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# Prenatal Undernutrition: Effects on Behavior, Brain Chemistry and Neuroanatomy in Rats[1]

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VILLESCAS, R , E. VAN MARTHENS AND R. P HAMMER, JR. *Prenatal undernutrition Effects on behavior, brain chemistry and neuroanatomy in rats.* PHARMAC BIOCHEM. BEHAV. **14**(4) 455–462, 1981.—The behavior and cerebral effects of prenatal protein-calorie undernutrition were investigated in newborn and postweanling rat pups. At birth, prenatally undernourished (PU) animals showed a deficit in body weight; however, by day 15 the difference was diminished and not significant On days 13–18, PU pups required more trials for reversal learning of a water-escape response in a T-maze than control (C) pups; however, on days 31–35, there was no difference between groups when trained to learn an active-avoidance response. Assessment of brain development showed that at birth, DNA and protein content were severely reduced in PU animals, however, normalization was noted in regional sections of the brain at 35 days of age. Brainstem reticular cells from PU pups at birth show less dendritic arborization and less terminal branching than cells from C pups. The results are discussed in terms of a delayed rate of maturation

DNA Protein Dendrites Behavioral inhibition Learning

ADEQUATE prenatal nutrition in the rat has been shown to be important for fetal growth and postnatal development. Alterations in maternal dietary intake during pregnancy, such as restriction in protein, calorie, or protein-calorie intake, and vitamin or mineral deficiency result in an altered fetal development (see review in ref. [20]).

At birth, prenatally protein undernourished animals show significant decreases in body weights [8], cerebral weights, cerebral DNA (cell number) and cerebral protein [21,22]. Neuromotor development and ontogeny of learning ability of prenatally undernourished (PU) animals have also been assessed by a variety of developmental tasks. Two previous studies [1, 12, 14] have reported that prenatal undernutrition results in significant age-delays in the first appearance of reflex responses (i.e., acoustic and righting response) and other developmental indices such as eye-opening and ear unfolding in suckling rat pups. Behavioral assessments of PU animals also show significant alterations in psychological development. At adulthood, PU-rehabilitated animals show heightened emotionality in the open field [9,13] as well as significant behavioral deficits in passive avoidance behavior [3,7], maze learning [6,9], and in the acquisition of an operant response [15].

In view of the fact that prenatal undernutrition has significant effects on neonatal brain parameters, and given that the maximal growth spurt for the rat brain covers the first three weeks postpartum [4, 5, 15], it is of interest to note that no data exists on the learning ability of PU rats during the lactation period, or early postweaning period. Based on the available literature, it appears that more exploratory studies are needed not only to investigate the ontogeny of learning ability, but more important, to study the possible relationships between behavioral parameters and biochemical-anatomical brain parameters of PU animals at the end of the growth spurt period.

Previous reports from this laboratory have dealt with the effects of protein deprivation during specific time periods of gestation on neonatal brain development [21] and on fetal body and brain development [17]. We have used fetal body weight, cerebral weight, cerebral DNA and protein content as assessment of fetal development. Normal neuron and glial cells at birth are essentially diploid and the amount of DNA per diploid cell of a given species is constant [18]. The actual number of cerebral cells could be calculated by dividing the total cerebral DNA by the constant DNA content per cell. It has been reported that in the newborn rat, the cerebrum contains predominantly neurons (neuroblasts) and that proliferation ceases at this time, with the possible exception of the short axon neurons [2]. Thus, the determination of cerebral DNA is a convenient and reliable quantitative method for cell, and possibly at birth, neuron enumeration in the rat.

