Effects of Maternal Nutrition on the Development of Rat Offspring: The Postnatal Period

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Summary The effects of a natural imbalanced diet on body maturity and brain composition of rats' offspring were studied from birth over the suckling period. Results were compared with those of a group of pups from mothers fed on a low protein diet and with pups from normal rats fed on a stock diet. Chemical maturity was measured as N/H2O ratio. Pups from mothers fed on the imbalanced diet showed retarded chemical maturity at the time of birth and until 14 days of age in spite of growth progress; they grew without increasing in chemical maturity but approached the chemical maturity of controls at 30 days of age. The chemical maturity of the low-protein progeny was preserved in spite of severe growth arrest. Brain/body ratio was normal in both groups and was not correlated with chemical maturity during the perinatal period, as would be normal. In the brain, low weight, low lipids and a DNA concentration higher than normal were the characteristics of the pups of the imbalanced group from birth until 14 days of age but, at 30 days, brain composition was normal, although it weighed less than it should have done. The brain of the progeny from the low-protein group showed low weight, low lipids and protein, and the protein/DNA ratio was significantly lower until 9 days of age. At 14 days of age there remained severe growth arrest although the brain composition was approaching normal. There was a high mortality in this group and it was impossible to continue the experiment over the 30-day period. These findings confirm that if the mothers are fed on an essential amino acid-imbalanced diet the brain and chemical maturity of the pups during the suckling period are affected in a different way when compared with low protein exposure, and confirmed the working hypothesis that protein quality plays a key role in development and that the effects of imbalanced diets cannot be merely ascribed to a relative protein deficiency.

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Within the topic of effects of maternal nutrition on the development of offspring, interest has centered primarily on the influence of malnutrition during pregnancy and lactation. Moreover, early maternal undernutrition has been mentioned among the factors that may impair offspring development. In spite of the fact that in humans malnutrition is generally prevalent in countries where dietary restriction is associated with the habitual feeding of low-quality protein sources, most of the experimental models used to study the interaction between maternal nutrition and pups’ development are focused on discrimination of the consequences of energy or protein deprivation, the quality of the protein being the subject of few reports.

Working along this line, we produced undernourished rats by feeding them a natural lysine-imbalanced diet, used as a model of low-quality protein, from the time they were weaned until the end of the experimental period.

We then observed that the body and brain maturity of the pups of these rats were dissociated at the time of birth. The effects of the imbalanced diet were essentially different from those produced by protein restriction and also from those reported for the offspring of rats in whose diet a particular indispensable amino acid was omitted. In this paper we have carried out a longitudinal follow-up of progeny of rats fed on an imbalanced diet from weaning in order to provide additional information about the effect of the dietary amino acid pattern in the maternal diet as a disturbing factor in the development of pups.

MATERIALS AND METHODS

Rats of the Wistar strain, undernourished from birth according to the procedure of Widdowson and McCance, were fed, from weaning, on one of the experimental diets outlined in Table 1. At 180 g weight, groups of six females were housed with pairs of males during a 4-day period. Those females in which mating was confirmed by the presence of vaginal plugs were housed individually. Normal groups (control), well nourished from birth and fed, from weaning, on the same stock diet as their dams, were run simultaneously. Diets and water were offered during the entire experimental period.

Diets. Imbalanced diet (I) contained 15.04% total protein of low-quality and lysine-deficient, and was obtained by mixing defatted egg yolk with wheat gluten so that each protein source provided 0.2 g available lysine/100 g diet; low protein control diet (B), containing 5.71% total protein, provided 0.4% available lysine from defatted egg yolk (containing 0.7% available lysine). Both diets contained the same amount of the limiting amino acid per 100 g, and consequently the same amount of “complete protein,” taken as the amount of the limiting amino acid with respect to the requirement pattern for the pregnant rat.
Table 1. Composition of diets.

