Biol. Neonate *32*: 205–210 (1977)

Differential Responses to Prenatal Malnutrition among Neonatal Rats

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Key Words. Maternal malnutrition · Neonatal rats · Differential response · Response to malnutrition

Abstract. Female rats were fed (A) protein-restricted diet 1 month prior to mating and throughout pregnancy, or (B) protein-free diet during 10–20 days of pregnancy. At birth, four parameters of the offspring, body weight, cerebral weight, cerebral DNA, and cerebral protein were well correlated with each other, and were significantly lower than in the controls fed stock diet. The malnourished population had on the average 30–70% 'outstanding low' individuals (parameter values more than 2 SD below the mean of the control); this is 13–20 times more than in the control. Even in malnourished populations a certain number of individuals escaped malnutrition (parameter values not lower than the mean of the control): they appear to be those which in normal populations would be well above the average (1.2–2.1 SD), but now, because of prenatal malnutrition, were merely just about average. These individuals escaped malnutrition not by taking advantage of their littermates: the latter, though malnourished, were still better than the average in the malnourished group. The mechanisms by which some of the malnourished females differentially provide enough nutrients for one of their fetuses, and more than the average for its littermates, may involve differential mobilization of maternal nutrient reserves among individual mothers, as well as differential blood supply to individual placentas or placental transfer to individual fetuses.

Introduction

In the previous work (7), 'outstanding' newborn rats, with parameter values more than 2 SD above the mean (OH) or below the mean (OL), were identified in a *normal* population of 720 animals and were studied for correlations between these parameters: body weight, brain weight, brain DNA (cell number), and brain protein. The animals OH on any one of these parameters were also higher than average on all the others, and some of these animals came from OH litters. The animals OL on one parameter tended to be OL on all the others, and

correlations between parameters were higher in this group than in the entire population. The correlations among the OH parameters were not the same as the correlations among the OL. It was postulated that the causes of the occurrence of OH and OL animals are more likely to be environmental than genetic.

In the present work, similar analysis has been applied to a population of newborn rats whose mothers were subjected to one of the two regimes of malnutrition, either (A) 1 month before pregnancy and throughout pregnancy – protein restriction (9, 12, 13), or (B) during 10–20 days of pregnancy only – total protein deprivation (11). In particular, we have studied in such populations the occurrence of 'outstanding low' (OL) individuals as well as the occurrence of individuals that escaped malnutrition (ESC).

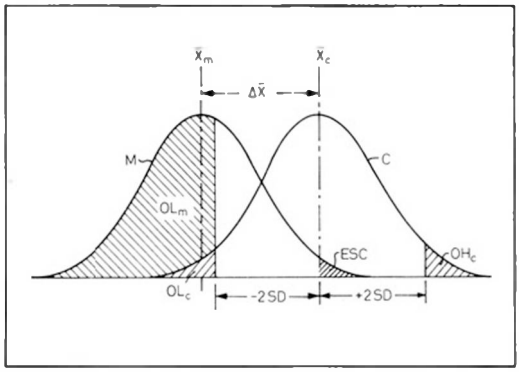

Fig. 1. Diagrammatic representation of normal distribution curves for any of the four studied parameters of the newborn population. C = Control; M = malnourished population; $\bar{X}_c$ and $\bar{X}_m$ = mean values of these populations. $\Delta\bar{X}$ difference between these means; OH_c = 'outstanding high'; OL_c = 'outsantding low' of control population; OL_m = 'outstanding low' of the malnourished population (more than 2 SD below the mean of the control); ESC = animals that 'escaped malnutrition' (individuals from malnourished population with parameter values not lower than the mean of the control).

Materials and Methods

The animals and their nutrition regimes were as described in the previous work (7, 9, 13). The rats were Sprague-Dawley derived. Virgin females, 3 months old and weighing 200–260 g, were mated; the presence of a vaginal plug was considered day 0 of pregnancy. The control group C was fed a pelleted stock diet containing 20.5% protein (Wayne Mouse-breeder Block, Allied Mills, Chicago, Ill.). The experimental animals were fed as follows: group E_1 was fed for 1 month before mating and throughout gestation a powdered diet containing 8% protein, supplied by Nutritional Biochemicals, Cleveland, Ohio; it contained 10% fat, 4% salts, 76% starch, 2.2% vitamin diet fortification mixture (Nutritional Biochemicals). Group E_2 was fed during 10–20 days of gestation a powdered protein-free diet, supplied by Nutritional Biochemicals, Cleveland, Ohio, which had the following composition (in %): corn starch, 70; Alphacel (cellulose), 15; vegetable oil, 10; salt mixture, USP XIV, 4; cod liver oil, 1. This diet was supplemented with vitamin B complex (Becotin, Lilly), consisting of (in mg): thiamin, 50; riboflavin, 50; pyridoxine, 20.5; niacinamide, 250; pantothenic acid, 125; and B_{12} 5 μg/1,000 g.

