IMPAIRMENT OF ANTIBODY RESPONSE IN PYRIDOXINE-DEFICIENT RATS

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(Received for publication, October 14, 1946)

In a preliminary communication (1) it was reported that rats immunized when in a state of pyridoxine deficiency developed serum antibody levels far below those of inanition controls and controls fed a complete diet *ad libitum.* The present investigation was undertaken to compare the effect on serum antibodies of single deficiencies in other B factors and in protein with that in pyridoxine deficiency. Observations were limited to deficiencies which appeared comparable in severity to the pyridoxine deprivation. The severity of a deficiency was judged by its effect on body weight gain.

Numerous attempts have been made in the past to demonstrate a relationship between dietary factors and antibodies. Cannon, Wissler, and their coworkers have succeeded in showing that prolonged protein depletion depresses the formation of antibodies (2), and that this depression can be reversed by feeding protein (3), or a mixture of crystalline amino acids (4). Less success has attended similar efforts to implicate single vitamins in the antibody-forming mechanism. In Robertson's extensive review the pertinent literature, up to 1934, is summarized (5). In the mass of conflicting reports cited there appears no unequivocal evidence to indicate that any particular vitamin is essential for the development of antibodies.

Recently, Spies and his coworkers (6, 7), and Ruchman (8) have found that in B complex deficiency antibody responses are markedly impaired.

Methods and Materials

Ninety rats of the Sherman strain, about 4 weeks of age, were divided into twelve groups of litter mates, as indicated in Table I. The animals were weaned on a basic diet to which were added crystalline B vitamins.

Diet:

	gm.
Cerelose	62.8
Casein (Labco)	30.0
Wesson's salt mixture (modified)	1.2
Bone ash	2.0
Cellu-flour	2.0
Carotene	0.1
Cottonseed oil	1.9
	100.0

Crystalline substances mixed into cellu-flour:

	mg.
Thiamin Hcl	1
Riboflavin	2
Ca-pantothenate	4
Nicotinic acid	4
Choline Cl	200
Pyridoxine HCl	1

The diet listed was regarded as complete and was fed to the control groups. The two groups on low protein intake received a modification of this diet, in which casein was reduced from 30 per cent to 10 per cent and cerelose was increased accordingly. Four other variants

TABLE I

Summary of Data on Growth, Thymus Weight, and Hemagglutinin Titers in Rats Fed Diets Low in Protein or Deficient in Single B Factors

Group*	Diet	Body weight		Thymus weight	Hemagglutinin titers		No. and sex of rats
		Initial	Final		Average	Range	
		gm.	gm.	gm.			
A	Low thiamin	37	57	0.027	1:85	1:8 –1:128	8♀
B	" "	77	41	0.041	1:64	—	1♂
B	" riboflavin	69	109	0.192	1:38	1:4 –1:128	8♂
B	" pantothenic acid	69	113	0.172	1:38	1:16–1:64	8♂
B	" pyridoxine	70	101	0.054	1:8	0 –1:32	8♂
C	" "	47	83	0.047	1:0.4	0 –1:4	9♂
A	" "	37	65	0.032	1:11	0 –1:32	4♀
B	" protein (10 per cent)	67	109	0.232	1:20	0 –1:64	8♂
A	" " " " "	36	86	0.201	1:35	1:16–1:64	6♀
C	Inanition control	46	78	0.182	1:64	1:32–1:128	7♂
C	Control	45	160	0.395	1:36	1:10–1:80	8♂
B	"	68	141	0.313	1:35	1:4 –1:64	7♂
A	"	38	116	0.266	1:85	1:64–1:128	6♀

* Similar letters indicate groups of litter mates.

of this diet were made by omitting from each a single one of the four vitamin B factors which are essential for the rat (thiamin, riboflavin, pantothenic acid, and pyridoxine).

Since it was found that nearly all rats fed a diet without added thiamin failed to survive more than 4 weeks, a daily supplement of thiamin chloride was given by mouth to animals deficient in this factor (3 γ per day for 11 days and 1.5 γ per day for the next 27 days).[1] An additional group of rats were given restricted amounts of the complete diet in order to duplicate the growth retardation observed in the pyridoxine-deficient animals (paired weighing).

During the 5th week of the dietary experiment all animals were immunized against sheep red blood cells (preserved in Alserver's solution). On 3 alternate days, 0.5 ml. of a 5 per cent saline suspension of washed sheep erythrocytes was injected intraperitoneally. 5 days after the last injection the animals were bled under ether anesthesia. Hemagglutinin determina-

[1] The daily intake of thiamin HCl in the control animals fed *ad libitum* was approximately 150 γ.

tions were carried out on individual sera after they had been inactivated in a water bath at 56° for 30 minutes. The serum dilutions were in twofold steps and the titer end point adopted was three plus. (Complete agglutination regarded as four plus.)

Immediately after exsanguination the thymuses were weighed and prepared for histological examination. Thymus weights and morphology were studied because they constitute an approximate measure of the state of the lymphoid tissue elsewhere.

