EXPERIMENTAL NUTRITION

THYMIC FUNCTION IN MALNUTRITION

Undernutrition of young rats causes thymic atrophy due to loss of small lymphocytes, but rehabilitation is followed by prompt recovery which is related more to the weight than the age of the animal. Corticosterone is bound more avidly by the thymus and spleen of undernourished animals than controls.

Key Words: malnutrition, newborn rats, thymus, spleen, cellular growth, corticosterone

Developments in the response of immune defense mechanisms to malnutrition have been reported in this journal in recent months.[1,2,3] It is apparent that malnutrition depressed both the cellular and humoral responses of human infants and thereby alters the clinical picture of infectious disease and its prognosis.[4] Sometimes this may appear to be to the benefit of the individual suffering, for example, from infectious hepatitis in an endemic environment,[3] but usually the change is part of the inimical spectrum that is malnutrition. Two recent studies have furthered our appreciation of this topic by examining in some detail the thymic response of the rat to malnutrition and the part played in this by glucocorticoids.

P. A. McAnulty and J. W. T. Dickerson[5] studied the chemical and histological development of the thymus following undernutrition of 24-day-old rats. Male pups raised in litters of constant size were weaned and placed on a balanced diet at the age of 21 days. At 24 days the amount of food intake of the experimental animals was reduced so that they had no significant weight gain over the next 28 days. At the end of this time some were sacrificed while others were rehabilitated on the same diet and sacrificed after three, seven, ten, and 16 days. Two types of control were used: "weight" controls which were normal animals of the same weight as the experimental group, and "age" controls which were normal animals of the same age as the experimental group during the period of rehabilitation. Ten animals in each group were used for analyses of thymic DNA, RNA, and protein content, and two were used for histological examination.

The control animals gained 147 g body weight between 24 and 52 days, whereas the experimental animals gained only 1.8 g. In 16 days of rehabilitation the undernourished rats put on 110 g whereas the weight of the control group rose by 83 g. During the period of undernutrition the weight of the thymus in the undernourished group fell to 19 percent of that at 24 days, whereas the thymus of the control pups grew steadily, more than doubling in weight by the age of 55 days but not changing thereafter. Rehabilitation caused rapid weight gain of the experimental thymus so that after 16 days the experimental and control groups were almost identical. The thymic/body weight ratio fell in both control and experimental groups but faster in the latter. After rehabilitation the ratio became normal for age in seven days and at 16 days was significantly greater than that of the age-control animals. When the recovering experimental animals were compared with weight controls they were found to regain a normal thymic/body weight ratio by ten days and to maintain this thereafter.

The increase in thymic weight of the control animals was accompanied by an increase in the total DNA content of the gland, whereas the thymic DNA content of the experimental animals fell. The proportionate decrease in DNA was greater than that in thymic weight, however, and was therefore associated with a rise in thymic weight or protein/DNA ratio. After refeeding, the thymic DNA of the experimental group increased dramatically, rising more than tenfold in 16 days to regain a normal value for the age of the animal and to become supranormal in comparison to a weight control. RNA concentrations behaved similarly to

DNA. The protein/DNA ratio of normal animals remained constant throughout the period of study, whereas that of the experimental group rose sevenfold by the age of 52 days, falling thereafter as rehabilitation proceeded to resume a normal value at seven days.

Histological examination of experimental thymus at 52 days revealed smaller lobules, loss of demarcation between the cortex and medulla, fewer lymphocytes, and thereby a preponderance of large cells in the gland.

The thymus is an interesting organ for the study of cell size and number by chemical techniques because it is made up of populations of cells of different sizes, some of which, the lymphocytes, are capable of being discharged from or accumulated by the gland. The normal thymus of an infant or young animal contains many small lymphocytes and a smaller proportion of large cells: lymphoblasts and epithelial cells.[6] Undernutrition of weanling rats in this study caused a large fall in thymic weight, a greater fall in DNA content and a considerable rise in protein/DNA ratio. Viewed simplistically, these findings might be interpreted as indicating loss of cells and a change in organ composition so that the cells remaining at 52 days were considerably larger than those at 24 days. In some respects this was so; undernutrition depleted the gland of small lymphocytes, and preferential loss of these cells is adequate to explain the type of change observed.

