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Effect of Maternal Undernutrition on the Growth and Composition of Young Rat Brain

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분만전후의 어미쥐의 영양부족이 새끼쥐의 뇌성장발육과 조성에 미치는 영향

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〈국문초록〉

분만전후 4주동안 Spraque Dawley 암컷 쥐에게 양적으로 식이를 제한했다. 임의로 먹인 control group의 1일평균섭취량의 1/2의 시중사료를 식이제한 group에게 주었다. Deficient Ⅰ group에서는 분만후부터 이유시까지 식이제한을 했고, deficient Ⅱ group에서는 임신 15일부터 이유시까지 계속 식이제한을 했다.

식이제한 group의 체중과 뇌무게는 control group의 새끼쥐보다 유의적으로 낮았지만, 체중에 대한 뇌무게의 비는 control group 보다 높았다. 이유시에는 두 식이제한 group사이에서 체중과 뇌무게의 유의적인 차가 나타났다.

뇌의 DNA, RNA 및 단백질함량은 control group보다 식이제한 group에서 유의적으로 낮았지만 RNA/DNA, 뇌무게/DNA 및 단백질/DNA은 control group보다 식이제한 group에서 높았다. 이것은 새끼쥐뇌에서 세포분열이 세포의 크기성장보다 이 기간중의 어미쥐의 식이제한에 의해 더욱 심한 영향을 받았음을 시사해준다. 뇌의 DNA와 RNA는 두식이제한 group간에 유의적인 차를 나타내지만, 단백질의 경우는 유의적인 차를 나타내지 않았다.

Introduction

Undernutrition is defined as malnutrition resulting from the consumption of inadequate amounts of food so that one or more of the essential nutrients are lacking in the diet[1]. Undernutrition during fetal period and infancy

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is a prevalent problem in many parts of the world, but very little information is available on the effect of this undernutrition on brain growth and composition. Animal studies provide a reasonable means of obtaining information relevant to this problem, and data from such studies will undoubtedly be helpful to understand the effect of undernutrition on the brain growth and composition of infants[2].

Animal studies have demonstrated that undernutrition in early life may cause a variety of abnormalities including; lower body and brain weight[3)4)], lower brain nucleic acid content[5-7)], abnormal cerebral protein metabolism[8)], abnormal brain ganglioside metabolism[9)], lower leveals of neurotransmitters[10-14)], and retarded behavior and learning ability[15)16)].

The mammalian brain is an enormously heterogeneous and structurally complex organ containing an inderterminately large number of chemically and functionally distinct populations of cells[17)18)]. Fewer than one-third of the total number are neurons; the remainder are mostly vascular elements and characteristic supporting cells known collectively as glia. Early malnutrition in rats retards cell devision in any brain area in which proliferation is occurring and permanantly affects any cell type proliferating. In human, cell devision is curtailed by early malnutrition. Later malnutrition will reduce the size of individual cells, but not the number of cells[7)17)19)].

The DNA content of the diploid nucleus in the cells of a given species has been shown be constant[5)]. Therefore, a change in total DNA content of a tissue or organ can be considered to indicate a change in cell number. The weight/DNA ratios and protein/DNA ratios are then indicative of the total amount of the protein per cell, respectively. These have used to estimate cell size[17)].

Cell division is time-dependent[20)]. Thus, reduction in cell number as a result of malnutrition results in permanent stunting of growth[17)].

Brain size and composition are readily affected by undernutrition during the preweaning period than the postweaning period. Winick and Noble[7)] reported that undernutrition of rats from birth until 21 days of age resulted in lower brain weight and DNA, RNA, and protein content. Culley and Mertz[21)] noted that undernutrition of rats from 5 until 21 days of age caused a significant reduction in brain weight. Guthrie and Brown[22)] found that undernutrition of rats during the preweaning period caused irreversible decrements in brain size and DNA content. Nevertheleses, not all workers have found that undernutrition during the preweaning period affected brain size and composition. Rajalakshmi et al.[23)] found that undernutrition from birth until 28 days of age did not affect rat brain size or its DNA, RNA or protein content. Benton et al.[24)] found that the effect of undernutrition on rat brain size could be overcome by ad libitum feeding from three to six weeks of age.

The purpose of this study was to determine the effect of a quantitative restirction of maternal diet during the third week of gestation and lactation, without changes in the quality of the diet, on the growth and composition of young rat brain. This paper deals with the changes in body weight, brain weight, brain DNA, RNA and protein content during the period of dietary restriction.

