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# DNA (Cell Number) and Protein in Rat Brain

## Second Generation ($F_2$) Alteration by Maternal ($F_0$) Dietary Protein Restriction[1]

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*Abstract.* Female rats ($F_0$) were maintained on a normal (C) or a protein-restricted (8%) diet (R) 1 month prior to mating and throughout pregnancy. Their offspring ($F_1$) were nursed by their natural or foster mothers on normal diet or protein-restricted diet. All offspring were maintained on normal diet after weaning; the females were mated with normal males, and one group of R males with normal females. The second generation ($F_2$) was maintained on normal diet from birth. Body weight and cerebral weight, DNA and protein were measured in $F_1$ and $F_2$, at birth and at 30 and 90 days. Previously-reported developmental deficiencies were observed in $F_1$ in all restricted groups at birth and at 30 days, but only in DNA of some groups at 90 days. The $F_2$ offspring from all experimental females had at birth significantly lower cerebral DNA (cell number); in most groups this deficiency in $F_2$ did not persist at 30 days. Cerebral deficiencies in newborn $F_2$ resulting from protein restriction in $F_0$ or $F_1$ were transmitted to the next ($F_2$) generation through females but not through males. Several explanations of this effect are offered.

*Key Words*
Rat
Dietary protein restriction
$F_2$ generation
Brain
DNA
Protein
Cerebral deficiency
Transmission through females

In our previous work [28] we have reported that when female rats were maintained on a low-protein (8%) diet 1 month prior to mating and throughout pregnancy, their offspring at birth had significantly smaller body weights, cerebral weights, cerebral DNA (cell number) and cerebral protein. Similar results were obtained by WINICK [16] and by ZEMAN and STANBROUGH [31].

The present work was designed to investigate whether a malnutrition-induced injury in one generation can be transmitted to the next generation,

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with particular reference to brain underdevelopment. The possibility of a transfer of maternal constraint (when she was a fetus), affecting body weight and behavior of her offspring, has been indicated in the past [5, 11, 15]; it has also been suggested that such constraint has a survival value at times of food shortage [11].

## *Experimental*

Sprague-Dawley-derived albino rats bred as a closed colony in our laboratory for 22 generations were used in the previous and present work [14, 22–28]. 60-day-old virgin females ($F_0$ generation) were randomly divided into an experimental and control group. The experimental group (R) was fed a protein-restricted diet (8%) and the control group (C) was fed a standard control pellet diet, as described previously [28]. Food intakes were calculated for both groups by feeding known amounts and weighing the remainder. At 90 days of age, all females were mated and maintained on their respective diets throughout gestation. The food intakes during the month prior to mating averaged 12.6 g/24 h for group R and 13.4 g/24 h for group C. During pregnancy the intake was 13.1 g/24 h for group R and 13.2 g/24 h for group C.

In the $F_1$ generation all litters at birth (within 6 h after delivery) were standardized to 8 newborns per female; the excess newborns were used for biochemical analysis (table I). After decapitation, the brains (cerebral hemispheres, without cerebellum and olfactory lobes) were immediately removed and weighed; they were then frozen and subsequently used for analysis. DNA was determined by a modification of the diphenylamine colorimetric method [10, 19, 21]; protein was determined by a modification of the Lowry colorimetric method [9].

For the $F_1$ generation groups C and R were further subdivided depending on the diet fed during weaning. The designations for these groups are as follows: CC, CR, RR, RC, C(R)R, and R(C)C. The first letter indicates the *pre-partum* diet of the biological mother, and the second letter indicates the type of maternal diet during weaning. Two groups were cross-fostered and the letter in parenthesis indicates the *pre-partum* diet of the foster mother.

During the weaning period the food intakes were calculated as 16.8 g/24 h for females on the control diet and 16.0 g/ 24 h for those on the protein-restricted diet. After weaning all $F_1$ animals were placed on the control diet for the remaining part of the experiment.

At maturity the $F_1$ females were mated with normal males, The males used for mating were fed control diet except that in one experiment, to test the nontransmission to $F_2$ of male deficiencies in $F_1$, adult males of group RC were mated with control females.

Biochemical analyses were performed on $F_1$ and $F_2$ newborns (table I), 30-days $F_1$ and $F_2$ males (table II), and adult $F_1$ and $F_2$ females (table III).

