Biochem. J. (1982) **204**, 757–764
Printed in Great Britain

Changes in the proportion of acetyl-CoA carboxylase in the active form in rat liver

Effect of starvation, lactation and weaning

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(*Received 21 January 1982/Accepted 18 March 1982*)

1. The activity of acetyl-CoA carboxylase (EC 6.4.1.2) in extracts of freeze-clamped liver samples from fed or 24h-starved virgin, pregnant, lactating and weaned rats was measured (i) immediately after preparation of extracts ('I activity'), (ii) after incubation of extracts with partially purified preparations of either rabbit muscle protein phosphatase 1 [Antoniw, Nimmo, Yeaman & Cohen (1977) *Biochem. J.* **162**, 423–433] or rabbit liver phosphatase [Brandt, Capulong & Lee (1975) *J. Biol. Chem.* **250**, 8038–8044] ('A activity') and (iii) after incubation with 20mM-potassium citrate before or after incubation with phosphatases ('C activity'). 2. Incubation of liver extracts at 30°C without any additions resulted in activation of acetyl-CoA carboxylase that was shown to be due to dephosphorylation of the enzyme by endogenous protein phosphatase activity. This latter activity was not stimulated by Ca^{2+} and/or Mg^{2+} but was stimulated by 1mM-Mn^{2+}. Incubation of extracts with either of the partially purified phosphatases (0.2–0.5 unit) resulted in faster dephosphorylation and activation. The activity achieved after incubation with either of the exogenously added phosphatases was similar. 3. The A and C activities increased during late pregnancy, were lower than in the virgin rat liver during early lactation and increased by 2-fold in liver of mid-lactating rats. Weaning of mid-lactating rats for 24h resulted in no change in A and C activities but after 48h weaning they were significantly lower than those in livers from suckled mothers. 4. The I activity followed a similar pattern of changes as the A and C activities during pregnancy and lactation such that, although the I/A and I/C activity ratios tended to be lower during late pregnancy and early lactation, there were no significant changes in I/A and I/C ratios between lactating and virgin animals. However, these ratios were significantly higher in liver from fed 24h-weaned animals. 5. Starvation (24h) resulted in a marked decrease in I activity for all animals studied except early-lactating rats. This was due to a combination of a decrease in the concentration of acetyl-CoA carboxylase in liver of starved animals (A and C activities) and a decrease in the fraction of the enzyme in the active form (lower I/C and I/A ratios). The relative importance of the two forms of regulation in mediating the starvation-induced fall in I activity was about equal in livers of virgin, pregnant and lactating animals. However, the decrease in I/A and I/C ratios was of dominating importance in livers of weaned animals. The A/C activity ratios were the same for livers from all animals studied. 6. The maximal activity of fatty acid synthase was also measured in livers and was highly and positively correlated with the A and C activities of acetyl-CoA carboxylase, suggesting that the concentrations of the two enzymes in the liver were controlled co-ordinately. 7. It is suggested that the lack of correlation between plasma insulin levels and rates of lipogenesis in the transition from the virgin to the lactating state may be explained by different effects of insulin and prolactin on the concentration of acetyl-CoA carboxylase in the liver and on the fraction of the enzyme in the active form.

0306-3283/82/060757-08$01.50/1

It is widely accepted that acetyl-CoA carboxylase (EC 6.4.1.2) catalyses a rate-limiting reaction for lipogenesis in the liver and other tissues (see, e.g., Lane *et al.*, 1974) and that the rate of hepatic fatty acid synthesis is dependent on the concentration of malonyl-CoA, the product of the reaction catalysed by acetyl-CoA carboxylase (Guynn *et al.*, 1972). The activity of acetyl-CoA carboxylase is regulated primarily by three mechanisms: through alterations in the concentration of enzyme in tissues (Allman *et al.*, 1965; Muto & Gibson, 1970); through the actions of effector molecules (e.g. citrate, long-chain acyl-CoA esters) (Denton *et al.*, 1977); and through phosphorylation and dephosphorylation of the enzyme (Kim, 1979; Brownsey *et al.*, 1977). Changes in activity and in the degree of phosphorylation of the enzyme in isolated rat liver cells and adipocytes have been demonstrated after exposure of these preparations to various hormones (Geelen *et al.*, 1978; Witters *et al.*, 1979; Brownsey *et al.*, 1979).