Experimental

1. Animal

Virgin female rats of Spraque Dawley strain supplied by animal breeding laboratory of Seoul National University were used in this experiment.

Commercial diet was obtained from Jeil Fodder Co.

2. Experimental design

Virgin female rats weighing 180 to 220 gm were mated with normal males(male : female =1 : 2). Pregnancy assumed to have begun when sperm were found in a vaginal smear (×200). The animal were fed a commercial diet. The composition of the diet is in Table 1.

The scheme of experimental design is shown in Figure 1. Twenty-one pregnant rats were individually housed in a plexiglass cage and divided into 3 groups.

Table 1. Composition of diet

Ingredients	%
Grain	55
Bran	11
Flour	20
Fish Meal	10
Mist	3

Content		%
Crude Protein	above	19.0
Crude Fat	〃	3.0
Crude Fiber	below	6.0
Crude Ash	〃	9.0
Ca	above	0.6
P	〃	0.4
DCP (digestable crude protein)	〃	16.5
TDN (total digestable nutrient)	〃	73.0

The above composition of diet was obtained from Animal Breeding Laboratory of Seoul National University, to which it was submitted by Jeil Fodder Co. as a guaranteed table of composition.

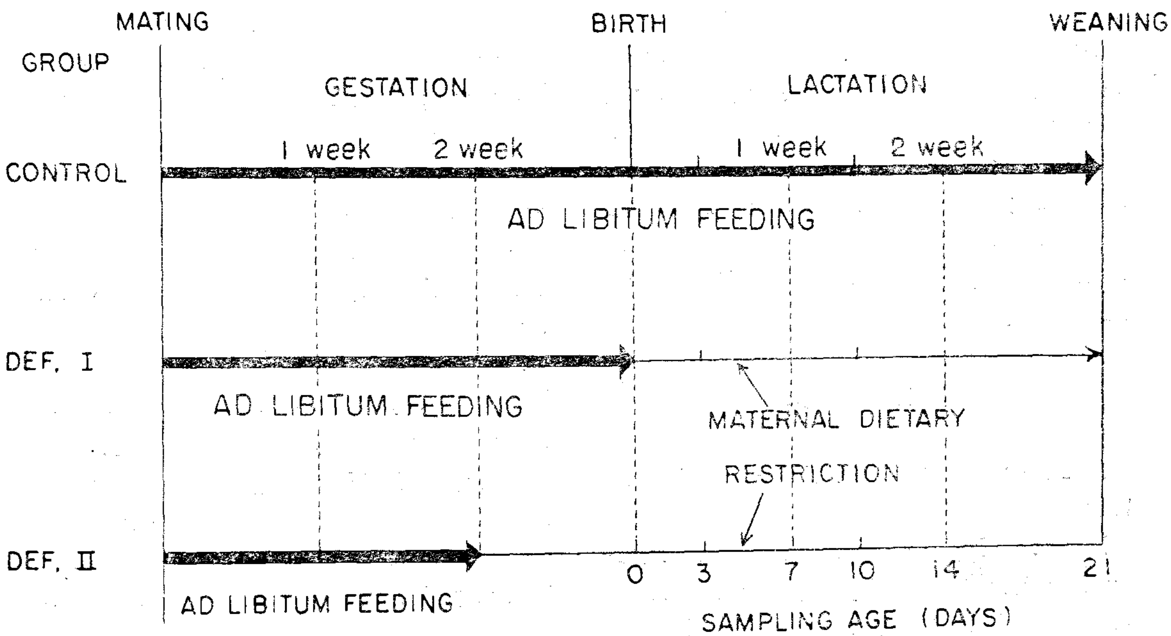

Fig. 1. The scheme of experimental design.

Control group; fed a diet ad libitum during all experimental period. Seven female rats were fed a balanced commercial diet ad libitum during gestation and lactation.

Deficient I group; quantitative restriction of diet from birth to 21 days of postnatal age. Eight lactating rats were fed 50% of diet (20 gm of commercial diet) that lactating rats of control group were fed ad libitum.

Deficient II group; dietary restriction was given from the 15th day of gestation to 21 days of postnatal age. Fifteen gram of diet per day was given to the 6 pregnant rats from the 15th days of gestation to the birth. After delivery, 20 gm of diet per day given to undernourished mother from birth to weaning. This regime represents approximately half of the normal average daily intake of a rat during gestation and lactation.