## *Results*

Tables I–III represent the effects of maternal protein restriction on neonatal, 30- and 90-day-old offspring in $F_1$ and $F_2$. Maternal ($F_0$) protein restriction to 8% 1 month prior to mating and throughout gestation resulted

*Table I.* The effect of maternal protein restriction on neonatal offspring in $F_1$ and $F_2$

| Group[1] | Genera-tion | Number | Body weight[2] g | Cerebrum[2] weight, mg | DNA, $\mu$g | protein, mg |
|---|---|---|---|---|---|---|
| CC | $F_1$ | 123 | 5.7 ± 0.6 | 172 ± 13 | 572 ± 44 | 9.7 ± 1.6 |
| | $F_2$ | 74 | 6.1 ± 0.5 | 170 ± 13 | 598 ± 33 | 8.8 ± 0.8 |
| RR | $F_1$ | 136 | 4.9 ± 0.9* | 149 ± 21* | 532 ± 46* | 8.1 ± 1.2* |
| | $F_2$ | 59 | 6.1 ± 0.8 | 168 ± 12 | 562 ± 43* | 8.8 ± 0.9 |
| RC | $F_1$ | same as group RR | | | | |
| | $F_2$ | 63 | 5.7 ± 0.5* | 158 ± 15* | 565 ± 35* | 8.3 ± 0.8** |
| R(C)C | $F_1$ | same as group RR | | | | |
| | $F_2$ | 59 | 5.9 ± 0.5 | 159 ± 16* | 555 ± 32* | 8.2 ± 1.1** |
| C(R)R | $F_1$ | same as CC | | | | |
| | $F_2$ | 50 | 6.3 ± 0.8 | 169 ± 17 | 557 ± 38* | 8.7 ± 0.9 |
| CR | $F_1$ | same as CC | | | | |
| | $F_2$ | 48 | 5.9 ± 0.7 | 160 ± 18** | 569 ± 41* | 8.3 ± 1.2 |

[1] See text (Experimental) for explanation of groups.

[2] Each value represents the mean ± SD.

* Significant at $p < 0.001$ level. ** Significant at $0.01 > p > 0.001$ level. Standard t-test. All comparisons made to the control (CC).

in significant decreases in all measured parameters (body weight, cerebral weight, DNA and protein) in their offspring ($F_1$) at birth (table I, groups RR, RC, R[C]C). $F_2$ newborns in groups RC and R(C)C had significant deficiencies in neonatal cerebral DNA and practically all other neonatal parameters. In these 2 groups (RC and R[C]C), at the age of 30 days, most of these deficiencies remained significant for $F_1$ but not for $F_2$, in which even a transient increase in DNA was observed for RC. Practically all deficiencies became nonsignificant at maturity (90 days) for both $F_1$ and $F_2$.

When group RC $F_1$ adult males were mated with control females, no significant deficiency of any parameter in $F_2$ could be demonstrated even for neonatal animals (body weight 6.7 g; cerebrum: weight 175 mg; DNA 587 $\mu$g, protein 8.02 mg).

Groups CR and C(R)R in the $F_1$ generation were significantly lower in all parameters at 30 days and in cerebral weight and cerebral DNA – at 90 days.

*Table II.* The effect of maternal protein restriction on 30-day-old male offspring in $F_1$ and $F_2$

| Group[1] | Generation | Number | Body weight[2], g | Cerebrum[2] | | |
|---|---|---|---|---|---|---|
| | | | | weight, mg | DNA, μg | protein, mg |
| CC | $F_1$ | 27 | 73 ± 9 | 1,031 ± 40 | 913 ± 52 | 80.1 ± 6.8 |
| | $F_2$ | 27 | 73 ± 9 | 1,031 ± 40 | 913 ± 52 | 80.1 ± 6.8 |
| RR | $F_1$ | 10 | 36 ± 11* | 904 ± 75* | 848 ± 45** | 71.6 ± 6.2** |
| | $F_2$ | 15 | 77 ± 14 | 1,031 ± 48 | 1,034 ± 81* | 79.2 ± 15.0 |
| RC | $F_1$ | 15 | 72 ± 18 | 968 ± 56** | 836 ± 48* | 71.7 ± 5.4* |
| | $F_2$ | 28 | 67 ± 15 | 999 ± 85 | 1,013 ± 62* | 78.6 ± 11.7 |
| R(C)C | $F_1$ | 16 | 60 ± 14** | 1,008 ± 50 | 846 ± 51* | 70.4 ± 4.2* |
| | $F_2$ | 12 | 77 ± 23 | 1,002 ± 79 | 916 ± 61 | 70.5 ± 6.6** |
| C(R)R | $F_1$ | 11 | 38 ± 19* | 879 ± 91* | 803 ± 58* | 63.0 ± 6.1* |
| | $F_2$ | 11 | 65 ± 12 | 975 ± 44** | 985 ± 62** | 74.1 ± 9.4 |
| CR | $F_1$ | 20 | 28 ± 6* | 882 ± 62* | 865 ± 58** | 69.7 ± 7.4* |
| | $F_2$ | 10 | 73 ± 15 | 1,012 ± 79 | 912 ± 42 | 70.6 ± 5.9** |

[1] Groups as in table I.
[2] As in table I.
* and **, see table I.

