The Effect of Food-Deprivation on Lactic Dehydrogenase Activity in Immature Rat Brain[1] (34755)

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In immature animals deprived of food, the brain weighs less than normal and assumes a higher percentage of body weight (1, 2). The total DNA concentration and total lipids, including cholesterol and phospholipids, are also decreased (1, 2). The decrease in cerebrosides is proportionately more than in other lipid fractions.

DNA concentration is uniform in the diploid nuclei of mammalian species, including the rat (3). Thus DNA concentration per organ is proportional to the number of nuclei. Determinations of organ weight, protein concentrations, and enzyme activity, together with the knowledge of the change in DNA concentration, can be used to monitor cellular changes (4). Increased DNA concentration represents growth by cell division. Increasing weight to DNA ratios represent growth by increased cell weight and probably size. Studies have shown that the rate of DNA accumulation in rat brain decreases on about the fourteenth day of life, with no further accumulation after the sixteenth day (5). However, Winick and Noble reported some later DNA accumulation (1, 4).

In the present study, total brain weight, DNA concentration, protein concentration, total lactic dehydrogenase activity, and activity of the five LDH isozymes were studied in food-deprived immature rats.

Methods and Materials. Newborn albino rats were divided into two unequal litters: 20 in the experimental group and 10 in the control group. Food deprivation was induced by the Widdowson–McCance method (6). Each group was placed with one dam. The dams were allowed an unrestricted balanced diet and nursed the litters until the end of the experiment. The diet of the deprived group was therefore balanced, but reduced in quantity. The animals were killed at 7, 14, and 21 days. The brain was studied in 10 deprived rats whose body weights were within one standard deviation of the group mean and in all control animals. A normal control group was studied at 120 days of age for brain LDH isozyme activity. A total of 100 rats were employed.

After decapitation, the brains were immediately removed in a room kept at 0–4°. Only the hemispheres were used. They were weighed, placed in a Teflon–glass homogenizer containing a 0.9% solution of sodium chloride, and homogenized for 2 min to yield a 20% (w/v) preparation. Accurately measured quantities were put aside for nucleic acid, enzyme, and protein studies. The material for enzyme studies was centrifuged immediately at 5000*g* for 10 min, and the supernatant was saved for determinations. All reaction rates were studied under optimal conditions (those at which the reaction rates are linear with time, and optimal for obtaining maximal rates with respect to substrate and enzyme concentrations).

The method of Wroblewski and LaDue (7) was used to determine total LDH activity. LDH isozyme activity was determined by the method of Dietz and Lubrano (8) which employs separation on polyacrylamide gel. Quantitation of the resultant stained bands was accomplished by use of a Canalco Model 12 densitometer. Protein analysis was carried out by the method of Lowry and associates (9). DNA concentration was determined

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TABLE I. Effect of Food-Deprivation on Brain and Body Weight and Brain DNA Concentration.

	Brain wt (g)	Body wt (g)	DNA/brain (mg)
7-day rats[a]			
Control	0.90 ± 0.01	18.4 ± 0.4	1.43 ± 0.04
Food-deprived	0.48 ± 0.02	7.1 ± 0.2	0.93 ± 0.04
14-day rats			
Control	1.29 ± 0.01	27.9 ± 0.4	2.56 ± 0.07
Food-deprived	0.96 ± 0.04	14.6 ± 1.1	1.82 ± 0.03
21-day rats			
Control	1.36 ± 0.02	35.7 ± 1.2	2.39 ± 0.04
Food-deprived	1.13 ± 0.02	19.0 ± 0.9	2.15 ± 0.03

[a] Each value represents mean and standard error of 10 rat brains.

by the method of Schneider (10), using a modification of the diphenylamine reaction (11). A commericial, highly polymerized calf thymus DNA[2] was used.

Results. In all the food-deprived rats, the brain weight was not as adversely affected as the body weight, but it was lower than that of the controls, as was the DNA concentration (Table I). At each age studied the protein concentration per brain was lower in the deprived groups.