| Diet components                  | B (g%) | I (g%) | C (g%) |
|----------------------------------|--------|--------|--------|
| Defatted egg yolk                | 7.56   | 3.78   | —      |
| Wheat gluten                     | —      | 15.85  | —      |
| Choline                          | 0.15   | 0.15   | —      |
| Corn oil                         | 10.00  | 10.00  | —      |
| Lysine-HCl                       | 0.30   | 0.15   | —      |
| Water-soluble vitamins           | 0.25   | 0.25   | —      |
| Fat-soluble vitamins             | 0.50   | 0.50   | —      |
| Minerals                         | 5.00   | 5.00   | —      |
| Dextrin                          | 76.24  | 64.32  | —      |
| Available lysine                 | 0.4    | 0.4    | 0.8    |
| Total protein (g%) (N x 6.25)    | 5.71   | 15.04  | 25.00  |
| Biological value                 | 86.7   | 46.0   | 71.2   |

* Stock diet Forramez (Molinos Rio de la Plata, Argentina) containing 15% fat, 7% ash, 4.5% fiber.  
  * Desyemal (Argentina) containing 75.5% protein (N x 6.25) and 4.27% available lysine.  
  * Containing 76.87% protein (N x 6.25) and 1.26% available lysine.  
  * According to Tagle et al. (1970): diets B and I contained: 0.62% calcium, 0.36% phosphorus, 0.75% potassium, 0.34% sodium, 0.05% magnesium, 86.5 ppm iron, 10 ppm copper, 75 ppm manganese, 18.5 ppm zinc, 1.2 ppm iodine, 0.07 ppm cobalt, 0.55 ppm aluminium, 0.116 ppm selenium, 3,200 IU% vit. A, 600 IU% vit. D, 100 mg vit. E, 0.5 mg% thiamine, 0.5 mg% riboflavin, 5 mg% niacin, 2 mg% calcium pantothenate, 0.25 mg% pyridoxine HCl, 0.02 mg% folic acid, 0.1 mg% menadione, 0.01 mg% biotin, 0.005 mg% vit. B12, 10 mg% inositol, 5.0 mg% ascorbic acid and 0.15% choline.  
  * Dextrina British gum 90010/201/4 (Refinerias de Maiz S.A.I.C. Argentina).  
  * According to Farina et al. (11).

In order to improve the survival of the new pups, some modifications in fat, vitamin and mineral composition were introduced in diets according to the figures proposed by Tagle and Donoso (10).

All the diets were controlled as previously described (6) for nitrogen and available lysine. Biological value tested by the short method of Farina et al. (11) was 86.7, 46.0 and 79.2 for the B, I and C diets, respectively.

Collection of the material. At the time of delivery, at least six pups, selected at random from each group, were killed (0 time). Of the remaining pups, no more than six by litter were nursed by their respective dam and each group was randomly assigned to an experimental period of 9, 14, 22 or 30 days. At the end of each experimental period, development was evaluated through changes in weight and chemical indicators of body and brain maturity.

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Pups were killed and, after removing the brain, were frozen until processed. The carcass was dried at 105°C for water determination. The dried body was powdered and extracted with chloroform–methanol (2:1) in a Virtis blender, and used for nitrogen determination by the method of Jones (12).

The whole brain was processed according to Culley and Lineberger (13); the entire chloroform-methanol filtrate was used for lipid evaluation. From portions of the nucleic acid extract, DNA was determined by the method of Burton (14) and RNA by the orcinol reaction (15). Nitrogen was evaluated from the remainder according to Jones (12) and the result was expressed as protein using the conventional factor of 6.25.

Results were analyzed by a variance test according to Scheffé (16) at a significance level of 1%.

RESULTS

In Table 2 the mean values for body weight, water, nitrogen and nitrogen-to-water ratio over the experimental period are summarized. As previously reported (6), the B pups’ weight at birth was significantly lower than that of I and C groups, whereas no differences were found between these last two groups. As a consequence of differences in weight and number of pups, the total weight of the litters was also significantly different for group B in comparison with I and C (22.3 g).