*Table III.* The effect of maternal protein restriction on 90-day-old female offspring in $F_1$ and $F_2$

| Group[1] | Generation | Number | Body weight[2], g | Cerebrum[2] | | |
|---|---|---|---|---|---|---|
| | | | | weight, mg | DNA, μg | protein, mg |
| CC | $F_1$ | 77 | 269 ± 41 | 1,197 ± 52 | 1,040 ± 105 | 93.8 ± 9.0 |
| | $F_2$ | 49 | 262 ± 43 | 1,206 ± 57 | 1,103 ± 71 | 98.6 ± 6.4 |
| RR | $F_1$ | 15 | 243 ± 35 | 1,084 ± 91* | 972 ± 72** | 87.9 ± 16.8 |
| | $F_2$ | 24 | 254 ± 33 | 1,167 ± 54** | 1,025 ± 57* | 97.3 ± 7.0 |
| RC | $F_1$ | 20 | 247 ± 45 | 1,129 ± 69* | 997 ± 77 | 100.2 ± 11.8 |
| | $F_2$ | 28 | 271 ± 32 | 1,210 ± 60 | 1,087 ± 47 | 98.9 ± 7.7 |
| R(C)C | $F_1$ | 15 | 280 ± 32 | 1,167 ± 70 | 1,001 ± 102 | 102.1 ± 14.0 |
| | $F_2$ | 6 | 276 ± 30 | 1,129 ± 86 | 1,004 ± 91 | 94.8 ± 7.5 |
| C(R)R | $F_1$ | 12 | 249 ± 23 | 1,097 ± 59* | 953 ± 77** | 92.6 ± 10.8 |
| | $F_2$ | 4 | 244 ± 15 | 1,115 ± 19** | 996 ± 47 | 89.2 ± 7.9 |
| CR | $F_1$ | 18 | 260 ± 36 | 1,127 ± 57* | 916 ± 67* | 92.5 ± 13.6 |
| | $F_2$ | 6 | 249 ± 15 | 1,197 ± 66 | 1,002 ± 40** | 102.7 ± 10.3 |

[1] See table I.
[2] See table I.
* and **, see table I.

In $F_2$ decreases were significant for cerebral DNA and/or cerebral weight at birth and 90 days, but not at 30 days when a transient increase in DNA in group C(R)R was observed.

## *Discussion*

With reference to neonatal parameters in $F_1$ the present work confirms the results previously reported by us [28] and confirmed by others [16, 31]: Maternal prenatal ($F_0$) protein restriction (8%) results in significant decreases in neonatal body weight, cerebral weight, cerebral DNA and cerebral protein. It is of interest that these decreases persist postnatally till at least 30 days even in groups RC and R(C)C where the lactating female was receiving control diet. As discussed in previous publications, the determination of brain DNA is a convenient and objective quantitative method for determination of total brain cell numbers [22, 23, 25]; neuron (neuroblast) proliferation in the rat ceases at or before birth [review in ref. 23 and 25], and normal glia (glioblast) proliferation – at weaning [review in ref. 16]. It has been reported that at birth the neuron/glia ratio reaches 5, and at 30 days 2 or 3 [2]; this suggests that the DNA (cell number) decrease observed at birth reflects the deficiency of neuroblasts themselves. The fact that the percentage decrease of DNA (cell number) remains essentially the same at 30 days (despite a 60-percent increase in total DNA) suggests that the number of glia (glioblasts), proliferating after birth, also suffered a deficiency, and that this deficiency essentially followed the deficiency in number of neurons (neuroblasts).

In addition to quantitative changes in number of cells, the cerebrum in $F_1$ also undergoes qualitative changes: At birth and at 30 days the deficiency in total cerebral protein is higher than the deficiency in the number of cerebral cells which signifies less protein per cell.

Thus, prenatal nutritional protein restriction alone (RC and R[C]C) is sufficient to sustain cerebral deficiencies past the age (21 days) at which the rat cerebrum is considered essentially mature [19].

