Biochem. J. (1982) **204**, 417–423
Printed in Great Britain

Effects of inhibition of protein synthesis by cycloheximide on lipogenesis in mammary gland and liver of lactating rats

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(*Received 16 November 1981/Accepted 28 January 1982*)

1. Administration of cycloheximide (an inhibitor of protein synthesis) to lactating rats raised the concentrations of amino acids, and in particular, the branched-chain amino acids (valine, leucine and isoleucine) in blood, liver and mammary gland. 2. Inhibition of protein synthesis increased the incorporation *in vivo* of L-[U-^{14}C]leucine into lipids of mammary gland and liver. 3. Cycloheximide treatment caused no immediate change in the overall rate of lipogenesis *in vivo* (measured with ^3H_2O) in mammary gland but increased the rate in liver 3-fold; this latter effect also occurred in livers of virgin rats. 4. The increased rate of hepatic lipogenesis was not accompanied by significant changes in the plasma insulin concentration or the activity of acetyl-CoA carboxylase. 5. Although cycloheximide decreased the entry of total triacylglycerol into the circulation it did not alter the rate of secretion of newly synthesized saponifiable lipid. 6. Cycloheximide slightly stimulated lipogenesis from endogenous substrates in isolated hepatocytes, but this effect was abolished when lactate was the exogenous substrate. 7. Administration of cycloheximide to virgin rats decreased liver glycogen and increased the hepatic content of glucose 6-phosphate, pyruvate and lactate. 8. It is concluded that (*a*) there is no short-term link between the rate of protein synthesis and lipogenesis in the lactating mammary gland and (*b*) the increased rate of hepatic lipogenesis in cycloheximide-treated rats is mainly due to stimulation of glycogenolysis, glycolytic flux and consequent increased availability of pyruvate.

During lactation the mammary gland of the rat is a major site of utilization of substrates present in the circulation, for example, glucose for lipogenesis (Hawkins & Williamson, 1972; Robinson & Williamson, 1977) and amino acids for protein synthesis (Viña & Williamson, 1981*b*). Recent experiments (Viña & Williamson, 1981*a*,*b*) have shown that certain amino acids, in particular L-leucine, can provide carbon for the formation of lipid in the gland as well as being incorporated into protein.

The original aim of the studies reported here was to examine whether inhibition of protein synthesis with cycloheximide leads to increased incorporation of L-leucine into lipid and whether there is a regulatory link between the synthesis of lipid and of protein in the lactating mammary gland. The results indicate that administration of cycloheximide not only increases the incorporation of L-[U-^{14}C]leucine into lipid in both mammary gland and liver *in vivo* but it also considerably increases the overall rate of hepatic lipogenesis as measured with ^3H_2O. The possible reasons for this latter effect have been examined in both lactating and virgin rats.

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Experimental

Materials

Rats. Lactating rats of the Wistar strain [295 ± 34 g (mean ± S.D.; $n = 47$)] with nine or ten pups were used at peak lactation (10–14 days); virgin rats weighed 196 ± 30 g ($n = 34$). Where indicated rats were anaesthetized with Nembutal (60 mg/kg body wt.; dissolved in 0.9% NaCl). The rats were fed *ad libitum* on Oxoid breeding diet (Oxoid, London S.E.1., U.K.).

Biochemicals. All enzymes, including collagenase (grade II) and coenzymes, were obtained from Boehringer Corp. (London).

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Radioactive compounds. L-[U-^{14}C]Leucine, $NaH^{14}CO_3$ and ^3H_2O were obtained from The Radiochemical Centre, Amersham, Bucks., U.K.

Methods

Studies in vivo. Where indicated cycloheximide (0.2% solution; 2mg/kg body wt.) was administered by intraperitoneal injection. L-[U-^{14}C]Leucine incorporation into lipid and protein of mammary gland and liver *in vivo* was measured as described by Viña & Williamson (1981*a*).

The rate of lipogenesis in mammary gland and liver *in vivo* was measured by the incorporation of ^3H_2O into saponified lipid essentially by the method of Stansbie *et al.* (1976) with the modifications described by Robinson *et al.* (1978).

