The Role of Nutritional Factors in the Antibody Responses of the Anamnestic Process

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Previous studies in this laboratory have demonstrated the significant role of the vitamins in the processes of antibody production. In these investigations, the effects of *specific*, individual vitamin deficiencies upon antibody production in the albino rat were determined. In early experiments with human erythrocytes as the antigen it was shown that antibody synthesis was markedly impaired in pantothenic acid, pyridoxine, and pteroylglutamic acid deficiencies.[1] Lesser degrees of inhibition were noted in other vitamin deficiency states; in vitamin D and vitamin B_{12} deficiencies no effects were apparent. It was also demonstrated repeatedly that simple inanition failed to modify the antibody response.

These experiments were subsequently extended to include purified, alum-precipitated, diphtheria toxoid as the antigen.[2] Again, the marked impairment of antibody response in various vitamin deficiency states was evident. With the exception of the deleterious action of the vitamin D deficiency, the trends observed with diphtheria toxoid were similar to those previously noted when red blood cells served as the antigen. Here too, simple inanition was without effect.

The experiments described in this report were designed to investigate the role of nutritional factors in the various phases of the anamnestic process.

METHODS AND MATERIALS

Male, weanling, albino rats of the Holzman strain were employed in all of the vitamin experiments. Rats weighing 90 g were utilized in the tryptophan study. Each animal was housed in an individual, wide-meshed cage. The individual vitamin deficiencies were produced by feeding the purified diets previously described.[2] The control rats in each instance received the basal, vitamin-deficient diet supplemented with the crystalline vitamin in question. For the production of a tryptophan deficiency, the casein of the control diet was replaced by acid-hydrolyzed casein and the diet supplemented with 0.6 per cent of DL-methionine. For the tryptophan controls this basal diet was further supplemented with 0.2 per cent of DL-tryptophan. All rats were fed *ad libitum* and were utilized in groups of 20 for each experimental procedure.

The intraperitoneal injection of 0.15 ml of purified, alum-precipitated, diphtheria toxoid (Lederle) served as the antigenic stimulus throughout these studies. Blood was obtained from nembutalized animals by cardiac puncture and serum antibody titers determined on the heat-inactivated sera by a sensitive, serial-dilution, hemagglutination technic which is dependent upon the agglutination of tannic acid-treated sheep erythrocytes coated with

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diphtheria toxoid.[3,4] The content of serum antibody is expressed as the reciprocal of the highest serum dilution capable of a positive hemagglutination reaction and, therefore, varies *directly* with the reciprocal titer.

EXPERIMENTAL PROCEDURE

A typical anamnestic response in normal rats to diphtheria toxoid is shown in Figure 1.

In all of the vitamin studies weanling rats were fed the experimental diets for four weeks prior to the primary injection of antigen. In the tryptophan experiment, the animals were maintained for two weeks on their respective diets prior to antigenic stimulation. The serum antibody titers determined three weeks after the primary injection constitutes the *primary response* and this three-week interval is designated as the *primary phase* of the anamnestic process. Lessening the time of the primary phase diminishes the magnitude of the secondary response. Immediately following the withdrawal of blood for the determination of the primary response, the animals were given a second or booster injection of antigen. The ensuing week is referred to as the *secondary phase* of the anamnestic process and the antibody titer at the end of this week is the *secondary response*. It is seen in Figure 1 that the booster injection produces a very sharp increase in antibody titer. The relatively low

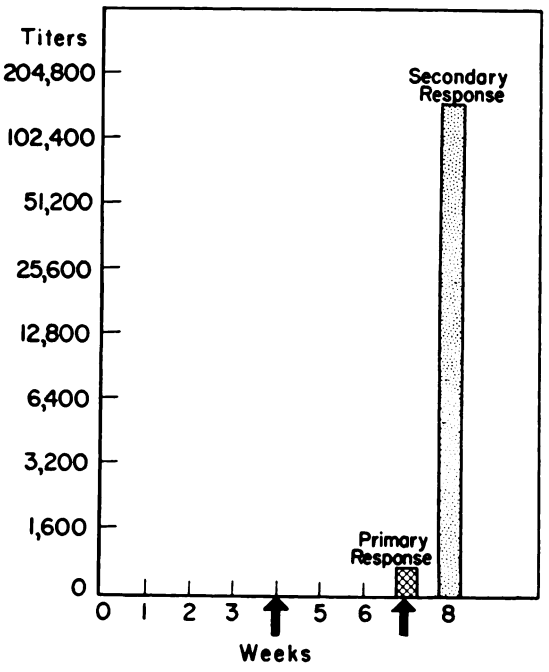

Fig. 1. The anamnestic response in control rats. Weanling rats were placed on experiment at zero weeks and the primary and secondary injections of diphtheria toxoid given at the times indicated by the arrows. Figures on the ordinate represent reciprocal titers.

