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Effects of lactation on circulating plasma metabolites in 'cafeteria-fed' rats

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1. The effects of 'cafeteria feeding' on primiparous Wistar rats during lactation have been studied by measuring circulating levels of glucose, amino acids, lactate, urea and ammonia as well as glycogen levels in liver and muscle.

2. No significant changes in glucose levels were observed despite alterations in blood glucose compartmentation.

3. Compared with controls, the dams given the cafeteria diet had higher liver glycogen stores which were more easily mobilized at the peak of lactation.

4. Rats given the cafeteria diet showed a lower amino acid utilization than controls and adequately maintained circulating levels, as determined by the lower circulating levels of ammonia and urea.

5. No significant differences in body-weight were observed in the period studied despite increasing dam weight after weaning in the cafeteria-fed group.

6. The size of pups of cafeteria-fed dams was greater than that of controls, and the differences were marked after weaning, when the metabolic machinery of the cafeteria pup maintained high protein accretion and body build-up using fat as the main energy substrate characteristic of the preweaning stage. The controls, however, changed to greater utilization of amino acids as an energy substrate and adapted to high-protein (low-biological-quality) diets with a significantly different pattern of circulating nitrogen distribution.

Lactation imposes a very severe burden on maternal energy homeostasis because the increased needs of nursing cannot be met by increased food intake alone, at least in the rat (Spray, 1950; Cripps & Williams, 1975). This situation results in diminishing fat and other stores in the mother and a progressive adaptation to lower nitrogen loss despite increased dietary amino acid utilization for energy purposes (Palou *et al.* 1982).

In the pups, the effect of decreasing milk availability with respect to the enormously increasing demands of their own growth results in the initiation of ingestion of solid food from day 12 onwards (Babický *et al.* 1975) and complete weaning by days 24–30; this change of diet is reflected at the biochemical level by an adaptation from a high-fat–high-quality-protein diet (milk) to a high-carbohydrate–low-quality-protein in the rat chow.

The presentation to the experimental animals of a mixed, highly palatable diet containing densely energy-packed high-quality nutrients, known as a 'cafeteria diet' (Sclafani & Springer, 1976), results in overfeeding (Rothwell & Stock, 1982) and increased energy disposal through thermogenesis and fat accretion (Rothwell & Stock, 1979), leading to increased body-weight. Feeding this diet results in increased food intake and increased foodstuff availability both for body growth and for energetic purposes. In the present study we have intended to obtain a general idea of the handling of circulating glucose and N compounds as well as the glycogen stores by dams and pups subjected to this diet compared with controls. In addition we have determined whether or not the increased energy ingested could counteract the demands of pup growth imposed on the dam's metabolism, and what effect a very rich diet could impose on the metabolic changes that occur at weaning.

* For reprints.

MATERIALS AND METHODS

Animals and diet

Primiparous Wistar rats aged about 12 weeks and weighing initially (day 1) 245 (SE 19)g were divided into two groups, the first received rat chow pellets (A04 type; Panlab, Barcelona) and tap water *ad lib.* as their only diet. The second group received the same as group 1 and, in addition, they were offered daily, banana, hazelnuts, cookies, cookies spread with liver paste, raw bacon, pastries ('croissants'), candy pastilles and chocolate, as well as whole cow's milk containing 250 g sucrose/l and 15 g of a protein–mineral–vitamin supplement/l (Gevral-Proteína), all of them *ad lib.*; these items were renewed daily at 08.00 hours. Control virgin rats were also given the rat chow or cafeteria diet described. The rats were housed in individual polypropylene cages with wood shavings as absorbent material, maintained in a temperature of 22·5–23·5° and light-controlled (light period 08.00–20.00 hours) animal room with 75–85% relative humidity.

Experimental procedure

Groups of dams from both dietary treatments were killed by decapitation (at 08.00–08.30 hours) on days 1, 5, 10, 14, 20, 30 and 40 after parturition. The animals were kept in individual cages with their litters, equalized to eight pups just after birth, until forced weaning on day 30. The pups were also killed on days 1, 5, 10, 14, 20 and 30.

