Mammary sensitivity to protein restriction and re-alimentation

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The present study tested the influence of protein undernutrition and re-alimentation on mammary gland size and secretory cell activity in lactating rats. During gestation, female Sprague–Dawley rats were offered a high-protein diet (215 g crude protein (N × 6·25; CP)/kg DM; H); litters were standardized to twelve pups at parturition. During lactation, two diets were offered *ad libitum*, diet H and a low-protein diet (90 g CP/kg DM; L). Lactational dietary treatments were the supply *ad libitum* of either diet H (HHH) or diet L (LLL) for the first 12 d of lactation, or diet L transferring to diet H on either day 6 (LHH) or 9 (LLH) of lactation. On days 1, 6, 9 and 12 of lactation, rats from each group ($n \geqslant 6$) were used to estimate mammary dry mass, fat, protein, DNA and RNA; the activities of lactose synthetase (*EC* 2.4.1.22) enzyme and Na^+,K^+-ATPase (*EC* 3.6.1.37) were also measured. Rats offered a diet considered protein sufficient (H) from day 1 of lactation showed a decrease in mammary dry mass and fat but an increase in DNA, RNA and protein on day 6, after which there was no further change, except for mammary protein which continued to increase. However, rats offered diet L showed a steady loss in mammary mass and fat throughout the 12 d lactation period and no change in mammary DNA, RNA or protein. Rats previously protein restricted for either the first 6 or 9 d of lactation had their mammary dry mass and mammary fat loss halted and showed a rapid increase in mammary DNA, RNA and protein on re-alimentation. Lactose production in group HHH, as measured by lactose synthetase activity, was similar on days 1 and 6 of lactation, after which a significant increase was seen. Protein-restricted rats showed no change in lactose synthetase activity during the 12 d experimental period. Changing from diet L to diet H led to a significant increase in lactose synthetase activity to levels comparable with those offered diet H from day 1. These results show that rats offered a protein-restricted diet during lactation suffer mammary underdevelopment, but this may be rapidly reversed by re-alimentation with a high-protein diet.

Lactation: Mammary gland: Dietary protein

Milk yield is a function of the number and activity of secretory cells present in the mammary gland. Development of this gland has been shown to occur during both gestation and early lactation, although development during lactation varies between species. For example, it has been shown that lactational development accounts for 41% of the total mammary dry mass in the rat (Griffith & Turner, 1961), 24% in the mouse (Brookreson & Turner, 1959), 44% in the rabbit (Lu & Anderson, 1973) and 6% for the hamster (Sinha *et al.* 1970). Data for ruminant species is less clear. However, mammary cell proliferation during lactation may account for up to 23% of the total in the goat (Knight & Peaker,

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1984) and 2% in the sheep (Anderson, 1975). Equivalent data from cattle, despite their economic importance, is remarkably lacking.

Altering a lactating animal's diet can change both the yield and composition of milk produced (Broster *et al.* 1969; Knight & Peaker, 1982*a*; Pine *et al.* 1994*b*, *d*). Despite the plethora of literature on mammogenesis, during both gestation and lactation little attention has been given to the effects of nutrition and, in particular protein malnutrition, on mammary development. Undernutrition in virgin rats (Srivastava & Turner, 1966) and during gestation (Sykes *et al.* 1948; Rosso *et al.* 1981) can reduce mammary weight and, therefore, lactational performance, as can offering protein-deficient diets during gestation (Pyska & Styczynski, 1979*a*) and lactation (Pyska & Styczynski, 1979*b*). The primary objective of the present experiment was to assess the sensitivity of the rat mammary gland, in terms of mammary cell number and cellular activity to protein undernutrition and re-alimentation, during lactation.

MATERIALS AND METHODS

Experimental protocol

Seventy-seven multiparous (second parity) female Sprague–Dawley rats (B & K Universal Ltd, Hull, Humberside), weighing on average 300 (SE 2·45) g, were housed in a room regulated at 22° with relative humidity between 45 and 65% and with a light period from 07.00 to 19.00 hours for a minimum of 2 weeks before breeding. During this period they were offered a standard rat chow (B & K Universal Ltd) *ad libitum*. At the appropriate time, females were placed individually in a wire-bottomed cage with a proven male breeder. Day 1 of gestation was the morning on which mating was confirmed through the presence of a vaginal plug, after which the females were caged individually in solid-bottomed, plastic cages for the remainder of the experiment.

