounts of insulin (Jones *et al.*, 1984).

A number of groups have presented convincing evidence that a considerable proportion of the glycogen synthesized in the livers of starved (24 h) non-lactating rats after administration of an oral or intragastric load of glucose is not derived directly from the exogenous glucose but instead is synthesized from C_3 intermediates via the gluconeogenic pathway (Newgard *et al.*, 1983, 1984; Niewoehner *et al.*, 1984; Sugden *et al.*, 1983). This implies that, after re-feeding, hepatic gluconeogenesis continues for a significant period, despite the availability of exogenous glucose.

In the present work we have addressed the question of whether hepatic gluconeogenesis continues when starved lactating rats are re-fed with a chow

* Present address: Professorial Medical Unit, University of Leeds, Leeds General Infirmary, Leeds LS1 3EX, U.K.

diet and whether this process contributes glucose for the 'switch-on' of lipogenesis in the lactating mammary gland. To provide information on this question, we have studied the effects of mercaptopicolinic acid, an inhibitor of gluconeogenesis, at the phosphoenolpyruvate carboxykinase (EC 4.1.1.49) step (DiTullio *et al.*, 1974) on the metabolic response of liver and mammary gland of starved lactating rats to natural re-feeding. The rationale for these experiments was the finding that mercaptopicolinate inhibits glycogen deposition and stimulates lipogenesis in livers of starved non-lactating rats given an oral or intragastric load of glucose (Sugden *et al.*, 1983; Newgard *et al.*, 1984).

Experimental

Materials

Rats. Lactating Wistar rats with nine or ten pups were used at peak lactation (10–16 days). The rats were fed *ad libitum* on commercial chow diet, PRM [E. Dixon and Sons (Ware) Ltd., Ware, Herts., U.K.] containing approx. 48% (w/w) carbohydrate and were housed in individual cages. The lighting was on in the animal house from 07:30 to 19:30 h. Starved rats had their food withdrawn for 24 h, but had free access to water and the pups were present.

Biochemicals. All enzymes, including collagenase (grade II) and coenzymes, were from Boehringer Corp. (London) Ltd., Lewes, Sussex, U.K. Mercaptopicolinic acid was a gift from Dr. H. L. Saunders, Smith Kline and French Ltd., Philadelphia, PA, U.S.A.

Radioactive compounds. $^3\text{H}_2\text{O}$ and D-[1- ^{14}C]-glucose were obtained from Amersham International, Amersham, Bucks., U.K.

Studies in vivo

Treatment of rats. All experiments were commenced between 09:00 h and 10:00 h. Where indicated, lactating rats were starved for 24 h and were then given 5 g of chow diet (equivalent to about 2.4 g of carbohydrate) as pellets; this was consumed within 10–15 min. Chow diet was chosen for re-feeding because oral administration of glucose after a period of starvation produces only a small increase in mammary-gland lipogenesis (Munday & Williamson, 1981; Bussmann *et al.*, 1984). Mercaptopicolinic acid [1 ml of 4% (w/v) neutralized with NaHCO_3] and glucose (3 ml of 2 M) were injected intraperitoneally as indicated.

Measurements of lipogenesis. The rate of lipogenesis in mammary gland and liver *in vivo* was measured by the incorporation of $^3\text{H}_2\text{O}$ into saponified lipid essentially by the method of Stansbie *et al.* (1976), with the modifications described by Robinson *et al.* (1978). At 30 min after

the start of re-feeding, rats were injected intraperitoneally with 3 mCi (0.3 ml) of $^3\text{H}_2\text{O}$ and were then dissected 60 min later under pentobarbitol anaesthesia (60 mg/kg body wt.; solution in 0.9% NaCl).

Tissue metabolites. Liver and mammary-gland metabolites were determined by rapid removal of a piece of tissue (about 2 g) from an anaesthetized rat and clamping it between light-alloy tongs cooled in liquid N_2 (Wollenberger *et al.*, 1960). The frozen tissue was then treated as described by Robinson & Williamson (1977), and the metabolites were determined in the neutralized tissue extract.

Collection of blood. Arterial blood was collected from the abdominal aorta into heparinized syringes. A sample (0.5 ml) for blood metabolites was added to 2 ml of 10% (w/v) HClO_4 and then treated as described previously (Robinson & Williamson, 1977). Plasma was separated by centrifugation at 3000 rev./min for 10 min.