The present study, therefore, was designed to investigate the effects of a mild protein-calorie restriction imposed thirty days prior and throughout gestation on (a) neuromotor development and ontogeny of learning ability in suckling rat

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TABLE 1
DESCRIPTION OF NEUROMOTOR TESTS

| Reflex | Testing age | Eliciting stimuli | Response | Score criteria |
|---|---|---|---|---|
| Negative geotaxis | 4– 5 | Pup is placed head down on a 25° slope | Turn face up the slope | A turn of 180° |
| Auditory startle | 9–12 | Sound stimulus mousetrap closing on wooden base | Sudden brief contraction of the nape of the neck | Response present |
| Climb an inclined plane | 12–14 | Pup is placed on a 70° inclined mesh plane | Climb a 45 cm long inclined plane | Latency to reach a platform at the top |
| Free-fall righting | 16–18 | Pup is suspended, back downwards, by the nape of the neck and tail, 20 cm above a cage filled with wood shavings | To turn in mid-air | To land on all four limbs |
| Descending on rope | 16–18 | Pup is placed at the top of a 36 cm long hanging rope | Descending on the rope | Percentage of animals descending |

Description of tests used for assessment of neuromotor development and reflex maturation of control (C) and prenatally undernourished (PU) rats during the suckling period. Pups randomly selected for these tests were not used in subsequent learning tests

pups, (b) newborn and postweanling biochemical brain parameters as well as neuromorphology of the neonatal brain, and (c) the possible relationships between behavioral and cerebral parameters of PU animals rehabilitated at birth.

## METHOD

### *Diets and Animals*

The rats used in this experiment were Sprague-Dawley derived, and have been bred in our closed colony for 42 generations. Virgin females, two months old were housed individually and their daily food intake restricted to two-thirds of the ad lib intake. Body weights were recorded every 10 days in the experimental group (n=60) and in the age-matched control group (n=48). At 90 days of age all females were mated and the presence of a vaginal plug was considered day 0 of pregnancy. During the time the females were housed (mating) with the males, all rats were on ad lib food intake; following positive mating, the experimental females were again restricted in their food intake until the birth of the offspring. All maternal body weights were recorded on days 0, 10, 15 and post partum. After birth, all rats were given food and water ad lib for the remainder of the experiment, thus resulting in two groups of litters: controls (C) and prenatally undernourished (PU). Litter size was adjusted to 6–8 pups and those with less than 6 were not used in the study. Pup body weights were recorded at birth and every 5 days until 35 days of age. Weaning was at 30 days. In this experiment, separate litters randomly selected from the two experimental groups were used for biochemical and behavioral assessment. Behavioral observations were made on male rats only.

### *Biochemistry and Dissections*

Following spontaneous delivery, a representative number of offspring (n=39 in experimental group; n=25 in control group) were weighed and decapitated; the brains were immediately removed, weighed, frozen and subsequently used for analysis. At birth, the "brain" was dissected as cerebral hemispheres (without cerebellum and olfactory lobes) and brainstem. In the 35-day-old rats, the "brain" was further dissected into the cortex and diencephalon, the cerebellum and the brainstem, and all brain regions were analyzed separately. DNA was determined by a modification of the diphenylamine colorimetric method [11], protein was determined by a modification of the Lowry colorimetry method [10].

### *Histology*

Sagittal hemisections of brainstem were placed directly into a solution of: glutaraldehyde (50%), 5 ml; formaldehyde (37%), 5 ml; chloral hydrate, 5 g; potassium dichromate, 5 g; distilled water, 100 ml; and dimethylsulfoxide (optional), 5 drops. Daily changes of this solution for 2–3 days were followed by 0.75% $AgNO_3$ solution for approximately 24 hours. The tissue was sectioned at 160 $\mu$m, dehydrated, cleared and mounted using synthetic resin and coverslip [16]. The results were recorded using 35 mm photomicrography or drawn directly from the microscope.

### *Apparatus*

A T-shaped water maze, with temperature maintained at 25°C, was used for training rat pups to establish a position preference for a water escape-response and for reversal

learning. The maze was made of dark Plexiglas (0.5 cm thick). The walls were 16 cm high and each arm measured 27×8 cm. The stem was 20 cm long and 8 cm wide. Overall, the crosspiece was 62 cm long. An escape ramp was provided in each arm of the maze.