The blood from the neck wound was collected in dry heparinized beakers and a portion used for the separation of plasma. Immediately after death, pieces of liver and hind-leg striated muscle were dissected and immediately used for isolation of glycogen (Good *et al.* 1983) and its estimation (Fraga, 1956). Blood was deproteinized by the Somogyi (1945) procedure and used for the estimation of whole-blood glucose (Bergmeyer *et al.* 1974). Portions of blood were deproteinized with acetone (Arola *et al.* 1977) and used for the measurement of total amino acid concentrations (Yemm & Cocking, 1955). The packed cell volume was also determined after 30 min centrifugation at 1500 ***g***. Plasma was used for the measurement of plasma proteins (Itzhak & Gill, 1964), free ammonia (Kalb *et al.* 1977) and urea (Chaney & Marbach, 1962). After deproteinization with acetone (Arola *et al.* 1977), total amino acids were measured (Yemm & Cocking, 1955).

Portions of plasma were also deproteinized with perchloric acid and then neutralized with potassium hydroxide–potassium bicarbonate, the supernatant fractions being used for the estimation of lactate (Hohorst, 1965) and glucose (Bergmeyer *et al.* 1974).

Differences between groups were estimated using Student's *t* test.

RESULTS

Fig. 1 shows body-weight, liver size and packed cell volume as well as protein concentrations of dams and their pups given the rat-chow pellets or cafeteria diet. Body-weight increased with lactation. No differences in weight were observed with the different diets; the cafeteria diet was associated with increased weights only from weaning. The differences in weight of the pups were also small but pups from cafeteria-fed dams had consistently higher weights. This was especially apparent from weaning, the differences being extreme and significant on day 30. Liver weight was relatively larger in lactating rats than in controls, with practically no differences between diets. Pup liver size was smaller than control adults on days 10 and 14, but higher on day 30; when the cafeteria diet was supplied to their dams liver size was greater on days 10 and 14 than that of control adults; there were differences between both groups on days 5 and 10. The packed cell volumes showed no differences between the dams and the control adults except post-lactation when there were higher

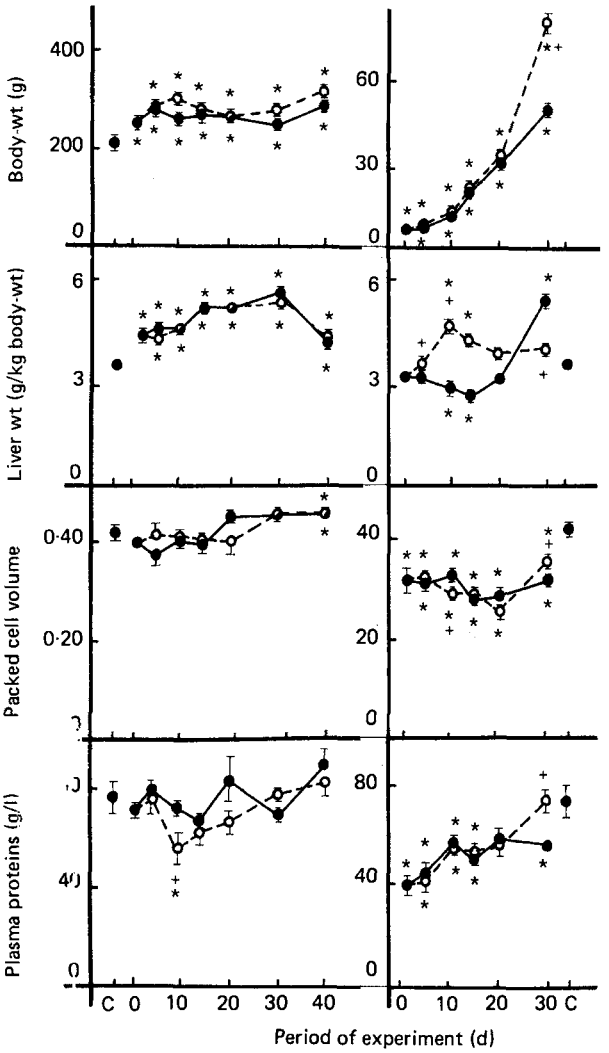

Fig. 1. Body-weight, liver weight (g/kg body-weight), packed cell volume and plasma protein concentrations in lactating dams (left) and nursing pups (right). (●——●), Control animals; (○---○), cafeteria-fed groups (for definition of dietary regimens, see p. 140). Points represent mean values with their standard errors for five to six animals. * Mean values were significantly different from those for virgin adult controls ($P < 0{\cdot}05$). † Mean values for control-diet group were significantly different from those for cafeteria-fed group ($P < 0{\cdot}05$). C, Values of control virgin rats.

packed cell volumes in both dietary groups. Pups had smaller packed cell volumes than the control adults, with significant differences between cafeteria-fed and rat-chow-fed groups for days 10 and 30. Plasma proteins in the dams were lower in the cafeteria-fed group than in those given the control diet on day 10. The differences were also appreciable in the 30 d pups, with plasma protein concentrations for control-diet pups significantly lower than those of the adults at all time-points studied. The difference was absent in the cafeteria-fed group from day 20.