Studies in vitro

Experiments with acini. Acini were prepared from the mammary glands as described by Robinson & Williamson (1977). The acini (about 100 mg wet wt.) were incubated in a total volume of 4 ml of Krebs–Henseleit (1932) saline containing [1- ^{14}C]-glucose (5 mM) and mercaptopicolinate (2 mM) where indicated. For further details see Robinson & Williamson (1977).

Determination of metabolites and insulin. Before analysis, the neutralized extracts of mammary tissue were filtered through 0.45 μm -pore-size type HA Millipore filters (Millipore S.A., Molsheim, France) to remove fat globules. The following metabolites were determined by enzymic methods: glucose (Slein, 1963); L-lactate (Hohorst, 1963); glucose 6-phosphate and ATP (Lamprecht & Trautschold, 1963); glycogen (Keppler & Decker, 1974); dihydroxyacetone phosphate, glyceraldehyde 3-phosphate and fructose 1,6-bisphosphate (Michal & Beutler, 1974).

Plasma insulin was determined by radioimmunoassay with a rat insulin standard (Albano *et al.*, 1972).

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

Statistics

Results are presented as means $\pm$ S.E.M. Statistical differences were tested by Student's unpaired *t* test.

Results and discussion

Effects of mercaptopicolinate on lipogenesis in vivo

Re-feeding chow diet (5 g) to lactating rats that had been starved for 24 h produced within 90 min the expected large increase (about 20-fold) in mam-

mary-gland lipogenesis *in vivo* (see also Robinson *et al.*, 1978; Munday & Williamson, 1981; Bussmann *et al.*, 1984), but no significant stimulation of hepatic lipogenesis (Table 1). Administration of mercaptopicolinate to starved re-fed rats considerably decreased the stimulation of mammary-gland lipogenesis, but stimulated hepatic lipogenesis by 140% (Table 1). A similar increase in hepatic lipogenesis was observed when starved non-lactating rats given an oral glucose load (0.36/100 g body wt.) were injected with mercaptopicolinate (Sugden *et al.*, 1983). When mercaptopicolinate was administered to fed lactating rats, there was no significant change in the rates of lipogenesis in mammary gland or liver (Table 1). These results suggest that the effects of mercaptopicolinate on lipogenesis are associated with re-feeding.

Stimulation of mammary-gland lipogenesis by infusion of physiological amounts of insulin results in a lowering of the mammary-gland content of glucose and glucose 6-phosphate, and this has been interpreted as indicating an activation of the glycolytic pathway at the level of phosphofructokinase (Jones *et al.*, 1984). In the present experiments, re-

feeding with chow diet produced a highly significant ($P < 0.001$) decrease in the mammary-gland content of these two metabolites compared with glands of starved rats, whereas administration of mercaptopicolinate attenuated this decrease (Table 2). However, there was no significant difference between the plasma insulin in the re-fed rats (14.7 ± 4.9 μ units/ml; $n = 8$) and those re-fed but treated with mercaptopicolinate (13.4 ± 5.1 μ units/ml; $n = 9$). In the fed state mercaptopicolinate did not significantly change the mammary-gland content of glucose or glucose 6-phosphate. The mammary-gland content of ATP or lactate did not alter significantly when mercaptopicolinate was administered in any of the situations studied (Table 2).

Lipogenesis in vitro

Previous studies have shown that there is a good correlation between rates of mammary-gland lipogenesis *in vivo* and the rates of this process in isolated mammary-gland acini incubated with glucose (see Robinson & Williamson, 1977; Munday & Williamson, 1981). We have therefore

Table 1. *Effects of mercaptopicolinate on rates of lipogenesis in vivo in mammary gland and liver of fed lactating rats and starved lactating rats re-fed with chow diet*

Three groups of lactating rats were studied: (a) fed, (b) starved (24h), (c) starved (24h) and then re-fed with 5.0g of chow diet (90min). The fed and starved-re-fed rats were injected intraperitoneally with either 1.0ml of 0.9% NaCl or 1.0ml of 4% mercaptopicolinic acid. For further details see the text. The results are expressed as μ mol of $^3\text{H}_2\text{O}$ incorporated into saponified lipid/h per g fresh wt. and are the mean values $\pm$ S.E.M. for the numbers of observations in parentheses. For the purposes of comparison, the values for starved (24h) rats have been taken from Jones *et al.* (1984). Values for the rats treated with mercaptopicolinate that are significantly different by Student's *t* test from the corresponding saline-treated rats are shown: ** $P < 0.001$.

