subcultures in broth. Evidence was obtained which indicated that appreciable numbers of streptomycin sensitive cells appeared in 2 of these strains: in one instance after 28 subcultures and in the other after 59 transfers. The 11 remaining strains appeared to retain their resistance.

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Circulating Antibodies in Vitamin-Deficiency States: I. Pyridoxin, Riboflavin, and Pantothenic Acid Deficiencies.*

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(Introduced by R. R. Mellon.)

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In the study of nutrition any measure of animal performance can be utilized for the evaluation of diets. Antibody production, the normal physiological response to an antigenic stimulus, might be expected to provide a measure of the nutritional status of an experimental animal. Inasmuch as relatively few conclusive studies of this type are reported in the literature, the ability to form antibodies seems a worthy supplement to other measures of the role of various dietary factors.

Few studies have appeared concerning the effect of specific vitamin B deficiencies upon antibody production in the rat. Ruchman[1] reported that neither riboflavin nor thiamin deficiencies affected antibody production. More recently, Stoerk and Eisen[2] found reduced serum antibody concentrations in pyridoxin deficiency. Stoerk, Eisen, and John[3] have reported normal serum antibody concentration in thiamin, riboflavin, and pantothenic acid-deficient rats. Washed sheep erythrocytes were employed as the antigen in these experiments.

The present report presents data concerning the effect of pyridoxin, pantothenic acid, and riboflavin deficiencies upon antibody production by the rat in response to human red blood cells as antigenic stimulus.

Experimental. Two series of experiments differing only in the immunization procedure were conducted. Male weanling albino rats of the Sprague-Dawley strain were distributed into groups of litter mates as indicated in Table I. The animals were housed individually in wide-mesh screen-bottom cages and weighed weekly. With the exception of the group which received a colony diet (Arcady Farms) *ad libitum*, the animals were placed on a dietary regimen which consisted of a basal diet and additional vitamins in the form of a daily pill. The basal diet had the following percentage composition: sucrose, 56.76; Labco "vitamin-free" casein, 25.00; salts,[4] 4.00; cod liver oil, 2.00; hydrogenated vegetable oil, 10.00; corn oil, 2.00; choline chloride, 0.20; *p*-aminobenzoic acid, 0.01; *i*-inositol, 0.03; and 2-methyl-1, 4-naphthoquinone, 0.001. Each of the pills given to the two control groups supplied the following vitamins: thiamin, 40 γ; riboflavin, 60 γ; calcium pantothenate, 200 γ; pyridoxin, 50 γ; biotin, 1 γ; folic acid, 1 γ; and nicotinic acid, 100 γ. For the pyridoxin, riboflavin, and pantothenic acid-deficient groups, the appropriate vitamin was omitted from the

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1 Ruchman, I., *J. Immunol.*, 1946, **53**, 51.

2 Stoerk, H. C., and Eisen, H. N., PROC. SOC. EXP. BIOL. AND MED., 1946, **62**, 88.

3 Stoerk, H. C., Eisen, H. N., and John, H. M., *J. Exp. Med.*, 1947, **85**, 365.

4 Jones, J. H., and Foster, C., *J. Nutrition*, 1942, **24**, 245.

TABLE I.
Summary of Growth and Food Consumption Records.

Group	No. of rats	Body wt*		Daily food consumption‡
		Initial	Final†	
	Series I.			
Pyridoxin-deficient	5	39	129	5.7
Riboflavin-deficient	5	39	48	
Pantothenic acid-deficient	5	41	100	
Inanition control	5	39	157	5.7
Control—*ad libitum*	5	40	329	
Colony diet	5	39	286	
	Series II.			
Pyridoxin-deficient	5	46	134	5.1
Riboflavin-deficient	6	42	59	3.2
Pantothenic acid-deficient	2	45	91	4.0
Inanition control	5	45	161	5.1
Control—*ad libitum*	5	43	348	11.9
Colony diet	5	43	289	