The rate of triacylglycerol entry into the circulation was measured by a modification of the procedure of Otway & Robinson (1967) using Triton WR1339 to prevent triacylglycerol removal from the plasma (Scanu, 1965). The modification consisted of the simultaneous administration of ^3H_2O to measure the rate of entry into the circulation of lipid synthesized *de novo* as well as triacylglycerols arising from esterification of fatty acids derived from adipose tissue or dietary fat (for details, see Agius *et al.*, 1981).

Liver and mammary-gland metabolites were determined by removal of a piece of tissue (about 2g) 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 determined in the neutralized tissue extract.

Milk production (transfer to pups) was measured by removing the pups for 3h and then returning to their mothers for 1h. The litters were weighed just before recommencement of suckling and then again 1h later; the difference in weight gives an indication of the amount of milk transferred (Cowie & Folley, 1947).

Studies in vitro. Preparation of isolated hepatocytes. These were prepared from fed rats essentially by the method of Berry & Friend (1969) as modified by Krebs *et al.* (1974).

Incubation procedure. Hepatocytes (60–90mg wet wt.) were incubated with constant shaking for 20, 40 and 60min in 4.0ml of Krebs–Henseleit (1932) saline containing dialysed albumin (low in long-chain fatty acid; final concentration 2.5%, w/v). The gas phase was O_2/CO_2 (19:1) and the temperature 37°C. For measurement of lipogenesis by the procedure of Harris (1975) ^3H_2O (0.5mCi) was added to the incubation flasks. Where indicated L-lactate (10mM) and cycloheximide (20μM) were also added.

Determination of metabolites. The following metabolites were determined by enzymic methods: glycogen (Keppler & Decker, 1974); glucose (Slein, 1963); glucose 6-phosphate (Lamprecht & Trautschold, 1963); L-lactate and pyruvate (Hohorst *et al.*, 1959); citrate (Dagley, 1963); 2-oxoglutarate (Bergmeyer & Bernt, 1963); phosphoenolpyruvate, 2-phosphoglycerate and 3-phosphoglycerate (Czok & Eckert, 1963). Whole blood and tissue amino acids were determined with an amino acid analyser (Jeol, model JLC-6AH).

Determination of hepatic acetyl-CoA carboxylase activity. For the determination of acetyl-CoA carboxylase activity freeze-clamped liver was powdered and homogenized by hand in 0.3M-mannitol/50mM-Tris/acetate/2mM-EDTA, pH7.4. The activity was determined in the supernatant after centrifugation in an Eppendorf centrifuge at 12000rev./min for 0.5min by measuring the incorporation of [^{14}C]bicarbonate into acid-stable material at 30°C for 1.5min (Majerus *et al.*, 1968; Halestrap & Denton, 1973). The assay incubation routinely contained 100mM-Tris/acetate, pH7.4, 0.5mM-EDTA, 10mg of defatted bovine serum albumin/ml, 1mM-glutathione, 20mM-magnesium acetate, 0.3mM-acetyl-CoA, 5mM-ATP, 20mM-$NaH^{14}CO_3$. Samples of homogenate were assayed immediately for 'initial' activity in the presence and absence of 20mM-sodium citrate. A further sample was assayed for 'total' activity after a 30min pre-incubation at 30°C in the presence of 20mM-sodium citrate. Reaction blanks containing no acetyl-CoA were also performed. One unit of enzyme activity represents 1μmol of substrate converted/min at 30°C.

Determination of insulin. Arterial blood was collected from the abdominal aorta into heparinized tubes. Plasma was separated by centrifugation at 3000rev./min for 10min. Plasma insulin was measured by radioimmunoassay with charcoal phase separation as described by Albano *et al.* (1972).

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

Expression of results

Rates of lipogenesis (measured with ^3H_2O) in isolated hepatocytes were obtained from graphical plots of the 20, 40 and 60min values. The results are expressed as μmol of ^3H_2O incorporated into saponified lipid/40min per g fresh wt. of hepatocytes. The rate of lipogenesis *in vivo* is expressed as μmol of ^3H_2O incorporated into saponified lipid/h per g fresh wt. of tissue. Incorporation of L-[U-^{14}C]leucine *in vivo* into lipids and proteins of mammary gland and liver is expressed as percentage of dose/g wet wt. of tissue at 20min after injection. Whole blood or tissue metabolite content is expressed as μmol/ml or per g fresh wt.