Marked changes in the hepatic lipogenic rate and in the partition of fatty acids between oxidation and esterification occur in the liver during the reproductive cycle (i.e. in the transition from the unmated animal through pregnancy, lactation and weaning) (see Williamson, 1981). These changes are associated with changes in the mitochondrial metabolism of fatty-acyl carnitine esters (Zammit, 1980) and in the activities of several enzymes (Smith, 1975; Zammit, 1981). The concentration of malonyl-CoA in the liver undergoes large changes in pregnancy and lactation and, in addition, the response of hepatic malonyl-CoA concentration to starvation is also highly dependent on the reproductive state of the animals (Zammit, 1981).

Previous studies on the activity of acetyl-CoA carboxylase in the liver of lactating rats involved the measurement of enzyme activity *in vitro* after this had been maximally stimulated by pre-incubation of extracts in the presence or absence of high concentrations of the activator citrate (Smith, 1975). Because this procedure is accompanied by complete polymerization of the enzyme (see Kim, 1979; Lane *et al.*, 1979) as well as by dephosphorylation (the present paper) the results obtained could not be used as a quantitative assessment of the activity of the enzyme *in vivo* and hence of the flux through the lipogenic pathway.

Therefore, we have measured the activity of acetyl-CoA carboxylase in extracts of freeze-clamped liver samples from animals in different stages of the reproductive cycle, before and after activation by dephosphorylation and/or treatment with citrate to ascertain the relative importance of the acute (covalent modification) and long-term (enzyme concentration) mechanisms involved in the regulation of the activity of the enzyme. In addition we have also measured the maximal activity of fatty acid synthase in the same extracts. Since this latter enzyme is not thought to be regulated by covalent modification (Hardie, 1980), a comparison of the changes of its maximal activity with the 'initial' and maximal activities of acetyl-CoA carboxylase should provide the possibility to distinguish between the importance of co-ordinate changes in the concentration of enzymes involved in fatty acid synthesis and of the acute regulation (via phosphorylation and dephosphorylation) of acetyl-CoA carboxylase in liver of animals in the various conditions studied.

Materials and methods

Animals

Source and treatments of rats were as described previously (Zammit, 1980, 1981). Rats were killed between 09:00 and 10:00 h.

Chemicals

Sources of chemicals were as described by McNeillie *et al.* (1981). Protein phosphatase 1 from rabbit skeletal muscle and rabbit liver protein phosphatase were partially purified up to the respective DEAE-Sephadex steps in the procedures described by Stewart *et al.* (1981) and Brandt *et al.* (1975). Highly purified rabbit muscle protein phosphatase 1 was a generous gift from Professor P. Cohen, University of Dundee, Dundee, Scotland, U.K. All phosphatase preparations were dialysed against extraction medium (see below) before use.

Preparation of homogenates

Frozen samples, obtained and powdered as described previously (Zammit, 1981), were homogenized (two periods of 10s) in 3vol. of ice-cold extraction medium containing 250mM-sucrose, 20mM-Tris, 20mM-Mops (4-morpholinepropanesulphonic acid), 2mM-EDTA and 2mM-EGTA, pH 7.4, using a polytron tissue homogenizer (Kinematika, Berne, Switzerland) fitted with probe OD10. After homogenization, albumin (fatty acid-poor and dialysed) was added to the extracts to give a final albumin concentration of 10mg/ml, and the extracts were centrifuged for 60s at 10000 ***g*** in an Eppendorf 5412 centrifuge.