Treatment of rats	Rates of lipogenesis	
	Mammary gland	Liver
Starved (24h) (4)	2.7 ± 0.4	4.0 ± 1.3
Re-fed (90min) (7)	60.7 ± 7.0	4.4 ± 0.6
Re-fed (90min) plus mercaptopicolinate (10)	$20.1 \pm 4.4^{**}$	$10.5 \pm 0.7^{**}$
Fed (5)	87.1 ± 19.6	17.9 ± 3.3
Fed plus mercaptopicolinate (5)	70.8 ± 4.6	23.6 ± 2.6

Table 2. *Effects of mercaptopicolinate on the contents of glucose, glucose 6-phosphate, lactate and ATP in mammary glands of fed lactating rats and starved lactating rats re-fed with chow diet*

For experimental details see Table 1 and the text. The results are mean values $\pm$ S.E.M. with the numbers of observations in parentheses. Metabolite contents are expressed as μ mol/g fresh wt. of mammary gland. For the purposes of comparison, the values for starved (24h) rats have been taken from Jones *et al.* (1984). Values for rats treated with mercaptopicolinate that are significantly different from the corresponding saline-treated rats are shown: * $P < 0.05$.

Treatment of rats	Glucose			
	Glucose	6-phosphate	Lactate	ATP
Starved (24h) (6)	2.02 ± 0.14	0.190 ± 0.012	2.22 ± 0.40	1.03 ± 0.19
Re-fed (90min) (9)	1.00 ± 0.13	0.098 ± 0.013	1.99 ± 0.25	1.07 ± 0.16
Re-fed (90min) plus mercaptopicolinate (8)	$1.40 \pm 0.14^*$	$0.148 \pm 0.016^*$	2.25 ± 0.72	1.06 ± 0.17
Fed (5)	1.38 ± 0.17	0.147 ± 0.026	0.90 ± 0.22	1.30 ± 0.04
Fed plus mercaptopicolinate (5)	1.57 ± 0.09	0.156 ± 0.012	1.72 ± 0.67	1.28 ± 0.10

examined whether the effects of mercaptopicolinate on mammary-gland lipogenesis persist *in vitro* and whether this compound inhibits the process in isolated acini (Table 3). There was no significant difference between the rate of glucose utilization by acini isolated from glands of re-fed rats and those from glands of re-fed rats injected with mercaptopicolinate (Table 3). However, the rate of conversion of [1- 14 C]glucose into 14 CO $_2$ and 14 C-labelled lipid was considerably lower (60%) in acini isolated from glands of starved-re-fed rats treated with mercaptopicolinate (Table 3), indicating lower activities of the pentose phosphate and lipogenic pathways. Addition of mercaptopicolinate (2mM) *in vitro* tended to decrease removal of [1- 14 C]glucose and its conversion into 14 CO $_2$ and 14 C-labelled lipid, but the small changes (less than 10%) were not significant even when the comparison was made by a paired *t* test. Lactate formation was significantly decreased (about 15%; $P < 0.05$) by the addition of mercaptopicolinate. In relation to this finding, it is of interest that in rat hemi-diaphragm mercaptopicolinate increased conversion of [1- 14 C]pyruvate into 14 CO $_2$ (Palmer *et al.*, 1984), suggesting a lack of specificity in its metabolic effects. The present results indicate that the inhibition by mercaptopicolinate of the stimulation of mammary-gland lipogenesis on re-feeding persists *in vitro* and that the inhibitor has no appreciable direct effect *in vitro*, when glucose is the substrate. MacDonald & Grewe (1981) found that mercaptopicolinate (0.5mM) did not inhibit lipogenesis in rat adipocytes incubated with glucose.