DNA was determined by a modification of the diphenylamine colorimetric method (4, 5); protein was determined by a modification of the Lowry colorimetric method (3).

Results and Discussion

Figure 1 represents diagrammatically normal distribution curves for any of the four studied parameters of the control (C) and for malnourished (M) populations. The actual numerical values are represented in tables I and II.

In control populations, the proportion of 'outstanding low' (OL_c) rats (with parameter values more than 2 SD below the mean of the

Table I. Identification of 'outstanding low'[1] individual newborns (rats)

Group[2]		Entire group				Outstanding low with respect to			
		body weight	cerebral weight	cerebral DNA	cerebral protein	body weight	cerebral weight	cerebral DNA	cerebral protein
C_1	number	720	720	720	720	27	23	16	15
	percent of total	100	100	100	100	3.75	3.19	2.22	2.08
	mean value (mg) ± SD	5,978 ± 715	161.5 ± 17.5	0.5597 ± 0.0344	8.75 ± 1.27	4,019 ± 471	115.5 ± 7.25	0.4765 ± 0.0147	5.77 ± 0.31
E_1	number	57	57	57	56	34	9	19	1
	percent of total	100	100	100	100	59.65	15.79	33.33	1.79
	mean value (mg) ± SD	4,570 ± 771.5	138.2 ± 9.72	0.5012 ± 0.0329	7.594 ± 0.69	4,026 ± 308	122.6 ± 3.12	0.4614 ± 0.0164	6.15 –
C_2	number	57	57	57	57	1	0	1	0
	percent of total	100	100	100	100	1.75	0	1.75	0
	mean value (mg) ± SD	6,100 ± 415	172.0 ± 8.46	0.5827 ± 0.0209	9.61 ± 0.69	5,100 –	–	0.529 –	–
E_2	number	82	82	82	82	66	50	53	47
	percent of total	100	100	100	100	80.49	60.98	64.63	57.32
	mean value (mg) ± SD	4,585 ± 800	149.7 ± 18.6	0.5225 ± 0.0348	8.09 ± 0.85	4,362 ± 731	139.4 ± 16.0	0.5014 ± 0.0218	7.54 ± 0.633

[1] Animals with parameter values more than 2 SD below the mean for the controls fed stock diet.

[2] C_1 = Control to E_1 which is 8% protein maternal diet, 1 month prior to mating and throughout pregnancy; C_2 = control to E_2 which is protein-free maternal diet, 10–20 days of pregnancy.

Table II. Individual newborns which escaped malnutrition (ESC)[1]

Group[2]		Escaped malnutrition as judged by			
		body weight	cerebral weight	cerebral DNA	cerebral protein
E_1	number[1]	2	0	1	3
	percent of total in the group	3.51	0	1.75	5.26
E_2	number[1]	0	10	4	2
	percent of total in the group	0	12.2	4.88	2.44

[1] Animals with parameter values equal or greater than the mean values for their controls.

[2] See table I.

Table III. Correlation coefficients for the entire population and for the OL_m and ESC animals; group C_1 combined with C_2, and E_1 with E_2

Parameters correlated	Control population		Experimental population	
	r	p	r	p
Total population, n	777		139	
Body weight–cerebral weight	0.749	<0.0001	0.661	<0.0001
Body weight–cerebral DNA	0.412	<0.0001	0.370	<0.0001
Body weight–cerebral protein	0.526	<0.0001	0.451	<0.0001
Cerebral weight–cerebral DNA	0.429	<0.0001	0.387	<0.0001
Cerebral weight–cerebral protein	0.651	<0.0001	0.704	<0.0001
Cerebral DNA–cerebral protein	0.445	<0.0001	0.509	<0.0001

r = Correlations for the entire population; r_1 = correlation observed in OL_m or ESC sample; p = significance levels; OL_m = outstanding low malnourished; ESC = animals which escaped malnutrition. See tables I and II for other explanations.