At the age of 0, 3, 7, 10, 14, and 21 days, the total body weight of all pups at the same age was weighed and mean value of each group was obtained as a body weight.

At 0, 3, 7, 10, 14, and 21 days of postnatal age, pups taken from each group randomly were decapitated and the whole brain was removed, weighed and stored frozen in a screw-capped bottle until used. The brain tissues from 0, 3, 7, 10, 14 and 21 day-old young of each group were assayed for DNA, RNA and total protein content.

Method

1) Determination of RNA and DNA in brain

DNA and RNA were determined by improved method[25] of diphenylamine reaction and orcinol reaction, respectively. In this method, precipitation of nucleic acids as their Cd (II) salts and modification of the orcinol and diphenylamine reaction can be minimize the effect of interferences, which applied to complex mixtures.

2) Determination of total Protein in brain

Total protein was determined by Coomassie Brilliant Blue G-250 dye-binding reaction[26]. The assay is reproducible and rapid with dye-binding process virtually complete in approximately two minutes with good color stability for 1 hr. There is little or no interference from cations such as sodium or potassium, nor from carbohydrate such as sucrose.

3) Statistical analysis

Experimental data from control and deficient groups were analyzed by Student t-test[27].

Results and Discussion

1. Body weight

Table 2 and Figure 2 show body weight of offsprings at 0, 3, 7, 10, 14, and 21 days of postnatal period. Body weight of offsprings of deficient groups was significantly lower than the control group. Differences were greater as they weaned.

At birth, the body weight of offspring of deficient II group (DEF. II group) was much lower than the control group.

At weaning, body weight of offsprings of DEF. II group was significantly lower than the deficient I group (DEF. I group). The offsprings of DEF. II group were nearly starved to death, and their hair was dark in color and eyes were closed, and their heart beat slightly. When they were decapitated, dark-red blood did not flow but nearly coagulated.

Table 2. Effect of maternal dietary restriction on body weight of offsprings (g)

Age \ Group	Control group	DEF. I group	DEF. II group
0 day	6.37±0.38[a] (151)[b]	6.37±0.38 (151)	5.45±0.30 (72)*
3 days	8.66±1.00 (78)	8.47±0.88 (68)	6.83±0.54 (56)*
7 days	13.99±1.34 (70)	11.78±1.69 (59)	9.22±1.18 (43)**
10 days	19.57±1.53 (57)	14.20±2.39 (37)**	11.31±1.12 (37)
14 days	28.01±1.34 (46)	15.67±2.05 (26)**	13.11±0.69 (23)**
21 day	45.86±2.11 (38)	17.74±1.90 (19)**	11.50±1.02 (4)** **c

a: Mean±S.D., b: Number of animals used for calculation

* P<0.05 Significantly different from control group

** P<0.01: Significantly different from control group

**c P<0.01: Significantly different from def. I group

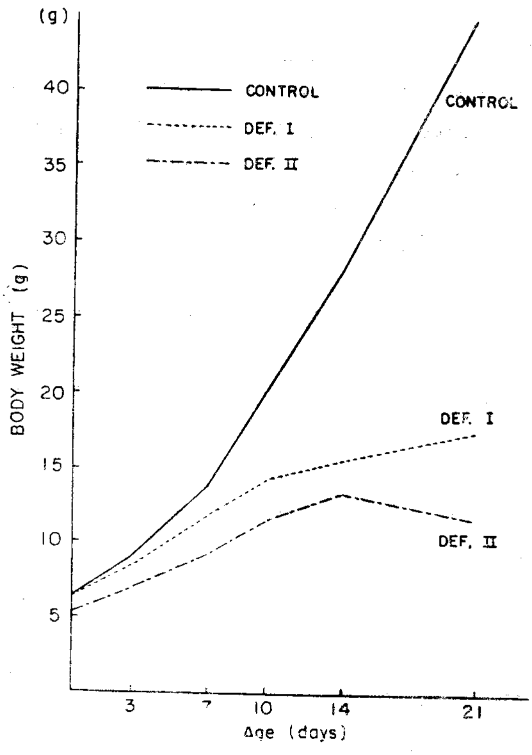

Fig. 2. Effect of maternal dietary restriction on body weight of offsprings.

Dams of deficient groups became more and more slender and a few of them finally died. Some of the offsprings were cannibalized by their dams. Since the 17th day of age, dams of deficient groups didn't nurse their offsprings, and then the offsprings of DEF. II group all died before or at 21 days of age. Most of the offsprings in DEF. I group could not open their eyes at 21 days of postnatal period and when they were left without their dams, they were all starved to death.

