Lipid metabolism during the initiation of lactation in the rat

The effects of starvation and tumour growth

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1. The effects of starvation *post partum* (24 h) and tumour growth *pre partum* on the initiation of lactation in the rat were studied. 2. Tumour growth decreased food intake at 24 h, but not at 2 days *post partum*. 3. Pup growth rate increased with hyperphagia; starvation and tumour burden decreased pup growth, and starvation decreased maternal body weight. 4. Starvation decreased gastrointestinal-tract mass; tumour growth decreased gastrointestinal-tract and mammary-gland mass. 5. Mammary-gland DNA-synthesis rate was high immediately *post partum*, but decreased by day 3 of lactation; starvation and tumour burden decreased this rate, and also decreased gastrointestinal-tract DNA-synthesis rate. 6. Arteriovenous differences for glucose and lactate across the mammary gland did not change with time, nor were they affected by the tumour. Starvation decreased arterial glucose and lactate, and the gland extracted less glucose but produced lactate. 7. Mammary-gland lipogenesis was sensitive to starvation and to tumour growth. 8. In contrast with the gradual development of mammary-gland lipogenic enzyme activities, lipoprotein lipase activity was high in the gland by 2 days *post partum*; starvation or tumour burden decreased the activity. 9. The mammary gland is sensitive *post partum* to decreased food intake, and to tumour presence. The effects of the latter are apparently independent of hypophagia.

INTRODUCTION

The transition from pregnancy to lactation is characterized by rapid changes in lipid metabolism in mammary gland, as well as other maternal organs (Spencer & Lowenstein, 1966; Mackall & Lane, 1977; Martyn & Hansen, 1980, 1981; Grigor *et al.*, 1982; Scow & Chernick, 1987). These changes include increased activities of lipogenic enzymes in mammary gland and liver (Spencer & Lowenstein, 1966; Mackall & Lane, 1977; Martyn & Hansen, 1981; Grigor *et al.*, 1982), and increased lipoprotein lipase (EC 3.1.1.34) activity in mammary gland, with concomitant decrease in adipose-tissue activity (Scow & Chernick, 1987). The factors responsible for co-ordinating these changes are poorly understood, although hormonal effects [e.g. progesterone (Kuhn, 1969; Bartholomeusz *et al.*, 1976; Martyn & Hansen, 1981), prolactin (Zinder *et al.*, 1974; Robinson & Williamson, 1977; Flint *et al.*, 1981) and insulin (Robinson & Williamson, 1977)] may be involved. Initiation of lactation in the rat is also associated with a rapid increase in food intake to support the increased metabolic demands of the active mammary gland (Cripps & Williams, 1975); gastrointestinal hypertrophy occurs at this time, although the causal relationship between the two events is not certain (Elias & Dowling, 1976). As this is a period of abrupt change in metabolic activity, with widespread alteration in tissue gene expression, it might be expected that physiological or pathological perturbation may have a profound effect on its regulation, and examination of this may provide some insight into the underlying mechanisms. Previous work from our laboratory has indicated that the presence of a rapidly growing tumour, the Walker-256 carcinosarcoma, during the *peripartum* period can adversely affect the normal hyperphagic response after parturition, and subsequent

lactational performance (Evans & Williamson, 1988a,b; Williamson *et al.*, 1988). It was therefore decided to investigate this effect in greater detail, and in particular to examine the possible role of food intake in the initiation of lactation *post partum*.

EXPERIMENTAL

Animals

Female Wistar rats (200–300 g) were fed on a chow diet consisting of (by wt.) approx. 52% carbohydrate, 21% protein and 4% fat; the residue was non-digestible material (Special Diet Services, Witham, Essex, U.K.). *Post partum* control ('fed') animals were fed *ad libitum* throughout and studied 1, 2 and 3 days *post partum*. In addition, some mid-lactating animals at 10–14 days *post partum* were studied for comparison. Starved animals were deprived of food 24 h before being killed, which was 1–2 days *post partum*. Again, some mid-lactating animals were studied for comparison. A third group of animals were inoculated with the Walker-256 carcinoma 1–3 days *pre partum*; a cell suspension of approx. 2×10^7 cells in 0.5 ml of 50 mM-phosphate (K_2HPO_4/KH_2PO_4)-buffered saline, pH 8.0, was injected subcutaneously on the left flank of the rats under light diethyl ether anaesthesia. Walker-256 carcinoma is a rapidly growing tumour with a volume doubling time of 0.86 days (Herzfeld & Greengard, 1972); the amount implanted ensured that the tumour mass was approx. 1 g at the time of the experiments. Tumour-bearing animals were fed *ad libitum* until experimentation at 1 or 2 days *post partum* (2–5 days of tumour burden). Food intake and mean pup mass were measured daily. All experiments were commenced between 09:00 and 10:00 h on the stated day *post partum*.

Biochemicals

All enzymes and coenzymes were from Boehringer Corp. (London) Ltd., Lewes, Sussex, U.K.

