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Effect of Early Pre- and Postnatal Acquired Malnutrition on Development and Sexual Behavior in the Rat

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MENENDEZ-PATTERSON, A., S. FERNANDEZ, J. FLOREZ-LOZANO AND B. MARÍN. *Effect of early pre- and postnatal acquired malnutrition on development and sexual behavior in the rat.* PHARMAC. BIOCHEM. BEHAV. 17(4) 659–664, 1982.—In this paper we attempt to elucidate the effects on the rat offspring of undernourishment during pregnancy and lactation on the following parameters were examined: weight at birth and its evolution over a three-month period, sexual behavior of adult males, vaginal opening and sexual cycle of females, blood sugar, blood proteins, hematocrit, natremia, potassemia, body weight and weight of testicles, seminal vesicles and adrenal glands in males 5 months of age. Our results indicate highly significant decreases in body weight in experimental animals over a period of three months. Vaginal opening in experimental females is significantly delayed and their estrous cycle is shortened. Male sexual behavior shows decreases in neuromotor activity and prolongation of the refractory period in experimental male animals. Of the parameters monitored at 5 months of age, only blood sugar levels were significant decreased in experimental male rats. In view of these results, it can be concluded that in utero and lactation period malnutrition affects fundamental parameters of both development and reproductive function in the offspring.

Prenatal malnutrition   Postnatal malnutrition   Sexual behavior   Development   Estrous cycle

PERINATAL malnutrition has been shown to reduce DNA content, modify acetylcholinesterase activity, alter cerebral biogenic amines [1, 18, 30, 36] and produces changes in bioelectrical activity of the brain [21]. Learning and behavioral changes are also common after malnutrition. Various authors [14,26] found a significant decrease in the learning capacity of adult rats subjected to a protein-deficient diet during gestation. Similar results were obtained by Barnes and coworkers [3] and by Cowley and Griesed [7], who found that early malnutrition in the rat permanently affects learning capacity.

In 1920, Jackson and Stewart [15] found that malnutrition induced prior to weaning caused a reduction in brain weight which persisted even after adequate nourishment had been restored to the experimental animals. These results were later confirmed by Dobbing and Widdowson [11], and Dobbing and Sands [10]. They further noted, that animals subjected to early malnutrition evidenced a disproportionately small brain, with a significant reduction in the number of nervous cells. Recently, Sanchez-Turet and coworkers [25], noted a significant reduction in the learning capacity of rats which had been undernourished during their gestational and lactation periods. Such learning defects, however, could be due to effects on motor coordination and exploratory activity [24,28]. Also, Levine and Wierner [19] report that rats deprived of adequate nourishment during the lactation period display increased emotionality and aggressiveness [22].

Clinically it has been shown that children with protein deficiency syndrome show psychomotor retardation, as well as abnormalities in language development, oculo-manual coordination, and categorization and visuo-perceptual abilities [8].

Other authors [12] have recently demonstrated that early nutritional and/or affective deprivation has a "braking affect" on growth and intellectual development, significantly delaying the age at menarche and limiting the intellectual level [4]. With respect to sexuality, malnutrition or early undernourishment seems to effect human reproductive processes. The components of fertility (age at menarche and postpartum amenorrhea) are clearly altered in women on protein-poor and calories-poor diets [4]. However, other variables which affect fertility (age at menopause, prevalence of permament sterility, regulation of ovulation, quality and quantity of sperm, intrauterine death, etc.) appear to be less altered by chronic malnutrition [4].

Based on these data, we attempted to study the effects of in utero and lactation period malnutrition in the rat, using metabolic, nervous, growth and behavioral parameters, including the sexuality of the animals themselves once they reached sexual maturity. We were particularly interested in the effects of malnutrition in utero and during lactation, given that previous studies investigated the effects of malnutrition solely during the lactation period and prior to sexual maturity.

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TABLE 1

EFFECT OF EARLY PRE- AND POSTNATAL ACQUIRED MALNUTRITION ON BODY WEIGHT (g)

	Control		Experimental				
Age	Males (A)	Females (B)	Males (C)	Females (D)		*t*	*p*
Birth	5.83 ± 0.06*	5.48 ± 0.10	4.86 ± 0.06	4.51 ± 0.09	A vs C	9.33	0.001
	(17)	(18)	(33)	(34)	B vs D	6.29	0.001
1 Week	13.15 ± 0.21	12.30 ± 0.21	8.35 ± 0.20	7.48 ± 0.23	A vs C	14.23	0.001
	(16)	(18)	(33)	(29)	B vs D	14.29	0.001
2 Weeks	23.43 ± 0.83	23.47 ± 0.80	11.71 ± 0.50	10.38 ± 0.54	A vs C	12.76	0.001
	(16)	(18)	(29)	(26)	B vs D	13.76	0.001
1 Month	75.06 ± 3.43	71.36 ± 2.73	39.96 ± 1.15	36.80 ± 1.27	A vs C	11.35	0.001
	(16)	(18)	(25)	(21)	B vs D	11.98	0.001
2 Months	240.50 ± 8.04	186.27 ± 3.73	187.30 ± 6.87	129.37 ± 5.96	A vs C	11.56	0.001
	(16)	(18)	(24)	(20)	B vs D	8.29	0.001
3 Months	332.93 ± 9.93	225.77 ± 3.29	264.10 ± 8.36	164.12 ± 6.59	A vs C	4.79	0.001
	(16)	(18)	(24)	(20)	B vs D	9.37	0.001