Table 2. Body composition of pups born from mothers fed on imbalanced or basal diets, compared to normal controls.

| Age (days) | 0  | 9   | 14  | 22  | 30  |
|-----------|----|-----|-----|-----|-----|
| Group     |    |     |     |     |     |
| I         | 5.2±0.1 b | 11.8±0.9 a,b | 15.7±0.7 a | 36.5±2.4 | 41.0±4.6 |
| B         | 4.3±0.1 a  | 5.5±0.7 a    | 11.7±0.7 a | —     | —    |
| C         | 5.9±0.2    | 16.3±0.2     | 23.4±2.1   | 35.0±3.5 | 48.3±2.2 |
| Water (%) | I  | 86.3±0.1 b | 78.3±0.8 | 72.8±0.9 | 75.1±1.1 | 77.1±1.9 |
| B         | 84.9±0.2   | 77.6±0.6     | 75.7±1.8   | —     | —    |
| C         | 84.4±0.2   | 79.6±0.3     | 73.1±0.7   | 79.3±0.2 | 78.3±0.2 |
| Nitrogen (%) | I  | 1.49±0.04 a | 1.37±0.17 a | 1.24±0.08 b | 1.99±0.12 a | 2.61±0.10 |
| B         | 1.68±0.04  | 1.91±0.09    | 2.06±0.28  | —     | —    |
| C         | 1.71±0.03  | 2.27±0.04    | 2.25±0.03  | 2.48±0.06 | 2.66±0.05 |
| N/water ratio (%) | I  | 1.73±0.04 a,b | 1.77±0.22 a | 1.71±0.13 a | 2.67±0.30 | 3.41±0.20 |
| B         | 1.98±0.05  | 2.47±0.13    | 2.74±0.45  | —     | —    |
| C         | 2.02±0.04  | 2.85±0.05    | 3.07±0.04  | 3.13±0.08 | 3.41±0.10 |

Values and mean±SE. a p<0.01 between the experimental group and C. b p<0.01 between the experimental groups I and B.

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against 56.1 g and 59.6 g respectively). The lack of difference between the weight of the litters of the imbalanced and control groups agrees with the findings of Jansen and Chase (4) who did not find that the size or total weight of the litters of rats fed white bread during the first pregnancy decreased. As the experiment progressed, the weight of rats in I and B groups differed from that normal for age, the B group being more severely affected. After 22 days the differences in weight between imbalanced and control groups were no longer significant whereas most of the pups of the B progeny died with a weight 50% below normal.

The only significant difference detected in the water content was at birth, when it was higher in the I group than in the B and C groups. The nitrogen concentration of B group was not significantly different from the normal group while pups remained alive. On the other hand, it tended to decrease from birth until 14 days of age in the I group. As a result of this abnormality, the most interesting finding was

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**Fig. 1.** Body and brain composition as percentage of the control group.

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related to the evolution of the N/H₂O ratio in this group, which differed from the normal process of maturity, showing values for the ratio not only lower than those of the controls but also lower than those of the low protein group. Therefore, the chemical maturity of pups from mothers fed on the imbalanced diet was retarded at the time of birth and arrested from birth until 14 days of age, the effects on chemical maturity being more severe than those on growth.

The body weight and brain composition of pups are shown in Fig. 1 as a percentage of the values of the control group. In the same way as body weight, brain weight was more affected by the low protein than by the imbalanced diet, but in both groups the brain weight to body weight ratio corresponds to the actual body weight (Fig. 2). The brain composition of both experimental groups differed from that of the control group at birth and during the perinatal period. Lower weight, low total lipids and protein, with a trend toward an increased protein/DNA ratio were the characteristics of the pups subjected to the imbalanced diet at birth; as time progressed, differences tended to decrease and at 30 days of age imbalanced and control groups were no longer differed whereas the brain of the protein-restricted progeny showed lower weight, low total lipids, protein and protein/DNA ratio until 9 days of age. At 14 days, brain composition was better preserved than the body weight and animals with 50% normal body weight tended to reach normal figures for brain composition, showing 80% of the normal values for weight, lipids, protein, DNA and RNA.

![Graph showing brain weight as function of body weight.](image)

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DISCUSSION

Effect of experimental diets on birth weight, growth and chemical maturity of offspring

The changes introduced in vitamins, minerals and fat in the experimental diets prevented the high neonatal mortality which impaired the longitudinal follow-up in a previous study (6) and the offspring development could be evaluated during a 30-day suckling period for the imbalanced group. The offspring of the basal group began to die after 14 days and, at 22 days of age, the number of live pups was insufficient for continuation of the experiment.