Therefore, the result suggests that normal growth of the offspring can't be achieved by maternal dietary restriction during gestation and lactation. The significant decrease in body weight gain is in agreement with the report of Winick et al[7]. who fed deficient group calorie-restricted diet consisted of the same quality as control group from birth to weaning. Many other investigators[2)3)7)14)28-31)] have also reported the similar results of effect of perinatal undernutrition on the body weight of offsprings.

It has been suggested[4)] previously that the offsprings of dietary restricted dams may remain smaller than the controls postnatally because they may be too weak to suckle adequately to obtain sufficient nourishment which, secondarily may result in a reduction in the amount of available milk.

2. Brain weight

Table 3. Effect of maternal dietary restriction on brain weight of offsprings (g)

Age (days)	Control group	Def. I group	Def. II group
0	0.207±0.024[a] (17)[b]	0.207±0.024 (17)	0.181±0.023 (17)
3	0.341±0.053 (7)	0.095±0.036 (10)	0.301±0.030 (11)
7	0.621±0.038 (6)	0.563±0.055 (6)	0.459±0.040 (7)*
10	0.820±0.115 (4)	0.736±0.116 (4)	0.665±0.074 (5)
14	1.186±0.069 (5)	0.762±0.072 (5)**	0.808±0.075 (5)**
21	1.435±0.018 (3)	1.166±0.118 (7)	0.754±0.035 (4)** **c

a: Mean±S.D., b: Number of animals sacrificed
* $P<0.05$: Significantly different from control group
** $P<0.01$: Significantly different from control group
**c $P<0.01$: Significantly different from def. I group

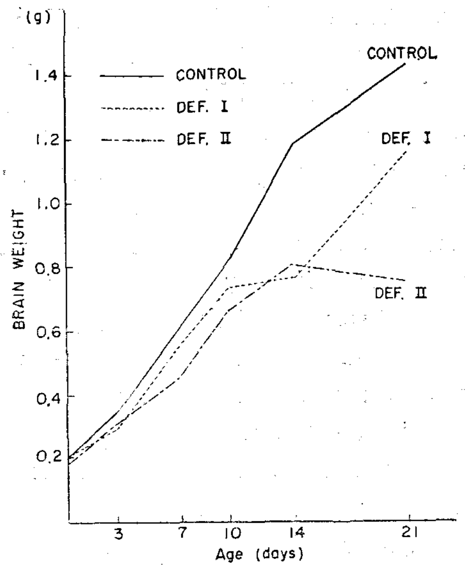

Fig. 3. Effect of maternal dietary restriction on brain weight of offsprings.

Brain weight of offsprings at 0, 3, 7, 10, 14, and 21 days of postnatal period are shown in Table 3 and Figure 3. The brain weight of offsprings of deficient groups was significantly lower than the control group, but was not as adversely affected as the body weight. At 14 days of age, the brain weight of offsprings of DEF. I was significantly lower than the control group. In DEF. II group, brain weight of offsprings was significantly lower than the control group from the 7th day. At weaning brain weight of offsprings of DEF. II group significantly lower than the DEF. I group, because offsprings of DEF. II group were nearly starved to death from the 17th day. Therefore, this result suggests that maternal dietary restriction during gestation and lactation can't give the offsprings the normal growth in brain, which is kept with previous studies[2)3)7)8)14)28-31)] on perinatal undernutrition.

3. Ratios

The relationship between brain weight and body weight is shown in Table 4. The offsprings of deficient groups had a brain representing a higher precentage of body weight than the control group. The brain of offsprings of deficient groups was smaller, but their body growth was still more stunted so that the brain of offsprings of deficient groups represented an increasing proportion of total body weight. This findings confirms the observation of earlier workers[2)3)8)30)31)] that the brain is protected to a greate rextent than the total body from the effect of

Table 4. Effect of maternal dietary restriction on various ratios of offsprings

Group	Age (days)	Brain weight/body weight (%)	RNA/DNA	Brain weight/DNA	Protein/DNA
Control group	0	3.25	1.594	156.3	45.98
	3	3.81	1.709	208.4	72.68
	7	4.44	1.179	190.0	46.21
	10	4.19	1.172	172.4	40.50
	14	4.23	1.471	169.5	45.52
	21	3.13	0.921	121.1	30.45
Def. I group	0	3.25	1.594	156.3	45.98
	3	3.48	1.708	224.7	69.92
	7	4.78	1.548	234.7	59.36
	10	5.18	1.298	190.9	48.81
	14	4.86	1.260	185.2	45.30
	21	6.29	1.179	146.5	29.67
Def. II group	0	3.32	1.738	189.3	57.91
	3	4.40	1.711	227.5	67.76
	7	4.98	2.209	262.9	69.65
	10	5.88	1.796	256.3	63.96
	14	6.16	1.919	238.0	61.44
	21	6.56	1.111	154.3	47.29

undernutrition.