From day 1 of gestation the females were offered *ad libitum* a high-protein diet (215 g crude protein (N × 6·25; CP)/kg DM; H), formulated to meet National Research Council (1978) requirements for vitamins and minerals, until parturition which was designated day 1 of lactation (for details see Pine *et al.* 1994*d*). On this morning, litters were standardized to twelve pups to ensure a uniform and high lactational demand. Litters which could not be standardized to twelve by cross-fostering with pups born on the same day were removed from the trial, along with their mothers.

During lactation two diets were offered *ad libitum*, both formulated to meet National Research Council (1978) requirements for vitamins and minerals, diet H and a low-protein diet (90 g CP/kg DM; L). The protein source for both diets was casein supplemented with DL-methionine (99:1, w/w) (for all dietary details, see Pine *et al.* 1994*d*). All diets were formulated to provide 21 MJ gross energy (GE)/kg DM, with a constant carbohydrate energy:fat energy ratio of 2·3:1. Lactational dietary treatments were the supply *ad libitum* of either diet H (HHH; *n* 6) or diet L (LLL; *n* 8) for the first 12 d of lactation or diet L with transfer to diet H after either 6 (LHH; *n* 6) or 9 (LLH; *n* 7) d lactation. This dietary allocation produced four groups of females (LLL, LLH, LHH and HHH), the first letter representing the dietary treatment from day 1 of lactation to day 6, the second from day 6 to 9 and the third from day 9 to 12.

Dam body weights and feed intakes were recorded at the same time each day throughout the experiment, as were standardized litter weights. All females were given free access to fresh drinking water. Dams were killed by decapitation on either day (1 (*n* 6), 6 (L *n* 6, H *n* 7), 9 (LL, *n* 7, LH *n* 6) or 12 of lactation when the mammary gland was dissected from all animals.

Lactose synthetase (EC *2.4.1.22*) *activity*

Rates of lactose synthetase activity were determined *in vitro*, based on the method described by Vonderhaar (1977) using fresh mammary tissue. Mammary tissue (approximately 400 mg) from the right inguinal gland was removed immediately after decapitation, chopped (20 μm slices) and homogenized using a hand-held, all-glass homogenizer in 2 ml Tris-HCl buffer (0·02 mol/l), pH 7·4 at 4°, containing $MgCl_2$ (0·01 mol/l) and β-mercaptoethanol (0·001 mol/l). Palmiter (1969) reported a loss in lactose synthetase specific activity for murine mammary tissue after several hours; to correct for this possible loss in activity in both our fresh samples and five samples which had to be frozen at −20° for 7 d before assay, an enzyme stability study was developed. Freshly dissected tissue was homogenized, a portion was removed and assayed at time 0 and hourly for a further 7 h, the homogenate being kept at 4° between sampling points. The remainder was immediately frozen at −20° and assayed daily for 12 d.

Measurement of mammary oxygen consumption

O_2 consumption rates of mammary tissue were measured polarographically as previously described by Pine *et al.* (1994*a*). The difference between the initial O_2 consumption (total respiration) and that following ouabain treatment (Na^+,K^+-ATPase (*EC* 3.6.1.37)-independent respiration) was termed the Na^+,K^+-ATPase-dependent respiration. The percentage inhibition of the original O_2 consumption associated with Na^+,K^+-ATPase activity was calculated using the value for Na^+,K^+-ATPase dependent respiration:total respiration.

Mammary analysis

Mammary dry mass was determined by freeze-drying to a constant mass. The freeze-dried mammary gland was stored at −20° for approximately 5 weeks before being cooled in liquid N_2 and ground to a fine powder using a pestle and mortar, and analysed for total protein, DNA, RNA and fat. Protein was determined by the method of Lowry *et al.* (1951) using bovine serum albumin as a standard. DNA was quantified using PicoGreen Nucleic Acid Quantitation Reagent (Molecular Probes Inc., Eugene, OR, USA), a fluorescent stain for quantifying double-stranded DNA. RNA was extracted using a total RNA Isolation Reagent (Advanced Biotechnologies Ltd, Leatherhead, Surrey) and quantified by measuring absorbance at 260 nm. Mammary fat was extracted using light petroleum (b.p. 40–60 °) using a Soxhlet flask and thimble. Cell number was calculated from total mammary DNA (DNA_t) using the equation of Winick & Noble (1965):

$$\text{cell number} = \frac{DNA_t(\text{mg})}{6{\cdot}2 \times 10^{-9}}.$$

Statistical analysis

The results were analysed by two-way ANOVA, with dam day-1 gestation body-weight as a covariate (Genstat5; Rothamsted Experimental Station, 1994), and calculation of least significant differences; *t* tests were used to compare sample means between dietary treatment groups.

