

Adaptations in protein metabolism during lactation in the rat

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1. The activities of two hepatic enzymes that participate in the regulation of amino acid oxidation and urea synthesis were measured in lactating rats (day 15 of lactation) and virgin controls. The enzymes were alanine aminotransferase (*EC* 2.6.1.2) and argininosuccinate synthase (*EC* 6.3.4.5). Carcasses of the dams were also analysed.

2. Changes in the activities of both enzymes in dams fed *ad lib.* on a diet containing an excess of protein indicated that amino acid oxidation was depressed. In dams restricted in protein to the level of intake of their controls but allowed to satisfy their needs for energy, enzyme activities were significantly reduced. In these animals lean tissue catabolism supplemented the dietary protein supply.

3. This adjustment in protein metabolism which effectively spares protein for milk-protein synthesis could be explained either by a reduction in the availability of substrate in the liver, or by the intervention of an anabolic hormone secreted in lactation.

During lactation in the rat the mobilization of body fat has been shown to make an important contribution to the additional energy cost of lactation, irrespective of the adequacy of the maternal diet (Naismith *et al.* 1982). When dietary protein was moderately restricted, maternal lean tissue was also catabolized, but to a very limited extent. Furthermore, the substantial increase in food consumption normally associated with lactation was not observed, and milk volume was greatly reduced. It would appear that when dietary protein is limited, the obligatory breakdown of fat disturbs the ideal balance between protein and energy supplies needed for the maintenance of maternal tissues and for milk formation, and so modulates both food intake and milk yield.

Another factor to be considered in this equation might be an augmented amino acid supply resulting from a reduction in amino acid catabolism. During pregnancy the efficiency of protein utilization is improved by the suppression of amino acid oxidation in the liver (Naismith, 1973). The hormone progesterone has been shown to reproduce this metabolic change when administered to virgin animals. Since it has been claimed (Sutter-Dub *et al.* 1974) that the plasma progesterone concentration remains elevated during lactation in the rat, Naismith *et al.* (1982) looked for evidence of a similar alteration in protein utilization during the second phase of reproduction. As an index of amino acid oxidation they measured the activity of hepatic argininosuccinate synthase (L-citrulline:L-aspartate ligase, *EC* 6.3.4.5). This enzyme is sensitive to the level of protein intake (Schimke, 1962) and is believed to be rate-limiting in the urea cycle (Brown *et al.* 1959; Das & Waterlow, 1974). At the moderate level of protein intake provided by the diet (110 g casein/kg), voluntary food intakes and therefore protein intakes of lactating rats and virgin controls were, fortuitously, identical. Nevertheless the activity of argininosuccinate synthase was reduced by 35% in the lactating animals.

In the present study further evidence of an adjustment in protein utilization was sought in animals receiving an adequate diet. We measured the activities of argininosuccinate synthase, and of alanine aminotransferase (L-alanine: 2-oxoglutarate aminotransferase, *EC* 2.6.1.2), an enzyme that has an important role in the final common pathway of amino acid oxidation, and changes in activity at the same rate and to the same extent in response to dietary and hormonal changes (Das & Waterlow, 1974).

EXPERIMENTAL

Animals and diets

Litters of rats of the Sprague-Dawley strain with a preponderance of females were reared on a laboratory stock diet until mean body-weights had reached 180 g. The females were then caged singly and provided *ad lib.* with a semi-synthetic diet of the following composition (g/kg): casein 200, maize starch 650, maize oil 150. To each kg of this basic mix was added 2.0 g DL-methionine, 40 g mineral mixture and 20 g vitamin mixture. The composition of the vitamin and mineral mixtures was as described by Naismith *et al.* (1969).