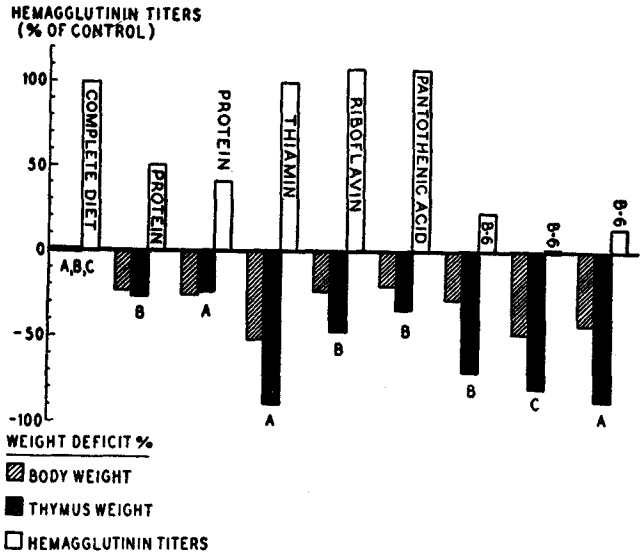

FIG. 1. Comparison of agglutinin titers of controls with those of rats maintained on a low protein diet (10 per cent casein) and of rats fed diets deficient in single B factors. Agglutinin titers, above the baseline, and deficits in thymus weights and in body weights, shown below the baseline, are expressed as percentages of the controls. Identical letters beneath the columns indicate groups composed of litter mates.

RESULTS

From the data summarized in Fig. 1 and in Table I it is apparent that the only groups having antibody titers lower than the controls were those deficient in protein or in pyridoxine. Animals consuming the low protein diet had serum antibody levels which were about 50 per cent below those of the controls. However, in view of the method employed such differences cannot be considered significant. The pyridoxine-deficient groups had titers which amounted to 1, 14, and 23 per cent of the control levels. Individual hemagglutinin titers of the animals in these three groups are compared with those of litter mate controls in the scatter chart (Fig. 2).

The effects of the various dietary deficiencies upon body weight gain can be estimated from the initial and final body weights given in Table I. The growth retardation exhibited by the pyridoxine-deficient rats and by those on the low

protein diet was no greater than that observed in litter mate animals which were deficient in riboflavin or in pantothenic acid or in thiamin, in all of whom the antibody response was unimpaired. That growth retardation, *per se*, had no influence on serum antibody levels was also evident from the findings in paired weighed controls, which formed antibody titers even higher than those of the full controls (see Table I, inanition controls).

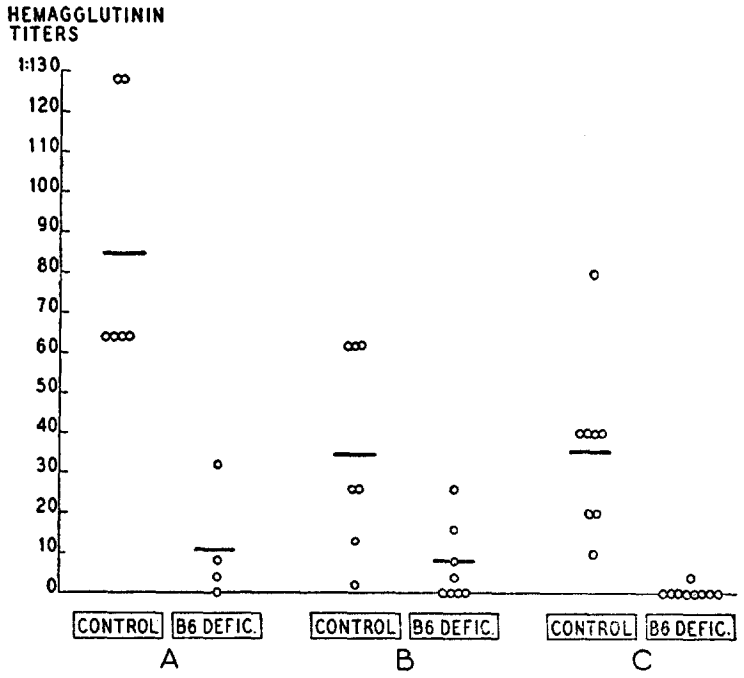

FIG. 2. Comparison of hemagglutinin titers in the three groups of rats fed a diet low in pyridoxine with those of litter mates on the complete diet.