RESULTS

Maternal body-weight changes, feed intakes and litter weight gains

The results for the lactation groups LLL, LLH, LHH and HHH are summarized in Table 1 and Figs. 1 and 2. By day 6 both groups LLL and HHH had lost body weight, but feeding the low-protein diet resulted in a continued weight loss and by day 12 these females were

Table 1. *Maternal body-weight change, feed intake and standardized litter weight gain of rats offered either continuous application of either a low (90 g crude protein (nitrogen × 6·25 : CP) /kg ; L)- or high (215 g CP/kg ; H)-protein diet for the first 12 d of lactation (LLL and HHH respectively) or diet L with transfer to diet H after either 6 (LHH) or 9 (LLH) d lactation**

Lactation dietary treatment...	Days of lactation	LLL	LLH	LHH	HHH	Pooled SED
Dam wt change (g)						
	1–12	−98·5[b]	−42·1[a]	−21·6[a]	−5·2[a]	19·04
	1–6	−40·3[b]	−45·0[b]	−35·1[b]	−6·9[a]	8·82
	7–12	−58·2[b]	2·9[a]	13·6[a]	1·7[a]	16·90
Dam feed intakes (g)						
	1–12	161·0[b]	193·0[b,c]	272·0[a,c]	310·0[a]	36·6
	1–6	69·5	69·6	69·5	101·5	86·1
	7–12	75·0[b]	124·0[b,c]	185·0[a,c]	209·0[a]	29·2
Litter wt gains (g)						
	1–12	59·7[b]	94·3[b]	166·4[a]	215·3[a]	20·10
	1–6	38·8[b]	35·2[b]	52·1[b]	75·6[a]	7·11
	7–12	20·9[b]	59·1[b]	114·2[a]	139·7[a]	17·24

[a,b,c] Means with different superscript letters within the same row were significantly different ($P < 0·05$).
* For details of dietary treatments, see p. 424.

significantly ($P < 0·01$) lighter than those continuously fed on diet H which had effectively regained their body weight. Dams previously offered diet L for either the first 6 or 9 d of lactation, showed a rapid, significant ($P < 0·05$) increase in body weight when transferred to diet H, returning to a weight comparable with group HHH by day 12.

Feed DM intakes of diets L and H followed a similar course for the first 3 d of lactation, after which they diverged. Consequently, this resulted in a much greater total feed intake (g DM) for females offered diet H compared with those offered diet L ($P < 0·01$; Table 1). Groups LHH and LLH showed an immediate increase in feed intake when offered diet H after days 6 and 9 respectively, and by day 12 the accumulated intake for females from group LHH was only slightly lower and not significantly different from that of females from group HHH. Lactational performance was estimated by the weight gain of a standardized litter and closely reflected DM intake (Figs. 1, 2). The greater supply of both dietary energy and protein received by dams offered diet H allowed their litters to achieve a greater weight gain during lactation when compared with that of those offered diet L. Changing from diet L to diet H after days 6 and 9 allowed both groups LHH and LLH to increase lactational performance as measured by daily litter weight gain, with significant improvements in litter weight gain being seen within 24 h of the dietary change.

Effect of lactational dietary treatments on mammary gland composition during lactation

The main effects of the lactation dietary treatments on mammary gland dry mass and composition are shown in Table 2 and Fig. 3. Mammary dry masses and total mammary fat values significantly decreased in all groups until day 6 of lactation, after which losses continued in rats on diet L, but were halted in those rats offered diet H and those transferred from diet L to H after both 6 and 9 d of lactation. Dams offered diet H showed a rapid and significant increase in DNA, RNA and protein between days 1 and 6 of lactation, after which the mammary protein continued to increase, but there was no further significant change in total DNA or RNA content. However, rats offered diet L showed no change in mammary protein or nucleic acids during the 12 d lactation period, but when