DNA Content of Brain

Brain DNA content of offsprings of each group is shown in Table 5 and Figure 4. DNA values, considered indicative of cell number[32], were highly significantly lower in brain of offsprings of deficient groups than the control group[2)5-8)22)28]. From 7 days to 21 days of age, brain DNA content of offsprings of DEF. II group was significantly lower than DEF. I group. This confirms that maternal dietary restriction during gestation and lactation decreased the total content of DNA in brain of offspring, which indicated

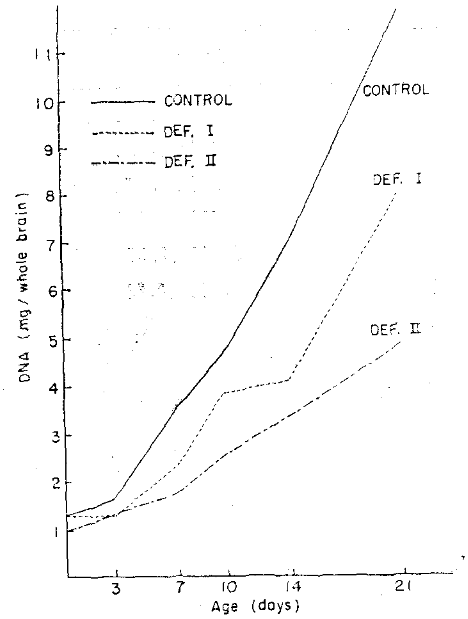

Fig. 4. Effect of dietary restriction on brain DNA of offsprings.

that a primary effect of dietary restriction is a reduction in the fermation of new cells[7)28]. It is also found that perinatal undernutrition had the severe effect on cell division, and especially prenatal undernutrition made the postnatal cell division more stunted[7].

It is noticable that DNA content in newborn rat brain of DEF. II group was significantly lower than the control group. This finding shows that dietary restriction from the 15th day of gestation had severe effect on cell division in the fetus, the rate of which was rapid in the third week of gestation[33]. This result is consistent with Winick[17] who reported as follows: Reduction of total calories from birth to weaning will retard the rate of cell division and result in a permanent deficit in the total number of brain cells. Either calorie restriction[7] or

Table 5. Effect of maternal dietary restriction on brain DNA of offsprings

(mg/whole brain)

Age (days)	Control group	Def. I group	Def. II group
0	1.324±0.011[a](17)[b]	1.324±0.011 (17)	0.956±0.038 (17)**
3	1.636±0. 32 (7)	1.313±0.048 (10)**	1.323±0.017 (11)**
7	3.268±0.074 (6)	2.399±0.051 (6)**	1.746±0.024 (7)** **c
10	4.756±0.023 (4)	3.856±0.029 (4)**	2.594±0.076 (5)** **c
14	6.955±0.060 (5)	4.115±0.018 (5)**	3.395±0.031 (5)** **c
21	11.853±0.031 (3)	7.960±0.057 (7)**	4.886±0.040 (4)** **c

a: Mean±S.D., b: Number of animals sacrified

** $P<0.01$: Significantly different from control group

**c $P<0.01$: Significantly different from def. I group

severe protein restriction during gestation will also reduce the number of brain cells at birth.

If an animal is exposed to both prental and postantal malnutrition, there is a much greater deficit in total brain cell number by the time of weaning than one would expect from just summing prenatal and postnatal effect alone. These data suggest that in addition to the time at which malnutrition begins, the duration of malnutrition is important during this critical period of rapid cellular proliferation.

This effect of prenatal malnutrition in the rat may be summarized by reduced number of cells in placenta, reduced birth weight of new-born, reduced brain-cell number at birth, reduced brain-cell number at weaning, and increased brain response to postnatal malnutrition[33].