Radioactive compounds

Glycerol tri[9,10(n)- ^3H]oleate, $^3\text{H}_2\text{O}$ and [methyl- ^3H]thymidine were obtained from Amersham International, Amersham, Bucks., U.K., and $\text{NaH}^{14}\text{CO}_3$ was from NEN Research Products, Dreieich, Germany.

Measurement of tissue DNA-synthesis rate *in vivo*

[methyl- ^3H]Thymidine (1 mCi/mmol; 0.6 mCi/kg body wt.) made up to 0.5 ml with saline (0.9% NaCl) was administered to rats by intraperitoneal injection. Then 1 h later, under pentobarbital anaesthesia (60 mg/kg body wt.), samples of inguinal mammary gland and heart were removed and freeze-clamped in liquid N_2 . The gastrointestinal tract was excised, rapidly cleaned with ice-cold saline, then blotted dry and weighed. Stomach, large intestine and small intestine were separately freeze-clamped (Wollenberger *et al.*, 1960) and stored in liquid N_2 .

Subsequently, samples were ground to a powder, and DNA was extracted by the method of Chung & Coffey (1971). A portion of the DNA solution was removed and its radioactivity measured in a scintillation counter. DNA concentration was measured by the method of Burton (1968), using calf thymus DNA as standard. Results for the rate of DNA synthesis are expressed as d.p.m./h per mg of DNA, but for the purposes of comparing rates between disparate groups, results are presented as a percentage of the rate obtained in heart (as a 'household' reference). The entire mammary gland was also dissected out and weighed.

Measurement of tissue lipogenesis *in vivo*

Lipogenic rate *in vivo* was determined by using $^3\text{H}_2\text{O}$ as previously described (Robinson *et al.*, 1978). Animals were injected with 0.3 ml (3.0 mCi) of $^3\text{H}_2\text{O}$ intraperitoneally 1 h before being killed; 5 min before death the animals were anaesthetized with pentobarbital (60 mg/kg body wt.). At 60 min after injection, blood samples were taken and duplicate samples of liver and inguinal mammary gland removed. The remaining carcass (minus gastrointestinal tract, liver and mammary gland) was autoclaved and then homogenized in a Waring blender. Tissue samples and carcass homogenate were saponified and fatty acids extracted by the method of Stansbie *et al.* (1976).

Lipogenic enzyme activities

Samples of mammary gland and liver were freeze-clamped (Wollenberger *et al.*, 1960) and stored in liquid N_2 . The tissues were ground to a powder with pestle and mortar. Enzymes were extracted by the method of Mackall & Lane (1977) and the high-speed supernatant [1:20 (w/v) homogenate] was used for enzyme assays. Fatty acid synthetase (EC 2.3.1.85) and ATP citrate lyase (EC 4.1.3.8) were assayed spectrophotometrically by the methods of Lynen (1962) and Srere (1959) respectively. Acetyl-CoA carboxylase (EC 6.4.1.2) was incubated for 30 min in the presence of 20 mM-citrate and 10% (w/v) bovine serum albumin at 37 °C to obtain maximal activation, and assayed as described by Munday & Williamson (1982). DNA concentration of the tissue

extracts was measured by the method of Burton (1968), and all enzyme activities are expressed as units/mg of cellular DNA, where 1 unit for fatty acid synthetase and ATP citrate lyase is equivalent to the transformation of 1 μmol of NADPH or NADH respectively per min at 25 °C, and 1 unit for acetyl-CoA carboxylase is equivalent to the incorporation of 1 nmol of $\text{H}^{14}\text{CO}_3^-$ into malonyl-CoA per min at 37 °C.

Lipoprotein lipase

Parametrial adipose tissue, mammary gland and heart samples were obtained and tissue lipoprotein lipase activities were measured by a modification of the technique of Nilsson-Ehle & Ekman (1977). Tissue samples were dried to a powder with acetone/ether, and then resolubilized and used in an assay system containing [^3H]triolein as substrate; ^3H -labelled fatty acids released after a 60 min incubation period were extracted and determined by the method of Nilsson-Ehle & Schotz (1976). Lipoprotein lipase activity is expressed as nmol of fatty acid released/min per mg of acetone-dried powder.

Blood metabolite concentrations

Mammary venous and aortic blood samples were obtained with heparinized syringes at the time of killing. The blood was deproteinized as described previously (Robinson & Williamson, 1977). Whole-blood glucose was determined by the method of Slein (1963) and lactate by the method of Hohorst (1963).

Insulin

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

RESULTS AND DISCUSSION

Previous work on the effects of tumour growth on lactation suggested that tumour presence *pre partum* may exert a deleterious effect on the initiation of lactation (Evans & Williamson, 1988b; Williamson *et al.*, 1988),

Fig. 1. Maternal food intake *peripartum*

Rats were fed *ad libitum* throughout pregnancy and lactation. Food intake refers to consumption during the previous 24 h, with parturition occurring at day 0. Results are expressed as mean values $\pm$ S.E.M. ($n = 10$); values at days -1 and 0 are significantly less than food intake in virgin animals [22.2 ± 0.51 g ($n = 15$); $P < 0.001$].