Pre-incubation of extracts

Portions (100μl) of supernatant were either diluted with 200μl of extraction medium and assayed for acetyl-CoA carboxylase immediately or incubated at 30°C after addition of 200μl containing one of the following: (i) 0.2–0.5 unit of rabbit liver protein phosphatase; (ii) 0.2–0.5 unit of rabbit muscle protein phosphatase 1 plus 1.5μmol of $MnCl_2$ (to give 1mM-free Mn^{2+} concentration); (iii) 1.5μmol of $MnCl_2$; (iv) 6μmol of potassium citrate (to give a final concentration of 20mM-citrate).

Portions were removed from the various incubations and assayed for acetyl-CoA carboxylase activity at the following times: (i) and (ii) at 30, 40 and 60 min; (iii) at 60, 80 and 100 min; (iv) at 20 and 30 min. These times were chosen to ascertain that the activation was maximal for the respective conditions used (see Fig. 1).

Assay of enzyme activities

Acetyl-CoA carboxylase was assayed at 30°C as described previously (McNeillie & Zammit, 1981; McNeillie *et al.*, 1981). The reaction medium contained a final volume of 0.475 ml. The reaction was initiated by addition of 25 μl of sample and terminated either at 30 or 60 s by addition of 0.1 ml of 6 M-HCl. Measurement of acid-stable radioactivity was made as described previously (McNeillie *et al.*, 1981). Appropriate controls were run concurrently and routinely gave negligible values.

Fatty acid synthase was assayed spectrophotometrically in 60000 ***g*** supernatants of homogenates by following the oxidation of NADPH at 340 nm using a Gilford 250 recording spectrophotometer. The reaction medium contained 125 mM-potassium phosphate buffer, pH 7.0, 2 mM-EDTA, 2 mM-dithiothreitol, 0.2 mM-NADPH, 0.15 mM-acetyl-CoA and 0.25 mM-malonyl-CoA. The reaction was initiated by addition of malonyl-CoA; controls from which malonyl-CoA was omitted were run concurrently.

Measurement of DNA

The DNA content of liver samples was measured in whole (uncentrifuged) homogenates by the method of Labarca & Paigen (1980) using calf thymus DNA dissolved in extraction medium containing albumin (10 mg/ml) as standard.

Measurement of incorporation of ^{32}P into acetyl-CoA carboxylase

These experiments were carried out as described previously (McNeillie *et al.*, 1981) and incorporation of ^{32}P into acetyl-CoA carboxylase was measured by densitometric scanning of radiographs of electropherograms performed on 4% sodium dodecyl sulphate/polyacrylamide gels (McNeillie *et al.*, 1981).

Expression of results

Three types of activities ('I', 'A' and 'C') are referred to below. The 'initial' activity measured immediately after preparation of the homogenates is denoted by 'I', the activity of carboxylase after activation by citrate (before or after dephosphorylation) by 'C' and the activity after complete dephosphorylation, in the presence of exogenous phosphatases, by 'A'. Consequently, the fraction of the enzyme in the active form is expressed as I/A or I/C activity ratios.

Activities are expressed on a unit-wet-weight basis as well as per unit DNA content of the liver so as to minimize the possibility of erroneous interpretation of data due to changes in liver composition during changes in the reproductive and nutritional state of the rats.

Results and discussion

Activation of acetyl-CoA carboxylase in liver extracts by exogenous and/or endogenous phosphatase(s)

Incubation of liver extracts at 30°C in the absence of any additions other than extraction medium resulted in a slow activation of acetyl-CoA carboxylase from the I activity measured immediately after preparation of the extracts. The activation reached a maximum after 60–80 min. Several workers have observed such 'heat activation' of acetyl-CoA carboxylase in tissue extracts, including those of liver (Allred & Roehrig, 1978; Witters *et al.*, 1979). In the case of crude extracts of adipose tissue (Brownsey *et al.*, 1979) and mammary gland (McNeillie *et al.*, 1981) and of partially purified carboxylase from adipose tissue (Krakower & Kim, 1980) this activation was found to be due to dephosphorylation by endogenous protein phosphatase activity of the preparation or extract. Several observations in the present study suggested that the activation of acetyl-CoA carboxylase in the liver extracts was due to dephosphorylation. Thus, activation was prevented by addition of KF (100 mM; see Fig. 1), whereas addition of a mixture of proteolysis inhibitors (phenylmethanesulphonyl fluoride, 0.5 mM; antipain, pepstatin, leupeptin and trypsin inhibitor, all at 0.1 mg/ml) did not affect the activation (results not shown), suggesting that proteolysis may not have been responsible for activation [limited proteolysis by trypsin has been shown previously to activate acetyl-CoA carboxylase from liver (Swanson *et al.*, 1967)].