An open-field constructed from plywood and measuring 75×75 cm with walls 9 cm high was used for measuring pup emotionality. The interior of the box was painted black with white lines dividing the floor into 25 equal size squares.

A two-chambered shuttle box, constructed from steel sheets (0.3 cm thick), was used for training young rats to learn a two-way active-avoidance response to electric shock. The walls of the box were 20 cm high; each chamber was 30 cm long and 15 cm wide at the top. The two side walls of each chamber were assembled in a V-shape against the middle partition and the two opposite end walls, resulting in a trough-shaped shuttle box with a steel floor 3 cm wide. The top of the box was covered with a clear Plexiglas lid to allow a top view of the subject. A Lafayette Master Shocker (Model 82400) was the source of electric shock.

### *Procedures for Neuromotor and Behavioral Assessment*

*Reflex tests.* A description of the reflex responses, the age of testing, and the score criterion is presented in Table 1. On each test day, the entire litter was removed from the home cage and maintained at room temperature in a separate cage containing wood shavings. Testing was done between 8:00 a.m. and 12:00 p.m. After each pup was tested, the entire litter was returned to the home cage.

*T-maze.* Daily inspections were made for determining the eye-opening age. At that time, individual pups were removed from their home cage and trained to establish a position preference for an escape response in a T-water maze. Training began by placing the pup in the start-box (stem) of the maze. After 10 seconds, the door of the start-box was opened and the pup was allowed to swim and escape through the ramp on the right or left arm of the maze. The intertrial interval (ITI) was 20 seconds. Training continued until the pup showed a position preference for the escape response. In this study, the criterion for a position preference was 9 of 10 escape responses to the same side. After preference was established, the escape ramp on the preferred side was removed and each pup was then given 30 trials to re-learn the escape response to the opposite arm. If the pup entered the wrong arm during reversal training, the pup was also allowed to swim to the opposite arm until it reached the escape ramp. ITI was the same as for position training. At the end of 30 trials, the pups were dried with paper towels and returned to their home cage. On day 2 of training, reversal learning continued for 30 trials. The behavioral parameters recorded were (1) number of trials to establish a position preference and (2) the number of correct responses on days 1 and 2 of reversal learning.

*Open-field.* Five days after eye-opening, all pups were tested in the open-field for 6 minutes. Individual pups were carried from the colony room to the adjacent test room in a half-gallon cardboard container. The test period was divided into two sessions. For the first 5 minutes, observations were conducted with the *light:off*. Testing began by placing the pup in the center area of the open-field. The behavioral parameters recorded were (1) latency to leave the center area, (2) number of squares entered, (3) number of rearings, (4) time spent self-grooming, and (5) number of boli excreted. A noise generator was used to provide white noise during the observation period. At the end of the 5-minute session, the pup remained in the open-field for a 1-minute test period under additional environmental stress. The stress consisted of the sudden presentation of 5 seconds of white light (60 W) every 10 seconds, and a loud alternating noise. The light source was located 1 m above the center of the open-field area. The behavioral parameters were (1) number of squares entered, (2) number of rearings, and (3) self-grooming. At the end of the session, the pup was returned to the home cage.

*Shuttle-box training.* Beginning at 31 days of age, male pups were trained to learn a two-way active-avoidance response to electric shock in a shuttle box. All training sessions were conducted in a dark room with red illumination. Trial 1 of avoidance learning began by placing the subject in the lighted left chamber of the shuttle box. A pilot light (40 mA, 28 V) located on the back wall of each chamber provided the white illumination. After 10 seconds, the white light was switched to the opposite chamber; *light:off* was the conditioned stimulus (CS). The CS remained for 10 seconds, during which the subject could make an avoidance response to electric shock by crossing over to the lighted chamber. If the subject did not cross over during the CS period, electric shock (0.35 mA), the unconditioned stimulus (UCS), was delivered through the steel floor until the subject crossed over and the trial was scored as an escape response. When the subject made an avoidance response into the safe lighted chamber, a 10-second period was again allowed before presentation of the CS. Avoidance training continued at 50 trials per day for 5 consecutive days. The behavioral parameters recorded were (1) the percentage of avoidance responses; and (2) the number of errors during each training session. In this study, an error was scored each time the subject failed to remain in the safe chamber of the box until presentation of the CS

## RESULTS

Analysis of the body weights and behavioral parameters was done by Student's $t$-test unless otherwise noted.