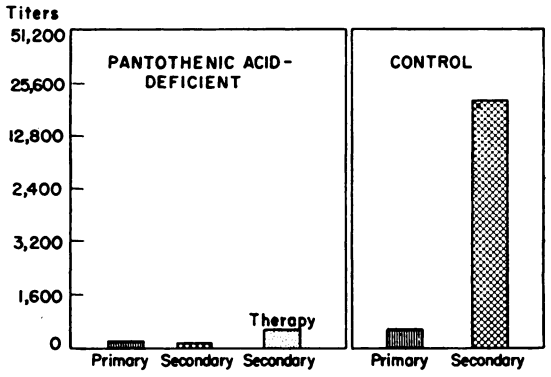

Fig. 2. Anamnestic responses to diphtheria toxoid in pantothenic acid-deficient and control rats. Figures on the ordinate represent reciprocal titers. The stippled bar represents antibody response in deficient rats which received the control diet and 1 mg of pantothenic acid daily by intraperitoneal injection only during the secondary phase.

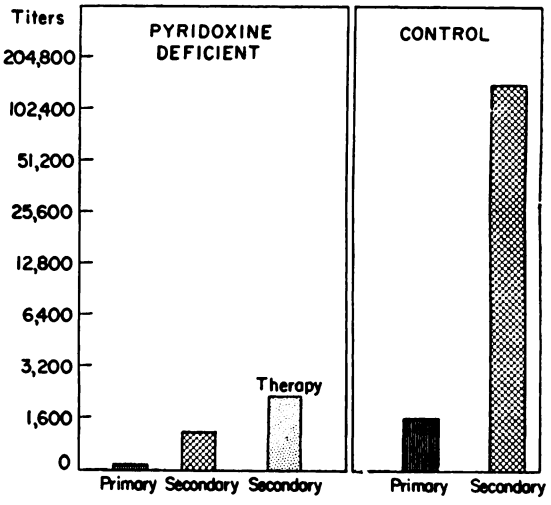

Fig. 3. Anamnestic responses to diphtheria toxoid in pyridoxine-deficient and control rats. Figures on the ordinate represent reciprocal titers. The stippled bar represents antibody response in deficient rats which received the control diet and 500 mcg of pyridoxine daily by intraperitoneal injection *only* during the secondary phase.

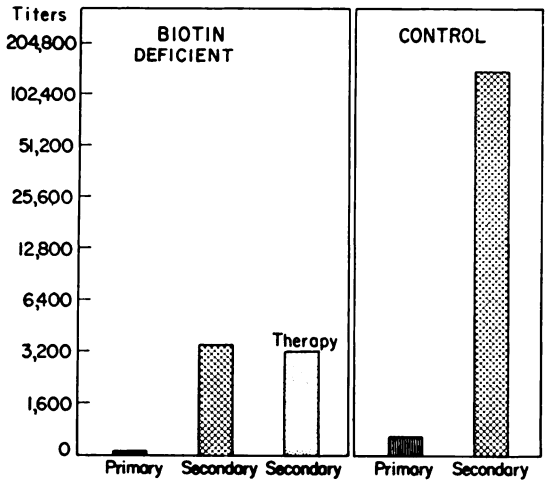

Fig. 4. Anamnestic responses to diphtheria toxoid in biotin-deficient and control rats. Figures on the ordinate represent reciprocal titers. The stippled bar represents antibody response in deficient rats which received the control diet and 10 γ of biotin daily by intraperitoneal injection *only* during the secondary phase.

primary response is maintained for several weeks in the absence of a secondary antigenic stimulus.

As will be illustrated later in this report, deficiencies of a number of nutritional factors during the *entire* experimental period inhibit both the primary and the secondary responses. The present experiments can be conveniently divided into two phases, each purporting to answer one of the following questions: (1) Can nutritional therapy *only* during the secondary phase elicit a normal secondary response in animals which have been nutritionally deficient during the period prior to the booster injection? and (2) Conversely, can a nutritional deficiency *only* during the secondary phase inhibit a secondary response in animals fed adequate diets prior to the booster injection?