The total brain LDH activity increased with maturation in both the control and experimental animals. LDH activity was less in the deprived animals. The LDH/DNA ratio also increased during maturation, but was not significantly different in the two groups at any of the ages studied.

The LDH isozymes in the control and food-deprived groups did not significantly differ from each other (Table II). There was an increase of the activity of LDH-1 with age. The study of LDH-1 isozyme activity in brain of 120-day-old rats is slightly increased over the 21-day-old animals, but is significantly increased over the 7-day-old animals. There is a slight decrease in LDH-4 isozyme activity from 7 to 120 days. Not much change is evident between the 7- and 21-day-old animals. However, there is a pro-

TABLE II. LDH Isozymes in Developing Rat Brain[a] (percentage of total LDH activity).

	Isozyme				
Age	1	2	3	4	5
7 days					
Control	12.5 ± 0.5	20.0 ± 0.6	18.1 ± 0.3	30.0 ± 0.5	19.6 ± 0.6
Food-deprived	13.1 ± 0.6	22.3 ± 0.4	18.1 ± 0.3	28.8 ± 0.3	17.7 ± 0.8
14 days					
Control	15.4 ± 0.4	19.4 ± 0.4	20.1 ± 0.9	31.1 ± 0.7	13.5 ± 1.1
Food-deprived	16.1 ± 0.4	21.1 ± 0.8	19.7 ± 0.8	31.7 ± 0.8	11.5 ± 1.3
21 days					
Control	18.2 ± 0.3	22.9 ± 0.6	21.6 ± 0.5	27.9 ± 1.0	9.4 ± 0.7
Food-deprived	16.9 ± 0.3	22.0 ± 0.5	21.6 ± 0.6	30.8 ± 0.7	8.7 ± 0.6
120 days					
Normal	20.2 ± 0.4	25.8 ± 0.5	21.2 ± 0.3	25.8 ± 0.3	7.0 ± 0.5

[a] Each entry represents mean of 10 animals ± SE.

[2] Mann Research.

nounced decrease in LDH-5 isozyme activity with maturation which begins early. Slight increases with age in isozymes 2 and 3 activity are noted.

Discussion. Increase in total LDH activity of brain with maturation has been reported by Kuhlman and Lowry (12). The total brain LDH activity increased with maturation in both the control and experimental groups. The increase in the enzyme/DNA ratios during maturation in both groups signifies increased activity per cell. Thus the increased total brain activity is due to increased activity per cell as well as increased numbers of cells.

There was a decrease in total LDH activity in the brains of the food-deprived group when compared to the control group. There was no significant change in the LDH/DNA ratio at any age studied between the groups, indicating a normal complement of LDH activity per cell. The decrease in total LDH activity between the two groups appears to be the result of a lower cell population as reflected in the DNA concentration values.

Bonavita and co-workers (13) noted that LDH isozyme activity in developing rat brain varied with the isozyme under study. In the present study LDH-1 isozyme activity increased markedly with maturation, and there were decreases in LDH-4 isozyme activity and LDH-5 isozyme activity with age. These findings parallel earlier studies except that LDH-4 isozyme activity did not fall to the same degree as reported by Bonavita and co-workers (13).

The lack of significant differences between the isozyme activities in the control and food-deprived animals indicates a normal qualitative maturational pattern of the LDH isozymes despite a decrease in total LDH activity in the food-deprived rat brain.

Summary. DNA concentration, weight, protein content, and LDH activity were studied in control and food-deprived rats at 7, 14, and 21 days. As in other studies, the DNA concentration, brain weight, and protein were decreased in the deprived animals.

Total brain activity of LDH was less in the food-deprived animals. The LDH activity per cell increased with maturation and was essentially the same in both groups. Increasing total brain activity of LDH during maturation is due to both increased activity per cell and increased numbers of cells.

Food deprivation in immature animals results in lowered total brain activity of LDH, primarily because of diminished cell multiplication.

The LDH isozyme pattern of development was not affected by food-deprivation despite decreased total LDH activity.

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