Fig. 2 shows the plasma and blood glucose concentrations as well as plasma lactate. Lactation induced a decrease in blood glucose in controls extending over the whole period

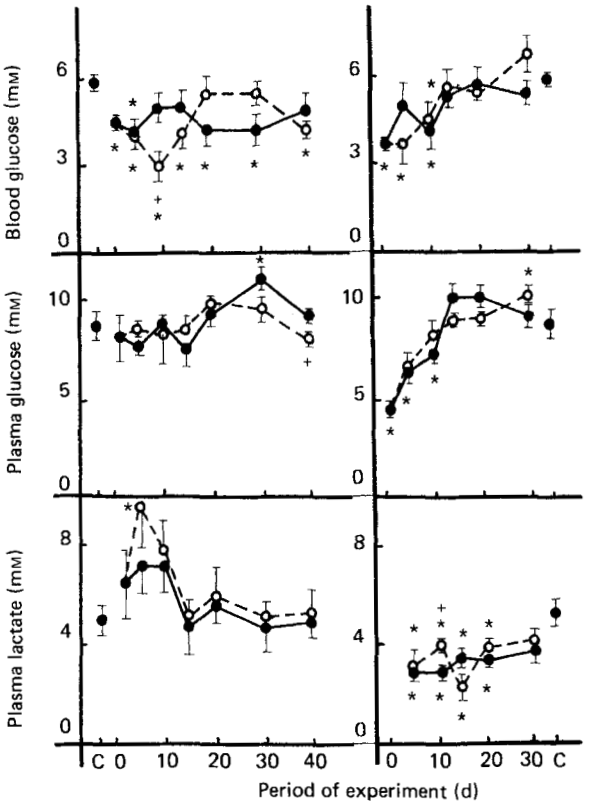

Fig. 2. Blood and plasma glucose and plasma lactate of lactating dams (left) and nursing pups (right) subjected to control or cafeteria diets. (●——●), Control animals; (○---○), cafeteria-fed groups (for definition of dietary regimens, see p. 140). Points represent mean values with their standard errors for five to six animals. * Mean values were significantly different from those for virgin adult controls ($P < 0{\cdot}05$). † Mean values for control-diet group were significantly different from those for cafeteria-fed group ($P < 0{\cdot}05$). C, Values of control virgin rats.

studied except days 10 and 14. However, in cafeteria-fed rats the only values not different from adults were found on days 20 and 30, when these were lower than those of virgin controls, and the day 10 value which was significantly different from the control-diet group. In the pups, the initial concentrations were lower, and the values increased from day 14 onwards when they were similar to those for the adult controls for both diet groups. Plasma glucose levels were maintained fairly constant with no differences between the dams and virgin controls except for a higher control value on day 30. The post-lactation glucose concentrations were significantly lower in the cafeteria-fed group than in the control-diet group. The plasma glucose pattern in the pups showed no significant differences from virgin control values.

Fig. 3 shows the liver and muscle glycogen concentrations. Total liver glycogen content, as well as glycogen levels, were higher in cafeteria-fed dams than in controls, with a maximum on day 10 followed by a decrease on day 14. On the other hand, controls showed only a transient decrease on day 10. No significant differences between both groups of pups were observed; their liver glycogen content increased steeply from day 20 to adulthood. The individual variation in muscle glycogen levels of the dams was considerable, showing lower values in the cafeteria-fed group on days 14–20. The trend was an increase in