Phase 1

In this phase of the investigations, deficiencies of pantothenic acid, pyridoxine, biotin, and tryptophan were induced and primary and secondary responses to diphtheria toxoid obtained as described above. Each experiment included a group of deficient animals which received intensive nutritional therapy during the one-week secondary phase as indicated in Figures 2, 3, 4, and 5. Typical signs of deficiency, in particular growth inhibition, were evident at the time of the secondary injection

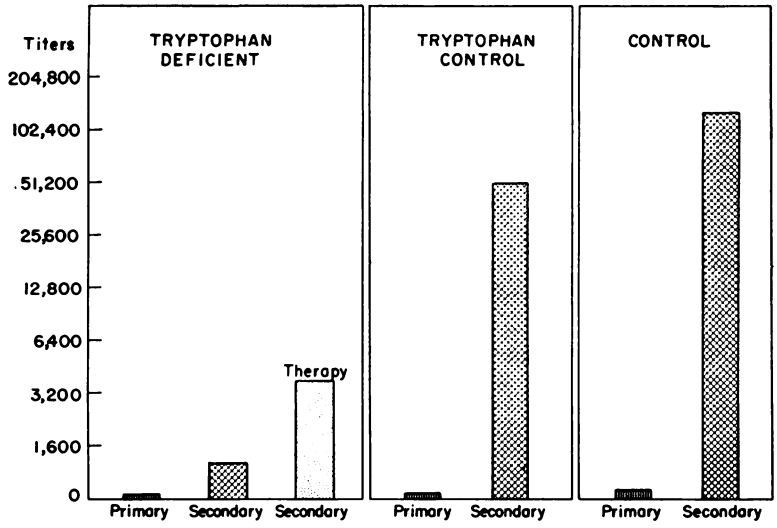

Fig. 5. Anamnestic responses to diphtheria toxoid in tryptophan-deficient, tryptophan-control, and control rats. Figures on the ordinate represent reciprocal titers. The stippled bar represents antibody response in deficient rats which received the tryptophan control diet *only* during the secondary phase.

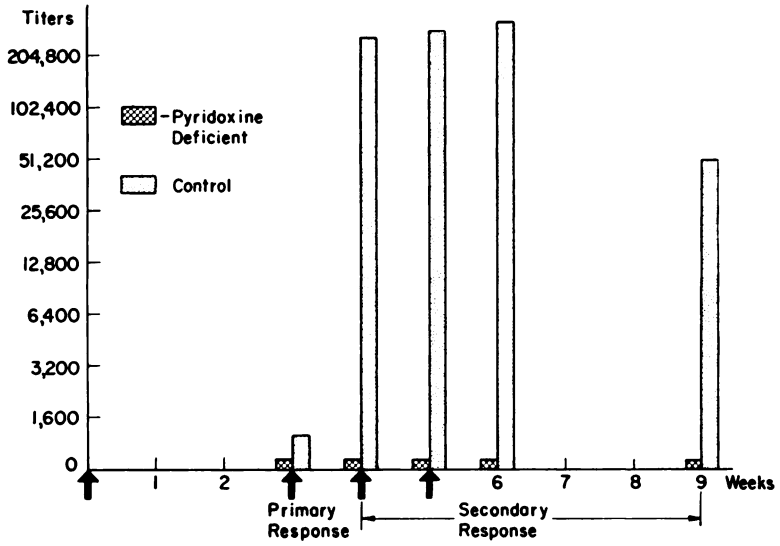

Fig. 6. Effect of repeated antigenic stimuli upon antibody response in pyridoxine-deficient and control rats. Injections of diphtheria toxoid given at times indicated by the arrows. Animals were fed the pyridoxine-deficient or the control diet during the entire course of the study, and were on experiment for four weeks prior to the first toxoid injection. Figures on the ordinate represent reciprocal titers.

and the usual immediate marked responses to the nutritional therapies were noted. Thus, weight gains of 30 to 40 g were observed during the week following therapy. In the tryptophan study an additional control group receiving the usual control diet (which contains unhydrolyzed casein as the source of protein) was utilized.

The results given in Figures 2, 3, 4, and 5 depict the inhibitory effects of the nutritional deficiency states upon the anamnestic process. The impairment of the secondary responses under these circumstances is most pronounced. Nutritional therapy during the secondary phase has very little, if any, effect upon the antibody response. In no case was an adequate anamnestic response seen in these supplemented animals despite their immediate and marked growth responses to nutritional therapy.

A subsequent experiment (Figure 6) demonstrated that repeated injections of diphtheria toxoid following the original booster injection failed to produce an antibody response in pyridoxine-deficient rats. Not shown in this figure are the observations that: (1) Pyridoxine therapy instituted concomitantly with the booster series failed to elicit an immediate anamnestic response but, after two weeks, did produce a mild increase in circulating antibodies comparable to the usual primary response. A true anamnestic response was seen in about 50 per cent of these animals one week later. (2) Pyridoxine-deficient rats not receiving any booster injections were unable to produce antibody despite pyridoxine therapy begun three weeks after the primary stimulus.