### *Maternal Body Weights*

Restriction of food intake during the 30 days prior to mating resulted in an average reduction of 14 percent in body weight at 90 days of age in the experimental group of females (Table 2). This deficit in body weight is not recovered during the time of ad lib feeding (during mating). Minimal weight gains are observed in the experimental group of females during pregnancy; however, on the 15th day of pregnancy the experimental females are significantly ($-25\%$, $p<0.001$) lighter than the control females (Table 2). The incidence of failure to maintain pregnancy was 34% in the experimental females as compared to 23% in the ad lib control rats.

### *Pregnancy*

The average length of gestation was identical ($22.0\pm0.6$ days) in both groups of animals. Average litter size was reduced by 13% in the experimental females; however, this decrease was not statistically significant—the incidence of stillbirth was also comparable between experimental and control females.

### *Newborn Biochemistry*

At birth all measured parameters, except wet weight of the brainstem, were reduced as compared to that of the con-

TABLE 2

EFFECTS OF PROTEIN-CALORIE UNDERNUTRITION ON MATERNAL BODY WEIGHTS PRIOR TO AND DURING PREGNANCY

| Groups | Prior to pregnancy—60–90 days | | | | | During pregnancy | | | |
|---|---|---|---|---|---|---|---|---|---|
| | N | 60 days (g) | 70 days (g) | 80 days (g) | 90 days (g) | N | Day 0 (g) | Day 10 (g) | Day 15 (g) | Post-partum (g) |
| Control | 33 | 148* ±17 | 177 ±24 | 199 ±31 | 206 ±26 | 27 | 225 ±23 | 251 ±26 | 270 ±27 | 265 ±27 |
| Experimental | 35 | 151 ±17 | 166 ±17 | 173 ±17 | 178 ±14 | 30 | 195 ±16 | 198 ±19 | 203 ±15 | 206 ±16 |
| %Difference† | | (2%) | (−6%)‡ | (−13%)§ | (−14%)§ | | (−14%)§ | (−21%)§ | (−25%)§ | (−22%)§ |

Body weights of control (C) and experimental (E) virgin females prior to and during pregnancy Randomly assigned E females were fed 2/3 of the ad lib food-intake of C females for 30 days prior to mating (60 to 90 days) and throughout pregnancy

*Mean ± SD

†Percent difference with respect to controls

‡$p<0.01$

§$p<0.001$

TABLE 3

EFFECTS OF PRENATAL UNDERNUTRITION ON NEWBORN BODY AND BRAIN PARAMETERS

| Groups | Body weight | Cerebrum | | | Brainstem | | |
|---|---|---|---|---|---|---|---|
| | (g) | Weight (g) | DNA (μg) | Protein (mg) | Weight (g) | DNA (μg) | Protein (mg) |
| Control (n=25) | 5.90* ±0 50 | 0 1648 ±0 0074 | 591 ±23 | 8 39 ±0 60 | 0 0712 ±0 0114 | 163 ±28 | 2 43 ±0 62 |
| Prenatal undernutrition (n=39) | 5 12 ±0 50 | 0 1464 ±0 0094 | 533 ±55 | 7 08 ±1 12 | 0 0711 ±0 0044 | 124 ±28 | 2 02 ±0 70 |
| % Difference† | (−13%)‡ | (−11%)‡ | (−10%)‡ | (−16%)‡ | 0 | (−24%)§ | (−17%)§ |

Effects of prenatal undernutrition (PU) on body weight and brain parameters of rat pups at birth PU pups were born to undernourished mothers fed 2/3 of the ad lib food-intake of control (C) mothers for 30 days prior to mating (60 to 90 days) and throughout pregnancy

*Mean ± SD

†Percent difference with respect to controls.

