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Cellular Antibody Synthesis in Thiamin, Riboflavin, Biotin and Folic Acid-Deficient Rats[1] (40068)

MAHENDRA KUMAR[2] AND A. E. AXELROD

Biochemistry Department, School of Medicine, University of Pittsburgh, Pittsburgh, Pennsylvania 15261

Decreased serum antibody titers in response to a variety of antigenic stimuli have been noted in a number of vitamin deficiency states (1). In view of the complexity of factors involved in the establishment of serum antibody levels (2, 3), it is apparent that a more direct approach is required for elucidation of the basic role of these vitamins in mediating these immune responses. Investigation of antibody synthesis at the cellular level affords such an approach. Accordingly, we undertook a series of studies on the effects of individual deficiencies upon the number of antibody-forming cells in the spleens of immunized rats. Our studies, to date, have demonstrated that the decreased serum antibody responses observed in vitamin B_6 and pantothenic acid-deficient rats can be attributed to the marked reduction in the number of individual antibody-forming cells in the spleens of these immunized deficient animals (4, 5).

Deficiencies of thiamin, riboflavin, biotin and folic acid affect serum antibody responses in the rat to varying degrees (6–10). In this paper, we report the effects of these specific deficiency states upon the development of splenic antibody-forming cells in immunized rats.

Materials and methods. Animals and diets. Male, weanling, 21-day-old rats of the Holtzman strain were used. The animals were housed individually in wide-meshed, screen-bottom, suspended cages and weighed weekly. Rats in the deficient groups were fed *ad libitum* the basal, semipurified diets previously utilized for production of thiamin, riboflavin, biotin and folic acid deficiencies (9). Control animals received the basal diet fed to the thiamin and riboflavin-deficient groups. In addition, each rat received daily a vitamin pill which, for control animals, supplied adequate quantities of the B-vitamins (9). For each deficient group, the corresponding vitamin was omitted from the pill. Rats in the riboflavin and biotin-deficient groups were fed the basal diets for 8–12 weeks prior to immunization. Corresponding periods for the folic acid and thiamin-deficient groups were 12–14 and 2–3 weeks, respectively.

Antigen. Sheep erythrocytes served as antigen in this study. Red blood cells were obtained commercially[3] in Alsever solution, washed and counted as described previously (4). Rats were immunized intravenously via the lateral tail vein by a single dose of 4×10^8 erythrocytes (4).

Measurement of antibody-forming cells (AFC) of spleens. The number of splenic cells capable of synthesizing hemolytic antibodies was determined by the method of Jerne *et al.* (11) with the modifications previously described (4). Briefly, this method involves removal of spleens from immunized rats and incubation of a suspension of splenic cells with sheep erythrocytes in agar. Addition of fresh complement subsequently causes lysis of the sensitized erythrocytes in the immediate vicinity of individual AFC. The clear plaques surrounding each AFC are counted.

Results. The effects of the various deficiency states on the number of splenic AFC in immunized rats are summarized in Table I. Since the number of AFC reaches a maximum value on the fourth day after immunization with sheep erythrocytes (4), AFC were determined only 4 days after immunization in the present study. It is apparent that the number of splenic AFC was reduced in all of

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[2] Present address: Montefiore Hospital and Medical Center, Albert Einstein College of Medicine, New York, N. Y.

[3] Grand Island Biological Company, Grand Island, New York.

