Uptake of (³H)Corticosterone and the Immune Response in Growth-retarded Rats

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Extract

Maternal rats were underfed during pregnancy and lactation. At 7 days of age their offspring received one injection of sheep erythrocytes. The immune response (hemolytic plaque-forming cells in spleen and thymus) to this antigen showed a peak at 15 days of age in undernourished rats, 2 days later than in normal animals. However, the size of this response at its peak was, in the undernourished group, at least appropriate for body weight. Growth retardation during the fetal and suckling periods resulted also in an increased rate of uptake of ³H-corticosterone into spleen and thymus *in vivo*.

Speculation

High rates of corticosteroid entry into spleen and thymus in undernutrition may tend to depress the immune functions of these organs independently of direct nutritional factors. During growth retardation the rate of certain immune responses may be impaired at least as much as their extent.

Introduction

Children who suffer from malnutrition appear to have defects in both humoral and cell-mediated immunity [19]. This may partly reflect impaired growth of thymus and spleen [7], two organs whose weights are known to be drastically reduced by both intrauterine and postnatal growth retardation in man [4, 14]. In addition, malnourished children have elevated levels of plasma cortisol [2, 15], which may be partly responsible for the impairment of thymolymphatic function, independently of direct nutritional effects [7, 8, 17].

This report is concerned with (1) hemolytic antibody response in normal and undernourished 7-day-old rats and (2) the effects of growth retardation on the rate of entry *in vivo* of labeled corticosteroid into different organs.

Methods

Animals

Rats of a black-and-white hooded strain were used. Maternal females had successfully reared at least one litter. The day of mating was determined by examination of vaginal smears for sperm. Litters were reduced to eight animals (four of each sex) at birth. All animals were killed by decapitation at 10.00-12.00 hr (the daily light cycle was 12 hr red, starting at 12.00 hr, and 12 hr white).

Diet

All animals received water *ad libitum* throughout. Control mothers had unlimited access to a good quality breeding diet, the composition of which has previously been given [18]. Undernourished mothers re-

Table 1. Biochemical results for 14-day-old control and undernourished rats¹

Biochemical variable	Control	Undernourished	P
Body weight, g	29.5 ± 4.1 (19)	12.1 ± 2.2 (23)	<0.001
Glucose in blood, mg/100 ml	117 ± 13 (12)	88 ± 14 (8)	<0.001
Serum albumin, g/100 ml	3.38 ± 0.33 (16)	2.75 ± 0.18 (8)	<0.001
Serum ceruloplasmin, $\Delta E_{530}/0.03$ ml/hr	0.166 ± 0.041 (16)	0.162 ± 0.048 (11)	n.s.
Serum total lipid, mg/100 ml	622 ± 77 (16)	695 ± 267 (10)	n.s.
Carbohydrate in liver, mg/g wet wt	34.8 ± 3.6 (6)	12.7 ± 5.4 (8)	<0.001
Fructose 1,6-diphosphatase activity, in liver, μ moles P_i /g wet wt/hr	342 ± 99 (6)	364 ± 19 (6)	n.s.
Glucose 6-phosphatase activity, in liver, μ moles P_i /g wet wt/hr	1,267 ± 167 (6)	1,576 ± 379 (8)	n.s.

¹ Each value is given as the mean $\pm$ SD of the number of rats indicated in parentheses. For each determination animals from at least two control and two undernourished litters were used. n.s.: not significant.

ceived the same diet but in a restricted quantity which represented approximately 50% of normal food intake during pregnancy and lactation. Thus, 10 g food/24 hr was given from mating to parturition (except for mothers of animals used for biochemical investigation (Table I) when undernutrition was begun on the 7th day after mating). All undernourished rats then received 15 g/24 hr during the 1st week of lactation, 20 g/24 hr during the 2nd week, and 25 g/24 hr thereafter.

Assessment of Nutritional Status

Serum albumin was determined using the biuret method after precipitation of globulins with ether [21]; bovine serum albumin was used as standard. Serum ceruloplasmin was determined using the copper oxidase method [16]. Blood glucose (glucose oxidase method) and total serum lipids were determined using test kits [25].

Liver carbohydrate was determined according to the method of Kemp and Van Heijningen [6].

For measurement of liver enzyme activities, homogenates (10%, w/v) in ice-cold 0.14 M KCl were prepared. Glucose 6-phosphatase (EC. 3.1.3.9) and fructose 1,6-diphosphatase (EC. 3.1.3.11) were determined on whole homogenates by the methods of Swanson [20] and McGilvery [12], respectively.

Immune Response to Sheep Erythrocytes

At 7 days of age rats received injections (i.p.) of sheep erythrocytes [26] (0.2 ml of a 40% (w/v) suspension in saline). Animals were subsequently killed at a specified time interval and the spleen and thymus were removed into sterile saline and washed three times to remove traces of blood. The total number of hemolytic

plaque-forming cells per organ was determined according to the method of Jerne and Nordin [5].

Uptake in Vivo of (³H)Corticosterone into Different Organs

Animals aged 15–17 days were used. Rats received injections (i.m.) of corticosterone-1 β , 2 β -³H [27] (30 μ Ci/g body wt) and were killed after different intervals (10, 20, 30, 60, 120 min). Brain, spleen, and thymus were solubilized in Hyamine hydroxide (10% (w/v) in methanol) and radioactivity was determined by liquid scintillation counting. Organs from animals killed without injection of labeled steroid gave negligible counts.

Results

Biochemical Studies

Rats subjected to nutritional growth retardation during the fetal and suckling periods to 14 days of age showed few severe biochemical abnormalities despite the large reduction in body weight (Table I). Total liver carbohydrate concentration was, however, reduced to 37% of normal in undernourished animals who nevertheless maintained a 75% of normal level of glucose in blood.