Results and discussion

Effects of cycloheximide on metabolism of L-*leucine in vivo*

Injection of cycloheximide (2mg/kg body wt.) into fed lactating rats resulted in increased concentrations of virtually all amino acids present in blood, liver and mammary gland (results not shown). The changes in the concentration of the branched-chain amino acids valine, leucine and isoleucine are given in Table 1. The increase in leucine concentration was 2.5-fold in blood and liver, and 9-fold in mammary gland. These increases in the tissue amino acid pools are presumably due to the inhibition of protein synthesis. As expected, cycloheximide (administered 1h before the radioactive tracer) decreased the incorporation of L-[U-^{14}C]-leucine into proteins in both mammary gland and liver (Table 2). This decrease was accompanied by an increase (about 2.5-fold) in the incorporation of radioactivity into lipids in mammary gland and liver. This increased incorporation of a trace amount of L-[U-^{14}C]leucine into lipid is likely to be an underestimate because of the expansion of the leucine pools on administration of cycloheximide (Table 1). These results do not therefore provide quantitative information on the conversion of L-[U-^{14}C]leucine into lipid or protein. In isolated mammary-gland acini L-[U-^{14}C]leucine (1mM) is incorporated into lipid at about 25% of the rate found with D-[6-^{14}C]glucose (calculated on a C_3-unit basis; Viña & Williamson, 1981*b*). So that even at the raised concentrations of L-leucine that occur after cycloheximide treatment (0.3mM; Table 1) the amino acid is only likely to make a minor contribution to the overall rate of lipogenesis. The results do, however, indicate that inhibition of protein synthesis leads to a greater conversion of L-leucine into lipid in liver and mammary gland. Presumably this finding also applies to other amino acids that can form acetyl-CoA.

Effects of cycloheximide on lipigenesis in vivo

Lipogenesis was measured *in vivo* in liver and mammary gland of lactating rats with ^3H_2O to examine whether inhibition of protein synthesis altered the overall rate of this process. In mammary gland there was no significant change in the rate of ^3H_2O incorporation into saponified lipid for at least 120min after administration of cycloheximide; between 180 and 240min the rate declined to about 20% of control values (Table 3). This decline may be due to loss of enzyme activity secondary to the

Table 1. *Effects of cycloheximide on concentrations of branched-chain amino acids in liver and mammary gland of lactating rats*

For details see the Experimental section. The rats were killed 2h after injection of cycloheximide (2mg/kg body wt.) or an equivalent volume of 0.9% NaCl. The results are means ± S.D. of three rats and are expressed as μmol/g of fresh tissue or μmol/ml of whole blood. Values for cycloheximide-treated rats that are significantly different from the control rats by Student's *t* test are shown: *$P < 0.05$; **$P < 0.005$.

	Valine		Leucine		Isoleucine	
	Saline	Cycloheximide	Saline	Cycloheximide	Saline	Cycloheximide
Liver	0.137 ± 0.027	0.431 ± 0.068**	0.125 ± 0.008	0.327 ± 0.042**	0.089 ± 0.010	0.167 ± 0.022**
Mammary gland	0.101 ± 0.045	0.480 ± 0.190*	0.032 ± 0.012	0.287 ± 0.125*	0.036 ± 0.027	0.133 ± 0.055*
Blood	0.187 ± 0.047	0.440 ± 0.058**	0.119 ± 0.020	0.308 ± 0.056**	0.079 ± 0.013	0.172 ± 0.022**

Table 2. *Effects of cycloheximide on the relative conversion of [U-^{14}C]leucine into lipid and protein in mammary gland and liver of lactating rats*

For details see the Experimental section. Rats were given an intraperitoneal injection of cycloheximide (2mg/kg body wt.) or 0.9% NaCl 60min before injection of L-[U-^{14}C]leucine (0.5ml containing 5μCi; sp. radioactivity of 300Ci/mol) into a tail vein. Incorporation of radioactivity (20min after injection) into lipid and protein of mammary gland and liver is expressed as percentage of dose/g wet wt. of tissue. The results are means ± S.D. for the numbers of observations shown in parentheses. Values that are significantly different by Student's *t* test from those for rats injected with saline are shown: *$P < 0.05$; **$P < 0.005$.