Table 1. Body composition, food intake and lactational performance during initiation of lactation

Control and tumour-bearing animals were fed *ad libitum*, except where otherwise stated. Food intake is for the last 24 h. Pup growth is the change in mean pup weight for the last 24 h. For further details see the Experimental section. The results are mean values $\pm$ S.E.M. with the numbers of observations in parentheses. Values for fed animals 2 and 3 days *post partum* that are significantly different by Student's *t* test from fed animals 1 day *post partum* are indicated by: *P < 0.05, **P < 0.01. Values for 24 h-starved or tumour-bearing animals that are significantly different from corresponding fed animals are indicated by: †*P* < 0.05, ††*P* < 0.01, †††*P* < 0.001.

State of rats	Food intake (g/24 h)	Pup growth (g/24 h)	Maternal body wt. (g)	Gastro-intestinal-tract mass (% of body wt.)	Mammary-gland mass (% of body wt.)
Fed					
1 day <i>post partum</i> (8)	18.5 $\pm$ 2.9	0.573 $\pm$ 0.154	263 $\pm$ 9.7	5.17 $\pm$ 0.28	4.52 $\pm$ 0.27
2 day <i>post partum</i> (10)	26.4 $\pm$ 2.4 (13)*	0.909 $\pm$ 0.106 (13)	245 $\pm$ 6.9	5.16 $\pm$ 0.20	4.23 $\pm$ 0.24
3 day <i>post partum</i> (5)	33.9 $\pm$ 2.0 (10)**	0.962 $\pm$ 0.090 (10)*	260 $\pm$ 13.4	4.99 $\pm$ 0.27	5.02 $\pm$ 0.37
24 h-starved					
1 day <i>post partum</i> (4)	0	-0.115 $\pm$ 0.178†	228 $\pm$ 8.9†	4.40 $\pm$ 0.13	4.42 $\pm$ 0.13
2 day <i>post partum</i> (4)	0	0.358 $\pm$ 0.131 (8)††	189 $\pm$ 12.0††	3.67 $\pm$ 0.12†††	5.37 $\pm$ 0.77
Tumour-bearing					
1 day <i>post partum</i> (8)	7.50 $\pm$ 1.9††	0.338 $\pm$ 0.144	243 $\pm$ 11.6	3.88 $\pm$ 0.22††	3.93 $\pm$ 0.48
2 day <i>post partum</i> (6)	21.9 $\pm$ 2.5	0.445 $\pm$ 0.122 (12)††	245 $\pm$ 10.5	3.70 $\pm$ 0.29†††	3.39 $\pm$ 0.26†

which did not occur during established lactation (Evans & Williamson, 1988a). Possible mechanisms for this effect include direct or indirect inhibition of the normal hyperphagia in lactation or some failure of mammary-gland terminal differentiation. It was therefore decided to examine the effect of tumour burden on food intake, mammary-gland development and lactational performance, including some aspects of mammary-gland lipid metabolism, and to compare and contrast these with a period (24 h) of starvation *post partum*.

Food intake and lactational performance

It is well documented that hyperphagia accompanies established lactation, with mean daily food intakes increasing from ~ 20 g/day in virgin animals to ~ 50 g/day at mid-lactation (12–14 days; Cripps & Williams, 1975). The present data (Fig. 1) indicate that there is a significant decrease in food intake 24 h before and 24 h after parturition. Wilde & Kuhn (1979) have briefly reported that food intake decreases in the last 4 days of pregnancy; Morgan & Winick (1981) and Moore & Brasel (1984) have examined food intake in the *peripartum* period, but in the former a semi-purified diet was offered and measured food intake was unusually low, whereas in none of these studies were statistical analyses presented. This observation argues against increased food intake itself as an initiating event for the

Fig. 2. Effect of starvation (24 h) on subsequent maternal food intake and pup growth in early lactation

One group of rats was fed *ad libitum* (●, ▲, *n* = 8); another group was starved for 24 h immediately *post partum*, then fed *ad libitum* (○, △; *n* = 11). Food intake indicates measured consumption for the previous 24 h; day 0 indicates the day of parturition. Results are mean values $\pm$ S.E.M.

Table 2. Comparative tissue DNA-synthesis rates during initiation of lactation

Tissue DNA synthesis was estimated as described in the text. Results are expressed as a percentage of the DNA-synthesis rate obtained in heart, and are mean values $\pm$ S.E.M. with the numbers of observations in parentheses. Values for fed animals 3 days *post partum* and in mid-lactation that are significantly different by Student's *t* test from fed animals 2 days *post partum* are indicated by: *P < 0.05. Values for 24 h-starved or tumour-bearing animals that are significantly different by Student's *t* test from corresponding fed animals are indicated by: †*P* < 0.05, ††*P* < 0.01.