Effects of mercaptopicolinate in hepatic metabolism

Re-feeding starved lactating rats for 90min increased the liver glycogen content about 6-fold; this increase was prevented by the administration

of mercaptopicolinate (Table 4). A similar effect of mercaptopicolinate was reported on the deposition of liver glycogen in starved non-lactating rats given an oral or intragastric load of glucose (Sugden *et al.*, 1983; Newgard *et al.*, 1984). In the present experiments the inhibition of glycogen synthesis was accompanied by decreased hepatic contents of glucose 6-phosphate and glucose. In addition, the difference between the hepatic and blood concentrations of glucose in starved-re-fed rats treated with mercaptopicolinate was 0.41 μ mol, whereas in the untreated rats it was 1.49 μ mol, which would favour a higher output of glucose from the liver. At first sight it may seem surprising that the blood glucose did not fall lower when mercaptopicolinate was administered to re-fed rats and hepatic gluconeogenesis was inhibited (Table 4). However, the rate of lipogenesis in the mammary gland was low (Table 1), indicating low carbohydrate consumption, and presumably sufficient glucose was still being absorbed by the intestinal cell to maintain the requirements of the peripheral tissues. Mercaptopicolinate had no significant effect on any of the parameters measured in livers of fed lactating rats (Table 4). It appears from these results that in lactating rats, as in non-lactating rats, the direction of flux in the liver on re-feeding after a period of starvation is towards glucose and glycogen synthesis from C $_3$ intermediates. A key question is whether, on re-feeding a lactating rat, glucose efflux from the liver continues.

Effects of intraperitoneal glucose

If production of glucose by the liver during re-feeding is important to supply the mammary gland and 'switch-on' lipogenesis, then the inhibition of this process by mercaptopicolinate should be relieved by a source of glucose independent of that

Table 3. *Effects of mercaptopicolinate on rates of glucose utilization and lipogenesis in mammary-gland acini isolated from starved lactating rats*

Starved (24h) lactating rats were re-fed with chow diet (5g), and were injected 10min later with either 1 ml of 0.9% NaCl or 1.0ml of 4% mercaptopicolinate, and were then killed 90min later. The isolated acini were incubated with [1- 14 C]glucose (5mM) and mercaptopicolinate (2mM) where indicated (+). Results are expressed as μ mol/min per 100mg defatted dry wt., and are mean values $\pm$ S.E.M., with the numbers of rats shown in parentheses. Values for mercaptopicolinate-treated rats that are significantly different from the corresponding values for acini from control rats are shown: * $P < 0.05$.

Measurement	Treatment of rats			
	Re-fed (90min) (4)		Re-fed (90min) plus mercaptopicolinate (4)	
	–	+	–	+
Glucose removal	1.09 $\pm$ 0.06	1.04 $\pm$ 0.10	0.89 $\pm$ 0.10	0.82 $\pm$ 0.11
Glucose $\rightarrow$ 14 CO $_2$	0.23 $\pm$ 0.01	0.21 $\pm$ 0.02	0.10 $\pm$ 0.04*	0.09 $\pm$ 0.04*
Glucose $\rightarrow$ 14 C-labelled lipid	0.23 $\pm$ 0.02	0.20 $\pm$ 0.01	0.10 $\pm$ 0.03*	0.09 $\pm$ 0.02*
Lactate formation	0.94 $\pm$ 0.19	0.82 $\pm$ 0.19	1.02 $\pm$ 0.16	0.83 $\pm$ 0.11

Table 4. Effects of mercaptopicolinate on contents of blood and liver metabolites in fed lactating rats and starved lactating rats re-fed with chow diet

For experimental details see Table 1 and the text. The results are mean values $\pm$ S.E.M., with the numbers of observations in parentheses. Metabolites are expressed as $\mu\text{mol/ml}$ of whole blood or $\mu\text{mol/g}$ fresh wt. of liver. For the purposes of comparison, the values for starved (24h) rats have been taken from Jones *et al.* (1984). Values for rats treated with mercaptopicolinate that are significantly different by Student's *t* test from the corresponding saline-treated rats are shown: ***P* < 0.001.