*Mean ± SE. In parentheses, the number of animals studied.

METHOD

Animals

Wistar rats from the Interfaculty Department of Physiology (Medicine and Science) of Oviedo University were used. The animals were randomly selected and maintained under standard conditions of light (12L–12D), and temperature (23±3°C); the animals had free access to drinking water and were fed a standard diet.

Procedure

A total of 15 females were placed in threes in separate cages, with one male per cage, for three days. After this time the male was removed from the cage. Nine of these females were used as experimental animals and six as controls. During their pregnancy the former were fed a daily diet of 14 grams of diet [25], while the latter had free access to food. The pups of all females were weighed and counted at birth.

The diet of the experimental females during the lactation period was 21 grams per day [25]. During this period, the mortality rate of the suckling animals was striking (31–34%). It was impossible to wean them at 20 days due to their marked state of undernourishment; therefore, the weaning was delayed ten days more. After this time (20 days), they were permitted free access to the standard diet. The weight of each animal was recorded at birth, and at ages of one week, two weeks, one month, two months, three months and immediately preceding sacrifice at five and six months of age. The age of vaginal opening was recorded in all animals, experimental and control. Their sexual cycle was also evaluated by studying vaginal cytology in lavages obtained for at least 20 consecutive days. In addition, this generation of females was mated with intact males selected at random, to observe their percentage of pregnancy and the effects of the undernourishment on the weight and number of their offspring. The sexual behavior of all four month old males was observed with females which had been injected with estradiol benzoate (10 μg) 48 hours before testing, to insure sexual receptivity.

The sexual behavior test was carried out by the method of Soulairac [29]. Behavior was observed in a box (62.5×60.5×30 cm) containing five males and four females. One hour was the standard test length and the following parameters were recorded: Number of intromissions per minute, number of ejaculations, refractory period and neuromotor activity. The neuromotor activity is represented by the relationship between the number of intromissions preceding ejaculation and the latency of ejaculation; that is, the time in minutes from first intromission until ejaculation occurs. It permits the objective evaluation of the variation in neuromotor activity.

The basal physical activity of animals was studied in an "Actisystem" apparatus (Panlab, Pb-0603). Control and experimental animals were placed in the center of the two sensory units of the Actisystem. They were studied simultaneously in groups of two in their habitual cages. The animals' movements were recorded on a central counter. The experiment was conducted in a closed room during the nocturnal cycle, thus achieving maximal silence during the 12 hours of observation. The sensitivity used was 3, a threshold which selected only for the most intense and rapid movements. The diverse groups of animals were retested in order to insure the greatest possible accuracy in the results.

All animals were sacrificed at the same time of the day, and immediately blood glucose levels were determined by the glucose oxidase method, and total blood proteins by the Biuret method. Sodium and potassium were measured by flame photometry.

RESULTS

The effects of malnutrition during fetal development and the lactation period on the weight of offspring from birth to three months of age are recorded in Table 1. This experimental situation produces a sharp decrease in weight throughout development. No significant differences in the number of pups at birth were observed: the number of males per litter was 5.66; the number of females was 6.00; and in

TABLE 2

EFFECT OF EARLY PRE- AND POSTNATAL ACQUIRED MALNUTRITION IN FEMALE RATS ON VAGINAL OPENING, ESTROUS CYCLE, PERCENTAGE OF PREGNANCY AND WEIGHT OF PUPS

	Control	Experimental		t	p
Vaginal Opening (days)	39.85 ± 0.90 (18)*	44.80 ± 0.85 (20)		3.97	0.001
Estrous Cycle	4.464 ± 0.091 (56)†	3.666 ± 0.16 (60)		4.20	0.001
Percentage of Pregnancy	83%	95%	Z	1.2	N.S.

Weight of Pups

	Control		Experimental				
Age	Males (A)	Females (B)	Males (C)	Females (D)		t	p
5 Days	10.96 ± 0.14* (55)	10.60 ± 0.20 (59)	9.71 ± 0.19 (102)	9.63 ± 0.18 (87)	A vs C	4.40	0.001
					B vs D	3.49	0.001

*Mean ± S.E. In parentheses, the number of animals studied.
†Mean ± S.E. In parentheses, the number of cycles studied.

experimental group the number of males per litter was 5.50 and number of females was 5.66.