State of rats	Tissue DNA synthesis (% of heart DNA synthesis)			
	Mammary gland	Stomach	Small intestine	Large intestine
Fed				
2 day <i>post partum</i> (6)	1117 $\pm$ 224	307 $\pm$ 66.6	1009 $\pm$ 219	842 $\pm$ 179
3 day <i>post partum</i> (5)	301 $\pm$ 110*	222 $\pm$ 6.7	642 $\pm$ 101	492 $\pm$ 65.6
Mid-lactation (3)	163 $\pm$ 66.1*	358 $\pm$ 28.5	898 $\pm$ 190	730 $\pm$ 104
24 h-starved				
2 day <i>post partum</i> (4)	76.2 $\pm$ 23.6††	125 $\pm$ 23.1†	434 $\pm$ 76.8†	390 $\pm$ 117
Tumour-bearing				
2 day <i>post partum</i> (5)	190 $\pm$ 38.5††	185 $\pm$ 57.6	532 $\pm$ 105	268 $\pm$ 91.5†

onset of lactation and suggests that it must be secondary to increased substrate demand. The food-intake pattern may serve as a useful guide to impending parturition and assist in timing experiments in the period *pre partum*.

Tumour-bearing animals showed a decreased food intake (40 %) at 1 day *post partum*, when the tumour had been present for only 2–4 days, but food intake was not significantly decreased by 2 days *post partum* (Table 1), indicating that the normal hyperphagic drive at this stage of lactation is not affected by low tumour mass (approx. 1 g); however, pup growth (a sensitive index of lactation) was decreased by 50 % at this time (Table 1), suggesting inhibition of some specific aspect of mammary-gland metabolism. For this reason, subsequent experiments were timed to occur at 2 days *post partum*.

Starvation for 24 h in the immediate period *post partum* caused a significant decrease in pup growth and maternal body weight (Table 1). This effect was seen even 1 day *post partum* (i.e. with 24 h starvation commencing immediately *post partum*); this is interesting, as control animals fed *ad libitum* are consuming less chow at this time, yet even this modest food intake is clearly critical to normal establishment of lactation and maintenance of maternal and pup mass. To test whether starvation at this time had a lasting effect, a group of animals were starved for 24 h immediately *post partum*, then fed *ad libitum*, and subsequent food intake and pup growth were measured daily. The results indicate that enhanced food intake and lactation (as measured by pup weight) commence as soon as food is re-introduced at 1 day *post partum* (Fig. 2), suggesting that food deprivation at this period of rapid mammary-gland growth and differentiation does not irreversibly prevent mammary-gland development and initiation of lactation. Others have shown that continued undernutrition *post partum* does affect lactational performance (Kliwer & Rasmussen, 1987; Sakanashi *et al.*, 1987).

Gastrointestinal-tract and mammary-gland development

In order to test the possible relationship between hyperphagia, gastrointestinal-tract hypertrophy and mammary-gland development, total masses of these excised organs were measured in the period *post partum*

(Table 1) and their growth rate was assessed by estimating the rate of DNA synthesis by the thymidine-incorporation method (Table 2). Results show that gastrointestinal-tract and mammary-gland masses do not change significantly during the first 3 days of normal lactation (Table 1), the period corresponding to a rapid rise in food intake (Fig. 1) and pup growth (Table 1). Remesar *et al.* (1981) found no *peripartum* change in gut weight in the brown rat. DNA-synthesis rate in stomach, small and large intestine was high at this time, but not significantly higher than during mid-lactation (Table 2). Mammary gland, however, demonstrated very high DNA-synthesis rates in early (day 2) lactation, but this had already significantly decreased by day 3 of lactation to only one-third of the initial value; further significant decreases were seen by mid-lactation (Table 2). Others have reported a peak of DNA synthesis in mouse mammary gland 2–3 days *post partum* (Knight & Peaker, 1982; Zwierzchowski *et al.*, 1984). These data suggest that mammary-gland and gastrointestinal-tract hypertrophy are early phenomena which occur at an initial stage, possibly *pre partum*, despite the low food intake at this time.

Tumour burden, preceding parturition, and starvation *post partum* both cause a significant decrease in gastrointestinal-tract mass. In starvation, this is apparent at 2 days *post partum*, but not at 1 day (Table 1). This again argues that gastrointestinal-tract hypertrophy commences before parturition at a time when food intake is low; it is therefore not surprising that starvation immediately *post partum* (1 day) has no significant effect on gastrointestinal-tract mass, as food intake is physiologically low at this time (Fig. 1). The fact that tumour growth significantly decreased gastrointestinal-tract mass at 1 day *post partum*, whereas starvation did not (Table 1), argues for some specific mechanism other than hypophagia for the inhibitory effect of tumour on gastrointestinal-tract mass. Previous demonstrations of gastrointestinal effects of tumour-associated cytokines (Patton *et al.*, 1987) provide a clue to the possible nature of this mechanism.