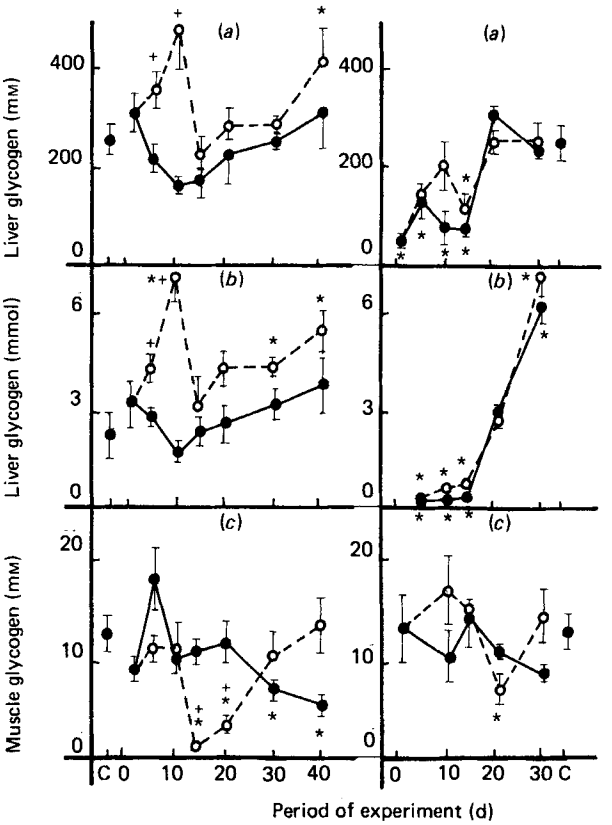

Fig. 3. Liver and muscle glycogen in lactating dams (left) and nursing pups (right) subjected to control or cafeteria diets. The concentrations of glycogen for liver (a) and muscle (c) are expressed in mmol glycosyl residues/kg tissue weight. (b), Glycogen content of the liver expressed in mmol glycosyl residues present in the whole organ. These values were calculated from those of liver glycogen concentration and the weights shown in Fig. 1. (●——●), Control animals; (○---○), cafeteria-fed groups (for definition of dietary regimens, see p. 140). Points represent mean values with their standard errors for five to six animals. * Mean values were significantly different from those for virgin adult controls ($P < 0{\cdot}05$). † Mean values for control-diet group were significantly different from those for cafeteria-fed group ($P < 0{\cdot}05$). C, Values of control virgin rats.

concentration, in contrast with the decreasing values for the controls after weaning. In the pups, no significant differences were observed, except for a lower day-20 value in the cafeteria-fed group compared with the controls.

In Fig. 4, the blood and plasma amino acids, plasma urea and plasma ammonia concentrations are shown. Blood amino acids in the cafeteria-fed dams were not different from those of the controls, except for a transient peak on day 10. No statistically significant differences were observed between the two dietary groups of pups. Plasma amino acids of both groups of dams were similar with higher values on day 40 than those of virgin controls. In the pups, after a significant decrease between days 5 and 10, the cafeteria-fed group attained the control values, which were not different from those of adult virgin females. Plasma urea increased on days 14–20 in the control dams, whereas the cafeteria-fed group showed a minimum on day 14, and decreased again on day 40. The plasma urea concentration of the pups decreased in the controls from a peak during days 0–10 to the low day-20 concentrations. Urea levels for the cafeteria-fed group were consistently lower