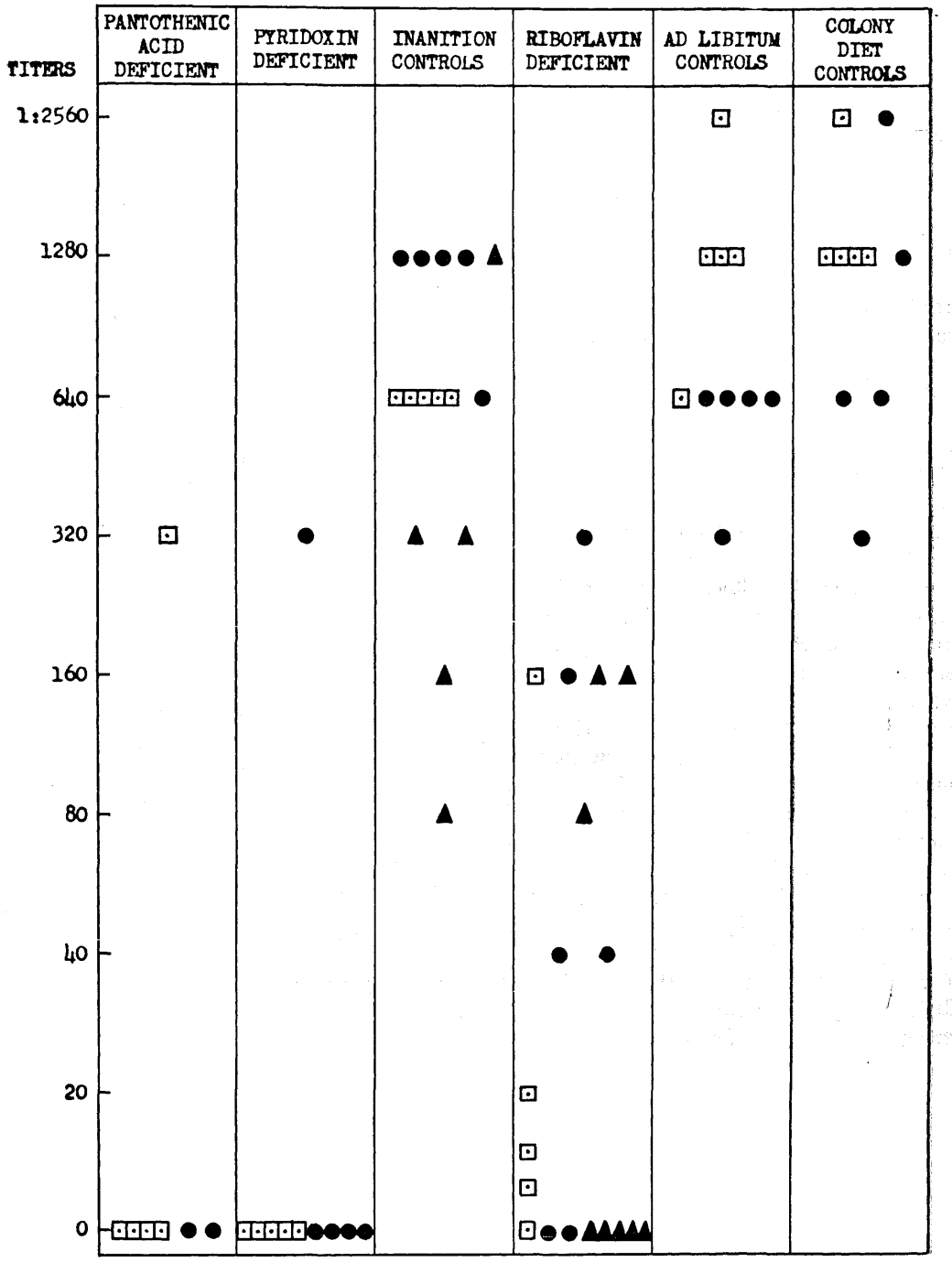
* Group average in grams.
†At the time of bleeding.
‡ Group average in grams.

pill. The basal diet was fed *ad libitum* to all groups except the inanition controls, in which case the daily food intake of each rat was restricted to that consumed during the previous day by its litter mate in the pyridoxin-deficient group. In Series II, the food consumption of the remaining animals was also determined as shown in Table I.

After 7 weeks on experiment, the animals in the 3 vitamin-deficient groups had plateaued in weight and immunization of all animals was instituted. A 10% suspension of washed Group O, Rh positive human erythrocytes in normal saline was given intraperitoneally as antigen. In Series I an initial dosage of 0.5 ml of the red cell suspension was followed by 1 ml inoculations. In Series II, 0.5 ml was also employed for the primary injection, but the dosage for the subsequent injections was 1 ml of red blood cell suspension per 100 g of body weight except that the maximum dosage employed was 2.0 ml. Inoculations were made on alternate days until a total of 6 injections had been given. Five days after the final injection the rats were bled under ether anesthesia and the serum collected. The serums were tested for agglutinin titer within 24 hours after collection. Serial, 2-fold dilutions, beginning with a 1:5 dilution, were made with normal saline. A 0.2 ml sample of each dilution was incubated in 10 x 75 mm tubes at room temperature for one hour with 0.2 ml of a 2% saline suspension of Group O, Rh positive cells of the same origin as those used for the inoculations. At the end of the hour, the tubes were centrifuged lightly and read using a three-plus titer for the endpoint.