Examination of DNA-synthesis rates in gastrointestinal tract shows that starvation at 2 days caused

Table 3. Blood metabolite concentrations during initiation of lactation

Control and tumour-bearing rats were fed *ad libitum*, except where stated. For further details see the Experimental section. The results are mean values $\pm$ S.E.M., expressed as $\mu\text{mol/ml}$ of blood, with the numbers of observations in parentheses. Values for fed animals 2 and 3 days *post partum* that are significantly different by Student's *t* test from fed animals 1 day *post partum* are indicated by: *P < 0.05, **P < 0.01. Values for 24 h-starved animals that are significantly different by Student's *t* test from corresponding fed animals are indicated by: †*P* < 0.05, ††*P* < 0.01.

State of rats	Blood metabolite concn. ($\mu\text{mol/ml}$)					
	Glucose			Lactate		
	Arterial	Mammary venous	Arteriovenous difference	Arterial	Mammary venous	Arteriovenous difference
Fed						
1 day <i>post partum</i> (8)	6.31 $\pm$ 0.27	4.80 $\pm$ 0.32	1.50 $\pm$ 0.19	2.11 $\pm$ 0.26	1.91 $\pm$ 0.23	0.199 $\pm$ 0.098
2 day <i>post partum</i> (14)	5.41 $\pm$ 0.19*	3.85 $\pm$ 0.16**	1.56 $\pm$ 0.14	2.14 $\pm$ 0.23	1.79 $\pm$ 0.11	0.348 $\pm$ 0.142
3 day <i>post partum</i> (9)	5.00 $\pm$ 0.45*	3.19 $\pm$ 0.35**	1.81 $\pm$ 0.26	2.78 $\pm$ 0.34	2.11 $\pm$ 0.19	0.672 $\pm$ 0.224
24 h-starved						
2 day <i>post partum</i> (8)	4.30 $\pm$ 0.25††	3.46 $\pm$ 0.32	0.84 $\pm$ 0.16††	1.53 $\pm$ 0.11†	1.63 $\pm$ 0.11	-0.105 $\pm$ 0.092†
Tumour-bearing						
2 day <i>post partum</i> (8)	4.78 $\pm$ 0.56	3.20 $\pm$ 0.39	1.58 $\pm$ 0.35	2.64 $\pm$ 0.26	2.25 $\pm$ 0.28	0.383 $\pm$ 0.260

Table 4. Tissue lipogenesis *in vivo* during initiation of lactation

Control and tumour-bearing rats were fed *ad libitum*, except where stated. For further details see the Experimental section. The results are mean values $\pm$ S.E.M., expressed as μmol of $^3\text{H}_2\text{O}$ incorporated into saponified lipid/h per g wt. of tissue. Values for fed animals 2 and 3 days *post partum* and in mid-lactation that are significantly different by Student's *t* test from fed animals 1 day *post partum* are indicated by: *P < 0.05. Values for 24 h-starved or tumour-bearing animals that are significantly different by Student's *t* test from corresponding fed animals are indicated by: †*P* < 0.05, ††*P* < 0.01.

State of rats	$^3\text{H}_2\text{O}$ incorporation into saponified lipid (μmol of $^3\text{H}_2\text{O/h}$ per g wet wt. of tissue)		
	Mammary gland	Liver	Carcass
Fed			
1 day <i>post partum</i> (6)	43.1 $\pm$ 17.0	14.8 $\pm$ 5.59	3.32 $\pm$ 0.562
2 day <i>post partum</i> (8)	58.8 $\pm$ 9.15	15.5 $\pm$ 3.33	3.73 $\pm$ 0.481
3 day <i>post partum</i> (4)	73.1 $\pm$ 7.92	16.1 $\pm$ 2.17	3.08 $\pm$ 0.325
Mid-lactation (6)	113 $\pm$ 24.4*	22.0 $\pm$ 3.17	6.07 $\pm$ 0.760*
24 h-starved			
2 day <i>post partum</i> (4)	4.43 $\pm$ 0.956††	2.56 $\pm$ 0.43†	1.86 $\pm$ 0.221†
Tumour-bearing			
2 day <i>post partum</i> (9)	33.1 $\pm$ 7.87†	10.1 $\pm$ 1.55	2.48 $\pm$ 0.186†

a significant decrease (halved rate) in the upper gastrointestinal tract (stomach and small intestine), whereas tumour burden significantly decreased the rate in only the lower tract (large intestine) (Table 2). Thus the decrease in gastrointestinal-tract mass consequent upon starvation is presumably a 'local' phenomenon caused by decreased 'work' hypertrophy, whereas the decrease in gastrointestinal-tract mass seen in association with tumour burden appears to be a time-specific effect independent of food intake (Table 1). Supporting this argument is the observation that starvation caused no decrease in mammary-gland mass (Table 1), despite a significant lowering of the expected high DNA-synthesis rate in the gland at this stage (Table 2), whereas tumour

growth did significantly decrease mammary-gland mass as well as gland DNA-synthesis rate (Tables 1 and 2). Thus tumour growth appears not to exert its deleterious effects simply via alterations in food intake.