Treatment of rats	Blood		Liver			
	Glucose	Lactate	Glucose		Glycogen	ATP
			Glucose	6-phosphate		
Starved (24h) (4)	5.03 $\pm$ 0.28	0.75 $\pm$ 0.04	6.00 $\pm$ 0.089	0.220 $\pm$ 0.089	8.0 $\pm$ 3.0	1.63 $\pm$ 0.66
Re-fed (90 min) (7)	5.32 $\pm$ 0.28	2.17 $\pm$ 0.43	6.81 $\pm$ 0.25	0.253 $\pm$ 0.048	46.9 $\pm$ 8.9	1.33 $\pm$ 0.30
Re-fed (90 min) plus mercaptopicolinate (10)	4.92 $\pm$ 0.21	2.53 $\pm$ 0.30	5.33 $\pm$ 0.22**	0.050 $\pm$ 0.009**	10.7 $\pm$ 4.7**	1.83 $\pm$ 0.16
Fed (5)	5.10 $\pm$ 0.24	2.43 $\pm$ 0.76	6.09 $\pm$ 0.57	0.381 $\pm$ 0.043	169 $\pm$ 23	2.12 $\pm$ 0.42
Fed plus mercaptopicolinate (5)	5.59 $\pm$ 0.21	1.94 $\pm$ 0.25	6.19 $\pm$ 0.57	0.459 $\pm$ 0.082	136 $\pm$ 24	1.53 $\pm$ 0.17
						2.30 $\pm$ 0.44
						2.07 $\pm$ 0.11

provided by the liver or the intestine. Intraperitoneal administration of glucose (1.08 g) to starved lactating rats gave a rate of mammary-gland lipogenesis that was not significantly different from that found on re-feeding starved lactating rats treated with mercaptopicolinate, although the blood glucose was increased by 124% (Table 5). The starved rats injected with glucose had a hepatic glycogen content which was not significantly different from the value (cf. Tables 4 and 5) for rats re-fed with chow, which indicates that synthesis of hepatic glycogen was able to proceed at a normal rate. That appreciable stimulation of mammary-gland lipogenesis did not occur despite an increase in plasma insulin (results not shown) and glucose availability implies that natural re-feeding might release additional signals which directly activate the mammary gland (Williamson *et al.*, 1983). Further support for this view is the finding that similar loads of glucose administered by gastric intubation are not as effective as natural re-feeding in 'switching-on' mammary-gland lipogenesis after a period of starvation (Munday & Williamson, 1981; Bussmann *et al.*, 1984). Surprisingly, intubation of powdered chow diet caused little stimulation of mammary-gland lipogenesis (Bussmann *et al.*, 1984), and this was ascribed to the possible importance of salivary amylase in initiating starch hydrolysis. It appears that for maximal stimulation of mammary-gland lipogenesis there is a requirement for natural re-feeding and a high rate of glucose delivery to the gland. It is likely that the higher blood glucose (11 mM) when glucose is injected intraperitoneally overcomes any limitation of hepatic glucokinase (Newgard *et al.*, 1983), and conversion of glucose into glycogen proceeds via the direct route. Support for this view is the finding that the deposition of hepatic glycogen was restored in starved-re-fed rats treated with mercaptopicolinic acid and intraperitoneal glucose (Table 5), where gluconeogenic flux is presumably still inhibited at the level of phosphoenolpyruvate carboxykinase. Intraperitoneal injection of glucose in starved lactating rats treated with mercaptopicolinate and re-fed with a chow diet produced a 3-fold stimulation of mammary-gland lipogenesis and a 2-fold increase in liver lipogenesis (Table 5); the rate of mammary-gland lipogenesis was not significantly different from that observed on re-feeding alone (Table 1). The stimulation of mammary-gland lipogenesis was accompanied by significant decreases in the gland contents of glucose and glucose 6-phosphate (Table 5) and of the [glucose 6-phosphate]/fructose 1,6-bisphosphate ratio from 4.4 ± 1.0 to 1.2 ± 0.1 ($n = 4$; $P < 0.005$), compared with glands from starved rats injected intraperitoneally with glucose. This latter finding suggests that stimulation

Table 5. *Effects of intraperitoneal administration of glucose on the inhibition by mercaptopicolinate of mammary-gland lipogenesis in starved lactating rats re-fed with chow diet*
 For experimental details see Table 1 and the text. The dose of glucose (intraperitoneal) was 3 ml of 2M. The results are mean values $\pm$ S.E.M., with the numbers of observations in parentheses. Metabolite contents are expressed as μ mol/g fresh wt. mammary gland or liver and rates of lipogenesis as μ mol of $^3\text{H}_2\text{O}$ incorporated into saponified lipid/h per g fresh wt. Results for starved rats re-fed with chow and injected with mercaptopicolinate have been given for comparison (Tables 1, 2 and 4). Values for re-fed rats treated with mercaptopicolinate and glucose that are significantly different from those for starved rats given only glucose are shown: * $P < 0.05$; ** $P < 0.001$.