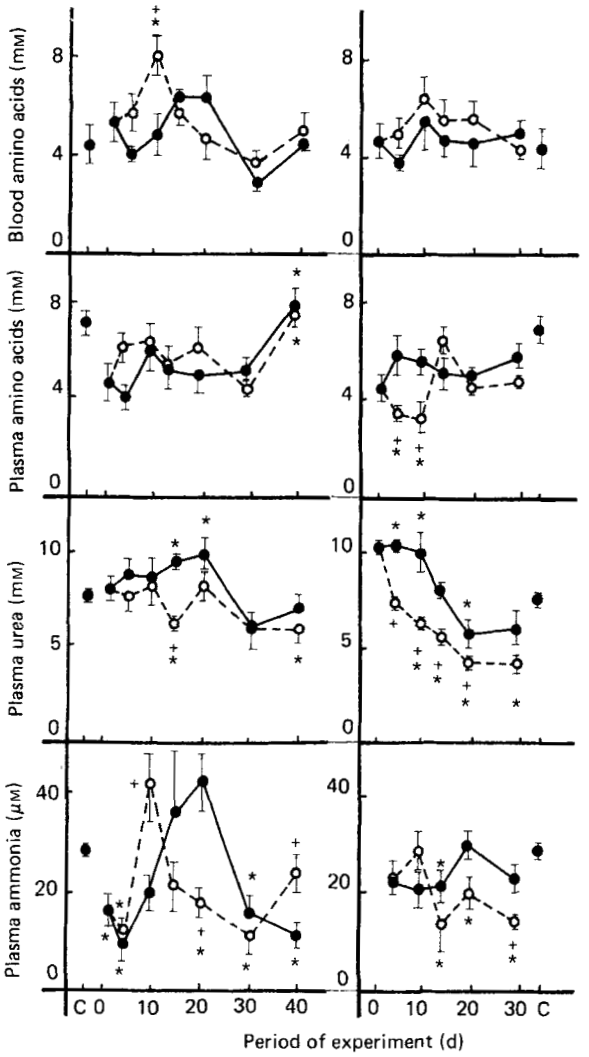

Fig. 4. Blood and plasma amino acids, plasma urea and plasma free ammonia in lactating dams (left) and nursing pups (right) subjected to control or cafeteria diets. (●——●), Control animals; (○---○), cafeteria-fed groups (for definition of dietary regimens, see p. 140). Points represent mean values with their standard errors for five to six animals. * Mean values were significantly different from those for virgin adult control ($P < 0{\cdot}05$). † Mean values for control-diet group were significantly different from those for cafeteria-fed group ($P < 0{\cdot}05$). C, Values of control virgin rats.

than the control values from day 5 onwards, and were lower than those for virgin female adults from day 10.

Plasma ammonia showed two minimum values on days 1–5 and 30–40 in control dams compared with virgin females. Cafeteria-fed dams followed a similar pattern, with a transient peak on day 10 and then lower values on days 20 and 30. All pups showed lower ammonia values than the adults on day 14, the cafeteria-fed group maintaining low concentrations whereas the controls reached the adult values from day 20 onwards.

DISCUSSION

The weight of cafeteria-fed dams did not increase to the same extent as occurs in non-lactating rats (Castellà, 1985), the differences with controls in this respect being minimal. This lack of body tissue accretion contrasts with the higher energy contained in the diet offered to cafeteria-fed rats. The cafeteria-fed dams suffered, however, to a lesser extent than controls, the effects of the drain of resources imposed on the dams by nursing. At the same time, the pups from cafeteria-fed dams increased their weight considerably more than controls, which is in agreement with increased milk production in the dams. This higher achievement in conveying to their pups part of the richer diet, maintaining at the same time their own energetic homeostasis, was correlated with a higher efficiency in N conservation; N was directed mainly into protein synthesis instead of being used for urea production. This trait in N conservation would then be a trait marking a significant difference between cafeteria-fed rats and controls as well as genetically-obese animals (Rolls & Rowe, 1982; Barber *et al.* 1984; Arola *et al.* 1984).

The cafeteria diet received by these animals is comparable to the self-selected diet picked up from the same offering of food by control female rats of the same strain; these animals consumed a mean 1·94 times the energy taken up by their controls given rat-chow pellets (Castellà, 1985). The protein energy content of this self-selected cafeteria diet was 134 J of protein/kJ ingested, compared with a mean 212 J of protein/kJ of control food (Castellà, 1985).

Thus, the cafeteria-fed rats received an adequate protein supply that probably allowed them to give a larger milk output, as adequate protein supply is needed for increased milk production (Roberts & Coward, 1985). This is in accordance with the increased pup weight found in cafeteria-fed dams.

These results, in accordance with those of Roberts & Coward (1985), contrasted with those of other authors (Rolls *et al.* 1980; Rolls & Rowe, 1982) in which obese or fat-supplemented-diet-fed dams showed poor lactating performance despite increased energy intake, probably because of insufficient dietary N availability for optimal milk output (Roberts & Coward, 1985).

During weaning, cafeteria-fed pups received a high-fat–high-energy diet rich in essential amino acids, whereas the control pups received the rat-chow diet containing mainly starches and low-biological-value plant protein. Thus control rats had to adapt their metabolism to the changing diet (Snell & Walker, 1973; Arola *et al.* 1984) and gave preference to essential amino acid conservation whilst actively-degrading non-essential amino acids for energy and fat synthesis (White & Miller, 1976). The cafeteria-fed pups, however, did not have to adapt to a changing diet and received a high-fat–high-quality-protein diet comparable to the milk they received in the fast-growing initial stages of lactation. They continued growing at a comparable rate maintaining the metabolic machinery of the lactating pup in an otherwise mature organism, with a predominance of N conservation because practically no excess non-essential amino acids remained after enhanced protein synthesis. This postulated higher protein build-up can be detected because of the larger size of cafeteria pups and faster achievement of the adult plasma protein levels in the cafeteria groups compared with the lower levels of the controls (Morgan, 1973; Palou *et al.* 1977). They had higher packed cell volume, together with a liver weight more similar to that of adults that could be observed in the 30 d cafeteria-fed pups, which were more anatomically mature but less biochemically adapted to the adult diet.