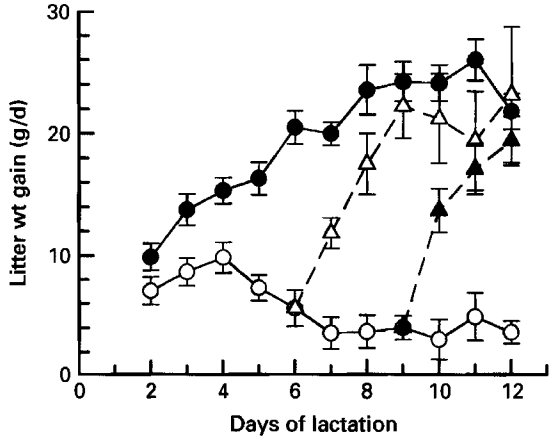

Fig. 1. Daily litter-weight gain (g) of rats offered either a low (90 g crude protein (nitrogen × 6·25; CP)/kg; L)- or high (215 g CP/kg; H)-protein diet for the first 12 d of lactation (LLL (○); *n* 8 and HHH (●); *n* 6, respectively), or diet L with transfer to diet H after either 6 (LHH (△); *n* 6) or 9 (LLH (▲); *n* 7) d lactation. For details of dietary treatments, see p. 424. Points are mean values with their standard errors represented by vertical bars, using dam day 1 gestation weight as a covariate.

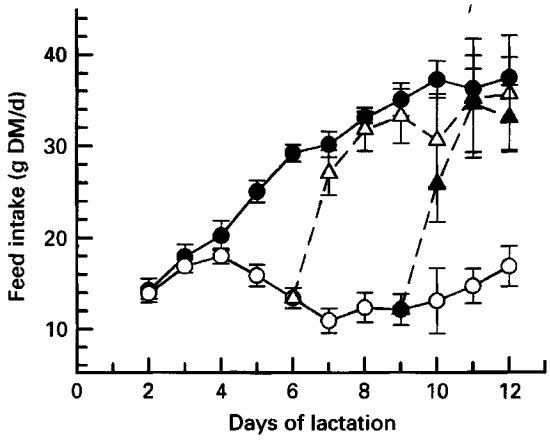

Fig. 2. Daily feed DM intake (g) of rats offered either a low (90 g crude protein (nitrogen × 6·25; CP)/kg; L)- or high (215 g CP/kg; H)-protein diet for the first 12 d of lactation (LLL (○); *n* 8 and HHH (●); *n* 6, respectively), or diet L with transfer to diet H after either 6 (LHH (△); *n* 6) or 9 (LLH (▲); *n* 7) d lactation. For details of dietary treatments, see p. 424. Points are mean values with their standard errors represented by vertical bars, using dam day 1 gestation weight as a covariate.

these rats were offered diet H after both days 6 and 9, groups LHH and LLH respectively, they showed a significant increase in nucleic acid and protein content.

Mammary cell activity

The responses of the mammary enzyme lactose synthetase to lactational dietary treatment and the O_2 consumption and associated Na^+,K^+-ATPase activity of the mammary gland are given in Table 3. The activity of lactose synthetase decreased in rats offered diet L and by day 9 the activities were significantly ($P < 0{\cdot}05$) lower than those on day 1. However, on day 12 the activities had increased to levels not different from those on day 1. Rats offered diet H showed no significant change in lactose synthetase activities over the first 6 d

Table 2. *Mammary composition on days 1, 6, 9 and 12 of rats offered either a low (90 g crude protein (nitrogen × 6·25; CP)/kg; L)- or high (215 g CP/kg; H)-protein diet for the first 12 d of lactation (LLL and HHH respectively), or diet L with transfer to diet H after either 6 (LHH) or 9 (LLH) d of lactation**

Lactation dietary treatment...	Day of lactation	LLL	LLH	LHH	HHH	Pooled SED
Dry mass (g)						
	1	11·10[d]			11·10[d]	
	6	6·44[e]			7·44[e]	0·884
	9	5·31[e,f]		7·05		
	12	4·03[b,f]	6·05[a]	6·47[a]	7·15[a,e]	
Total protein (g)						
	1	1·94			1·94[d]	
	6	1·902			2·564[d]	0·281
	9	1·776[a]		2·624[b]		
	12	1·674[b]	2·575[a]	2·899[a]	3·262[a,e]	
Total fat (g)						
	1	8·89[d]			8·89[d]	
	6	4·21[e]			4·70[e]	0·743
	9	3·17[e,f]		4·21		
	12	2·03[f]	3·27	3·08	3·68[e]	
Total RNA (mg)						
	1	55·8			55·8[d]	
	6	62·7			104·7[e]	16·52
	9	53·6[a]		97·5[b]		
	12	43·4[b]	86·9[c]	132·9[a]	119·1[a,c,e]	
RNA:DNA						
	1	5·13			5·13	
	6	6·07			5·79	1·16
	9	5·25		6·67		
	12	3·68[b]	5·09[a]	6·74[a]	7·01[a]	