Phase 2

In these experiments, a study was made of the effect upon antibody production of an acute pyridoxine deficiency induced during the secondary phase of the anamnestic process with the aid of the pyridoxine antagonist, desoxypyridoxine. During the primary phase the animals received a control diet furnishing ten instead of the usual 50 μg of pyridoxine daily. This lower amount of pyridoxine permitted good growth (25 g per week) and antibody production and was utilized in order to facilitate the action of the desoxypyridoxine. Administration of the desoxypyridoxine to rats fed a "pyridoxine-free" diet produced an

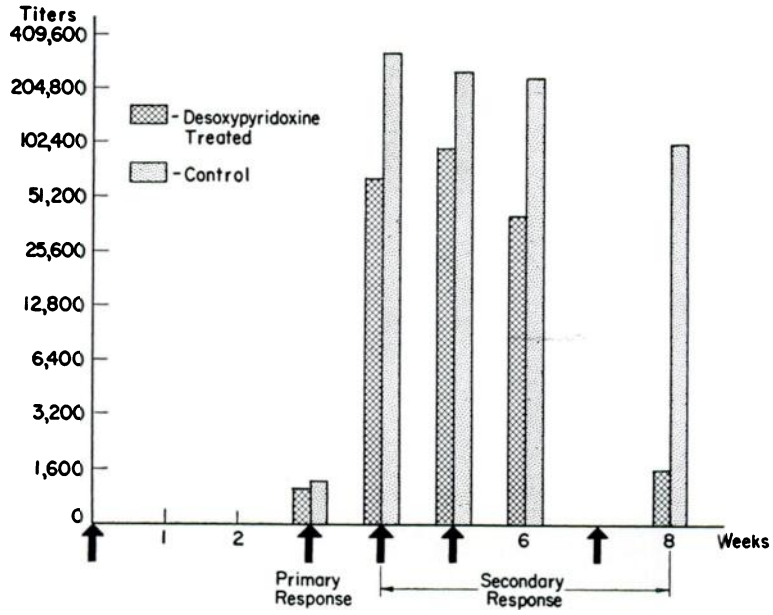

Fig. 7. Effect of desoxypyridoxine-treatment during the secondary phase upon antibody response. Injections of diphtheria toxoid were given at the times indicated by the arrows. *All* animals were fed a control diet furnishing 10 μg of pyridoxine daily for four weeks prior to the first injection of the toxoid and during the subsequent three-week primary phase. Following the determination of the primary response, the animals were divided into two groups; one continued to receive the control regimen while the other was fed the basal "pyridoxine-free" diet and given daily intraperitoneal injections of 5 mg of desoxypyridoxine per rat. Figures on the ordinate represent reciprocal titers.

immediate weight decline and the appearance of typical pyridoxine deficiency symptoms within a week. During a five-week period of this acute pyridoxine deficiency the animals lost an average of 80 g.

The results of the first experiment in this series are shown in Figure 7. In the control group the typical pronounced secondary response was observed in every animal one week following the initial booster injection and was maintained at a high level during the succeeding four weeks. In contrast, the secondary response of the desoxypyridoxine-treated group was significantly diminished. Thus, 40 per cent of these rats failed to show a true secondary response one week following the first booster injection and a marked fall in antibody titer occurred at the fifth week despite continued antigenic stimulation.

The second experiment (Fig. 8) was designed to obtain further information on the time course of the secondary response to a single booster injection and thus to delineate more sharply the effects of a pyridoxine deficiency upon this response. The plan of this experiment was similar to the preceding one with the exception that only a *single* booster injection was given and antibody titers were determined three, six, and nine days later. In order to ascertain the specificity of the desoxypyridoxine, one group was supplemented with pyridoxine during the period of desoxypyridoxine administration in the secondary phase.