‡$p<0.001$

§$p<0.01$

TABLE 4

EFFECTS OF PRENATAL UNDERNUTRITION ON BODY WEIGHT AND BRAIN PARAMETERS OF 35-DAY-OLD MALE RAT PUPS

| Groups | Body weight | Cortex | | | Diencephalon | | |
|---|---|---|---|---|---|---|---|
| | (g) | Weight (g) | DNA (μg) | Protein (mg) | Weight (g) | DNA (μg) | Protein (mg) |
| Control (n=14) | 96 25* ±7 55 | 0 7941 ±0 0444 | 681 ±37 | 59 57 ±4 15 | 0 2600 ±0 0198 | 242 ±20 | 21 25 ±1 58 |
| Prenatal undernutrition (n=19) | 94.32 ±12 23 | 0.7774 ±0 0757 | 661 ±52 | 56 88 ±5.12 | 0 2335 ±0.0234 | 27 ±21 | 18 03 ±1 88 |
| % Difference† | (−2%) | (−2%) | (−3%) | (−5%) | (−10%)§ | (−10%)§ | (−15%)‡ |

Body weight and brain parameters of 35-day-old control (C) and prenatally undernourished (PU) male rats PU rats were born to mothers fed 2/3 of the ad lib food-intake of C mothers for 30 days prior to mating and throughout pregnancy Postnatally and until time of sacrifice, all rats were fed a stock diet ad lib and weaned at 30 days of age

*Mean ± SD.

†Percent difference with respect to controls.

‡$p<0.001$

§$p<0.01$

trol offspring. The cerebrum was significantly ($p<0.001$) reduced in its weight, cell number (DNA) and protein content. In comparison to the cerebrum the brainstem appeared not to be as severely affected by the maternal protein-calorie restriction; no decrease in wet weight was observed; however, DNA and the protein content were reduced. The results are summarized in Table 3.

*Biochemistry, 35 Days*

At 35 days of age some offspring were sacrificed and were analyzed. The results are presented in Table 4. As can be seen, only the diencephalon demonstrated persistent significant decreases in weight, cell number and protein content.

*Newborn Neuromorphology*

Cells of the nuclei reticularis gigantocellularis and pontis caudalis have been examined and contrasted in experimental and control animals. The dendritic arborizations appear more profuse with greater terminal branching in control tissue. At this age, dendrites are extending into the surrounding neuropil and presynaptic extensions develop from the membrane in the form of dendritic protospines. Dendritic varicosities, which may represent burgeoning internode branches or spines, and decrease in number during maturation, occur more frequently upon undernourished cells

*Pup Body Weights*

The pup weights and the results of the group comparisons are summarized in Table 5. The group means represent the body weights of experimental animals used for behavioral testing, plus the weights of additional control (C) and prenatally undernourished (PU) animals. Separate $t$-tests on the weights of animals tested for behavior still showed that there was no significant difference between the body weights of C and PU animals at the same time of testing. During the preweaning period, the experimental group had a 31% mortality rate as compared with an 18% mortality rate in the control group.

*Reflex Ontogeny*

The parameters on reflex ontogeny in C and PU animals are presented in Table 6. In order to minimize the amount of experimenter handling during the first postnatal week of life, the animals tested for reflex ontogeny were not used for the learning tests. Overall, our results demonstrate that while there were no significant differences between groups in terms of the age at which a response first appeared, fewer PU animals exhibited the response on that particular day.

*Behavior in the T-maze*

The parameters of pup learning in the T-maze are presented in Table 7. There was no significant difference between groups in the number of trials required to establish a position preference for an escape response. However, when escape training was reversed on days 1 and 2 (that is, the escape ramp was placed on the previously non-reinforced arm of the maze), PU pups made significantly fewer correct responses (reversals) than did the controls. PU pups exhibited more perseverance to the previously reinforced arm.