[a,b,c] Means with different superscript letters within the same row were significantly different ($P < 0·05$).
[d,e,f] Means with different superscript letters within the same column for each variable were significantly different ($P < 0·05$).
* For details of dietary treatments, see p. 424.

of lactation, after which activities significantly increased ($P < 0·05$). Changing from diet L to H on either day 6 or day 9 led to a significant increase ($P < 0·05$) in lactose synthetase activity to levels comparable with those fed on diet H from day 1.

The enzyme stability study showed no significant loss of specific activity over 7 h at 4°. However, the activities of the five samples kept for 7 d at −20° showed a decline with time and were corrected using linear regression analysis (loss in specific activity 78·8 disintegrations/min per d; r^2 0·93; equivalent to 9·8 %/day of initial activity).

The mammary-gland total respiration and both Na^+,K^+-ATPase-dependent and -independent activities of the mammary gland tended to decline in rats fed on both diets H and L over the 12 d lactation period, although the reductions were not statistically significant ($P > 0·05$; Table 3). Rats previously offered diet L showed a significant ($P < 0·05$) increase in total respiration rate from 690 to 1199 nmol O_2/mg DNA per min when offered diet H after 6 d. However, it was only a temporary increase and by day 12 the total cellular O_2 consumption was similar to that of rats offered diet L for the full 12 d. The increase in total respiration rate from 549 nmol O_2/mg DNA per min on day 9 to

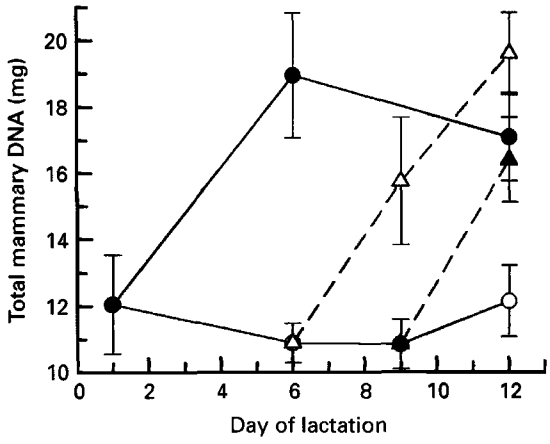

Fig. 3. Total mammary gland DNA (mg) of rats offered either a low (90 g crude protein (nitrogen × 6·25; CP)/kg; L)- or high (215 g CP/kg; H)-protein diet for the first 12 d of lactation (LLL (○) and HHH (●) respectively), or diet L with transfer to diet H after either 6 (LHH (△)) or 9 (LLH (▲)) d lactation. For details of dietary treatment, see p. 424. Points are mean values with their standard errors represented by vertical bars using dam day 1 gestation weight as a covariate.

841 nmol O_2/mg DNA per min on day 12 when diet H was offered after 9 d of *ad libitum* diet L was not significant. O_2 consumption attributable to Na^+,K^+-ATPase activity (Na^+,K^+-ATPase dependent) followed a similar pattern to that of total cellular O_2 consumption, but the increases seen on refeeding were more pronounced. As a consequence, the increases in Na^+,K^+-ATPase-independent respiration were smaller on refeeding diet H and no significant difference was seen between treatments or time.

DISCUSSION

The primary objective of the present work was to assess the sensitivity of the rat mammary gland to dietary protein restriction and re-alimentation during lactation, in terms of mammary cell number and cellular activity. The results show that the mammary gland is extremely sensitive to re-alimentation, and that rapid increases in both cell mass and cellular activity were seen. The rapidity of response was remarkable and has not been reported previously. Similarly, the responsiveness of the gland to nutrition at a relatively advanced stage of lactation is an important observation.