In Table 2, the effect of undernourishment on vaginal opening, estrous cycle and percentage of pregnancies is recorded. This experimental situation results in a highly significant delay in the timing of vaginal opening. The sexual cycles, which, although very regular, are shown to be significantly shortened in relation to the control animals due to shortened diestrus. No significant differences exist in the percentage of pregnancies. The offspring of the experimental rats displayed significantly lower weights than those of the control offspring.

The parameters of male sexual behavior are presented in Tables 3 and 4. Although a decrease in the number of ejaculations is observed in experimental males, it is not significant. There are significant differences in the following parameters: false intromissions (f_1 and f_2, decreased), neuromuscular activity (ANM_1 and ANM_4, decreased) and refractory period (Pr_3 and Pr_4 prolonged).

TABLE 3

EFFECT OF EARLY PRE- AND POSTNATAL ACQUIRED MALNUTRITION ON SEXUAL BEHAVIOR OF MALE RATS. FIRST AND SECOND EJACULATION

	Control	Experimental	t	p
NE	4.40 ± 0.63 (10)*	3.90 ± 0.47 (10)	0.75	N.S.
Li	16.20 ± 4.23 (10)	10.50 ± 3.68 (10)	1.01	N.S.
Le_1	11.00 ± 1.17 (10)	10.60 ± 0.97 (10)	0.26	N.S.
f_1	16.40 ± 2.31 (10)	7.00 ± 1.24 (10)	3.57	0.001
v_1	10.90 ± 1.76 (10)	9.40 ± 1.46 (10)	0.65	N.S.
Pr_1	3.50 ± 0.40 (10)	6.30 ± 0.87 (10)	2.91	0.001
ANM_1	2.55 ± 0.22 (10)	1.58 ± 0.19 (10)	3.30	0.001
Le_2	4.33 ± 0.31 (9)	4.33 ± 0.72 (9)	0.00	N.S.
f_2	6.44 ± 0.93 (9)	2.44 ± 0.54 (9)	3.70	0.001
v_2	5.33 ± 0.47 (9)	5.77 ± 0.49 (9)	0.65	N.S.
Pr_2	4.44 ± 0.54 (9)	5.77 ± 0.51 (9)	1.77	N.S.
ANM_2	2.70 ± 0.22 (9)	2.17 ± 0.32 (9)	1.35	N.S.

*Mean ± S.E. Number of tests in parentheses.
NE = Number of ejaculations during 60 min.
Li = Intromission latency (min).
Le = Ejaculation latency (min).
f = False intromission.
v = True intromission.
Pr = Refractory period.
ANM = Neuromotor activity $\left(\frac{f+v}{Le}\right)$.

TABLE 4

EFFECT OF EARLY PRE- AND POSTNATAL ACQUIRED MALNUTRITION ON SEXUAL BEHAVIOR OF RATS. THIRD AND FOURTH EJACULATION

	Control	Experimental	t	p
Le	5.37 ± 0.93 (8)*	4.12 ± 0.90 (8)	0.95	N.S.
f_3	10.62 ± 3.41 (8)	4.25 ± 1.54 (8)	1.69	N.S.
v_3	5.75 ± 0.94 (8)	5.87 ± 0.62 (8)	0.11	N.S.
Pr_3	4.62 ± 0.43 (8)	7.12 ± 0.83 (8)	2.65	0.01
ANM_3	3.00 ± 0.21 (8)	2.54 ± 0.10 (8)	1.91	N.S.
Le_4	3.62 ± 0.52 (8)	4.42 ± 0.60 (8)	1.00	N.S.
f_4	5.25 ± 1.79 (8)	3.28 ± 1.08 (8)	0.90	N.S.
v_4	5.37 ± 0.52 (8)	6.85 ± 0.62 (8)	1.83	N.S.
Pr_4	5.25 ± 0.42 (8)	7.57 ± 0.60 (8)	3.22	0.001
ANM_4	3.14 ± 0.33 (8)	2.28 ± 0.20 (8)	2.10	0.05

*Mean ± S.E. Number of tests in parentheses.
Le = Ejaculation latency (min).
f = False intromission.
v = True intromission.
Pr = Refractory period.
ANM = Neuromotor activity $\left(\frac{f+v}{Le}\right)$.