	Proportion of dose of ^{14}C incorporated into lipid (%)		Proportion of dose of ^{14}C incorporated into protein (%)	
Treatment of rats	Mammary gland	Liver	Mammary gland	Liver
Saline (10)	0.14 ± 0.08	0.084 ± 0.03	0.83 ± 0.37	0.39 ± 0.14
Cycloheximide (6)	0.31 ± 0.12**	0.22 ± 0.14**	0.27 ± 0.20**	0.22 ± 0.15*

Table 3. *Effects of cycloheximide on the incorporation of ^3H_2O into lipid in vivo in liver and mammary gland of lactating rats*

For details see the Experimental section. Rats were injected with either cycloheximide (2mg/kg body wt.) or an equivalent volume of 0.9% NaCl and then at 0min, 60min or 180min they were injected with ^3H_2O (4mCi; 0.5ml) and killed 60min later. The results are means ± S.D. for the numbers of observations indicated in parentheses, and are expressed as μmol of ^3H_2O incorporated into saponified lipid/g wet wt. of tissue per h. Values for cycloheximide-treated rats that are significantly different by Student's *t* test from those for saline-treated rats are shown: $^{**}P < 0.005$.

	Liver			Mammary gland		
Treatment of rats	0–60min	60–120min	180–240min	0–60min	60–120min	180–240min
NaCl	21.0 ± 6.0 (4)	22.6 ± 13.1 (6)	29.7 ± 11.1 (3)	85.9 ± 20.1 (4)	96.7 ± 34.7 (6)	118 ± 45.9 (3)
Cycloheximide	70.5 ± 3.9** (6)	72.0 ± 24.8** (6)	25.2 ± 4.9 (4)	103.6 ± 8.9 (6)	107.9 ± 49.8 (6)	20.6 ± 4.7** (4)

inhibition of protein synthesis. For example, pyruvate dehydrogenase in mammary gland of lactating rats is inactivated after administration of cycloheximide (Baxter *et al.*, 1979). Alternatively, the decline may be connected with the inhibition of milk production and/or milk transfer. Measurements of changes in pup weight showed that litters (10 pups) of rats injected with saline gained 3.4g (mean of three litters), whereas litters of cycloheximide-treated rats lost 1.8g (mean of three litters) (for details of timing see the Experimental section). The mammary tissue of the cycloheximide-treated rats did, however, contain an appreciable amount of a milk-like fluid. In marked contrast, the hepatic rate of lipogenesis was increased 3-fold for up to 120min after administration of cycloheximide and then it decreased to control values between 180 and 240min (Table 3). Cycloheximide also increased the rate of lipogenesis in livers of virgin rats (at 60–120min; controls, 10.8 ± 2.3 (means ± S.D.; $n = 6$) μmol of ^3H_2O incorporated into saponified lipids/g fresh wt. per h; cycloheximide-treated, 54.3 ± 6.3 ($n = 6$; $P < 0.005$). For this reason some experiments were also carried out in virgin rats. It seemed unlikely that this large stimulation of lipogenesis was entirely due to increased conversion of amino acids into lipid, especially as in the lactating rat the effect was confined to liver. Further experiments were therefore designed to examine other possible reasons for the effect of cycloheximide on hepatic lipogenesis.

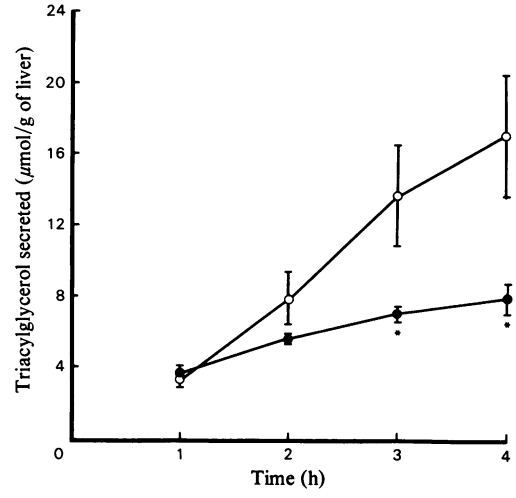

Fig. 1. *Effect of cycloheximide on the secretion of triacylglycerol into plasma by livers of fed lactating rats*

Fed lactating rats were injected with saline (○) or cycloheximide (●) (2mg/kg body wt.) at 0min, with simultaneous injection of 1ml of 10% Triton WR1339 into a tail vein. Blood samples were collected hourly for 4h and plasma [triacylglycerol] was estimated. Points are means of six animals per group. Vertical bars represent ± S.E.M. Values for cycloheximide-treated rats that are significantly different from saline-treated rats by Student's *t* test are shown: $^{*}P < 0.05$.