*Behavior in the Open-field*

Analyses of pup behavior in the open-field during the first (5 minutes) and second (6th minute) test sessions showed that there were no significant differences between groups in either (1) latency to leave center area, (2) total entries, (3) rearings, or (4) time spent self-grooming. The only significant difference between groups was the number of fecal boli dropped during both test sessions. Prenatally undernourished animals defecated more often (mean=2.0) than controls (mean=0.6), $p<0.02$.

*Avoidance Learning in Shuttle Box*

A 2 (Nutrition)×5 (Days) Analysis of Variance was used to analyze the animals' learning performance in the shuttle box. The results show a significant Nutrition effect, $F(1,31)=7.78$, $p<0.009$, on the number of errors, and a significant Nutrition×Days effect, $F(1,124)=4.84$, $p<0.001$, on

TABLE 4 (Continued)

| Cerebellum | | | Brainstem | | |
|---|---|---|---|---|---|
| Weight (g) | DNA (μg) | Protein (mg) | Weight (g) | DNA (μg) | Protein (mg) |
| 0.2269 ±0.0116 | 1387 ±69 | 18.61 ±1.14 | 0.1688 ±0.0097 | 151 ±12 | 15 11 ±0 86 |
| 0.2158 ±0.0203 | 1357 ±92 | 17 72 ±1 35 | 0.1656 ±0 0147 | 146 ±16 | 15.47 ±1.37 |
| (−5%) | (−2%) | (−5%) | (−2%) | (−3%) | (+2%) |

TABLE 5

BODY WEIGHTS OF CONTROL AND PRENATALLY UNDERNOURISHED RAT PUPS DURING THE WEANING PERIOD

| Group | Body weight (g) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Day 1 | Day 6 | Day 10 | Day 15 | Day 20 | Day 25 | Day 30 | Day 35 |
| Control | 6.19* | 11 50 | 19.86 | 29.29 | 40.21 | 58.07 | 74.50 | 96 25 |
| | ±0.63 | ±1.65 | ±3 40 | ±3 79 | ±5 72 | ±4.26 | ±5 88 | ±7.55 |
| | (23)† | (23) | (23) | (23) | (23) | (14) | (14) | (14) |
| Prenatal undernutrition | 4 94 | 8.60 | 16 46 | 27 16 | 38 26 | 57 00 | 73.78 | 94 32 |
| | ±0 77 | ±2 80 | ±4 43 | ±5 98 | ±7 12 | ±9 83 | ±11 23 | ±12 23 |
| | (19) | (19) | (19) | (19) | (19) | (19) | (19) | (19) |
| Significance | $p<0.001$ | $p<0$ 001 | $p<0.02$ | | | | | |

Changes in body weight of control (C) and prenatally undernourished (PU) rats during the weaning period PU rats were born to mothers fed 2/3 of the ad lib food-intake of C mothers Postnatally, all dams with pups were fed ad lib and litters weaned at 30 days of age.

*Mean ± SD

†Figures in parentheses indicate the number of subjects

TABLE 6

EFFECTS OF PRENATAL UNDERNUTRITION ON NEUROMOTOR AND REFLEX MATURATION IN SUCKLING RAT PUPS

| Group | Reflexes | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Negative geotaxis | | | Startle response | | | Time (sec) to climb an inclined plane | | | Descending on rope | | | Righting reflex | | |
| | day | | | day | | | day | | | day | | | day | | |
| | 4 | 5 | 6 | 11 | 12 | 13 | 12 | 13 | 14 | 16 | 17 | 18 | 16 | 17 | 18 |
| Control | 81* | 95 | 89 | 16 | 67 | 90 | 80 | 79 | 71 | 37 | 44 | 49 | 59 | 83 | 86 |
| Prenatal undernutrition | 56† | 81‡ | 93 | 10 | 63 | 87 | 76 | 80 | 81 | 20† | 40 | 57 | 13† | 86 | 97† |
| | | (63, 57)§ | | | (73, 77) | | | (78, 75) | | | (70, 75) | | | (59, 74) | |