RNA Content of Brain

Brain RNA content of offspring of each group at 0, 3, 7, 10, 14 and 21 days of age is shown in Table 6 and Figure 5. RNA values in brain of offsprings of deficient groups were significantly lower than the control group[2)5-8)22)28)]. From the 7th day of postnatal period, brain RNA content of offspring of DEF. II group was significantly lower than DEP. I group.

The ratios of RNA to DNA are shown in Table 4. The ratio of RNA to DNA of deficient groups was higher than the control

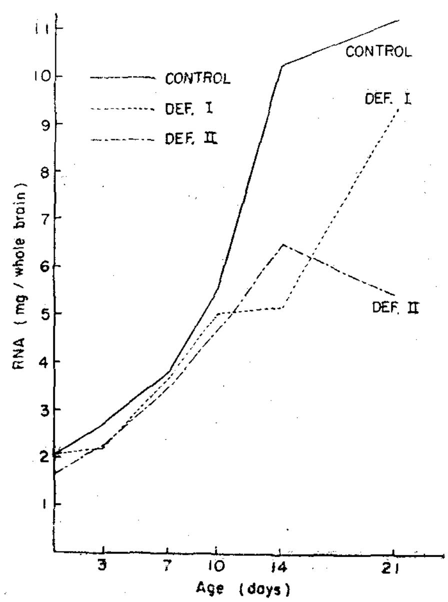

Fig. 5. Effect of maternal dietary restriction on brain RNA of offsprings.

Table 6. Effect of maternal dietary restriction on brain RNA of offsprings

(mg/whole brain)

Age(days)	Control group	Def. I group	Def. II group
0	2.110±0.019[a] (17)[b]	2.110±0.019 (17)	1.663±0.040 (17)** **c
3	2.796±0.041 (7)	2.242±0.027 (10)**	2.264±0.064 (11)**
7	3.852±0.012 (6)	3.716±0.034 (6)*	3.857±0.024 (7)** **c
10	5.576±0.037 (4)	5.004±0.058 (4)**	4.658±0.045 (5)** **c
14	10.30±0.080 (5)	5.182±0.043 (5)**	6.514±0.072 (5)** **c
21	10.91±0.148 (3)	9.388±0.119 (7)**	5.429±0.037 (4)** **c

a: Mean±S.D., b: Number of animals sacrificed

* $P<0.05$: Significantly different from control group

** $P<0.01$: Significantly different from control group

**c $P<0.01$: Significantly different from def. I group

group. This result suggests that cell size is less affected by perinatal undernutrition in the brain of offspring of deficient groups, but cell number was more reduced than cell size by maternal dietary restriction during gestation and lactation. Similar result in ratio of RNA to DNA content of whole brain with age have been noted by others[2)34)].

To confirm this result related to cell size, the ratios of brain weight to DNA obtained from data are shown in Table 4. This ratio is similar to that of RNA to DNA ratio. The brain weight to DNA of offspring of deficient groups was higher than the control group. Therefore, it is found that brain DNA (cell number) was more reduced than brain weight by the maternal dietary restriction during gestation and lactation[2)5-8)].

Total Protein Content in Brain

The total protein content in whole brain of offspring at 0, 3, 7, 10, 14 and 21 days of age are shown in Table 7 and Figure 6. The total protein content in brain of offsprings of deficient groups was significantly lower than the control group. There was no significant difference between DEF. I group and DEF. II group.

Changes in brain protein of offsprings of each groups were closely related to changes in brain RNA content which confirms that RNA is related to protein synthesis[2)3)]

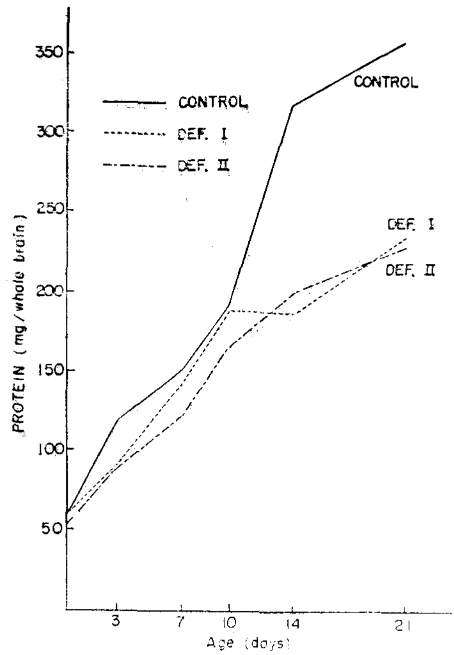

Fig. 6. Effect of maternal dietary restriction on brain protein of offsprings.

