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Effect of Maternal Protein Deprivation on Enzymatic Development in Newborn Rats[1] (38862)

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In rats, maternal protein deficiency during gestation (or during gestation and lactation) can result in permanent growth stunting and increased mortality of the offspring (1, 2). Pups from malnourished dams show a deficit in body weight (2–6), in the weights of organs such as liver, brain, kidneys, heart, thymus (5, 7), and in DNA, total protein, and total RNA content of organs (3, 5, 7). Development of the skeleton (5, 6) and the gastrointestinal tract (8) is retarded. Histochemical estimates have indicated that the activities of enzymes in several organs are depressed (9).

The activity of rat liver tyrosine aminotransferase (TAT), which is absent or very low during fetal life, increases explosively on birth reaching two to three times the adult value 12 hr after birth (10). Glucose-6-phosphatase (G-6-Pase) activity, also very low in fetal rat liver, begins to accumulate in late fetal life and rises very rapidly after birth to reach a value higher than in the adult by the second day of life (11). The rapid appearance of these enzymes is also brought about by premature surgical delivery and by injection of glucagon during late fetal life (12). The postnatal appearance of these enzymes, which may be an adaptation to postnatal hypoglycemia (11), is due to the synthesis of enzyme protein rather than to activation of existing apoenzyme (12, 13). The present study was undertaken to determine whether maternal protein deficiency during gestation which was severe enough to impair reproductive performance would also impair the neonatal induction of these enzymes.

Materials and Methods. Female Sprague–Dawley rats (287 ± 36.5 g) mated between 1 and 2 PM were received the next morning (day 1). They were housed in individual cages and were fed ad lib. one of the following diets[2] throughout gestation; 18% casein[3] diet supplemented with 0.3% of L-methionine[4] (18% S) as control diet, 6% casein diet supplemented with 0.3% of L-methionine and 0.2% of L-threonine[4] (6% S), 6% casein diet (6%), or 4% casein diet (4%). The diets used initially were supplemented with methionine and threonine to improve the quality of the protein. The experimental diets were tested in sequence in order of decreasing protein quality and content. Since reproductive performance of rats fed the 6% S diet was satisfactory, the amino acid supplements used to improve the quality of the low-protein diet were then deleted. As the weights of pups in the first two litters from dams fed the unsupplemented diet were only slightly below control values, the protein content of the deficient diet was further reduced to ensure that protein depletion of the dams would be severe enough so that pups used in the enzyme studies would weigh significantly less than control pups.

The major comparison in this study is between pups from dams fed the standard, highly adequate, methionine-supplemented 18% casein diet and those from dams fed the clearly inadequate 4% casein diet. Although the numbers of those fed the intermediate diets were small, information about them is included in the text for comparison.

On the morning of day 21, pups were delivered by cesarean section under ether anesthesia. Upon delivery, pups were kept without feeding in an incubator maintained

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[2] Components of diets have been described previously (14). Supplementation with amino acids was done at the expense of carbohydrates.

[3] NBC, Cleveland, Ohio. N content: 14.53%.

[4] NBC, Cleveland, Ohio.

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TABLE I. REPRODUCTIVE PERFORMANCE OF DAMS FED DIETS CONTAINING DIFFERENT AMOUNTS OF PROTEIN DURING GESTATION.

Diet	Initial wt (g)	Body wt gain (g)	Daily food intake (g)	Liver wt (g)	Liver protein concentration (mg/g)	Total liver protein (g)	No. pups in litter	Litter wt (g)	Wt of individual pups (g)
18% S (10)[a]	278.1	148.3	21.2	13.4	201.4	2.68	9.6	52.1	5.5
	±14.1[b]	±8.83	±0.76	±0.50	±2.96	±0.07	±1.17	±6.17	±0.10
6% S (4)	302.7	166.0	25.5	14.0	173.7	2.44	10.7	62.4	5.8
	±15.8	±5.75	±1.14[c]	±0.60	±1.75[d]	±0.12	±0.74	±4.17	±0.10
4% (9)	279.3	32.0	17.8	8.2	179.5	1.45	8.8	39.9	4.6
	±11.1	±6.57[d]	±1.45	±0.61[d]	±2.09[d]	±0.10[d]	±0.93	±3.61[c]	±0.14[d]

[a] Number of dams in parentheses.
[b] Mean ± SEM.
[c] Significantly different from control, $P < 0.01$.
[d] Significantly different from control, $P < 0.001$.

at 37°. One to three pups were killed at 0, 4, 8, and 12 hr after delivery. Pooled livers were immediately frozen on dry ice and kept in a freezer until assayed for enzyme activity the next day.

TAT activity was assayed by the method of Rosen *et al.* (15). G-6-Pase activity was assayed either by the method of Harper (16) or by the method of Yeung *et al.* (17). Liver protein was determined by the method of Lowry *et al.* (18).

The significance of differences was assessed by Student's *t*-test (19).