Direct evidence that activation under the conditions used was due to dephosphorylation by endogenous protein phosphatase activity was obtained by following the release of ^{32}P from acetyl-CoA carboxylase (see the Materials and methods section) after the enzyme had been labelled with ^{32}P by incubation of extracts with 2 mM-[γ-^{32}P]ATP (a process that decreased enzyme activity to about 5% of maximal within 3 min; results not shown). Subsequent removal of ATP with glucose and hexokinase (see McNeillie *et al.*, 1981) resulted in rapid release of ^{32}P from acetyl-CoA carboxylase and activation of the enzyme even in the absence of exogenously added phosphatases (results not shown), suggesting that endogenous phosphatase activity was responsible for activation of acetyl-CoA carboxylase in liver extracts incubated at 30°C.

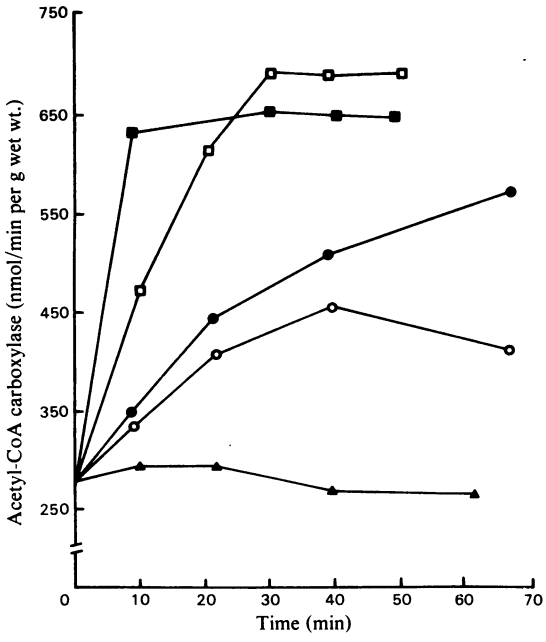

Fig. 1. *A representative time course of changes in acetyl-CoA carboxylase activity in an extract of liver from a fed mid-lactating rat*

Liver extract was prepared as described in the Materials and methods section and incubated at 30°C either with extraction medium alone (○) or under one of the following conditions: 100mM-KF (▲); 5mM-$MnCl_2$ (●); 0.2 unit of rabbit liver protein phosphatase (□); 0.5 unit of rabbit muscle protein phosphatase 1 plus 5mM-$MnCl_2$ (■). Routinely, time courses similar to those shown were obtained.

Addition of exogenous purified protein phosphatases accelerated the release of ^{32}P, whereas fluoride prevented loss of ^{32}P from the enzyme (results not shown).