Arteriovenous differences for glucose and lactate across the mammary gland

Lactating mammary gland utilizes glucose and lactate for lipid and lactose synthesis in the fed state, but this is rapidly decreased after starvation (Katz & Wals, 1972; Robinson & Williamson, 1977). Arteriovenous concentrations of these blood metabolites have not been examined in the early period *post partum*. Under control (fed) conditions, there was a significant decrease in

arterial glucose, despite low initial but rapidly increasing food intake over the same time period (Fig. 1), which was paralleled by a comparable decrease in mammary-venous glucose, so that the arteriovenous difference remained constant (Table 3). However, it is likely that mammary-gland blood flow increases during early lactation in the rat, and this would imply that the rate of extraction increases. Lactate, also an important lipogenic precursor for the mammary gland, did not change in concentration significantly in arterial or mammary-venous blood, but its extraction (arteriovenous difference) by the gland tended to increase during the period *post partum* (Table 3).

Tumour-bearing animals demonstrated a similar metabolic profile to fed controls at 2 days *post partum* (Table 3), implying that there was no defect in precursor supply or utilization caused by the presence of the tumour. Starvation (24 h) at the same stage *post partum* did, however, cause a significant arterial hypoglycaemia and decreased extraction of glucose by the gland. Similarly, arterial lactate was decreased in starved animals, and the arteriovenous difference for this metabolite became negative, implying lactate production by the mammary gland (Table 3). This effect of starvation in early lactation on glucose and lactate extraction is similar to that observed at peak lactation (Robinson & Williamson, 1977).

Tissue lipogenesis during early lactation

Mammary gland has a very high rate of lipogenesis *in vivo* at mid-lactation (Robinson *et al.*, 1978; Table 4), and this is acutely sensitive to alterations in food intake (Robinson *et al.*, 1978; Munday & Williamson, 1983; Busmann *et al.*, 1984). We have examined the development of the lipogenic rate in the first 72 h of lactation (Table 4). The rate at 24 h *post partum* was approx. 30 % of that at peak lactation and gradually increased over the next 2 days (Table 4). Since this time course is mirrored by the increasing food intake (Fig. 1), there may be a causal relationship. Hepatic lipogenesis was unchanged throughout the period. Starvation (24 h) during this period of establishment of lactation significantly decreased the mammary-gland lipogenic rate, as did tumour burden 2 days *post partum* (Table 4). These results agree with our previous work (Evans & Williamson, 1988b), which suggested that decreased mammary-gland lipogenesis is a feature of tumour implantation *pre partum*. However, the earlier studies examined a large proportion of animals at 1 day *post partum* (Evans & Williamson, 1988b), when hypophagia certainly exists (Table 1) and low mammary-gland lipogenesis may therefore be expected (Robinson *et al.*, 1978). Consequently, a correlation was sought in our present, more rigorous, data (where tumour burden caused no significant hypophagia at 2 days *post partum*) between food intake, mammary-gland lipogenesis and pup growth (Tables 1 and 4). For fed (control) animals, food intake correlated positively with mammary-gland lipogenesis ($r = 0.84$) and pup growth ($r = 0.83$), and pup growth with mammary-gland lipogenesis ($r = 0.71$) ($n = 16$). Similarly, in tumour-bearing animals fed *ad libitum*, at 2 days *post partum* food intake correlated positively with mammary-gland lipogenesis ($r = 0.72$) and pup growth ($r = 0.77$), and pup growth with mammary-gland lipogenesis ($r = 0.76$) ($n = 7$). Thus an initial decrease in food intake at 24 h *post partum* in tumour-bearing animals (Table 1; Evans & Williamson, 1988b) is

Table 5. Activities of lipogenic enzymes during initiation of lactation

Control and tumour-bearing rats were fed *ad libitum*. For further details see the Experimental section. Results are mean values $\pm$ S.E.M., with the numbers of observations in parentheses. Specific activities are expressed as units per mg of cellular DNA. For ATP citrate lyase and fatty acid synthetase, 1 unit is equivalent to the transformation of 1 μ mol of NADH or NADPH per min at 25 °C; for acetyl-CoA carboxylase, 1 unit is equivalent to the incorporation of 1 nmol of $H^{14}CO_3$ into malonyl-CoA per min at 37 °C. Values for fed animals 2 and 3 days *post partum* and in mid-lactation that are significantly different by Student's *t* test from fed animals 1 day *post partum* are indicated by: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Values for 24 h-starved or tumour-bearing animals that are significantly different by Student's *t* test from corresponding fed animals are indicated by: † $P < 0.05$, †† $P < 0.01$, ††† $P < 0.001$.