Results and Discussion. Reproductive performance. Dams fed the 4% casein diet gained only 22% as much as the control rats (Table I) and actually lost body weight during pregnancy when the weight of fetuses is considered. Liver weight at the time of delivery of rats fed the 4% casein diet was significantly lower than that of control rats. Liver protein concetration was significantly reduced in all the experimental groups compared to the control rats. Dams fed the 6% S diet consumed significantly more food and gained more weight during pregnancy than the control rats. Due to the large size of liver, total liver protein content of rats fed the 6% S diet was not significantly different from that of control rats.

Pups from dams fed the 6% S diet were not significantly smaller than control pups. When a 6% casein diet without amino acid supplements was tested with two rats (average weight = 329.5 g), no significant difference from the controls was observed in the number of pups per litter nor in the weight (average = 5.1 g) of pups, although the weight gain of dams (average = 58.5 g) was considerably less than that of control dams. However, when the casein content of the diet was further decreased to 4%, the average weight of pups (4.6 g) was significantly less than that of the controls (5.5 g), indicating that the maternal protein deficiency had affected the size of the pups.

Other studies have shown that a relatively severe nutritional stress imposed on the mother during pregnancy is shared by the fetus (1–9). However, the severity of the protein deficiency required to produce an effect on the offspring in the present study supports earlier findings (20, 21) that the fetus behaves as a parasite and is protected to a considerable degree from the adverse effects of maternal malnutrition.

The frequency of resorption, expressed as a percentage of the total number of fetuses conceived, was 10% for the 4% casein groups compared to 8.6% for the control. No resorption occurred in the 6% S group. Frequency of resorption is reported to be higher in rats fed a low-protein diet prior to and during gestation (2, 22). Despite the slightly higher rate of resorption for the low-protein group, there were no significant differences among the numbers of pups per litter. Reports on the effect of maternal nutrition on the size of litter are contradictory. In some studies the number of pups per litter was reduced to a greater extent than the average weight of the pups by maternal protein deprivation (22, 23), whereas in other studies, although the weights of litters from mal-

nourished dams were lower than for litters from well-fed dams, litter size was not significantly affected by maternal diet (2, 20, 21).

The pups from dams fed the 4% casein diet were larger than those reported in some other studies. The average weight of pups from dams fed a 6% casein diet during gestation has been reported to be 4.2 g (5, 7, 24). This difference might be due in part to the larger size of dams used in the present investigation (average = 279 g) compared to those used in the other studies (180–200 g). Thomson (25) concluded from observations on women that the weight of the mother was much more important in determining the birth weight of the offspring than the caloric value of the maternal diet. Barnes *et al.* (26) reported that the average weight of pups from dams fed a 7% casein diet throughout pregnancy was 5.3 g, which is considerably higher than the weight of pups fed a 6% casein diet in Zeman's study (5). Barnes *et al.* did not report the weight of dams.

The effect of the size of mother on the birth weight of pups is shown by the study of Macomber (27). Rats fed 20, 16, 10, and 5% casein diets gave birth to pups with average weights of 5.25, 5.25, 5.57, and 4.98 g, respectively. The size of dams was not reported, but the same rats were fed the four diets in sequence and, therefore, the size of dams must have been progressively larger as the level of casein in the diet was lowered. In our study the dams fed the control and the 4% casein diet had the same average weight.

Nelson and Evans (28) reported that feeding rats a 6% casein diet throughout pregnancy resulted in 30% resorption, maternal weight gain of only 11 g and pups weighing 4.7 g, performance slightly below that of rats fed the 4% casein diet in our study. The protein content of casein used in their study was 83%, whereas that in our study was 91% ($N \times 6.25$). Nelson and Evans reported a greatly improved reproductive performance by adding vitamin B_{12} and doubling the amount of water-soluble vitamins, which indicates that the poor reproductive performance in their study was partly due to insufficient vitamin supply. The size of dams in their study was about 200 g which is considerably smaller than the size of those we used. The difference in size may be an important reason why even rats fed a 30% casein diet (25% protein) in their study neither consumed as much food (16.5 g/day) nor gained as much weight (120 g) as our control rats fed the 18% casein diet supplemented with 0.3% of methionine. Zeman (1) reported a weight gain of 33 g in 180- to 200-g rats fed a 6% casein diet throughout pregnancy, which is higher than that reported by Nelson and Evans but similar to that of the larger dams fed only 4% casein in our study. Zeman also found that food intake of dams and survival rate of fetuses in utero for groups fed 24% or 6% casein diets were not significantly different.

Daily food intake of control rats did not change considerably throughout pregnancy. The food intake of the 4% group was about the same as that of the controls during the first one-third of pregnancy but fell progressively below that of the controls thereafter. This observation is in general agreement with that of Zeman (1) except that control rats fed 24% casein diet in Zeman's study consumed more food during the second half than during the first half of pregnancy whereas food intake of our control rats did not change during pregnancy.

Enzyme development. In all groups TAT activity rose relatively slowly until 4 hr after birth and then more rapidly thereafter (Fig. 1). It was observed that TAT activity continued to rise, at least up to 16 hr after birth, in contrast to the report by Sereni *et al.* (10) that enzyme activity reached the maximum at 12 hr, after which it decreased slowly. G-6-Pase activity rose steadily in all groups during the first 12 hr after birth (Fig. 2).