The observation that the endogenous protein phosphatase in liver extracts was active in the presence of 2mM-EDTA and 2mM-EGTA (see the Materials and methods section) and in the absence of added bivalent metal ions was at variance with the previously reported properties of the endogenous protein phosphatases, which activate acetyl-CoA carboxylase in extracts of rat mammary gland (McNeillie & Zammit, 1981; McNeillie *et al.*, 1981) and adipose tissue (Brownsey *et al.*, 1979), both of which require Mg^{2+} and Ca^{2+} for activity. Addition of Ca^{2+} (free Ca^{2+} concentration, 20–50μM; see McNeillie & Zammit, 1981) and/or Mg^{2+} (5mM) did not affect the rate or extent of activation of acetyl-CoA carboxylase by endogenous phosphatase in liver extracts (results not shown). However, addition of Mn^{2+} (free Mn^{2+} concentration, 1mM) resulted in an increased rate of activation of acetyl-CoA carboxylase by endogenous phosphatase as well as a significant increase in the extent of activation (Fig. 1). This latter observation and the results of preliminary studies that showed that endogenous-phosphatase-activated acetyl-CoA carboxylase activity was not a fixed proportion of the maximal activity measured after the enzyme was activated with citrate suggested that the endogenous phosphatase in liver extracts was not sufficiently active to achieve complete dephosphorylation during up to 60min of incubation time (after which loss of activity was observed). Consequently, it was necessary to incubate extracts with exogenous, purified protein phosphatases to achieve complete dephosphorylation.

Incubation of extracts with 0.2–0.5 unit of either rabbit muscle protein phosphatase 1 (which was active only in the presence of Mn^{2+}; compare results of Stewart *et al.*, 1981) or rabbit liver protein phosphatase gave very similar activation of acetyl-CoA carboxylase (Fig. 1). The activation of the dephosphorylated enzyme thus achieved was much more rapid and 20–40% higher than that achieved by endogenous protein phosphatase activity alone (Fig. 1). This difference between the two values was negatively correlated ($r = 0.87$) to the magnitude of the I/A activity ratio. This correlation suggested that dephosphorylation by the endogenous phosphatase was proportionately less complete the larger the amount of phosphorylated enzyme substrate present in the extracts. Conversely, the A/C activity ratio was the same (about 83%) for extracts of livers from all the animals studied, suggesting that complete dephosphorylation by exogenous phosphatases resulted in a reproducible change in enzyme structure with respect to the completely polymerized enzyme (after citrate activation). The activation achieved after incubation with partially purified protein phosphatase 1 and a highly pure preparation of the phosphatase was the same.

Activation of acetyl-CoA carboxylase by citrate

Incubation of liver extracts with potassium citrate resulted in a severalfold activation of acetyl-CoA carboxylase. Maximal activation was obtained after 20min incubation at citrate concentrations above 10mM: 20mM-potassium citrate was used routinely to obtain values for C activity. The enzyme activity after activation by citrate was the same irrespective of whether the enzyme was dephosphorylated before incubation with citrate (compare with data of McNeillie *et al.*, 1981).

Relationship between I, A and C acetyl-CoA carboxylase activities

Since liver extracts were prepared from samples freeze-clamped *in situ* the activity of acetyl-CoA carboxylase measured immediately after prepara-

Table 1. *Activities of acetyl-CoA carboxylase in extracts of lvier from fed or starved (24 h) rats at different stages of the reproductive cycle*

Values are means ± S.D. and the numbers of different rats used for each determination are shown in parentheses. Statistical significance (Student's *t* test) of the difference between values for fed and starved animals in any one stage of the reproductive cycle is denoted by *($P < 0.05$) and **($P < 0.01$) and that between fed mid-lactating animals and fed animals in other stages is denoted by †($P < 0.05$) and ††($P < 0.01$). This statistical treatment could not be applied to values of I/C ratios due to non-normal distribution.