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TABLE I. ANTIBODY-FORMING CELLS (AFC) IN THE SPLEENS OF THIAMIN, RIBOFLAVIN, BIOTIN AND FOLIC ACID-DEFICIENT IMMUNIZED RATS.[a]

Group	No. of rats	AFC/10^6 Splenic cells		Body weight (g)[b]	
		Average	Range	Initial	Final
Control	8	160	146–200	56 ± 1	242 ± 9
Thiamin-deficient	7	59	39–81	64 ± 5	76 ± 6
Riboflavin-deficient	6	16	5–42	57 ± 6	105 ± 23
Biotin-deficient	6	4	1–6	60 ± 5	130 ± 18
Biotin-deficient, treated[c]	5	54	49–63	60 ± 5	166 ± 16
Folic acid-deficient	8	10	2–54	62 ± 8	177 ± 32
Folic acid-deficient, treated[d]	5	34	6–62	62 ± 8	185 ± 38

[a] Sheep erythrocytes, 4×10^8 per rat, were administered intravenously 4 days before removal of the spleens.
[b] Body weights are expressed as average ± SE. Final body weight was recorded at the time of sacrifice.
[c] Each rat received a daily intraperitoneal injection of 10 μg of biotin beginning 3 days before immunization and continued until the end of the experiment.
[d] Each rat received a daily intraperitoneal injection of 50 μg of folic acid beginning 3 days before immunization and continued until the end of the experiment.

the deficiency states under study, with the least effect in thiamin deficiency. Daily administration of biotin or folic acid for 7 days beginning 3 days prior to immunization partially restored the ability of the corresponding deficient animals to produce AFC.

Discussion. The present study has demonstrated the varying effects of thiamin, riboflavin, biotin and folic acid deficiencies upon AFC formation. We have previously reported that the production of circulating antibodies following the administration of diphtheria toxoid was also reduced to variable degrees in these deficiency states (9). Of particular note is the observation that thiamin deficiency which had the least effect upon formation of AFC also had the least effect upon the production of circulating antibodies. Direct comparisons of the effects of these deficiencies upon circulating antibody and AFC formation are difficult since both the antigens and the mode of assessment of the immune responses differed in the two studies. Whereas the cellular response reported in the present paper is associated with IgM formation (12, 13), the serum antibody titers determined 3–4 weeks after immunization with diphtheria toxoid (9) reflects both IgM and IgG concentrations (14).

The adverse effects of the deficiency states of thiamin, riboflavin and biotin may not be attributed to the delayed formation of AFC after immunization since the number of splenic AFC in the deficient rats was less on day 5 than on day 4 after immunization. Thus, the number of antibody forming cells 5 days after immunization in thiamin, riboflavin, and biotin-deficient rats was 47, 8 and 1, respectively. Previous studies with inanition controls have demonstrated conclusively that inanition does not have a deleterious effect upon the formation of AFC (4, 5). A similar lack of effect of inanition *per se* upon immune responses has been noted frequently (1). The effects of the deficiency states upon the formation of AFC in the present study can, therefore, not be attributed to inanition.

In contrast to the failure to restore completely the capacity to fabricate AFC with short-term therapy with either biotin or folic acid, this decreased cellular immune response was restored to normal by similar administration of vitamin B_6 or pantothenic acid to deficient animals (4, 5).

The precise function of the B-complex vitamins in the development of AFC is not known although the adverse effects of vitamin B_6 deficiency have been linked to the role of pyridoxal phosphate in the formation from serine of one-carbon unit required for the synthesis of DNA and RNA involved in antibody formation (15, 16). The immune response is a complex process involving the discrete steps of antigen processing, differentiation of progenitor cells, replication of plasma cells, antibody synthesis and transport of newly-synthesized antibodies into the circulation. Much further study will be required to delineate those steps whose rates are controlled by the metabolic activities of the coenzymatic forms of these vitamins. Current investigations in this laboratory involve studies

of the effects of these deficiency states upon the quantitation of various cell types involved in antibody synthesis and their interactions.

Summary. The formation of splenic antibody-forming cells was determined by the Jerne agar-plaque technique in normal rats and in rats deficient in thiamin, riboflavin, biotin or folic acid immunized with sheep erythrocytes. The number of antibody-forming cells was reduced in all deficiency states with thiamin deficiency having the least effect. This decreased cellular immune response in biotin and folic acid deficiencies was partially restored to normal by administration of biotin or folic acid, respectively, shortly before immunization.

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