What is not so easy to understand is the degree of change in protein/DNA ratio. The authors do not say in what units they measured this parameter, so the reader cannot compare their estimates of cell size with other figures in the literature. The increase in this ratio in the experimental group was sevenfold and implies a mean change in cell diameter by approximately a factor of two. The heavy preponderance of small lymphocytes in the normal gland and the dramatic histological change to a gland composed of large cells after malnutrition is compatible with this chemical finding.

In experiments of similar general design, B. P. F. Adlard, J. Hamid, R. Labedz, and H. McFarlane[7] have examined the immune responses of perinatally undernourished rats and their handling of glucocorticoids. Plasma steroid levels are known to be raised in malnutrition[8,9] and this is probably responsible for much of the lymphatic atrophy which occurs and of which thymic shrinkage is an important part.[10]

The mothers of the experimental animals were fed approximately 50 percent of a normal food intake during pregnancy and lactation. The pups were studied when aged 14 days. At this time their mean body weight was 12.1 g compared with 29.5 g in control animals. Biochemical analyses showed the experimental animals to have lower blood glucose, hepatic carbohydrate, and serum albumin levels than controls, while no significant difference between the groups was noted for serum ceruloplasmin, total lipid, or in liver fructose 1, 6-diphosphatase or glucose 6-phosphatase activities. As in the first study reviewed here, thymic growth retardation was greater than that of the body and the deficit in splenic weight was greater still.

The immunologic competence of the thymus and spleen was tested by giving an intraperitoneal injection of sheep erythrocytes at the age of seven days. At different time intervals thereafter animals were sacrificed, the spleen and thymus removed, washed carefully to remove traces of blood, and then the total number of hemolytic-plaque forming cells in each organ was measured.[11] Plaque formation in both spleen and thymus was maximal in control animals six days after the injection of antigen, whereas the undernourished animals had their peak response two days later. The maximum response of the experimental group was less than that of the controls but was appropriate to the body weight of the animal. This description of the immune responses describes the trends observed but the differences in the peak response or the time to achieve peak response do not appear to be significant because of variation between animals.

Animals aged 15 to 17 days were injected intramuscularly with ^3H corticosterone in a dose proportionate to body weight and sacrificed at intervals up to two hours. Brain, spleen, and thymus were solubilized and the radioactivity in them was determined by liquid scintillation counting. Radioactivity of the three organs was maximum ten minutes after injection in all except the thymus of the undernourished group, which peaked at 20 minutes. Specific radioactivity in the brain was much lower than that in either of the two lymphatic organs and did not differ between control and experimental animals. In contrast both the thymus and the spleen of the undernourished rats took up more radioactivity than those of control animals. The differences were significant at 20 and 30 minutes after injection and possibly also at other times, though the authors make no specific statement on this point.

The work of Adlard et al. is more interesting for the ideas it produces than for the points proven. No analysis of the nature of the specific radioactivity was made, so it may not be accurate to assume that counts in an organ represent corticosterone. In both the measurements of plaque counts and of specific radioactivity, differences between experimental and control groups were less impressive than the trend of changes. Taking trends at face value, the reader may conclude that undernutrition produces a slower but quantitatively normal hemolytic antibody response and that more corticosterone is bound by the spleen and thymus of the undernourished animal. If steroid binding is a property of the "large" cells of the organ rather than the small lymphocytes, the latter finding is quite compatible with the observations of McAnulty and Dickerson. The two papers illustrate how complex is the effect of undernutrition on the immune competence of an animal and are useful indicators as to what may be occurring in malnourished children.

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