Effects of cycloheximide on triacylglycerol entry into the circulation

As cycloheximide inhibits protein synthesis it also prevents the formation of lipoprotein and secretion of very-low-density lipoproteins; this may explain the fatty liver, which occurs after administration of cycloheximide (Bar-On *et al.*, 1972). To test whether the apparent increase in the rate of hepatic lipogenesis was due to inhibition of secretion of the newly synthesized lipids, the rate of entry of triacylglycerol into the circulation was measured (Agius *et al.*, 1981). This procedure allows simultaneous measurement of the entry of total triacylglycerol and also of that synthesized *de novo*. Triacylglycerol entry in control rats was virtually linear between 1 and 4h, whereas in cycloheximide-treated rats the rate of entry decreased significantly ($P < 0.05$) between 2 and 3h after administration of the cycloheximide (Fig. 1). There was,

however, no significant difference between the rate of appearance of ^3H_2O in saponified plasma lipids between control and cycloheximide-treated rats (Fig. 2). This latter result suggests that the apparent higher incorporation of ^3H_2O into saponified lipids of livers of cycloheximide-treated rats is not merely due to inhibition of secretion of the newly synthesized lipid. Further evidence in support of this conclusion is that little ^3H-labelled lipid is secreted within 60min in control rats (Fig. 2) and therefore measurements in liver over this period of time can be taken to indicate the rate of hepatic lipogenesis.

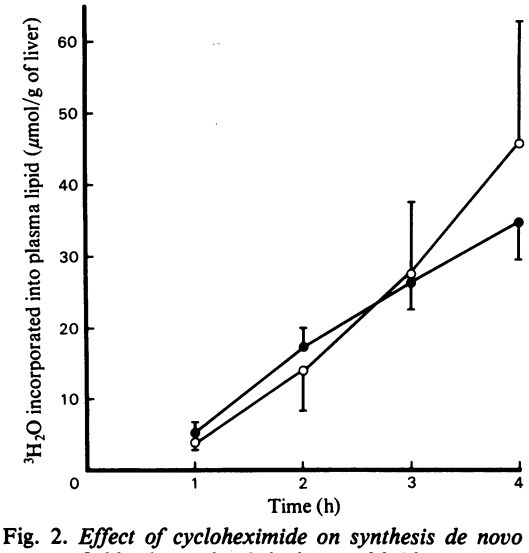

Fig. 2. *Effect of cycloheximide on synthesis de novo of saponifiable plasma lipids by livers of fed lactating rats*
Fed lactating rats were injected with saline (○) or cycloheximide (●) (2mg/kg body wt.) at 0min, with simultaneous injection of 4mCi of ^3H_2O (intraperitoneally) and 1ml of 10% Triton WR1339 (intravenously). Blood samples were collected hourly and ^3H_2O incorporation into saponifiable plasma lipids was estimated. Points are means of five animals per group. Vertical bars represent ± S.E.M.

Cycloheximide and acetyl-CoA carboxylase activity

Two factors that might lead to a stimulation of hepatic lipogenesis are an increase in plasma insulin and/or an increase in the activation state of acetyl-CoA carboxylase (Stansbie *et al.*, 1976). Cycloheximide caused no significant increase in plasma insulin in either virgin or lactating rats (Table 4). There was, however, a significant decrease in blood glucose in both groups of rats after cycloheximide administration (Table 4). There was no significant alteration in the 'initial' or total activity of acetyl-CoA carboxylase in livers of lactating or virgin rats treated with cycloheximide (Table 5).