Effects of prenatal undernutrition (PU) on neuromotor and reflex maturation of rat pups during the suckling period PU rats were born to mothers fed 2/3 of the ad lib food-intake of control mothers. Postnatally, all dams with pups were fed ad lib and litters weaned at 30 days of age

*Values represent the percentage of animals exhibiting the response

†$p<0.01$

‡$p<0$ 05

§Number of subjects in the control and undernourished groups, respectively.

the acquisition of the avoidance response.

Prenatally undernourished animals made fewer errors (mean=4.8) than controls (mean=7.7). The data on the acquisition of the active-avoidance response by both groups across days are summarized in Table 8. An analysis of variance on the slopes calculated from the regression curves for each animal indicated a significant difference between groups, $F(1,31)=6.68$, $p<0.05$. The slope values were 10.71 and 3.10 for the C and PU groups, respectively.

## DISCUSSION

The purpose of the present study was to investigate the effects of prenatal undernutrition on the growth, ontogeny of learning ability and biochemical brain development of pre- and post-weanling rat pups.

### *Growth and Biochemical Parameters*

During the first 10 days of life, PU animals weighed significantly less than the C animals; however, by the end of the second week (day 15), these weight deficits were not significant (Table 5). Our results demonstrate that even when nursed by their natural mothers, the pups are able to regain weight deficits resulting from this type of prenatal undernutrition.

The biochemical data from this study demonstrates that even a mild protein-calorie restriction prior to and during pregnancy is sufficient to affect offspring brain development. At birth, the cerebrum and brainstem contained significantly less cells (DNA) and protein; however, most of these deficits disappeared when the offspring were examined at 35 days of age. That the cerebellum was not affected by the prenatal dietary restriction is not surprising in view of the well known

TABLE 7

PERFORMANCE OF CONTROL AND PRENATALLY UNDERNOURISHED RAT PUPS ON ACQUISITION AND REVERSAL LEARNING OF A WATER-ESCAPE RESPONSE IN A T-MAZE

| Group | N | Eye-opening age (days) | Behavioral parameters | | |
|---|---|---|---|---|---|
| | | | Trials to establish position preference | Day 1 % of correct responses during reversal training | Day 2 % of correct responses during reversal learning |
| Control | 23 | 15.1* ±1 0 (13–17)† | 17.6 ±8 6 | 52.9 ±26.2 | 64.8 ±19.5 |
| Prenatal undernutrition | 19 | 16.3 ±0.9 (15–18) | 18.2 ±11.2 | 35.5 ±20.1 | 35 0 ±24.6 |
| Significance | | $p<0$ 01 | | $p<0.005$ | $p<0.005$ |

Effects of prenatal undernutrition (PU) on acquisition and reversal learning of a water-escape response in a T-maze. Training began at eye-opening age. PU rats were born to mothers fed 2/3 of the ad lib food-intake of control mothers. Postnatally, all dams with pups were fed ad lib and litters weaned at 30 days of age

*Values represent group Mean ± SD.

†Values in parentheses represent the minimum and maximum scores for that parameter

TABLE 8

LEARNING PERFORMANCE OF 31- TO 35-DAY-OLD CONTROL AND PRENATALLY UNDERNOURISHED RATS ON THE ACQUISITION OF A TWO-WAY ACTIVE-AVOIDANCE RESPONSE

| Group | N | Percentage of avoidance responses (50 trials/day) | | | | |
|---|---|---|---|---|---|---|
| | | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 |
| Control | 14 | 32 0* ±11 0 | 35.4 ±21.2 | 43.5 ±26.7 | 56.8 ±25.0 | 56.4 ±25.4 |
| Prenatal unernutrition | 19 | 23.6 ±12.4 | 53.3 ±25 7 | 56.7 ±29.3 | 59 5 ±29 2 | 62.3 ±28.8 |
| Significance | | | $p<0.0005$ | $p<0.009$ | | |