Table 7. Effect of maternal dietary restriction on brain protein of offsprings (mg/whole brain)

Age (days)	Control group	Def. I group	Def. II group
0	60.88± 3.62[a] (17)[b]	60.88± 3.62 (17)	55.36±1.13 (17)
3	118.9± 5.63 (7)	91.80± 9.26 (10)*	89.65±3.91 (11)**
7	151.1±13.49 (6)	142. 4±17.80 (6)	121.6±14.7 (7)
10	192.6±25.30 (4)	188. 2±14.53 (4)	165.9±15.1 (5)
14	318.4±17.86 (5)	186. 4±11.1 (5)**	208.6±19.5 (5)**
21	360.9±19.86 (3)	236. 2±17.9 (7)**	231.1±16.16 (4)**

a : Mean±S.D.,
b : Number of animals sacrificed
* : $P<0.05$, Significantly different from control group
**: $P<0.01$, Significantly different from control group

Total protein contents in whole brain of offsprings of deficient groups were less affected than DNA and RNA contents by maternal dietary restriction during gestation and lactation.

There was no significant difference of brain in total protein between control and DEF. II group of brain of newborn rats which indicates that protein synthesis during the third week of gestation was not affected by maternal dietary restriction.

It is of interest that protein synthesis in deficient groups was severely affected by maternal dietary restriction after 2 weeks of postnatal period[2)3)5-8)14)28)31)].

The ratios of total protein to DNA content in whole brain are shown in Table 4. These ratios show that perinatal maternal dietary restriction had little effect on the cell size but greatly to the reduction of cell number in the brain of offsprings.

The above results were not in agreement with those of Rajalakshmi et al.[23)] who found that undernutrition from birth to the 28th day of age did not affect rat brain size of its DNA, RNA or protein content.

Summary

A quantitative restriction of maternal diet without changes in quality of diet was given to the Sprague Dawley rats during the third week of gestation and lactation. Half the normal average daily intake of control group was given to deficient groups in this period. Female rats of control group were fed a commercial diet ad libitum throughout the experimental period. Dietary restriction started from birth to weaning in deficient I group and from the 15th day of gestation to weaning in deficient II group.

Body and brain weight of offsprings of deficient groups were significantly lower than control group, but the ratios of brain weight to body weight in deficient groups were higher than the control group. Significant difference between deficient groups (I and II) was noticed at weaning.

Brain DNA, RNA and total protein of offsprings of deficient groups were significantly lower than control group, but RNA/DNA, brain weight/DNA, and total protein/DNA

show that cell number were more affected than the cell size by the maternal dietary restriction during the third week of gestation and lactation. Between the deficient groups, there was a significant difference in brain DNA and RNA, but no significant difference in total brain protein.

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References

1) Stenesh, J.: Dictionary of biochemistry, John wiley & Sons. New York. pp. 370.

2) Culley, W.J., and Lineberger, R.O.: Effect of undernutrition on the size and composition of the rat brain. J. Nutr. 96 : 375—381, 1968.

3) Tyzbir, R.S., Coupain, J.G., and Beecher, G.R.: Influence of dietary protein levels on rat brain enzyme activities during early development J. Nutr. 107 : 1094—1101, 1977.

4) Zeman, F.J.: Effect on the young rat of maternal proctein restriction J. Nutr. 93 : 167—173, 1967.

5) Zeman, F.J., and Stanbrough, E.C.: Effect of maternal protein deficiency on cellular development in the fetal rat. J. Nutr. 99 : 274—282, 1969.

6) Zamenhof, S., Marthens, E.V., and Margolis, F.L.: DNA (cell number) and protein in neonatal brain: Alteration by maternal dietary protein. Science 160 : 322—323, 1968.

7) Winick, M., and Noble, A.: cellular response in rats during malnutrition at various ages. J. Nutr. 89 : 300—306, 1966.

8) Zamenhof, S., Marthens, E.V., and Grauel, L.: DNA (cell number) and protein in neonatal rat brain: Alteration by timing of maternal dietary protein restriction. J. Nutr. 101 : 1265—1270, 1971.

9) Tyzbir, R.S., and Dain, J.A.: Effect of maternal protein deficiency on ganglioside metabolism in neonatal rat brain. Adv. Ext. Med. Biol. Neonat. 19 : 571—579, 1972.