Cycloheximide and lipogenesis in isolated hepatocytes

To test the effects of cycloheximide on lipogenesis *in vitro* hepatocytes were isolated from control or cycloheximide-treated virgin rats and incubated in a medium containing ^3H_2O. Addition of cycloheximide (20μM) caused a small but significant ($P < 0.05$) increase in the rate of lipogenesis from endogenous substrates (mainly glycogen) in hepatocytes from control and cycloheximide-treated rats (Table 6). Addition of lactate (10mM) as exogenous substrate increased the rate of lipogenesis (80 and 160% respectively), but cycloheximide no longer stimulated the process (Table 6). The absence of an effect of cycloheximide when lipogenesis is not limited by the supply of substrate (on addition of lactate) is analogous to the findings with two hormones, vasopressin and insulin, which only stimulate lipogenesis when the rate of the process is submaximal (Assimacopoulos-Jeannet *et al.*, 1981). Vasopressin stimulates glycolysis (Hue *et al.*, 1981; Assimacopoulos-Jeannet *et al.*, 1981) and the effects of cycloheximide on hepatic lipogenesis could be explained if it also increased glycolytic flux.

Cycloheximide and hepatic metabolites in vivo

It has been reported that administration of cycloheximide to fed rats activates phosphorylase and depletes liver glycogen (Cecil & Bitman, 1966;

Table 4. *Effects of cycloheximide on blood glucose and plasma insulin*
For details see the Experimental section. Fed virgin or lactating rats were injected with cycloheximide (2mg/kg body wt.) or 0.9% NaCl and then anaesthetized with Nembutal 2h later. Blood was collected from the abdominal aorta. Results are means ± S.D. for the numbers of observations given in parentheses. Values for cycloheximide-treated rats that are significantly different by Student's *t* test from those for saline-treated rats are shown: *$P < 0.05$.

	Virgin rats		Lactating rats	
Treatment of rats	Blood glucose (μmol/ml)	Plasma insulin (μunits/ml)	Blood glucose (μmol/ml)	Plasma insulin (μunits/ml)
NaCl	6.85 ± 0.43 (7)	29.2 ± 7.7 (7)	4.90 ± 0.57 (3)	4.7 ± 1.9 (3)
Cycloheximide	6.07 ± 0.63 (7)*	38.9 ± 14.6 (7)	3.94 ± 0.61 (4)*	6.7 ± 1.3 (4)

Table 5. *Effects of cycloheximide on the activity of acetyl-CoA carboxylase in livers of virgin and lactating rats*
Fed virgin or lactating rats were injected with cycloheximide or 0.9% NaCl and killed 2h later. Acetyl-CoA carboxylase activity was measured as described in the Experimental section. Where indicated activity was measured in the presence of 20mM-sodium citrate. Results are means ± S.D. for the numbers of observations shown in parentheses.

	Virgin rats (9)			Lactating rats (5)		
Treatment of rats	Initial activity (% of total)		Total activity (m-units/min per mg of protein)	Initial activity (% of total)		Total activity (m-units/min per mg of protein)
	No citrate	Plus citrate		No citrate	Plus citrate	
NaCl	19.8 ± 6.2	51.2 ± 5.9	2.40 ± 1.70	17.9 ± 3.1	60.8 ± 20.2	4.15 ± 1.71
Cycloheximide	23.4 ± 9.6	73.2 ± 14.2	3.60 ± 2.11	17.5 ± 11.5	53.1 ± 24.7	5.62 ± 3.00

Table 6. *Effects of cycloheximide on lipogenesis in isolated hepatocytes from virgin rats*
For details see the Experimental section. Hepatocytes were isolated from fed virgin rats that had been injected with cycloheximide (2mg/kg body wt.) or 0.9% NaCl and then killed after 90min. Where indicated lactate (final concn. 10mM) and cycloheximide (final concn. 20μM) were added to the incubation flasks. The rate of lipogenesis is expressed as μmol of ^3H_2O incorporated into saponified lipid between 20 and 60min incubation per g fresh wt. of hepatocytes and the results are means ± S.D. of three separate experiments. Values obtained in the presence of cycloheximide that are significantly different by Student's paired *t* test from those in its absence are indicated by: $^*P < 0.05$.