After a 7 d period of adjustment, food intakes were measured weekly until body-weights exceeded 220 g. Littermate triplets were then selected on the basis of similarity in growth rate, body-weight and food intake, and assigned randomly to three different treatments; two animals from each triplet were mated with males of the same strain, pregnancy being dated from the appearance of a mating plug in the food-spill trays below the cages, and the third served as a control. Seven litters of rats were used. One group of animals was fed on the experimental diet *ad lib.* throughout pregnancy and for 2 weeks of lactation. The control (unmated) group was also fed *ad lib.* for an equivalent period (5 weeks). The second group of pregnant animals received the same diet *ad lib.* during pregnancy, but was restricted to the level of intake of the control animals during lactation. However, in order to avoid an energy restriction as well as a protein restriction during lactation, these animals had free access to a supplement composed of maize starch (800 g/kg) and maize oil (200 g/kg).

The rats were housed individually in screen-bottomed wire-mesh cages under thermostatic conditions (22°). Food consumption was measured weekly throughout the experiment. During lactation the group pair-fed with their littermate controls received their protein-containing diet twice daily in the form of a thin paste to minimize spillage. Their consumption of the protein-free diet, offered in powder form, was recorded.

On the morning of the 15th day of lactation (or the equivalent for the virgin controls) the animals were killed by cervical dislocation. Their livers were rapidly excised and weighed. A portion was taken immediately for analysis for argininosuccinate synthase activity. The remainder was frozen until used for the measurement of alanine aminotransferase activity, which is unaffected by short-term storage at -20°.

The kidneys, spleen, uterus and gastrointestinal tract were removed from each carcass, and the eviscerated carcasses were weighed, then dried to constant weight in an oven (105°) before analysis for fat and protein.

Analytical methods

A portion of fresh liver (1:20, w/v) was homogenized in cetyl-trimethyl ammonium bromide (1 g/l) for the assay of argininosuccinate synthase by the method described by Naismith *et al.* (1982). Unit activity is defined as the amount of enzyme catalyzing the formation of 1 μmol urea per h at 38°.

A portion of thawed liver was homogenized in ice-cold 0.25 M-sucrose solution (1:20, w/v). Alanine aminotransferase activity was then determined by the method of Segal & Matsuzawa (1970). Unit activity is defined as the amount of enzyme catalyzing the formation of 0.55 μmol pyruvate per min at 37°.

Carcasses were analysed for water, fat and protein by the procedures described previously (Naismith *et al.* 1982).

For the statistical treatment of the results a two-way analysis of variance was used. The critical difference is the smallest difference which would attain significance at $P < 0.05$

Table 1. *Body-weights (g) of control rats, of dams during pregnancy and lactation and of their offspring. During lactation rats were either fed ad lib. or restricted in protein intake*
(Mean values with their standard errors for seven matched triplets of rats)

Group	Pregnancy				Lactation		No. of pups		Individual pup wt	
	day 1		day 22		day 15				day 15	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Control	248	4.9	275	6.8	296	5.1	—	—	—	—
Lactating	245	4.4	377	7.6	301	4.0	8.9	1.0	18.3	1.0
Lactating (protein restricted)	247	4.9	389	11.4	260	11.3	10.0	1.7	17.9	1.8
Critical difference ($P < 0.05$)	8.9		23.6		22.5					

* For details of dietary regimen, see p. 534.

given the observed variance (Campbell, 1974). Where appropriate, related samples were compared using the paired t test.

RESULTS

The mean initial body-weights of the three groups of rats were essentially the same (Table 1). Weight gain in both groups of pregnant animals, as expected, greatly exceeded that of the controls, the group that was later to be limited in protein intake showing a slightly greater gain. This is no doubt explained by their higher mean litter size (see Table 1). After 14 d of lactation, the mean body-weight of the rats fed *ad lib.* had almost returned to that of the virgin controls; the group restricted in protein had, however, lost a substantial amount of weight.

Intakes of energy and protein are given in Table 2. Both experimental groups were fed *ad lib.* during gestation and their food intakes did not differ significantly at any stage. During lactation, the food intake of the *ad lib.* fed group rose continuously, reaching a mean value in the 2nd week almost double that of their controls. A level of consumption of the protein-containing diet by the 'restricted' group very close to that of the controls was achieved. Although during the 1st week of lactation the total energy intake of this group almost equalled that of the group fed *ad lib.* (Table 2), resulting from the compensatory ingestion of the protein-free supplement, parity was not maintained during the 2nd week, and a significant deficit in total energy intake developed. Protein intake marginally exceeded that of the controls.