Voluntary intake of a diet with a low protein:energy ratio is considerably reduced in rats when compared with that of a diet with a higher protein:energy ratio. This results in an impaired lactational performance, as indicated by the daily litter weight gain of standardized litters, in agreement with previous experiments from our laboratory (Pine *et al.* 1994*a*, *c*, *d*). A reduced intake of a diet with a low protein concentration (diet L) must lead to a protein deficiency, and hence also an amino acid deficiency, in addition to a shortfall of other nutrients and energy when compared with the performances of rats receiving diet H.

DNA is located almost entirely within the nucleus, and its mass is constant within the diploid nucleus of the rat mammary cell (Griffith & Turner, 1957; Tucker & Reese, 1962). Mirsky & Ris (1949) suggested that total tissue DNA content (DNA_t) may be used as an accurate indicator of cell number and, therefore, development. This was later confirmed by Tucker (1987). For assessment of mammary-gland secretory mass, the assumption that changes in DNA mass are due only to changes in the mammary epithelial cell population

Table 3. *Mammary lactose synthetase* (EC *2.4.1.22*) *activity* (*log* (*nmol lactose formed/mg DNA per min*)) *and oxygen consumption* (*nmol/mg DNA per min*) *on days 1, 6, 9 and 12 of rats offered either a low* (*90 g crude protein* (*nitrogen* $\times 6{\cdot}25$; *CP*)/*kg*; *L*)- *or high* (*215 g CP/kg*; *H*)-*protein diet for the first 12 d of lactation* (*LLL and HHH respectively*), *or diet L with transfer to diet H after either 6* (*LHH*) *or 9* (*LLH*) *d of lactation**

Lactation dietary treatment...	Day of lactation	LLL	LHH	LLH	HHH	Pooled SED
Lactose synthetase activity						
	1	1·989[d]			1·989[d]	
	6	1·708[d,e]			1·977[d]	0·1369
	9	1·545[a,e]	2·200[b]			
	12	1·778[b,d,e]	2·162[c]	2·207[a,e]	2·342[a,e]	
Total respiration						
	1	714			714	
	6	690			673	167·3
	9	549[a]	1199[b]			
	12	644	841	854	577	
Na^+,K^+-ATPase†-dependent respiration						
	1	155			155	
	6	132			122	56·7
	9	110[a]	390[b]			
	12	173[a,e]	193[a,e]	261[b,e]	100[a]	
Na^+,K^+-ATPase†-independent respiration						
	1	560			560	
	6	558			551	124·8
	9	439[a]	809[b]			
	12	471	647	593	477	
Percentage inhibition						
	1	22·0			22·0	
	6	17·3			16·3	4·51
	9	19·9[a]	33·4[b]			
	12	24·9[a,b]	23·4[a,b]	30·6[b]	16·7[a]	

[a,b,c] Means with different letter superscript letters within the same row were significantly different ($P < 0{\cdot}05$).
[d,e,f] Means with different superscript letters within the same column for each variable were significantly different ($P < 0{\cdot}05$).
* For details of dietary treatments, see p. 424.
† *EC* 3.6.1.37.

must also be made. Paape & Sinha (1971) showed that the adipose and connective tissue cell population is determined largely before first conception and, therefore, any change in DNA_t noted during lactation may be ascribed to the secretory cell population. It is essential to base growth studies on total DNA because changes in cell size greatly affect DNA concentration.

Although feeding diet H resulted in a decrease in mammary dry mass (36%) over the 12 d period it did promote an increase (58%) in DNA_t and, therefore, secretory tissue mass. The overall loss of dry mass could mostly be ascribed to the loss of fat over this period. Cell number had reached a maximum by day 6, which is in agreement with other data for rats (Griffith & Turner, 1961) and also for mice (Knight & Peaker, 1982*b*). Knight & Peaker (1982*b*, *c*) suggested an exponential increase in murine mammary secretory cells, from day 12 of gestation until day 5 of lactation, with a doubling time of 6 d. As day 6 was our first