Treatment of rats	Rate of lipogenesis			Mammary-gland metabolites			
	Mammary gland	Liver	Blood glucose	Liver glycogen	Glucose	Glucose 6-phosphate	Fructose 1,6-bisphosphate
Glucose only (5)	26.1 $\pm$ 5.0	4.7 $\pm$ 0.5	11.0 $\pm$ 1.30	58.5 $\pm$ 12.5	4.15 $\pm$ 1.29	0.135 $\pm$ 0.024	0.030 $\pm$ 0.006
Re-fed (90 min) plus mercaptopicolinate (10)	20.1 $\pm$ 4.4	10.5 $\pm$ 0.7	4.92 $\pm$ 0.21	10.7 $\pm$ 4.7	1.40 $\pm$ 0.14	0.148 $\pm$ 0.016	—
Re-fed (90 min) plus mercaptopicolinate plus glucose (6)	67.0 $\pm$ 5.7**	21.8 $\pm$ 6.1**	7.78 $\pm$ 1.03	54.6 $\pm$ 6.6	1.57 $\pm$ 0.36*	0.044 $\pm$ 0.007**	0.041 $\pm$ 0.010

of mammary-gland lipogenesis is accompanied by activation of phosphofructokinase and/or glucose 6-phosphate dehydrogenase, perhaps through the action of the extra signal(s) suggested above.

Conclusions

The evidence presented here, in particular the reversal of the mercaptopicolinate inhibition of the 'switch-on' of mammary-gland lipogenesis by intraperitoneal administration of glucose, suggests that in the immediate period after re-feeding a chow diet to a starved lactating rat the liver remains in a gluconeogenic mode and that glucose is still released from the liver to supply peripheral tissues, especially the lactating mammary gland. The alternative explanation, that mercaptopicolinate depresses absorption of dietary glucose, would seem to be ruled out by the fact that it has no significant effect on mammary-gland lipogenesis in the fed state, where dietary carbohydrate is the main source of substrate for lipogenesis in the gland.

Despite recent findings in rat hemi-diaphragm which indicate that mercaptopicolinate has effects on glucose metabolism which cannot solely be explained by inhibition of phosphoenolpyruvate carboxykinase (Palmer *et al.*, 1984), the evidence presented here strongly suggests that the observed effects are the result of inhibition of this enzyme in liver.

A key question is the source of carbon for the continuation of hepatic gluconeogenesis on re-feeding with the chow diet. Possible precursors include the dietary glucogenic amino acids, the production of lactate from glucose in the intestine during absorption (Nicholls *et al.*, 1983), and the release of lactate (and pyruvate) from peripheral tissues. The present experiments provide no direct information on the relative importance of these sources; however, there is evidence for the continued release of lactate and pyruvate by the mammary gland. After 24 h starvation the activation state of pyruvate dehydrogenase in mammary gland is greatly decreased (Kankel & Reinauer, 1976; Baxter & Coore, 1978), and lactate and pyruvate are released (Hawkins & Williamson, 1972; Robinson & Williamson, 1977) at an appreciable rate (about 400 μ mol/h per total mammary tissue; Williamson & Robinson, 1977). Short-term re-feeding (2 h) with a chow diet does not appear to bring about complete re-activation of pyruvate dehydrogenase in mammary tissue, because pyruvate is still released from the gland (Robinson & Williamson, 1978). Thus continuation of hepatic gluconeogenesis during re-feeding allows the removal of pyruvate produced by the gland (and other tissues), which might otherwise accumulate if the liver was immediately switched to a glycolytic mode. The high glucagon/insulin ratio in the

plasma of the lactating rat (Robinson *et al.*, 1978) would favour this mechanism and also the continued release of glucose from the liver. The reason why lactate and pyruvate do not accumulate when gluconeogenesis is inhibited with mercaptopicolinate is presumably the diversion of carbon to hepatic lipogenesis and complete oxidation.

We thank Mr. S. Mercer for help with some of the experiments and Mrs. M. Barber for secretarial assistance. R. G. J. held an M.R.C. Training Fellowship, and D. H. W. is a member of the M.R.C. External Scientific Staff.