The influence of lactation on liver weight and on the activities of two enzymes involved in the regulation of amino acid catabolism is shown in Table 3. In animals fed *ad lib.* during lactation, liver weight was significantly increased. Those restricted in protein intake, however, failed to demonstrate the hypertrophy of the liver normally seen in adequately nourished animals (Kennedy *et al.* 1958). The activities of the enzymes were expressed as total activity per liver. Both alanine aminotransferase and argininosuccinate synthase were depressed in activity, by 40% ($P < 0.02$) and 38% ($P < 0.01$) respectively, in the livers of the 'restricted' lactating rats, despite their total protein intake being as great as that of the controls. During the 2nd week of lactation, the dams fed *ad lib.* consumed 85% more protein than did their virgin controls. Nevertheless the mean value for the activity of alanine aminotransferase was lower, although the difference from the control value was not statistically significant. Argininosuccinate synthase activity was, however, raised by 35%.

Table 2. *Energy and protein intakes of control rats, and lactating rats fed ad lib. or restricted in protein during lactation**

(Mean values with their standard errors for seven matched triplets)

Stage of lactation ...	Energy (kJ/d)				Protein (g/d)			
	1-7 d		8-14 d		1-7 d		8-14 d	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Control	320	13	319	15	2.7	0.1	2.7	0.1
Lactating	460	38	590	49	3.9	0.3	5.0	0.4
Lactating (protein restricted)	456	33	489	34	2.8	0.1	2.8	0.1
Critical difference ($P < 0.05$)	87		96		0.6		0.7	

* For details of dietary regimen, see p. 534.

Table 3. *Effect of lactation, and of protein restriction* during lactation on liver weight and activities of alanine aminotransferase (EC 2.6.1.2) and argininosuccinate synthase (EC 6.3.4.5) of rats*

(Mean values with their standard errors for seven matched triplets)

Group	Liver wt (g)		Alanine aminotransferase (units/liver)		Argininosuccinate synthase (units/liver)	
	Mean	SE	Mean	SE	Mean	SE
	Control	9.88	0.4	211	22.0	145
Lactating	13.52	0.6	186	22.8	196	17.9
Lactating (protein restricted)	10.93	0.6	127	7.8	90	13.2
Critical difference ($P < 0.05$)	1.7		53		40	

* For details of dietary regimen, see p. 534.

Table 4. *Effect of lactation for 14 d on body composition of rats fed ad lib. or restricted in protein**

(Mean values with their standard errors for seven matched triplets)

Group	Carcass wt (g)		Carcass composition (g)					
			Water		Fat		Protein	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Control	258.4	5.2	143.9	3.2	47.0	5.3	48.2	1.5
Lactating	258.6	5.1	157.3	9.5	33.1	2.4	47.5	1.5
Lactating (protein restricted)	218.4	10.7	137.4	6.8	24.7	2.9	43.2	1.4
Critical difference ($P < 0.05$)	21.1		20.2		10.2		2.8	

* For details of dietary regimen, see p. 534.

Table 4 illustrates the effects of lactation on body composition. At 15 d post-partum, the mean weight of the eviscerated carcasses of the lactating rats was identical with that of the controls. The increase in the water content of their carcasses, however, masked a substantial (30%) loss of body fat ($P < 0.01$). Carcass protein was unaffected, indicating that the diet had been adequate to sustain optimal lactation. In the two lactating groups the protein: water ratio was the same. In the animals limited in protein intake, not only was the loss of carcass fat more marked (47%, $P < 0.01$) but a significant amount of protein had also been mobilized.

The similarity in weight of the two groups of pups (Table 1) around the time of weaning, i.e. creep feeding, showed that the restriction in dietary protein had not appreciably impaired lactational performance.

DISCUSSION

The aim of this investigation was to determine whether the conservation of protein achieved in pregnancy by the suppression of amino acid oxidation in the liver persists during lactation. The *in vitro* measurement of the activities of two enzymes, alanine aminotransferase, which regulates the availability of amino groups for entry into the urea cycle, and argininosuccinate synthase, the rate-limiting enzyme of the urea cycle, were used to assess the extent of amino acid degradation.