sample point after parturition it is not possible to determine the exact time cell number reached a maximum or a doubling time. Feeding diet L, which was considered inadequate in terms of protein but not energy concentration, to dams in group LLL from day 1 of lactation inhibited any increase in the secretory cell population of the mammary gland. Although diet L was isoenergetic with diet H, over the 12 d lactation period their accumulated feed DM intake was 48% less than that of rats fed on diet H for the equivalent period. Thus, rats fed on diet L received approximately 78% less dietary protein but also 48% less gross energy than rats fed on diet H, and consequently it is not known whether secretory cell mitosis was inhibited due to a protein or an energy shortage. Whether this absolute nutrient shortfall acts directly on the gland or indirectly via a disturbance of the dam's metabolic and endocrine processes cannot be determined from the present study. However, the accumulated feed DM intakes until day 4 of lactation, a period of rapid cell proliferation in the dams of group HHH, were not significantly different for groups HHH and LLL (47·90 (SE 6·13) and 42·93 (SE 4·77) g DM respectively) whereas the CP intakes were significantly different (15·27 (SE 1·54) g and 5·10 (SE 0·40) g; $P < 0·001$ respectively) which may suggest protein was the limiting factor.

Carrick and Kuhn (1978) estimated lactose synthetase activities *in vivo* and reported that, like many metabolic variables, the enzyme exhibits diurnal fluctuations, with synthesis highest during the early morning and lowest during early evening. For this reason, all samples were taken at a similar time during the morning, kept at 4° and analysed as quickly as possible. The daily increase in litter growth rate throughout the 12 d lactation period reflects the demand for milk imposed on the dam during this period. As the total secretory cell population remained static from day 6, this increase in milk production must be achieved by an increase in milk synthesis per cell. When diet H was offered the cells' capacity to synthesize lactose apparently remained constant for the first 6 d of lactation; however, it must be remembered that no intermediary sample was taken. Although lactose production per cell remained constant over this period for these rats, cell number was increasing rapidly and, therefore, total lactose production per gland was also increasing. The second half of lactation showed a significant, positive increase in lactose synthetase activity and by day 12 the dams were producing approximately 2 g lactose/d. This compares favourably with the data of Carrick & Kuhn (1978) who calculated that Wistar rats fed on a standard chow would produce approximately 1·4 g lactose/d, but at peak synthesis rates, which our measurements were taken from, they would be producing 1·6 g/d. Our slightly increased production rate may have been due to a diet containing a higher protein concentration. Mansaray & Grimble (1983) fed Wistar rats on a diet containing a comparable protein concentration (200 g/kg DM) to that of diet H used in the present study and, from their measured lactose synthesis rates on day 14 of lactation, it can be calculated that their rats would have been producing 1·92 g lactose/d. Lactational malnutrition has been reported to suppress lactose synthesis (Carrick & Kuhn, 1978; Wilde & Kuhn, 1979; Mansaray & Grimble, 1983) and our results confirm this. Lactose synthetase specific activity was significantly ($P < 0·05$) suppressed by day 9 when compared with day 1, although this significance was lost by day 12.

Since mammary RNA is intimately related to the biosynthesis of protein, this nucleic acid may be used as an indication of the cell's synthetic potential (Winick & Noble, 1965). Dams offered diet H for the entire 12 d period showed a significant increase in total mammary RNA (RNA_t) over the first 6 d, after which no further increase was seen, in agreement with findings of Pine *et al.* (1994*c*). This pattern of development was similar to that of DNA, and as a result the RNA content per cell, expressed as RNA:DNA, did not change over the lactational period. Dams offered diet L throughout the experimental period showed a slight, but not significant, reduction in RNA_t (22%); by day 12 they had

approximately 64% less RNA than dams from group HHH on the same day, implying a dramatic shortfall in protein synthetic capacity. Of perhaps more interest is the RNA:DNA ratio, which was not significantly reduced on days 6 or 9 when compared with day 1, which may indicate that the cells were able to preserve an adequate quantity of RNA for protein synthesis but the actual rate of protein synthesis was limited by substrate availability. By day 12 the cellular RNA had significantly decreased in the LLL group, suggesting that the mammary epithelial cells now had a reduced protein synthetic capacity. Although the total mammary protein values for this group were significantly less than those for group HHH ($P < 0.01$) on day 12, there was no significant difference within group LLL over the 12 d period (Table 2). This may suggest that by day 12 of lactation the secretory cells' reduced capacity for protein synthesis in group LLL had not, as yet, become noticeable. It is also possible, however, that milk protein synthesis may have been impaired, since previous authors have demonstrated that dietary protein restriction during lactation reduces milk protein concentration (Crnic & Chase, 1978; Sturnman *et al.* 1986; Pine *et al.* 1994*b*) and cellular protein synthesis in these cells had been spared. Specific activity of the enzyme lactose synthetase was not dramatically suppressed on day 12 when compared with day 9 and in fact showed a slight increase in activity, reflecting a slight increase in feed intake, although neither of these increases were significant. This would also suggest that the cells were restricted through lack of substrate rather than RNA.