State of rats	Lipogenic enzyme specific activity (units/mg of cellular DNA)					
	ATP citrate lyase		Fatty acid synthetase		Acetyl-CoA carboxylase	
	Mammary gland	Liver	Mammary gland	Liver	Mammary gland	Liver
Fed						
1 day <i>post partum</i> (5)	0.265 $\pm$ 0.071	0.250 $\pm$ 0.097	0.061 $\pm$ 0.013	0.161 $\pm$ 0.063	22.3 $\pm$ 3.97	29.7 $\pm$ 6.57
2 day <i>post partum</i> (9)	0.304 $\pm$ 0.058	0.114 $\pm$ 0.023	0.166 $\pm$ 0.054	0.146 $\pm$ 0.028	49.2 $\pm$ 5.04**	65.3 $\pm$ 5.25**
3 day <i>post partum</i> (7)	0.361 $\pm$ 0.065	0.218 $\pm$ 0.052	0.308 $\pm$ 0.083*	0.205 $\pm$ 0.015	61.9 $\pm$ 6.58***	82.6 $\pm$ 8.35***
Mid-lactation (5)	1.29 $\pm$ 0.124***	0.942 $\pm$ 0.172**	1.26 $\pm$ 0.155***	0.504 $\pm$ 0.062**	164.7 $\pm$ 13.6***	118.7 $\pm$ 20.3**
24 h-starved						
2 day <i>post partum</i> (5)	0.295 $\pm$ 0.076	0.014 $\pm$ 0.009†	0.072 $\pm$ 0.016	0.138 $\pm$ 0.009	27.8 $\pm$ 2.87†	26.2 $\pm$ 5.06†††
Tumour-bearing						
2 day <i>post partum</i> (7)	0.354 $\pm$ 0.054	0.109 $\pm$ 0.039	0.141 $\pm$ 0.041	0.094 $\pm$ 0.021	46.2 $\pm$ 4.67	40.8 $\pm$ 6.63†

associated with the expected decreased mammary-gland lipogenesis (Robinson *et al.*, 1978). Tumour burden does not therefore interfere with the normal food-intake-mammary-gland-lipogenesis regulatory mechanism (at least at this period of lactation), a function which may be at least partly dependent on gastrointestinal-tract hormones (Page, 1989).

Starvation significantly decreased liver and carcass lipogenesis in early lactation, but tumour burden only decreased carcass lipogenesis (Table 4).

Mammary-gland lipogenic enzyme activity

The normal development *post partum* of mammary-gland and liver lipogenic enzyme activities has been examined previously (Mackall & Lane, 1977; Martyn & Hansen, 1981; Grigor *et al.*, 1982). In the present work the effects of tumour growth and starvation on the development of lipogenic enzymes were studied. The enzymes, ATP citrate lyase, fatty acid synthetase and acetyl-CoA carboxylase, were assayed under optimum conditions, and the results are expressed relative to DNA concentration (Table 5). In control animals fed *ad libitum*, the initial relatively low food intake for the first 3 days of lactation (Fig. 1), which correlates with the relatively low lipogenic rate *in vivo* (Table 4), is also associated with low activities of tissue (mammary gland and liver) lipogenic enzymes (Table 5). ATP citrate lyase showed no significant increase in either tissue during the immediate period *post partum* (3 days), whereas fatty acid synthetase was significantly increased in mammary gland by day 3 of lactation (though still significantly less than its activity at mid-lactation). Acetyl-CoA carboxylase activity showed a significant increase by day 2 of lactation in mammary gland and liver, but was still significantly less than mid-lactation values (Table 5); the data presented do not take into account possible changes in activation state of this rate-controlling enzyme (Hardie *et al.*, 1984). These findings for control rats are in general agreement with work of others (Mackall & Lane, 1977; Martyn & Hansen, 1981; Grigor *et al.*, 1982), except that Martyn & Hansen (1981) reported an increase in ATP citrate lyase activity over the first 3 days.

Starvation had no significant effect on the activity of ATP citrate lyase or fatty acid synthetase in mammary gland, but decreased acetyl-CoA carboxylase activity by nearly 50% in this tissue (Table 5), implying that the observed decrease in mammary-gland lipogenesis (Table 4) may be due to the changes in acetyl-CoA carboxylase concentration and/or substrate supply to the gland (see Table 3). Starvation decreased ATP citrate lyase as well as acetyl-CoA carboxylase activity in liver (Table 5), which may explain the decreased hepatic lipogenesis *in vivo* (Table 4). Tumour burden at 2 days *post partum* had no effect on mammary-gland lipogenic enzyme activity, but caused a modest decrease in hepatic acetyl-CoA carboxylase (Table 5). These findings are in keeping with the measurements of lipogenic rates *in vivo* already discussed above (Table 4).