Animals which were deficient in protein or pantothenic acid or riboflavin had thymuses which were smaller than those of the controls. These thymus weight deficits were, however, roughly proportional to the body weight deficits (Fig. 1). A similar relationship obtained in the paired weighed (inanition) controls. In contradistinction, the thymic atrophy observed in the pyridoxine-deficient and in the thiamin-deficient groups was disproportionately great. This effect was anticipated for the pyridoxine-deficient rats (9) but it is not characteristically found in thiamin deficiency. However, previous experience has shown that body weight deficit due to weight loss is usually associated with greater thymus weight deficit than that which accompanies body weight deficit due to

retarded growth. During the last 2 weeks of the experiment weight losses did occur in all animals on low thiamin consumption, whereas those rats deficient in pyridoxine and other B factors grew continuously, although at a reduced rate. Although the thymuses of thiamin-deficient and of pyridoxine-deficient rats were equally small, histological examination disclosed a difference: In the thymuses of thiamine-deficient animals there were well preserved lymphocytes, and distinct corticomedullary differentiation was retained. However, in most of the pyridoxin-deficient animals the thymuses were totally depleted of lymphocytes, the cortex had disappeared, and the glands consisted almost exclusively of epithelial cells and stroma. Similarly there was less lymphoid atrophy in lymph nodes of thiamin-deficient animals.

A pooled sample of serum from two pyridoxine-deficient animals was found on electrophoretic analysis[2] to have lower percentages of alpha and of gamma globulin than similar samples from animals deficient in other B factors and controls. This reduction in gamma globulin, if confirmed by further studies, would agree well with the deficit in serum antibodies.

DISCUSSION

From the data presented it appears that the consumption of a diet low in pyridoxine produces considerable impairment of the antibody response in rats. The depressant effect of pyridoxine deficiency is consistent with descriptions of a similar effect in B complex deficiency reported by Spies *et al.* (6, 7) and by Ruchman (8). The latter author also noted the failure of single deficiencies in riboflavin and in thiamin to influence the levels of circulating antibodies. In respect to inanition, the data reported here differ from those of Ruchman (8) who found extremely low antibody concentrations in the sera of underfed animals. However, the food restriction employed by the latter author must have been extreme since many of his animals died in less than 3 weeks. In contrast, the present inanition controls with growth retardation comparable to that in pyridoxine deficiency, survived the experiment in apparently good condition and had antibody levels which were even higher than those of the full controls. This enhancement of the antibody response by calorie restriction has been observed previously in unpublished experiments.

The depressant effect of pyridoxine deficiency on antibody responses was thought originally to follow from excessive lymphoid atrophy (1). This belief was based upon the hypothesis that the lymphocyte may be the site of antibody formation (10). Since rats deficient in pyridoxine exhibit a striking loss of fixed and circulating lymphocytes (11) their defective antibody responses might be attributable to a lack of lymphocytes. Some doubt is thrown upon this explanation by the fact that thiamin-deficient rats exhibiting marked

[2] We are grateful to Dr. D. H. Moore for doing the analyses.

lymphoid atrophy, though less extensive than pyridoxine-deficient animals, had serum antibody levels as high as those of the controls.

It is possible that the suppressive effect of pyridoxine deficiency on the antibody response is exerted through an entirely different mechanism. Pyridoxine has recently been shown to function as the coenzyme of several amino acid decarboxylases (12) and of glutamate-aspartate transaminase (13), and also to be intimately related to tryptophane metabolism (14). Thus this vitamin apparently plays an important rôle in protein metabolism. Since antibodies are globulins, it may be through interference with some phase of amino acid metabolism that a deficiency in pyridoxine produces the observed depression of antibody responses. It is conceivable that in a similar manner pyridoxine deficiency interferes with the formation of hemoglobin (15) and of serum globulins. It is also possible that globulins are necessary for the formation of normal lymphocytes in a manner analogous to the participation of hemoglobin in the formation of normal erythrocytes. Thus, the lymphoid atrophy occurring in pyridoxine deficiency could conceivably be a consequence of defective globulin synthesis rather than its cause.

It has been assumed in this discussion that a low antibody titer in serum is synonymous with defective antibody formation in the tissues. This assumption may not be valid since serum levels probably reflect a balance between synthesis and release of antibody on the one hand, and its degradation on the other. In pyridoxine deficiency the low serum antibody levels may mean diminished synthesis, as assumed above, or might equally well reflect an augmented rate of antibody degradation. In the absence of information about these specific processes, the mechanism responsible for the depressant effect on serum antibodies of pyridoxine deficiency remains undetermined.

SUMMARY

Striking impairment of the antibody response to sheep erythrocytes was found in pyridoxine-deficient rats. Deficiencies in the three other B factors required by the rat, and low protein feeding, having effects on body weight comparable to pyridoxine deficiency, failed to influence the antibody response studied.

Confirming previous observations, a striking loss of thymic and lymphoid tissue occurred in the pyridoxine-deficient animals. A marked deficit in thymus weight also resulted from the feeding of a diet low in thiamin. Histologically, however, lymphoid atrophy was less pronounced in thiamin-deficient rats than in those deprived of pyridoxine.

We wish to thank Miss Rose-Ruth Tarr and Miss Ruth Johnson for valuable assistance.

The expenses of this study have been in part defrayed by the Odlum Grant for the Study of Myasthenia Gravis, and by the Bertrand Fund.

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