Mammalian cellular mechanisms require O_2. Two of the most demanding are protein synthesis and the membrane transport system Na^+,K^+-ATPase, which plays an essential role in the transport of both sugars and amino acids across cell membranes (Milligan & Summers, 1986). The mammary epithelial cells of lactating rats are highly active cells and it would be expected that differences in cellular activity would be reflected in the utilization of greater quantities of O_2 and the proportion of tissue energy expenditure associated with Na^+,K^+-ATPase activity. Our results do not support this possibility and are in agreement with those of Pine *et al.* (1994*a*; Table 4). Mammary epithelial cells of dams offered only diet H or only diet L had similar tissue respiration rates, and the proportion associated with Na^+,K^+-ATPase activity on the whole remained unchanged. It should be remembered, however, that group HHH showed considerable hypertrophy in terms of cell number, which would result in a considerable increase in total and Na^+,K^+-ATPase-dependent energy expenditure. Dams offered diet L with transfer to diet H after 6 d (LHH) and 9 d (LLH) showed a temporary increase in both total respiration and the O_2 consumption attributable to Na^+,K^+-ATPase, suggesting a period of rapid cellular activity, perhaps in advance of an increase in cell number.

Dams initially protein restricted, by offering diet L during lactation, and then re-offered diet H on day 6 or 9 (groups LHH and LLH respectively) showed a rapid, significant increase in feed intake to levels comparable with those of dams offered diet H from day 1. This rapid increase in nutrient supply in turn led to an equally rapid and significant increase in daily weight gain of standardized litters from these groups. This improvement in lactational performance was due to a rapid, positive response in mammary cell mass, lactose synthetase activity, RNA_t, and a temporary increase in total cellular respiration rate and Na^+,K^+-ATPase-dependent energy expenditure. This would suggest that the mammary gland of rats whose endogenous labile protein reserves may be assumed to have been depleted (Pine *et al.* 1994*c*) is still capable of becoming fully functional after 9 d of severe dietary protein restriction during lactation. These results also show that although mammary cell proliferation is complete by day 6 of lactation in dams offered a diet of favourable protein:energy ratio, it is still possible to trigger mitosis in dams offered a diet with a low-protein:energy ratio, after this time. Re-alimentation with a diet of high protein concentration, diet H, led to an increased feed intake, which would lead to a greater supply

of metabolites for utilization by the mammary gland. This may also be further enhanced by an increased blood flow to the gland (Williamson, 1984). It is not clear what is inhibiting mitosis and reducing secretory cell activity in the dams offered a high-energy low-protein diet. This low protein:energy diet, for whatever reason, resulted in a significantly reduced DM intake, which led to a period of severe protein and, hence, amino acid restriction. This would have certainly led to an amino acid deficiency; however, it is not known whether this amino acid deficiency or any other nutrient and/or energy shortfall inhibited mitosis and suppressed cellular activity. Therefore, it is not known whether the reversal was due to the greater supply of amino acids, energy-yielding or other nutrients acting directly on the gland, or through other factors, for example the production of a blood-borne signal, hormone or metabolite. One peptide inhibitory factor, mammary-derived growth inhibitor (MDGI), has been shown to inhibit mitosis in several mammary cell lines (Bohmer *et al.* 1984) and is expressed *in vivo* in differentiated cells (Kurtz *et al.* 1990). This has led Burgoyne & Wilde (1994) to the suggestion that MDGI may be a regulator of cell proliferation. However, our work did not assay for MDGI or any other factor.

In summary, it can be concluded that lactating rats offered a diet with a low protein:energy value reduced feed DM intake, and thus subjected themselves to a period of severe protein restriction, which resulted in poor lactational performance. Poor lactational performance was a consequence of an absence of mammary secretory cell proliferation during lactation combined with a low activity of these cells. When a diet with a higher protein concentration was offered, feed intake increased rapidly and there was a corresponding increase in lactational ability. This increase in lactational ability was a result of rapid secretory cell proliferation and a marked increase in cellular activity.

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