control) (7) was on the average approximately 2.3% (table I). In contrast, in malnourished populations the proportion of 'outstanding low' (OL_m) animals (defined also as those with parameter values more than 2 SD below the mean of the *control*) was on the average 70% with respect to body weight, 38% brain weight, 33% DNA, and 30% protein; the differences between OL_m and OL_c were statistically highly significant ($p < 0.0001$) for all parameters (except for cerebral protein in group E_1). In other words, malnourished populations have 13–20 times more individuals that would be considered 'outstanding low' by normal standards.

Even in malnourished populations a certain number of individuals escaped malnutrition (ESC in figure 1 and table II), i.e., they exhibit parameter values no lower than the mean of the control.

Table III shows that for control as well as experimental populations all the parameters were highly correlated ($p < 0.0001$) (compare also 1, 2, 6, 7). The correlations were as good or better in the OL_m population; thus, also in malnourished populations an animal outstanding low on one parameter tended to be outstanding low on all the others. The ESC population was too small for a valid correlation study, but the data available (table III) tend to indicate that as far as correlations are concerned, this population was essentially no different from the others indicated above.

Closer analysis of the data on ESC individuals revealed that only 1 out of 10 individuals came from a litter that escaped malnutrition as a whole; possibly, in this 1 case, the pregnant female was able to mobilize sufficiently her stored nutrient reserves to counteract malnutrition for all of her fetuses (10). In all other cases only 1 individual in a malnourished litter managed to retain parameters as good as those of the controls. The proportion of ESC animals corresponds to the proportion of animals that

OL_m or ESC with respect to							
body weight, r_1		cerebral weight, r_1		cerebral DNA, r_1		cerebral protein, r_1	
OL_m	ESC	OL_m	ESC	OL_m	ESC	OL_m	ESC
100	2	59	10	72	5	48	5
0.755	–	0.798	0.878	0.703	0.814	0.810	−0.632
0.396	–	0.480	0.331	0.214	0.914	0.459	−0.568
0.607	–	0.558	0.225	0.458	0.654	0.542	−0.741
0.307	–	0.548	0.339	0.314	0.872	0.422	0.386
0.774	–	0.742	0.201	0.781	0.484	0.685	0.968
0.428	–	0.489	0.308	0.411	0.331	0.440	0.292

Table IV. Littermates of newborns which escaped malnutrition (ESC) (E_1 and E_2 combined)

		Littermates of those newborns which escaped malnutrition as judged by			
		body weight	cerebral weight	cerebral DNA	cerebral protein
Total number		16	9	33	24
Mean values (in %) of mean values for the total population $E_1 + E_2$	body weight	120	97	99	107
	cerebral weight	106	107	100	111
	cerebral DNA	101	99	102	101
	cerebral protein	104	107	103	105

would be, depending on parameter studied, 1.2–2.1 SD above the mean in the control population (7, 8). Thus, it is likely that almost all of the individuals that escaped malnutrition were those which in normal population would be well above the average, but now, because of prenatal malnutrition, were merely just about average.

Table IV represents an attempt to answer the question whether the above ESC animals escaped malnutrition by taking advantage of their littermates. If so, then the mean values of the parameters of these littermates would be lower than for the entire malnourished population. As can be seen from table IV, this is not the case: on the contrary, these littermates

appear to be better than an average malnourished animal. This again suggests that the ESC animals were those which in normally nourished population would be well above the average: indeed, previous study (7) has shown that the littermates of superior rats are also above the average.

The mechanism by which some of the malnourished females differentially provide enough nutrients for one of their fetuses and more than the average (though still not enough) for its littermates, may be quite complex: it may involve the differential degree of mobilization of maternal nutrient reserves among individual mothers as well as the differential blood supply to individual placentas or placental transfer to individual fetuses.

Acknowledgements

This study was supported by grants HD-05615, HD-08927 and AG-00162 from the National Institutes of Health, US Public Health Service. Computing assistance was obtained from the Health Science Computing Facility, UCLA, sponsored by NIH Special Research Resources grant RR-3. We thank Dr. *Edith van Marthens* for the biological data.

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