		DNA (mg/g wet wt.)	Acetyl-CoA carboxylase activity (nmol/min per g wet wt. at 30°C)			Acetyl-CoA carboxylase activity (nmol/min per mg of DNA at 30°C)			Activity ratio	
			I	A	C	I	A	C	I/A	I/C
Virgin	Fed (4)	4.05 ±0.20	130†† ±17	322†† ±14	390†† ±56	32 ±4	79 ±3	96 ±14	40.2 ±3.6	33.4 ±0.7
	Starved (5)	5.71 ±0.36	70** ±4	250** ±23	317 ±37	12** ±1	44** ±4	56** ±7	28.2** ±2.9	22.8 ±3.2
19-day pregnant	Fed (5)	3.97 ±0.26	179†† ±59	526 ±97	656† ±107	45 ±14	102 ±14.1	165 ±27	33.9 ±7.8	27.4 ±7.8
	Starved (4)	4.16 ±0.07	78** ±22	341** ±36	430** ±26	20** ±5	61** ±7	103** ±7	23.1 ±7.0	18.1 ±4.3
2–4-day lactating	Fed (4)	3.82 ±0.08	101†† ±21	320† ±59	376†† ±57	26 ±5	63 ±10	98 ±15	32.7 ±3.2	27.8 ±1.5
	Starved (4)	4.34 ±0.17	70 ±0.12	352 ±44	398 ±50	16 ±3	56 ±15	92 ±11	19.9** ±1.1	17.6 ±1.3
10–14-day lactating	Fed (5)	3.56 ±0.18	282 ±29	709 ±99	853 ±88	79 ±8	199 ±28	240 ±25	40.3 ±6.5	33.4 ±5.4
	Starved (6)	4.20 ±0.17	91** ±26	463** ±102	577** ±148	22** ±6	110** ±24	137** ±35	19.4** ±2.5	15.7 ±1.0
Weaned (24 h)	Fed (5)	3.63 ±0.16	376†† ±32	792 ±73	946 ±122	98 ±9	218 ±20	260 ±20	48.6 ±8.2	41.6 ±4.7
	Starved (5)	4.17 ±0.16	138** ±50	623 ±132	764 ±164	33** ±12	149** ±32	183** ±39	22.1** ±5.1	18.0 ±4.3
Weaned (48 h)	Fed (5)	3.81 ±0.24	270 ±70	568 ±150	640† ±163	71 ±18	149 ±39	168 ±42	53.9† ±11.4	47.8 ±8.3
	Starved (4)	4.79 ±0.22	125** ±23	582 ±80	699 ±77	26** ±5	122 ±17	146 ±16	21.5** ±3.2	17.9 ±2.4

tion of homogenates (i.e. the I activity) should represent the activity of the enzyme as determined by its state of phosphorylation *in vivo* at the moment the tissue was frozen. Conversely the A and C activities should give a measure of the activity of the enzyme after dephosphorylation and polymerization respectively.

Activity changes during pregnancy, lactation and weaning in fed animals. The transition from the virgin state through pregnancy, early lactation (2 days *post partum*) and mid-lactation (10–14 days) was accompanied by a biphasic change in the I activity of carboxylase (Table 1). The activity increased in liver of pregnant rats compared with that of virgin animals and then fell by 40% in livers of 2-day lactating animals. At peak lactation the I activity increased again to a value 2-fold higher than that in the liver of virgin animals. These changes tended to be more pronounced when the activity was expressed per unit DNA content of the liver (Table 1). Premature removal of the pups for 24 h from mothers at peak-lactation resulted in a further increase in I activity over that found in livers of suckled mothers. However, after 48 h-weaning the I activity returned to values observed in suckled animals.

Although the I/A and I/C activity ratios tended to be lower in pregnant and early-lactating rats they were not significantly different from those found for extracts of livers from lactating animals. This observation suggested that the changes in I activity were mediated primarily via changes in the enzyme concentration in the liver rather than through changes in the proportion of the enzyme in the active form. In livers of weaned animals the I/A and I/C ratios were higher than those found in other fed animals, suggesting that the amount of enzyme in the active form had increased.

Effects of starvation. Starvation of animals for 24 h resulted in a significant decrease in I activity of carboxylase in the livers of animals at all stages of the reproductive cycle except during early lactation

Table 2. *Maximal activities of fatty acid synthase in extracts of livers from rats in different stages of the reproductive cycle*

Activities were measured at 30°C and are means ± S.D. The numbers of different animals used for each determination are given in parentheses. Statistical significance for the difference between values for fed and starved animals in any one stage of the reproductive cycle is denoted by * ($P < 0.01$) and that between fed mid-lactating animals and fed rats in other stages is denoted by † ($P < 0.01$).