In the control rats, the appearance of circulating antibodies was noted three days after the booster injection and the titers increased to a maximum value at nine days. In agreement with the preceding experiment, the antibody responses were diminished in the pyridoxine-deficient group. This inhibition was detectable three days after the booster injection. Antibody titers similar to those of the control group were observed in the desoxypyridoxine-treated rats supplemented with

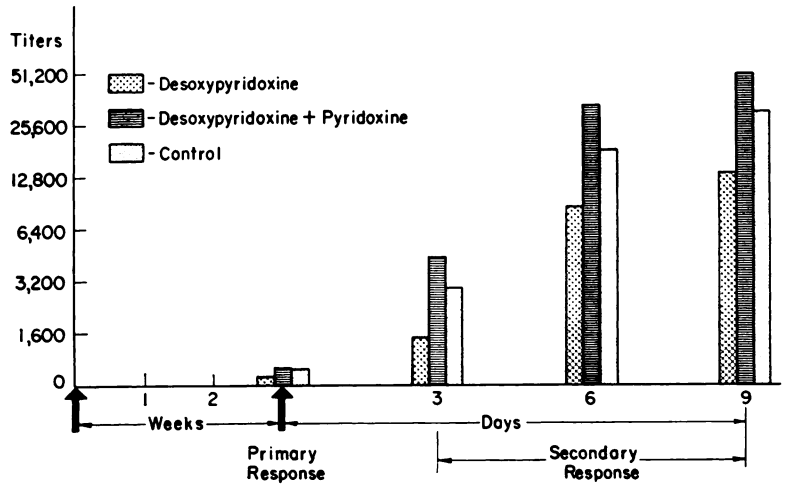

Fig. 8. Effect of desoxypyridoxine-treatment during the secondary phase upon antibody response. Experimental details were essentially those described in Figure 7 with the exception that 5 mg of desoxypyridoxine were given twice daily. An additional desoxypyridoxine-treated group received 1 mg of pyridoxine daily by intraperitoneal injection.

pyridoxine. All other symptoms attributable to desoxypyridoxine were relieved by pyridoxine.

DISCUSSION

The precise mechanism of the anamnestic process is not known with certainty. A number of theories, the discussion of which is outside the scope of this paper have been proposed and have met with varying degrees of acceptance. Whatever the mechanism, it is apparent that events, e.g. template or adaptive enzyme formation, transpire during the primary phase which are triggered by the secondary stimulus to initiate or, more likely accelerate, the processes of antibody synthesis. What then is the significance of the present findings when viewed within this framework of the anamnestic process?

It had been previously established in this laboratory that the primary antibody responses of the albino rat to the antigenic stimulus of diphtheria toxoid were markedly impaired in deficiencies of pyridoxine, pantothenic acid and biotin. The present study clearly demonstrates that the inhibitory effects of these deficiencies upon the secondary responses to this antigen are even more pronounced. Similar results were noted in a tryptophan deficiency. In these cases, the administration of the missing nutritional factor resulted in immediate weight gains but had very little effect upon antibody production. It seems, therefore, that the elaboration of the primary events of the anamnestic process does not occur in the absence of these nutritional factors and, accordingly, it is not surprising that the secondary stimuli are ineffective under these circumstances. The failure of nutritional therapy during the secondary phase to promote antibody production further emphasizes the absolute need for adequate nutrition *during the primary phase* in order to ensure the attainment of the complete anamnestic process. Continued nutritional therapy eventually permits the initiation of a typical primary response.

The second series of experiments demonstrated that a pyridoxine deficiency induced by administration of desoxypyridoxine during the secondary phase could significantly inhibit the secondary response. This inhibition was apparent three days after the booster injection. It should be stressed that the events of the primary phase proceeded in these experiments while the animals were in a state of good nutrition. Thus, a pyridoxine deficiency in the secondary phase can inhibit the sequence of events normally resulting from the trigger-

ing action of the secondary antigenic stimulus. This harmful effect of the deficiency is evident despite the existence of a normal functional mechanism elaborated during the primary phase. The delayed inhibitory action of the pyridoxine antagonist can be attributed to the partial stimulation of the secondary response occurring prior to the full impact of the deficiency state. This is evident from the failure to maintain the elevated anamnestic titers of circulating antibody during the prolonged, acute pyridoxine deficiency.

The above results are in conformity with those obtained by Stoerk[5] and can most likely be ascribed to the deleterious effects of the acute pyridoxine deficiency upon the lymphoid elements (cf. Mushett *et al.*[6] and Stoerk).[7] Stavitsky[8] has shown that the popliteal lymph node and the spleen are definitely involved in the enhanced response to a second injection of diphtheria toxoid in the rabbit. In the same species, Dixon[9] has demonstrated that the secondary response to a variety of particulate antigens is radiosensitive and he suggests that the radiosensitive process involves the preparation of particulate antigens by the host for antibody synthesis. Numerous experiments involving organ extirpation, cell transfer and *in vitro* culture have implicated the lymphoid apparatus in the processes of antibody formation.[8,10–13]

CONCLUSION

The successful attainment and maintenance of a satisfactory anamnestic response to diphtheria toxoid in the albino rat requires a state of adequate nutrition during both the primary and secondary phases of this process.

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