TABLE 5

EFFECT OF EARLY PRE- AND POSTNATAL ACQUIRED MALNUTRITION IN MALE RATS ON BODY WEIGHT AND PROPORTIONATE WEIGHT OF TESTICLES, SEMINAL VESICLES AND ADRENALS†

Weights	Control	Experimental	t	p
Body Weight (g)	373.80 ± 13.85* (15)	374.70 ± 11.43 (24)	0.05	N.S.
Testicles (% Bw)	0.85 ± 0.02 (15)	0.84 ± 0.01 (24)	0.22	N.S.
Seminal Vesicles (% Bw)	0.14 ± 0.003 (15)	0.15 ± 0.00 (24)	0.00	N.S.
Adrenal Glands (% Bw × 10^3)	10.12 ± 0.83 (15)	11.04 ± 0.47 (24)	1.03	N.S.

*Mean ± S.E. In parentheses, the number of the animals studied.
†The animals were sacrificed at the 5th month.

TABLE 6

EFFECT OF EARLY PRE- AND POSTNATAL ACQUIRED MALNUTRITION IN MALE RATS ON BLOOD SUGAR, PROTEINS, HEMATOCRIT, SODIUM AND POTASSIUM IN BLOOD†

	Control	Experimental	t	p
Blood Sugar (mg/dl)	99.60 ± 4.47 (13)*	84.66 ± 3.36 (24)	2.66	0.05
Proteins (g/dl)	6.34 ± 0.29 (15)	6.04 ± 0.09 (24)	1.15	N.S.
Hematocrit	47.93 ± 0.86 (15)	47.41 ± 0.43 (24)	0.59	N.S.
Sodium (meq/l)	157.55 ± 2.96 (15)	155.53 ± 2.38 (22)	0.53	N.S.
Potassium (meq/l)	5.65 ± 0.14 (15)	5.89 ± 0.10 (19)	1.38	N.S.

*Mean ± S.E. In parentheses, the number of determinations.
†The animals were sacrificed at the 5th month.

These are significant statistical differences in regard to basal physical activity, with undernourished animals displaying greater motor activity than control animals (t=2.40; p≤0.05).

Table 5 shows the weight of the animals at sacrifice, and the weight (expressed in % of body weight) of the testicles, seminal vesicles and adrenal glands. No significant differences are observed.

In Table 6 the values for blood sugar, total proteins, hematocrit, sodium and potassium at the time of sacrifice are recorded. Significant differences are present only in the blood sugar, which is higher in the control animals.

DISCUSSION

The model of undernourishment utilized in our work produces a highly significant decrease in the weight of all experimental animals, from birth up to three months of age (Table 1). Similar effects have been found by other authors [14,16]. However, in the case of the males at five to six months of age (Table 5) these differences have disappeared which coincides with the results of others [16].

Sexual maturity of the females, measured by the age when vaginal opening is noted (Table 2) is significantly delayed by undernourishment in utero and during the lactation period. The role of physical growth in sexual maturity is quite well known [13,20]; changes that slow down physical growth delay sexual maturity in humans as well as in rats [14]. Many have concluded that onset of puberty is more closely related to body size than to a particular age, and still more closely related to rate of growth than to a preestablished weight. However, other data on the timing of vaginal opening do not all agree with our results [2, 13, 17, 23, 34]. Nonetheless, we must bear in mind that results can be affected by various factors, such as laboratory conditions, technique for causing undernourishment and even the age at which these conditions are applied. The majority of researchers produce undernutrition during birth and lactation while we have applied it during pregnancy as well. Also, if we observe the duration of the sexual cycle (Table 2), we can see that it is significantly shortened in experimental animals.

These results could be explained by higher levels of plasma FSH noted in undernourished rats [14].

Regarding male sexual behavior (Tables 3, 4), as measured by the number of ejaculations, experimental animals show a decrease when compared to control animals, but this is not significant. In fact the model of sexual behavior remains practically unchanged. Also we must bear in mind that three months have elapsed since the animals discontinued the malnutrition diet, and at the end of this period, their body weight is restored, as is weight of the genital and extragenital structures (Table 5). Nonetheless, some parameters, such as neuromotor activity (ANM_1, ANM_4) and refractory period (Pr_1, Pr_3 and Pr_4) are altered by undernourishment. Neuromotor activity between ejaculations is decreased and the refractory period increased. These modifications could indicate possible neurological disorders, bearing in mind the significance of these parameters [29]. This coincides with other data, which point out that, while the effects of early undernourishment can be corrected by reinstituting a nutritious diet, the animals' reduced brain weights and neural alterations remain [32, 33, 35]. Perhaps the brain can adapt itself to short periods of undernourishment delaying certain types of development. But if this delay takes place during a period of rapid brain growth, the damage could be irreversible [6]. In addition, possible cerebral changes could be indicated by increased basal motor activity recorded in the experimental animals; this coincides with the data found in the literature [27, 31, 33].

With regard to the metabolic parameters studied (blood sugar, proteins, hematocrit, sodium and potassium), statistically significant modifications were found only in blood sugar (Table 6) which was decreased in the experimental animals. Low blood sugar can cause a decreased metabolic activity in neurons of undernourished animals [9,10].

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