Additional postnatal deprivation (group RR) adds considerably to the decreases in body weight and cerebral weight at 30 days, but does not alter decreases in cerebral DNA (neuron and glia number) and cerebral protein; thus, it appears that for these 2 cerebral parameters the postnatal nutritional insult did not augment the effects of the prenatal one. It is of interest that WINICK [17] did observe augmentation in a different type of pre-weaning malnutrition.

Between 30 and 90 days, cerebral DNA (controls) increases by an additional 14%. This increase might be due to further proliferation of non-neuronal cells[2] or further conversion of diploid cells into polyploid [7]. It is of interest that most of the deficiencies present at 30 days in groups RC and R(C)C, disappear at the age of 90 days [compare also ref. 4 and 12]. Conceivably, in these experimental animals, there is more proliferation of non-neuronal cells, or even glial cells, to fill the deficient volume; the latter is known to occur after brain injuries. Thus, disappearance of deficiencies at 90 days does not mean that the animals are rehabilitated: The deficiency of neurons (final at birth) may persist even though total DNA becomes normal. In group RR, the added postnatal restriction of $F_1$ suffices to carry their DNA deficiency to at least 90 days.

Entirely unexpected results were obtained when the females that appeared essentially normal in $F_1$ at 90 days (groups RC and R[C]C) were mated with normal males: Their offspring at birth still had significant decreases in almost all parameters. This effect was transmitted only through females: The $F_1$ males of group RC, mated with normal females, produced normal offspring. Thus, the effect does not follow Mendelian genetics. Chromosome abnormalities, produced by malnutrition, have recently been reported [1]; in the present study, however, the transmission appears to be due not to genetic but to environmental effects on the organism of the $F_1$ mothers.

The nature of this effect is, at present, not clear. One possible explanation could be that the effect on $F_2$ neonatal cerebra was due to poor lactation of $F_0$-nursing mothers that were protein-restricted before and during pregnancy (group RC). That this was not the case is apparent from group R(C)C, in which the effects on the neonatal cerebral parameters were essentially the same as in RC, even though the nursing mothers were never protein-restricted. Also, when the nursing $F_0$ mothers were protein-restricted before and during pregnancy as well as during the lactation period (group RR), the effect on cerebral DNA (cell number) in $F_2$ was not greater than in the case of protein restriction before and during pregnancy alone (groups RC and R[C]C).

Another explanation is based on the possibility that due to protein restriction of $F_0$ mothers before and during pregnancy, the $F_1$ animals were born handicapped, not only with regard to the brain [28] but also in other respects. ZEMAN [30] and HALL and ZEMAN [6] have reported that the off-

[2] The estimated proportion of nonneuronal cells in cerebral cortex of young adult rats is 30–35% [2].

spring of rats similarly protein-restricted during pregnancy suffer from retardation of kidney development and altered kidney function. They may also lack the vigor to suckle [29]. LEE and CHOW [8] have reported that the restricted progeny showed reduced feed efficiency and low nitrogen balance; they excreted more amino acids than the controls. Thus, such progeny ($F_1$) may indeed suffer from *cryptic* malnutrition, even when postnatally given full access to normal food (groups RC and R[C]C), and consequently their $F_2$ progeny would have a deficiency in all parameters.

Another possibility is that the handicap of $F_1$ animals involved their endocrine glands, as demonstrated recently by STEPHAN *et al.* [13]; in this case their deficiency would be not of the direct nutritional nature as postulated above, but would rather involve hormonal regulation. The glands affected (underdeveloped?) could be the hypothalamus, known to be sensitive to amino acid levels, and the pituitary [13], known to affect placental development [for a recent discussion, see ref. 25]; the latter might be correlated with brain development [20]. Thus, cerebral development in $F_2$ offspring would be limited by maternal (but not paternal) endocrine gland development in $F_1$.

Since the above deficiencies are demonstrated in neonatal ($F_2$) cerebra, they are likely to reflect primarily the deficiency in the number of neurons, as previously discussed. After the birth of $F_2$, placental $F_1$ underdevelopment becomes immaterial, and evidently the injury is of a different nature or different extent than in the previous generation, because the deficiencies cannot be demonstrated even at 30 days (groups RC and R[C]C). At 30 days (RC) there is even an unexplainable transient increase in DNA [compare also ref. 4], possibly due to proliferation of nonneuronal cells. Again, this does not imply that the animals are now rehabilitated because the neuron deficiency is likely to persist even if postnatal total DNA becomes normal or elevated.

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