Tissue lipoprotein lipase activity

The other main source of lipid for milk is the uptake of fatty acids derived from chylomicrons or very-low-density lipoproteins by the action of lipoprotein lipase (see Scow & Chernick, 1987). In agreement with previous findings (Hamosh *et al.*, 1970), high activity of this enzyme occurred in mammary gland from control animals at day 2 of lactation (Table 6). It is noteworthy that this was at a time when activities of lipogenic enzymes and lipogenesis *in vivo* in this tissue were still relatively low compared with mid-lactation values (Tables 4 and 5); in fact there was a significant decrease in lipoprotein lipase activity in mammary gland (by mid-lactation) to only two-thirds the value *post partum* (Table 6). These findings suggest that in the normal physiological establishment of lactation, milk lipid is initially principally supplied by gland assimilation of circulating endogenous (very-low-density lipoproteins) or exogenous (chylomicron) lipoproteins rather than synthesis *de novo* within the gland, whereas by mid-lactation the latter has assumed greater importance. Support for this view is the steady increase in the milk content of medium-chain fatty acids (products of synthesis *de novo*) compared with long-chain fatty acids as lactation proceeds (Martyn & Hansen, 1980).

Table 6. Tissue lipoprotein lipase activity during initiation of lactation

For full details see the Experimental section. The results are mean values $\pm$ S.E.M. with the numbers of observations in parentheses. Values for fed animals 3 days *post partum* and in mid-lactation that are significantly different by Student's *t* test from fed animals 2 days *post partum* are indicated by: **P < 0.01, ***P < 0.001. Values for 24h-starved or tumour-bearing animals that are significantly different by Student's *t* test from corresponding fed animals are indicated by: †††*P* < 0.01, ††††*P* < 0.001.

State of rats	Lipoprotein lipase activity (nmol of fatty acid released/min per mg of acetone-dried tissue)		
	Mammary gland	White adipose tissue	Heart
Fed			
2 day <i>post partum</i> (5)	1.27 $\pm$ 0.053	0.362 $\pm$ 0.076	0.420 $\pm$ 0.075
3 day <i>post partum</i> (4)	1.27 $\pm$ 0.077	0.394 $\pm$ 0.043	0.431 $\pm$ 0.015
Mid-lactation (6)	0.848 $\pm$ 0.055***	0.264 $\pm$ 0.042	0.169 $\pm$ 0.015**
24 h-starved			
2 day <i>post partum</i> (4)	0.665 $\pm$ 0.033†††	0.186 $\pm$ 0.022	0.343 $\pm$ 0.065
Tumour-bearing			
2 day <i>post partum</i> (5)	0.694 $\pm$ 0.125††	0.275 $\pm$ 0.062	0.444 $\pm$ 0.119

Starvation or tumour growth significantly decreased lipoprotein lipase activity in mammary gland (about 50 %; Table 6). The effect of starvation on the activity of this enzyme is in agreement with previous work from this laboratory on food deprivation during mid-lactation (Oller do Nascimento & Williamson, 1988). In contrast, it has been reported that 24 h starvation at peak lactation did not alter mammary-tissue activity of lipoprotein lipase (Clegg, 1988). Tumour growth in the present study did not significantly decrease food intake (Table 1), and this further supports the concept of a specific tissue-dependent effect, direct or indirect, of tumour burden on mammary-gland metabolism.

Conclusions

Parturition in the rat is associated with a physiological decrease in food intake, and therefore it is perhaps not surprising that starvation (24 h) immediately after birth does not cause an irreversible impairment of the establishment of lactation. In fact, the effects of 24 h starvation after birth are similar to those that occur at peak lactation, and which are likewise completely reversed by refeeding. In contrast, the effects of tumour growth in the *peripartum* period are more marked in that pup growth is rapidly impaired (Table 1), and this impairment continues throughout lactation (Williamson *et al.*, 1988). The present experiments provide some information on the mechanisms for this impairment in lactational performance. Firstly, the mass of mammary tissue is decreased by the presence of the tumour (approx. 20 %; Table 1); secondly, the activity of lipoprotein lipase is decreased (both on a g-wet-wt. basis and on a total-activity basis; Tables 1 and 6); thirdly, mammary-gland lipogenesis is decreased (Table 4). The possibility that lactose and protein synthesis are also impaired by tumour growth *peripartum* has not been investigated in the present work.

A point of considerable interest is why the effects of tumour growth on food intake, mammary-gland mass and lactational performance only occur if the tumour is implanted before parturition (Williamson *et al.*, 1988; Evans & Williamson, 1988a). A possible explanation is that the tumour or its presence results in the production of a factor which impairs the differentiation of the mammary gland around parturition and consequently diminishes the rate of lactation. Some support for this view is our finding that the presence of the tumour also inhibits the rapid 'switch-on' of lipoprotein lipase activity and lipogenesis in white adipose tissue on cessation of lactation (Evans & Williamson, 1988a). Putative effectors may be tumour necrosis factor α (Semb *et al.*, 1987; Evans & Williamson, 1988c), interleukin-1 (Argilés *et al.*, 1989) or specific tumour products.

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