Treatment of rats	Endogenous	Endogenous + cycloheximide	Lactate	Lactate + cycloheximide
NaCl	6.9 ± 0.65	8.1 ± 1.0*	11.4 ± 2.2	11.8 ± 4.0
Cycloheximide	6.0 ± 1.9	8.2 ± 2.6*	16.0 ± 3.5	19.0 ± 2.1

Wititsuwannakul & Kim, 1978). Since cycloheximide appears to lower blood glucose rather than increase it (Table 4) the stimulation of glycogenolysis is likely to result in an increase in glycolytic flux. The effects of cycloheximide on the content of certain glycolytic metabolites in livers of virgin rats were therefore measured (Table 7). Glycogen decreased by approx. 25% at 60min after administration of cycloheximide, confirming the findings of Wititsuwannakul & Kim (1978). This decrease was accompanied by significant increases in [glucose 6-phosphate], [pyruvate] and [lactate] (Table 7); these metabolite changes are consistent with increased glycolytic flux. The decrease in phosphoenolpyruvate and accumulation of pyruvate in the livers of cycloheximide-treated rats suggests increased activity of pyruvate kinase in addition to the activation of phosphorylase. Hepatic [glycogen] decreased and [pyruvate] increased in livers of lactating rats injected with cycloheximide (results not shown). Wititsuwannakul & Kim (1978) found that cycloheximide increased cyclic AMP in rat liver slices and considered that this was responsible for the activation of phosphorylase. It is well-established that in rat liver cyclic AMP inhibits glycolysis (Harris, 1975) and lipogenesis (Capuzzi *et al.*, 1974; Harris, 1975) and therefore cycloheximide must be able to overcome the inhibitory action of cyclic AMP.

Table 7. *Effects of cycloheximide on the hepatic content of glycolytic metabolites*
For details see the Experimental section. Fed virgin rats were injected with either cycloheximide (2mg/kg body wt.) or 0.9% NaCl and then anaesthetized with Nembutal 60min later. Liver was freeze-clamped and the hepatic metabolites extracted. Results are means ± S.D. for the numbers of observations indicated in parentheses and are expressed as μmol/g fresh wt. of liver. Values that are significantly different from the saline-treated rats by the Student's *t* test are shown: $^*P < 0.05$; $^{**}P < 0.005$.

		Treatment of rats	
Metabolite		Saline	Cycloheximide
Glycogen	(7)	309 ± 58	236 ± 57*
Glucose	(4)	7.07 ± 1.05	5.77 ± 0.55*
Glucose 6-phosphate	(4)	0.150 ± 0.04	0.220 ± 0.03*
3-Phosphoglycerate	(4)	0.329 ± 0.023	0.339 ± 0.042
2-Phosphoglycerate	(4)	0.042 ± 0.003	0.047 ± 0.001
Phosphoenolpyruvate	(4)	0.125 ± 0.006	0.101 ± 0.011*
Pyruvate	(4)	0.186 ± 0.029	0.429 ± 0.018**
Lactate	(4)	1.60 ± 0.49	3.63 ± 0.41**
Oxoglutarate	(4)	0.129 ± 0.011	0.220 ± 0.077*
Citrate	(4)	0.266 ± 0.084	0.333 ± 0.089

Conclusions

The work presented in this paper indicates that inhibition of protein synthesis in lactating mammary

gland does not result in any immediate alteration in the rate of lipogenesis *in vivo*. This suggests that there is no close link between the rate of production of protein and lipid in the gland. The lactose content of the gland is unaltered in cycloheximide-treated rats (A. F. C. Roberts & D. H. Williamson, unpublished work).

Inhibition of protein synthesis leads to increased incorporation of L-[U-^{14}C]leucine into lipid of mammary gland and liver. Presumably other amino acids can also act as lipogenic precursors in situations where net protein synthesis is depressed and amino acid availability is increased.

The unexpected finding that cycloheximide considerably increases the rate of hepatic lipogenesis appears to be mainly due to the ability of cycloheximide to stimulate glycogenolysis and increase glycolytic flux. This finding re-enforces the potential importance of hepatic glycogen as a lipogenic precursor during the immediate post-absorptive period. It is noteworthy that the rate of hepatic lipogenesis in cycloheximide-treated rats returned to control values between 180 and 240 min, which coincides with the virtual depletion of liver glycogen (Wititsuwannakul & Kim, 1978). It is also of interest that the increase in the rate of lipogenesis apparently occurs without any change in the state of activation of acetyl-CoA carboxylase.

This work was supported by the Medical Research Council, U.K., and the U.S. Public Health Service (Grant no. AM-11748). M. R. M. holds an M.R.C. Studentship and D. H. W. is a member of the External Staff of the Medical Research Council, U.K.

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