State of animal		Activity of fatty acid synthase at 30°C (μmol/min per g wet wt.)	Activity of fatty acid synthase at 30°C (μmol/min per mg of DNA)
Virgin	Fed (5)	1.89 ± 0.23†	0.43 ± 0.06†
	Starved (3)	0.83 ± 0.17*	0.15 ± 0.03*
19-day pregnant	Fed (4)	2.50 ± 0.25†	0.62 ± 0.06
	Starved (3)	0.72 ± 0.36*	0.17 ± 0.09*
2–4-day lactating	Fed (7)	1.45 ± 0.31†	0.36 ± 0.08†
	Starved (4)	0.50 ± 0.27*	0.13 ± 0.06*
10–14-day lactating	Fed (7)	4.51 ± 0.38	1.29 ± 0.11
	Starved (12)	1.97 ± 0.51*	0.47 ± 0.12*
Weaned (24 h)	Fed (5)	3.73 ± 0.21†	1.02 ± 0.08
	Starved (5)	3.36 ± 0.30	0.73 ± 0.08*

(when the activity of the enzyme in the fed state was already very low; see Table 1). This starvation-induced decrease in I activity was due to the combined effects of a decrease in the proportion of the enzyme in the active form (i.e. lower I/A and I/C activity ratios) as well as a decrease in the A and C activities of the enzyme. A comparison between the fractional decrease in I activity with that in the A or C activities after starvation of animals in different reproductive states suggested that the relative importance of regulation through changes in enzyme content and that through covalent modification of carboxylase during starvation varied depending on the reproductive state of the animals. In virgin, pregnant and lactating animals both types of regulation were equally important in mediating the observed decrease in I activity, but in weaned animals regulation by covalent modification accounted for most (in 24 h-weaned animals), if not all (48 h-weaned), of the decrease in I activity. Thus, whereas in livers of mid-lactating animals the contributions of changes in enzyme concentration and of the I/C (or I/A) ratio towards the decreased I activity after starvation were 46% and 54% respectively, in livers of 24 h-weaned animals the respective values were 24% and 76%. In livers of 48 h-weaned animals the decrease in I activity after starvation was entirely due to covalent modification of the enzyme.

Activity of fatty acid synthase. The maximal activity of fatty acid synthase measured *in vitro* was about 10-fold higher than the I activity of acetyl-CoA carboxylase for livers of all the animals studied (Table 2). Therefore, unless it is severely restricted *in vivo* by factors other than substrate concentration, the activity of fatty acid synthase is not likely to be rate limiting for lipogenesis. However, it is noteworthy that the maximal activities of fatty acid synthase and of acetyl-CoA carboxylase always changed strictly in parallel (the two parameters were highly correlated, $r = 0.92$), suggesting that the hepatic concentrations of the two enzymes were induced in a co-ordinate manner (compare with the data of Allman *et al.*, 1965; Muto & Gibson, 1970). Since fatty acid synthase is not thought to be regulated by covalent modification (Hardie, 1980) this correlation also highlights the importance of changes in I/A and I/C activity ratios in the regulation of acetyl-CoA carboxylase activity *in vivo* during starvation and, especially, in weaned animals.

General discussion. The use of incubation with citrate to activate acetyl-CoA carboxylase is recognized as a method to obtain a measurement of maximal activity of the enzyme *in vitro* (see, e.g., Assimacopoulos-Jeannet *et al.*, 1981). However, in the light of recent studies (see Denton *et al.*, 1981, for review) suggesting that the enzyme (at least in adipose tissue) can be phosphorylated at multiple sites and that phosphorylation at one of these sites is associated with activation, the interpretation of the activities obtained after dephosphorylation of acetyl-CoA carboxylase by exogenous phosphoprotein phosphatase(s) (the site specificity of which is unknown) has to be made with caution. Consequently, although I/C ratios can be assumed to give a valid indication of the 'initial'/'total' activity ratio, the same may not be necessarily true for I/A ratios. However, it is noteworthy that the A/C ratios were the same for livers of all animals studied suggesting that phosphatase-mediated activation always resulted in a reproducible alteration in

enzyme activity in relation to the fully activated (polymerized) state attained after incubation with citrate.