Reproductive efficiency, birth weight and survival were related to the mother’s diet: the number of pups born from mothers fed on diet I was similar to that of controls (10.8 and 10.1, respectively) but the mortality at birth was 18% against 0% in the control group. The average number of pups was significantly lower in the B group (5.2), with a neonatal mortality of 14%. The perinatal mortality was significantly high in this group. These findings are similar to those reported by Zamenhof et al. in their studies on the effects of chronic undernutrition over generations (17).

At birth, pups from I and B groups weighed 2.4% of their respective mother’s weight at the time of mating; this percentage was significantly lower than that of the control group C (3.6%) which was similar to the figure found by other investigators, using the same rat strain (18).

In the perinatal period, imbalanced pups grew without increasing in chemical maturity, but differences tended to decrease as body weight approached that of the control group, probably as a consequence of the changes in the requirement of the limiting amino acid. On the other hand, the chemical maturity of the control protein-restricted progeny was preserved while the pups were alive in spite of the severe growth arrest.

All these effects are similar to those described previously for the first generation of undernourished rats fed on imbalanced or low-protein diets (19): the normal process of chemical maturity was delayed when rats were fed natural imbalanced diets with a decrease in the N/H2O ratio, tending to be normal at 90 days of age, while rats fed low-protein diets showed a normal N/H2O ratio. When compared with the pups from protein-restricted mothers, this finding stressed the idea of a specific impairing effect of imbalanced diet which is transferred from the mothers to the second generation. Moreover, we think that in rats, the same as in humans (20), the mother’s dietary pattern modifies the pattern of plasma amino acids during lactation, pointing to some possible specific modification of the milk which might produce effects on the pups at least as severe as those observed in their progenitors fed imbalanced diets from weaning.

Body and brain development interrelationships

When compared with previous studies (6), concomitantly with the improved neonatal survival figures obtained after supplementing experimental diets with fat,
vitamins and minerals, a noticeable improvement in the brain cellularity of both experimental groups was observed at birth. The normal DNA content with low weight and protein increased DNA concentration, which seems to be inversely correlated with the N/H₂O ratio: low protein and control groups, with a similar N/H₂O ratio showed a similar DNA concentration, which was lower than that of the imbalanced group, whose N/H₂O ratio was significantly low.

The fall in DNA concentration in the brain, a time-dependent process associated to its biochemical maturity, and the changes in the N/H₂O ratio are linked to body chemical maturity; the way both variables were related was analyzed by plotting brain DNA concentration as a function of N/H₂O ratio (Fig. 3a). From this figure it is clear that the evolution of low protein and control groups follows a similar pattern with no significant differences between curve slopes. The brain DNA concentration of the B group corresponded in every respect to the N/H₂O ratio and this finding is similar to that reported previously for newly born rats (6). On the other hand, the DNA concentration of the I group would be located at birth on the theoretical prolongation of the control pups' curve, but the dilution of DNA continued in spite of the arrest in chemical maturity and only depended on body

Fig. 3. DNA as function of N/H₂O ratio (a) and of body weight (b).
weight (Fig. 3b).

The findings of this study confirm that a maternal dietary amino acid imbalance affects the body and brain development of pups not only at birth but also during the suckling period. However, in this study maturation of the brain seemed to be independent of body maturation and tended to be less affected than body maturity.

Since pups were fed exclusively by suckling, the results of this study tend to indicate that some modification of the milk may induce the characteristics of the imbalance described in previous paper (18). When compared with the low protein, the effects of the imbalanced diet were shown to be very different and confirmed the working hypothesis: protein quality plays a key role in development and the effects of imbalanced diets cannot be merely ascribed to a relative protein deficiency.

We would like to draw attention to an interesting fact: as a result of the modifications in diet of the dams, the results found in the progeny at birth differed from the results of our previous paper (6). Although in the progeny of rats fed on the imbalanced diet, body and brain maturity were dissociated, some of the constituents of the supplement in the diet seem to have reversed the specific deleterious effects on the brain, the effects on body maturity being more severe. The nutrient in the dietary pattern responsible for these changes in the pre-natal period deserves to be investigated.

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