References

- Albano, J. D. M., Ekins, R. P., Maritz, G. & Turner, R. C. (1972) *Acta Endocrinol. (Copenhagen)* **70**, 487–509
- Baxter, M. A. & Coore, H. G. (1978) *Biochem. J.* **174**, 553–561
- Bussmann, L. E., Ward, S. & Kuhn, N. J. (1984) *Biochem. J.* **219**, 173–180
- Carrick, D. T. & Kuhn, N. J. (1978) *Biochem. J.* **174**, 319–325
- DiTullio, N. W., Berkoff, C. E., Blank, B., Kostos, V., Stack, E. J. & Saunders, H. L. (1974) *Biochem. J.* **138**, 387–394
- Hawkins, R. A. & Williamson, D. H. (1972) *Biochem. J.* **129**, 1171–1173
- Hohorst, H. J. (1963) in *Methods of Enzymatic Analysis* (Bergmeyer, H. U., ed.), pp. 215–219, Academic Press, New York and London
- Jones, R. G., Ilic, V. & Williamson, D. H. (1984) *Biochem. J.* **223**, 345–351
- Kankel, K.-F. & Reinauer, H. (1976) *Diabetologia* **12**, 149–154
- Keppler, D. & Decker, K. (1974) in *Methods of Enzymatic Analysis* (Bergmeyer, H. U., ed.), pp. 1127–1131, Academic Press, New York and London
- Krebs, H. A. & Henseleit, K. (1932) *Hoppe-Seyler's Z. Physiol. Chem.* **210**, 33–66
- Lamprecht, W. & Trautschold, I. (1963) in *Methods of Enzymatic Analysis* (Bergmeyer, H. U., ed.), pp. 543–551, Academic Press, New York and London
- MacDonald, M. J. & Grewe, B. K. (1981) *Biochim. Biophys. Acta* **663**, 302–313
- Michal, G. & Beutler, H.-O. (1974) in *Methods of Enzymatic Analysis* (Bergmeyer, H. U., ed.), pp. 1314–1319, Academic Press, New York and London
- Munday, M. R. & Williamson, D. H. (1981) *Biochem. J.* **196**, 831–837
- Newgard, C. B., Hirsch, L. J., Foster, D. W. & McGarry, J. D. (1983) *J. Biol. Chem.* **258**, 8046–8052
- Newgard, C. B., Moore, S. V., Foster, D. W. & McGarry, J. D. (1984) *J. Biol. Chem.* **259**, 6958–6963
- Nicholls, T. J., Leese, H. J. & Bronk, J. R. (1983) *Biochem. J.* **212**, 183–187
- Niewoehner, C. B., Gilboe, D. P. & Nuttall, F. Q. (1984) *Am. J. Physiol.* **246**, E89–E94
- Palmer, T. N., Caldecourt, M. A., Warner, J. P. & Sugden, M. C. (1984) *Biochem. J.* **224**, 971–976
- Robinson, A. M. & Williamson, D. H. (1977) *Biochem. J.* **168**, 465–474
- Robinson, A. M. & Williamson, D. H. (1978) *Biochem. Soc. Trans.* **6**, 1316–1318
- Robinson, A. M., Girard, J. R. & Williamson, D. H. (1978) *Biochem. J.* **176**, 343–346
- Slein, M. W. (1963) in *Methods of Enzymatic Analysis* (Bergmeyer, H. U., ed.), pp. 117–123, Academic Press, New York and London
- Stansbie, D., Brownsey, R. W., Crettaz, M. & Denton, R. M. (1976) *Biochem. J.* **160**, 413–416
- Sugden, M. C., Watts, D. I., Palmer, T. N. & Myles, D. D. (1983) *Biochem. Int.* **7**, 329–337
- Wilde, C. J. & Kuhn, N. J. (1979) *Biochem. J.* **182**, 287–294
- Williamson, D. H. & Robinson, A. M. (1977) *Biochem. Soc. Trans.* **5**, 829–834
- Williamson, D. H., McKeown, S. R. & Ilic, V. (1975) *Biochem. J.* **150**, 145–152
- Williamson, D. H., Munday, M. R., Jones, R. G., Roberts, A. F. C. & Ramsey, A. J. (1983) *Adv. Enzyme Regul.* **21**, 135–145
- Wollenberger, A., Ristau, Q. & Schoffa, G. (1960) *Plügers Arch. Gesamte Physiol. Menschen Tiere* **270**, 399–412