TAT activities per gram of liver for the malnourished groups were not different ($P < 0.05$) from those for the control groups at any time after birth, and the postnatal upsurge of TAT was not impaired in any of the low-protein groups.

The postnatal increase of aspartate aminotransferase, which develops more slowly than TAT or G-6-Pase and reaches a maximum 10 days after birth, was delayed in intrauterine growth-retarded rats (29). Intrauterine growth retardation was induced by ligating the blood vessels of one uterine horn at day 17 of pregnancy. This resulted in more severe

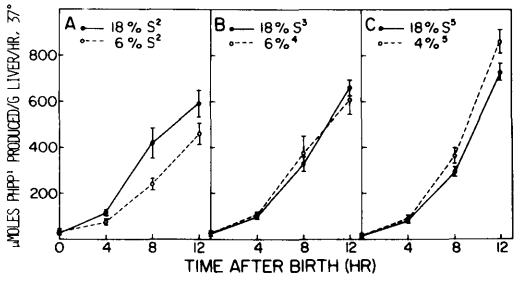

FIG. 1. Development of rat liver TAT after birth in offspring of rats fed diets of different level of protein during gestation. 1. *p*-Hydroxyphenylpyruvate. 2. Mean ± SEM of three to four determinations. 3. Mean ± SEM of 18% S from A and C. 4. Mean and range of two determinations. 5. Mean ± SEM of five to eight determinations.

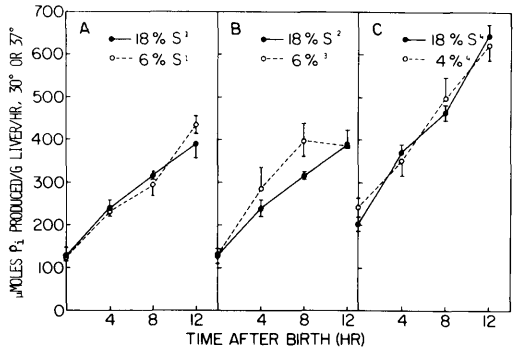

FIG. 2. Development of rat liver G-6-Pase after birth in offspring of rats fed diets of different level of protein during gestation. 1. Mean ± SEM of three to four determinations by the method of Yeung *et al.* (16). 2. Mean ± SEM of 18% S from A. 3. Mean and range of two determinations by the method of Yeung *et al.* (16). 4. Mean ± SEM of five to eight determinations by the method of Harper (15).

growth stunting than was observed in the present study probably owing to restriction of flow of all nutrients to the fetus. Nevertheless, the response of aspartate aminotransferase activity per gram of liver was delayed for only the first 24 hr of life. After day 3, the enzyme activity of the stunted rats was the same as that of the controls.

Shrader and Zeman (9) found that the concentrations of liver alkaline phosphatase, ATPase, succinic dehydrogenase, glucose-6-phosphate dehydrogenase, and DPN and TPN diaphorases were lower in liver of newborn rats of dams fed a 6% casein diet during gestation than in offspring of dams fed a 30% casein diet. They reported that enzymatic abnormalities occurred only in pups whose birth weights were less than 4.5 g. The average birth weight of pups in the 4% casein group in the present study was higher than this. They employed a histochemical method for the enzyme assay and did not measure units of enzyme activity quantitatively; thus, their results cannot be compared directly with those of others.

For both TAT and G-6-Pase, total activities in liver were lower in the protein-restricted than in the control pups due to smaller liver size of the protein-restricted pups rather than to lower concentrations of enzymes. Restriction of total food intake to 50% of that of ad lib.-fed controls during gestation produces similar results.[5] Synthesis of liver and kidney G-6-Pase and of alkaline phosphatase, and of renal phosphoenolpyruvate carboxykinase expressed as total activity per organ were lower in food-restricted rats than in the controls during early life due to the smaller number of cells present. As the number of cells per organ approached the control value, the deficit in total enzyme activity was corrected. It may be concluded that neither the restriction of total food intake by 50% nor restriction of protein intake of dams to less than the maintenance level during gestation impairs the ability of liver cells of newborn pups to synthesize several enzymes.

Summary. Postnatal development of liver tyrosine aminotransferase and glucose-6-phosphatase, expressed as activity per gram of liver, occurred as rapidly in pups from pregnant rats fed throughout gestation a diet containing only 4% of casein as in pups from those fed the control diet containing 18% of casein supplemented with 0.3% of L-methionine. Total enzyme activity per liver was lower in pups from protein-restricted dams due to their smaller liver size. Dams fed the 4% casein diet during pregnancy gained much less weight than those fed the control diet and produced pups that weighed significantly less than control pups. Reproductive performance of a few dams fed 6% casein diets with or without amino acid supplements during gestation was comparable to

[5] Roeder, L. M. (1972) Effect of perinatal undernourishment on cell proliferation and enzyme syntheses. Fed. Proc. **31**, 669.

that of dams fed the control diet. Litter size was not significantly reduced by the low-protein intake.

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