Effects of prenatal undernutrition (PU) on acquisition of a 2-way active avoidance response by 31–35 day-old-rat pups in a shuttle box. PU rats were born to mothers fed 2/3 of the ad lib food-intake of control mothers Postnatally, all dams with pups were fed ad lib and litters weaned at 30 days of age

*Values are group Mean ± SD

fact that the cerebellum does develop mainly during postnatal life in the rat. What was surprising, was the severe reduction in the development of the diencephalon observed 35 days postnatally. With regard to the structural composition of brainstem reticular neurons in PU animals at birth, the significant changes in dendritic terminal branching and the greater number of internode spines observed in these neurons suggest that these characteristics of reticular neuron development can be useful as indices of the status of cerebral development in early malnourished animals.

*Behavioral Development*

The results of the open-field behavior tests showed that the only significant difference between C and PU animals was the number of fecal boli dropped during both test sessions. The behavioral parameters of activity level and frequency of rearings, as well as body weights at the time of testing, did not reflect the pups' prenatal exposure to this type of maternal undernutrition. Overall, our data demonstrate that prenatal protein-calorie undernutrition does not produce abnormalities in the open-field behavior of suckling rat pups.

As far as the ontogeny of learning is concerned, the PU animals in our study showed a significant learning deficit (inability to inhibit a learned response) when trained for reversal learning at 2 weeks of age in the T-maze. However, the data also demonstrate that such a learning deficit was not present in a more difficult task. For example, in active-avoidance learning, there was no significant difference between C and PU animals in terms of the overall percentage of correct responses during acquisition learning. Our data also

demonstrated that there was no significant difference between the body weights of C and PU animals at the time of behavioral testing. Overall, the learning data does not show a permanent learning deficit in PU suckling animals. In the present study, the objective of the learning tests used was to observe the appearance of behavioral inhibition in young PU animals. The perseverative behavior that was observed in PU animals in the T-maze suggests that the prenatal undernutrition may have delayed the maturation of a behavioral (neural) inhibitory system. Although the data show that the learning deficit in the T-maze was not permanent, the finding is consistent with the interpretation of a previous study [19] from this laboratory. In that study it was suggested that the poor learning performance of undernourished animals may reflect a specific behavioral deficit (inability to inhibit a learned response) rather than a deficit in general learning ability.

In terms of reflex maturation, the data from this study shows that a mild prenatal undernutrition results in significant but not consistent differences between C and PU animals. The significant differences (Table 6) are in terms of percentage of animals exhibiting a particular response in a given day, and not in the age at which a response was first seen in both groups. Simonson *et al.* [12] and Smart and Dobbing [14] have reported that prenatal undernutrition is sufficient to produce an age-delay in the maturation of some neuromotor responses in the offspring. With the exception of the significant delay of eye-opening in the PU animals, the data from our study do not show age-delays in maturation of the neuromotor responses measured. However, it should be noted that different dietary regimens were used. In this study, the potential mothers were undernourished for 30 days prior to mating and throughout gestation, in contrast to the 2nd and 3rd weeks of gestational undernutrition reported in two previous studies [12,14], respectively.

In summary, our study has demonstrated that prenatal protein-calorie undernutrition results in significant but transient deficits in the learning ability of rat pups Furthermore, the results also show that while prenatal undernutrition may delay the maturation of a behavioral inhibitory system, it does not impair the ontogeny of an eventual learning ability and the normalization of biochemical brain parameters. The behavioral data from this study is consistent with the interpretation of a previous report [19] from this laboratory and further supports the idea that the poor learning performance of undernourished animals reflects a delay in maturation and/or a specific behavioral deficit and not a deficit in general learning ability.

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