In a nitrogen-balance experiment in which pregnant rats were pair-fed with non-pregnant littermates, a progressive reduction in N excretion was associated with a striking fall in the activities of these enzymes and in the plasma urea concentration (Naismith, 1973). The precise relation between N output and the activities of hepatic amino transferases and enzymes of the urea cycle was demonstrated in the elegant experiments of Das & Waterlow (1974). All enzymes were found to change to the same extent and at the same rate in response to changes in the level of protein ingestion, the time-course coinciding with changes in urinary N output.

Lactation in the rat fed on a diet adequate in protein entails a large increase in food intake, and consequently a rise in protein intake. To overcome this problem lactating rats were pair-fed with virgin controls on a diet containing 140 g protein/kg, but allowed to satisfy their additional need for energy. A second lactating group was fed on the diet *ad lib*. The concentration of protein in the diet was judged to be adequate to promote normal lactation when progressively diluted by consumption of the protein-free energy source. In the event the pair-fed animals failed to maintain the energy intakes of their *ad lib*. fed littermates. Even a moderate restriction below the recommended intake of protein for the lactating rate (120 g/kg; (US) National Research Council, 1978) has been shown to affect the spontaneous rise in food consumption during lactation and to impair lactational performance (Naismith *et al.* 1982). There was, however, no evidence in the present study of poor growth in the pups suckled by the protein-restricted dams, although the mean protein concentration of the total food consumed during lactation was reduced to 10%. The deficit in dietary protein and, to a lesser extent, dietary energy, was compensated in these animals by the catabolism of lean tissue, which was not observed in the group fed *ad lib*. and an enhanced mobilization of body fat.

The activities of the enzymes were expressed in the most meaningful way, as total activity per liver rather than as concentrations. The values thus reflect the potential capacity of the liver to degrade amino acids and to form urea. In the lactating rats allowed free access to the protein-containing diet the activity of alanine aminotransferase was reduced, although the difference from the control value did not reach statistical significance, and argininosuccinate synthase activity was moderately (35%) raised. These rats, however, had

more than doubled their daily protein intake by day 15 of lactation, when enzyme activity was measured.

In their study Das & Waterlow (1974) found a proportional relation between these variables; a fourfold change in protein intake induced a fourfold change in enzyme activities. A much greater rise in the activity of argininosuccinate synthase might therefore have been anticipated in the lactating animals. Conclusive evidence of adaptation in amino acid metabolism during lactation was derived from measurements on the dams pair-fed with their controls with respect to protein. Both enzymes showed a marked reduction in activity, indicating that protein was being spared for synthesis of milk protein.

There are clearly two probable explanations for this phenomenon. The vigorous uptake of amino acids from the circulation by the mammary gland (Mephram, 1977) might so reduce the flux of amino acids to the liver that enzyme activity declines in response to lack of substrate. Secondly, hormones secreted in the lactational phase of reproduction might inhibit enzyme activity.

Progesterone, which is secreted in increasing amounts throughout pregnancy has been shown to depress the secretory activity of the adrenal cortex and so induce a fall in the level of amino acid oxidation (Naismith, 1973). It has been claimed (Sutter-Dub *et al.* 1974) that during lactation the plasma progesterone concentration rises from an initial low level to a value similar to that found in pregnancy. It is possible, therefore, that progesterone continues to exert an anabolic effect on protein metabolism throughout the whole period of reproduction. Growth hormone, which was shown by McLean & Gurney (1963) to depress the activity of urea cycle enzymes, has also been reported to rise in concentration in the plasma of rats following suckling (Chen *et al.* 1974). The most likely putative agent of protein conservation in lactation is, however, prolactin. In the rat the serum prolactin concentration rises from the 1st day post-partum, and begins to fall only on the 15th day of lactation owing to the reduced suckling stimulus when the young begin to creep feed (Amenomori *et al.* 1970).

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