10) Shoemaker, W.J., and Wurtman, R.J.: perinatal undernutrition: Accumulation of catecholamines in rat brain. Science 171 : 1017—1019, 1971.

11) Shoemaker, W.J., and Wurtman, R.J.: Effect of perinatal undernutrition on the metabolism of catecholamines in the rat brain. J. Nutr. 103 : 1537—1547, 1973.

12) Marichich, E.S., and Molina, V.A., and Orsingher, O.A.: Persistent changes in cental catecholaminergic system after recovery of perinatally undernourished rats. J. Nutr. 109 : 1045—1050, 1979.

13) Detering, N., Collines, R.M., Hawkins, R.L., Ozand, P.I., and Karanasan, A.: Comparative Effects of ethanol and malnutrition on the development of catecholamine neurons: Changes in neurotransmitter levels. J. Neurochem. 34 : 6 : 1587—1593, 1980.

14) Ahmad, G., and Ranman, M.A.: Effect of undernutrition and protein malnutrition on brain chemistry of rats. J. Nutr. 105 : 1090 : 1103, 1975.

15) Cowley, J.J. and Griesel, R.D.: The effect on growth and behavior of rehabilitating first and second generation low protein rats. Anim. Behav. 14 : 506—517, 1966.

16) Bernes, R.H., Moore, A.A., and Reid, S.M.: Learning behavior following nutritional deprivation in early life. J. Am. Diet. Assoc. 51 : 34—39, 1967.

17) Winick, M.: Nutrition and nerve cell growth. Fed. proc. 29 : 4 : 1510—1515, 1970.

18) Wurtman, R.J., and Fernstrom, J.D.: Effect of the diet on brain neurotransmitters. Nutr. Rev. 32 : 7 : 193—200, 1974.

19) Dobbing, J., and Widdowson, E.M.: The effect of undernutrition and subsequent rehabilitation on myelination of rat brain as measured by its composition. Brain 88 : 377—366, 1965.

20) Pike, R.L., and Brown, M.L.: Nutrition: An integrated approach. 2nd ed. pp729—7566, John

wiley & Sons Inc., 1975.

21) Culley,W.J., and Mertz, E.J.: Effect of restricted food intake on growth and composition of preweaning rat brain. Proc. Soc. Exp. Biol. Med. 118 : 233—235, 1965.

22) Guthrie, H.A., and Brown, M.L.: Effect of severe undernutrition in early life on growth, brain size and composition in adult rats. J. Nutr. 94 : 419—426, 1968.

23) Rajalakshmi, R., Ali, S.Z., and Ramakrisnnan, C.V.: Effect on inanition during the neonatral period on discrimination learning and brain biochemistry in albino rats. J. Neurochem. 14 : 29—34, 1967.

24) Benton, J.W., Moser, H.W., Dodge, P.R., and Carr, S.: Modification of the schedule of myelination in the rat by early nutritional deprivation. Pedratrics. 38 : 801—807, 1966.

25) Hatcher, D.W. and Goldstein: Improved methods for determination of RNA and DNA. Anal. Biochem. 31 : 42—50, 1969.

26) Bradford. M.M,: A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72 : 248—254, 1976.

27) Choi, K.W. Analytical. Chemistry, Bak-young Inc., Seoul. pp. 25, 1975.

28) Swiman, K.F. Daliiden, J.M. and Wolfe, R.W.: The effect of food deprivation on enzyme activity in developing brain. J. neurochem. 17 : 1387—1391, 1970.

29) Winick, M.: Nutrition and fetal development. John Wiley & Sons., pp. 25, 1974.

30) Adlard, B.P.F. and Dobbing, J.: Vulnerability of developing brain III. Development of four enzymes in the brains of normal and undernourished rats. Brain research, 28 : 97—107, 1971.

31) Gopinath, G., Bijlani, V. and Deo., M.G.: Undernutriction and the developing cerebellar cortex in the rat. J. Neuropath. and exp. neur. 35 : 2 : 125—135, 1976.

32) Santon, R.J. and Agranoff, B.W.: Studies on the estimation of deoxyribonucleic acid and ribonucleic acid in rat brain. Biochem. Biophys. Acta 72 : 25, 1963.

33) Winick, M.: Nutrition & fetal development. John Wiley & Sons. pp. 20, 1974.

34) Zeman, F.Z.: Effect of protein deficiency during gestation on postnatal cellular development in the young rat. J. Nutr. 100 : 530—538, 1970.