Cafeteria-fed animals as well as obese rodents, are known to be hyperinsulinaemic and often hyperglycaemic (Martin, 1976; Bray, 1977; Cunningham *et al.* 1983). There were no differences in plasma glucose in the controls given rat chow either in the dams or their pups,

despite a significant difference in the pattern of blood glucose in cafeteria-fed dams compared with controls. This finding suggests important changes in blood glucose compartmentation during lactation in both groups studied. Intracellular glucose was lowest at the peak of lactation and increased with weaning, whereas the intracellular glucose was practically zero in the dams during weaning, in agreement with previous reports (Palou *et al.* 1980). Plasma lactate utilization by cafeteria-fed rats was less effective than that of controls.

The most striking differences between cafeteria- and control-fed dams were found in the liver and muscle glycogen levels. Liver glycogen stores were much higher in cafeteria-fed rats than in controls, the differences being increased just before the peak of lactation, coinciding with a low blood glucose availability. The high demands of milk production depleted more severely the added glycogen stores on days 14–20 in the cafeteria-fed group than in controls. This increased depletion was also reflected in muscle glycogen, which practically disappeared in cafeteria-fed rats on day 14. From these findings it can be suggested that the utilization of glycogen stores is much more contained in controls than in cafeteria-fed rats, at least during lactation. Cafeteria-fed dams could build up larger glycogen stores in liver than controls and mobilize them much more intensively and faster, carrying with them the low muscle glycogen stores.

The day-10 peak in blood amino acid concentration of the cafeteria-fed rat was not actually correlated with a similar peak in the plasma levels, which are similar but generally slightly higher in cafeteria-fed rats than in the controls. This suggests a significant build-up of intracellular amino acids, similar to that encountered during fasting (Soley *et al.* 1982), and seems to correspond with the build-up of liver glycogen and a maximum in ammonia production in cafeteria-fed rats, with unchanged urea levels. This important change seems to be a continuation of the preparation of the rat against the demands of the peak of lactation, similar to that found in the last third of pregnancy (Arola *et al.* 1982) when the rat builds up its own reserves to face lactation properly.

The continuation of a high-energy–high-nutrient-quality diet in these initial phases of lactation could allow the rat longer to build up its own reserves in addition to a small increase in body-weight and a higher milk output. This prolongation of the storage period ends abruptly because of the much increased demands at the peak of lactation, on days 14–20, because of larger pup size (and thus pup needs). The pattern of the N metabolism at this point was significantly different in both groups, as the peak in urea production of controls (because of higher amino acid utilization in energy production) is countered by a low point in urea production in cafeteria-fed dams, with lowered ammonia circulation and maintained circulating amino acids.

This results in improved blood glucose maintenance and reduced amino acid utilization in the dam which slowly increased its reserves towards the end of the period studied. In contrast, the controls ended this period metabolically exhausted (Freinkel *et al.* 1972; Arola *et al.* 1982).

No differences appeared in pup glucose concentrations except at the end of weaning, when the cafeteria-fed group achieved a marked degree of maturity. The blood amino acid: plasma amino acid value suggested a predominance of intracellular compartment in cafeteria-fed pups up to the beginning of weaning, because of the very-low plasma amino acid values; no variations were observed in the controls. The low urea levels in cafeteria-fed pups compared with the controls, in agreement with the low urea synthesis of the liver (Barber *et al.* 1984), together with lower ammonia levels, suggest that the relative drop in circulating amino acids is not actually a consequence of increased amino acid metabolism but increased amino acid utilization for protein synthesis. The relative stabilization of plasma glucose, urea, amino acids and ammonia levels attained by the controls from weaning seems to take

a little longer for the cafeteria-fed rats, which maintain lower urea levels, lower ammonia concentrations and comparable amounts of glycogen on day 30. At this stage, the diet the animals received was different in both groups and differed also from that received during lactation, when the differences in weight gain and maturation could be attributed mainly to the amount of milk received and not to its quality.

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