Results. The individual hemagglutinin titers are recorded in the scatter chart. It is evident that there was a marked diminution in the content of circulating antibodies in the pantothenic acid and pyridoxin-deficient groups. Thus, only one animal in each of these 2 groups possessed a measurable agglutinin titer. The fact that the agglutinin titers of the rats in the inanition control groups were equal to those of both the control animals which were fed *ad libitum* and the colony diet controls indicates that the lowered content of circulating antibodies in the pantothenic acid and pyridoxin-deficient groups cannot be ascribed to inanition *per se*. This is also borne out by the observation that, despite their poor nutritional state, the riboflavin-deficient rats possessed a higher content of circulating antibodies than either the pantothenic acid or pyridoxin-deficient rats. The content of circulating antibodies in the riboflavin-deficient rats was found to be intermediate between that of the controls and that of the pantothenic acid and pyridoxin-deficient rats. That the increased titer in the riboflavin-deficient group was not due to the

INDIVIDUAL HEMAGGLUTININ TITERS



□—Series I animals.
●—Series II animals.
▲—Comparable animals in which only 3 injections (0.5, 1.0, and 1.0 ml) of cells were used as antigenic stimulus. Inanition controls were pair fed with the riboflavin-deficient group.

higher dosage of antigen per unit of body weight is evident from the results of Series II where the dosage per 100 g of body weight was the same for all three groups. The reproducibility of these observations is demonstrated by the close agreement between the results of Series I and Series II.

Controlled hemolysin tests, using guinea pig complement, were run on serums from Series I. Hemolysin production was consistently low but followed the same pattern as the agglutinin production.

Discussion. A decrease in the content of circulating antibodies in pyridoxin deficiency was reported by Stoerk and Eisen.[2] This work is confirmed in our experiments. Our results, however, differ from those of Stoerk, Eisen, and John[3] with regard to the effects of pantothenic acid and riboflavin deficiencies. While these workers observed a normal antibody titer, we have noted a decreased titer, particularly in the pantothenic acid-deficient rats. The explanation for this difference may lie in the fact that Stoerk, Eisen, and John utilized sheep erythrocytes, whereas we employed human red cells as the antigen. The immunization procedures also differed. Since the agglutinin titers of our normal controls were considerably higher than those of Stoerk and co-workers, it is evident that our immunization procedure with human erythrocytes furnished a far stronger antigenic stimulus for agglutinin production. Sheep erythrocytes, in our hands also, have proved to be a poor stimulus for hemagglutinin production in the rat although they are effective antigens for hemolysin production. It is possible, therefore, that pantothenic acid and riboflavin do not become limiting factors for hemagglutinin production when the antigenic stimulus is of a low order of magnitude. It is also evident from our data that riboflavin is not as critical as pantothenic acid in the production of circulating antibodies.

There is considerable basis for linking pyridoxin and riboflavin to the processes of amino acid metabolism and the role of these vitamins in antibody production may be related to these functions. To our knowledge, a similar role of pantothenic acid in protein metabolism has not been suggested. The present demonstration of the decreased antibody concentration in pantothenic acid deficiency furnishes evidence for the participation of this vitamin in protein metabolism.

As emphasized in the paper of Stoerk, Eisen, and John,[3] the decreased antibody titer in serum is not unequivocal proof for an impairment of antibody synthesis in the tissues. The possible effect of the vitamin deficiency upon the processes of antibody distribution and destruction must also be taken into account before the relationship between vitamins and antibody production can be established.

Summary. 1. Hemagglutinin production in response to inoculation with human erythrocytes has been investigated in pyridoxin, pantothenic acid, and riboflavin-deficient rats. 2. Severe impairment of antibody response was observed in the pantothenic acid and pyridoxin-deficient rats. Variable but low titers were observed in the riboflavin-deficient groups. Consistently high titers were noted in both the inanition controls and the *ad libitum*-fed animals.

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