Serum albumin concentration was reduced by 19% in undernourished animals. There were no significant changes in serum ceruloplasmin, serum total lipid, or in liver fructose 1,6-diphosphatase or glucose 6-phosphatase activities.

Immunologic Studies

The body weights for animals used for measurements of the immunologic response to sheep erythrocytes are given in Fig. 1. The body weight deficit (49–

Fig. 1. Body weights of rats used for determination of the immune response to sheep erythrocytes. Each point represents the mean ($\pm$ SEM) of four or five animals.

Fig. 2. Plaque-forming cells in spleen after injection of sheep erythrocytes at 7 days of age. Each point represents the mean ($\pm$ SEM) of four or five animals.

63%) in growth-retarded animals varied little with age. The deficit in thymus weight (52–71%) was at all ages somewhat greater than that of body weight while the deficit in spleen weight (64–78%) was even more marked.

Control animals showed in both spleen (Fig. 2) and thymus (Fig. 3) a peak immunologic response at 13 days of age, 6 days after injection of antigen. This is comparable with the rate of response in adult or weanling rats [9]. Growth-retarded animals showed their peak response 2 days after that of controls and this delay was apparent in both organs.

Despite the altered timing of the immunologic response in undernourished animals, the size of the response at its peak was probably at least appropriate for

body weight in this group. Thus, at the peak of the response the total number of plaque-forming cells in undernourished animals (15 days of age) was reduced by only 39% (spleen) and 33% (thymus) compared with a greater deficit in body weight (Fig. 1).

Steroid Uptake

The body weight deficit of animals in the undernourished group used for determination of steroid uptake was 59%. The corresponding organ weight deficits were brain 24%, spleen 71%, thymus 76%.

With (^3H)corticosterone injected in a dose proportional to body weight, the specific radioactivity in both spleen and thymus was higher in undernourished animals than in controls (Figs. 4 and 5) at most points 10–120 min after injection. A similar effect was not clearly shown in brain, although steroid uptake into

Fig. 3. Plaque-forming cells in thymus after injection of sheep erythrocytes at 7 days of age. Each point represents the mean ($\pm$ SEM) of four or five animals.

Fig. 4. Uptake of (^3H)corticosterone *in vivo* into thymus and brain. Each point represents the mean ($\pm$ SEM) of four animals.

Fig. 5. Uptake of (^3H)corticosterone *in vivo* into spleen. Each point represents the mean ($\pm$ SEM) of four animals.

brains of undernourished animals 10 min after injection was significantly ($P < 0.05$) greater than in controls. Brain showed much lower specific radioactivities than did spleen or thymus.

Discussion

Serum albumin, but not serum ceruloplasmin, was somewhat reduced by undernutrition (Table I). The reduction was, however, small compared with that seen in protein-calorie-malnourished children [10, 13, 23, 24], who also show a low ceruloplasmin [11]. This suggests a milder degree of protein deficiency in animals undernourished by the present method.

The activities of two key enzymes of liver gluconeogenesis were not affected by undernutrition. It therefore appears that the capacity for a high rate of liver gluconeogenesis characteristic of the suckling rate [22] was not impaired in growth-retarded animals although it is possible that the *in vivo* availability of gluconeogenic amino acids could be limited.

Rates of uptake of labeled corticosterone from blood could clearly be influenced by differences in endogenous steroid levels, thus giving different degrees of dilution in plasma. However, since growth-retarded rats, at least at 22 days of age, show higher than normal plasma corticosterone concentrations [1], such an artifact would lead to decreased organ specific radioactivities and cannot explain the increased radioactivities observed here (Figs. 4 and 5). It is therefore suggested that the findings represent an increased rate of uptake

of corticosterone into spleen and thymus in growth retardation.

In undernourished children the immune response seems to be severely depressed only in those with protein deficiency [19], although it should be borne in mind that certain vitamin deficiencies may also impair immunity [3]. Since the growth-retarded rats used in the present work were probably not grossly protein deficient, it is therefore perhaps not surprising that the immune response examined appeared to be quantitatively adequate. It may be that the major defect in hemolytic antibody response in growth retardation without specific protein deficiency involves a reduced rate of response (Figs. 2 and 3) and it would be worth examining this question in detail.

It is well known that glucocorticoids cause atrophy of lymphoid tissue, particularly the thymus. Undernutrition causes an elevation in plasma corticosteroids in both suckling rats [1] and children [2, 15], and such elevations are known to involve, in addition, an increase in the proportion of steroid which is not bound to plasma protein [17]. This implies an increase in biologically active steroid which is proportionately greater than the increase in total plasma steroid. The mechanism of these changes is not clear but may involve a reduction in corticosteroid-binding globulin and an impairment in hepatic steroid degradation rather than increased adrenocortical activity [2].

In undernutrition a marked increase in biologically active plasma glucocorticoid may cause a depression of thymus function in addition to purely nutritional factors. Once an impairment of thymus growth is established an increased rate of steroid entry into this organ (Fig. 4) would enhance the thymolytic effects of circulating corticosteroids. Such a cycle of events may explain why growth of the thymolymphatic system is particularly sensitive to nutritional deprivation.

Summary

Rats undernourished during the fetal and suckling periods showed, at 2 weeks of postnatal age, significant reductions in glucose levels in blood, in serum albumin, and in total concentrations of carbohydrate in liver. These changes were associated with a higher than normal rate of uptake of ^3H -corticosterone into spleen and thymus *in vivo*. Growth restriction decreased the rate of immune response to sheep erythrocytes (hemolytic plaque-forming cells in spleen and thymus) when assessed during the period of 1–3 weeks of age.

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