Changes in I activity of acetyl-CoA carboxylase that occur during the reproductive cycle in fed rats appear to be due primarily to changes in the maximal activity of the enzyme rather than to changes in the proportion of the enzyme in the active state. However, during starvation regulation of the initial activity of the enzyme by covalent modification becomes important, especially in weaned animals. The hormonal mechanisms underlying these regulatory mechanisms require investigation since initial activity of acetyl-CoA carboxylase in the liver has been shown to be under the control of at least three hormones: glucagon (inhibition), insulin and vasopressin (activation). In mid-lactating rats, serum insulin concentration is lower than in virgin animals (Robinson *et al.*, 1978). In view of previous reports on the effects of insulin and anti-insulin serum on acetyl-CoA carboxylase activity in hepatocytes *in vitro* and the liver *in vivo* (Geelen *et al.*, 1978; Stansbie *et al.*, 1976) the lower levels of insulin during lactation would be expected to result in lower I/A and I/C activity ratios during lactation. Although the present results show some evidence of decreased ratios in early lactation, the I/A and I/C ratios were identical in fed virgin and mid-lactating animals. However, they decreased markedly during starvation, a condition associated with decreased insulin levels, and they were highest in fed weaned animals in which insulin levels have been shown previously to be elevated at least 2-fold (Agius *et al.*, 1979; Agius, 1980).

There is an apparent paradoxical relationship between the rates of hepatic lipogenesis in virgin, lactating and weaned rats and the respective insulin levels in these conditions. Thus lipogenesis in liver of mid-lactating rats is twice as high as that in liver of virgin animals and increases further after 24h-weaning (Agius, 1980). However, plasma insulin levels are lower in lactating rats than in virgin animals, although they increase markedly on weaning (see above). It is suggested that the apparent lack of correlation between plasma insulin levels and hepatic lipogenesis may be explained by different effects of insulin and prolactin on the acute (changes in I/A and I/C ratios) and long-term (changes in A and C activities) modes of regulation of acetyl-CoA carboxylase respectively. The high rates of lipogenesis during lactation (high prolactin, low insulin) are primarily due to an increased concentration of acetyl-CoA carboxylase in the liver, whereas the increased rate of hepatic lipogenesis in weaned animals (high insulin, low prolactin) is achieved through an increase in the fraction of the enzyme in the active state (higher I/A and I/C ratios). Hence high rates of hepatic lipogenesis are possible both in a low plasma insulin and in a high insulin situation through an alternation in the importance of the two regulatory mechanisms.

If this suggestion is valid, the question arises as to why maximal activities of acetyl-CoA carboxylase and fatty acid synthase are not decreased after 24h-weaning when prolactin levels are very low. It is known that long-term hormonal regulation of concentrations of lipogenic enzymes in the liver is achieved through changes in the rates of synthesis (Craig *et al.*, 1972) and that the rates of degradation are very slow except during early stages of starvation (Craig *et al.*, 1972). Thus it is possible that, whereas increasing prolactin concentrations (from early to mid-lactation) result in a gradual induction of lipogenic enzymes, the sharp fall in prolactin on weaning is not followed immediately by a decrease in lipogenic enzyme concentrations in the liver. In addition the transiently increased levels of insulin in the serum of weaned animals (see above) would tend to maintain synthesis of lipogenic enzymes (Nepokroeff *et al.*, 1974). It is evident that the concentration of hepatic acetyl-CoA carboxylase responds much more slowly than that in the mammary gland, which decreases by over 90% during the first 24h of weaning (McNeillie & Zammit, 1982).

We thank Dr. R. A. Clegg for performing the gel electrophoresis, Professor M. Peaker and Dr. W. W. Christie for their interest and encouragement and Professor P. Cohen for the gift of purified phosphoprotein phosphatase 1.

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