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Received April 15, 1952. P.S.E.B.M., 1952, v80.

Complement-Fixing Murine Typhus Antibodies in Vitamin Deficiency States.* III. Riboflavin and Folic Acid Deficiencies. (19638)

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(Introduced by F. S. Cheever.)

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The purpose of this investigation, and others in progress, is an attempt to find an explanation for the increased susceptibility and mortality that occurs in malnourished animal and human populations exposed to typhus(1) and other rickettsial and bacterial infections. Wertman and Sarandria(2) reported that pantothenic acid and thiamine deficiencies influenced the antibody response when a relatively small amount of immunizing material was injected. However, the influence was not as apparent when relatively large amounts of the antigenic material was injected. In addition, they also reported(3) a severe impairment of antibody response in pyridoxine deficient rats when a rickettsial antigen (*Rickettsia typhi*) containing 0.0073 mg N was injected. However, rats deficient in nicotinic acid did respond and produce complement-fixing antibodies when injected with the same amount of antigen. Stoerk, Eisen, and John(4) reported normal serum antibody titers in thiamin, riboflavin and pantothenic acid deficiencies. However, Axelrod *et al.*(5) noted a greatly decreased titer in pantothenic acid, pyridoxine deficiencies and variable but lower titers in riboflavin deficiencies. Ludovici and Axelrod(6) reported severe impairment of antibody response in the pteroylglutamic acid-deficient rats and a moderate impairment in vit. A and niacin-tryptophan deficiencies. These investigators employed a 10% suspension of washed Group O, Rh positive human erythrocytes in physiological saline for antibody stimulation and measured the hemagglutinin production. The present paper deals with the effects of riboflavin, and folic acid deficiencies upon complement-fixing antibody production under similar experimental conditions(2,3).

Experimental. Male weanling albino rats of the Sprague-Dawley strain, distributed into groups as indicated in Table I, were housed individually in wide-meshed, screen-bottom cages and weighed at regular intervals. The ingredients used in preparing the different diets were obtained from General Biochemicals, Inc., and Nutritional Biochemicals Co. All ingredients were of the highest purity obtainable and each basal diet was formulated so that only a minimum amount of the B complex vitamins to be studied were present. The basal diet for the riboflavin groups was as follows: Sucrose, 58.75%; casein (vitamin-free), 25.00%; vegetable oil (hydrogenated), 10.00%; salt mixture No. 2 (U.S.P.), 4.00%; corn oil (Mazola), 2.00%; choline chloride, 0.20%; i-inositol, 0.03%; paraminobenzoic acid, 0.01%; dl-alpha-tocopherol acetate, 0.01%; and 2-methyl-1,4-naphthoquinone, 0.001%. The basal diet for the folic acid groups was as follows: Sucrose, 57.77%;

* This investigation was partially supported by a research grant from the Upjohn Co., Kalamazoo, Mich.

TABLE I. Complement-Fixation Titers and Body Weight Change of Rats in Riboflavin and Folic Acid Deficiency States.

Diet and immunization	No. rats	Complement-fixation titers									Body wt	
		0	1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:1280	Initial avg, g	Final avg, g
Riboflavin deficient	26											
1 inj.		26	—	—	—	—	—	—	—	—		
3 "	*	—	—	—	1	1	2	11	8	1	51	93
Riboflavin control (paired weighed)	10											
1 inj.		1	1	1	4	2	1	—	—	—		
3 "		—	—	—	—	—	—	—	6	4	49	92
Riboflavin control *(Ad lib.)*	9											
1 inj.		5	1	1	2	—	—	—	—	—		
3 "		—	—	—	—	—	—	4	2	3	53	296
Folic acid deficient	25											
1 inj.		25	—	—	—	—	—	—	—	—		
3 "	†	1	—	1	6	8	1	—	1	—	39	126
Folic acid control (paired weighed)	10											
1 inj.		2	4	3	1	—	—	—	—	—		
3 "		—	—	—	—	—	—	1	4	5	38	121
Folic acid control *(Ad lib.)*	10											
1 inj.		3	2	4	1	—	—	—	—	—		
3 "		—	—	—	—	—	—	3	3	4	38	311

* 2 rats died. † 7 rats died.

casein (vitamin-free), 25.00%; vegetable oil (hydrogenated), 10.00%; salt mixture No. 2 (U.S.P.), 4.00%; corn oil (Mazola), 2.00%; sulfasuxidine (succinylsulfathiazole), 1.00%; choline chloride, 0.20%; i-inositol, 0.03%; dl-alpha-tocopherol acetate, 0.01%, and 2-methyl-1,4-napthoquinone, 0.001%. In addition, 2 drops of Haliver oil (3,000 U.S.P. of vit. A and 24 U.S.P. of vit. D) were given by dropper to each rat once a week. The rats in the riboflavin and folic acid groups received their supplemental vitamins daily in the form of a pill. The riboflavin-deficient group had riboflavin omitted from the pill and the folic acid-deficient group had folic acid omitted. Each pill for the riboflavin-deficient group was compounded to supply the following vitamins (7): Thiamine, 40 γ; pyridoxine, 50 γ; calcium pantothenate, 150 γ; nicotinic acid, 150 γ; biotin, 1 γ; folic acid, 1 γ. Each pill for the folic acid-deficient group was compounded to supply the following vitamins(6,7): Thiamine, 40 γ; riboflavin, 60 γ; pyridoxine, 50 γ; calcium pantothenate, 300 γ; nicotinic acid 150 γ; and biotin, 3 γ. The folic acid control group received daily 20 γ of folic acid in the vitamin pill daily and the riboflavin control group received 60 γ of riboflavin in addition to the daily vitamin supplement made available to the deficient animals. In addition to the control groups that were given the complete basal diet plus the entire vit. B requirement, inanition control groups were included to determine the influence of a reduced food intake upon antibody production when all the necessary B vitamins were present. Ten animals for each group were paired-weighed against 10 rats in the deficient groups. At the end of the period necessary to establish the deficiency, each rat was injected intraperitoneally with 0.5 cc (0.0073 mg N)† of washed, formalinized suspensions of *Rickettsia typhi* (murine typhus).‡ One cc inoculations were repeated at 7-day intervals until each rat had received a total of 2.5 cc (0.0365 mg N). During this period, the animals were bled 7 days after inoculations of murine typhus vaccine to determine if the amount of antigenic stimulus played a role. The com-

† Nitrogen content determined by Biophysics Department, University of Pittsburgh.

‡ Purified antigens supplied by Dr. Herald Cox, Lederle Laboratories Division, American Cyanamid Co., Pearl River, N. Y.

plement-fixation technic employed in this study was identical to that of Plotz(8), Plotz and Wertman(9), and Wertman(10). Fixation was allowed to take place for 18 hours at 4 to 6°C. The sensitized cells (0.25 ml sheep cells 3% and 0.25 ml amboceptor containing 3 MHD) were added and the tubes incubated for 30 minutes at 37°C in a water bath. The tests were read following secondary incubation and only 4^+ and 3^+ fixations were accepted as end-points. The controls necessary to insure valid results and reproducibility were included in all the titrations and serum dilution tests.

Results. The riboflavin and folic acid control rats at the end of the feeding period, all had gained weight, appeared healthy and possessed smooth even coats. The rats fed on the riboflavin and folic acid diets showed the signs which might be expected to accompany their respective deficiency. The inanition control groups showed approximately the same weight gain as their respective deficiency groups. The initial average and final average weight of each group are recorded in Table I. The riboflavin deficient group and the folic acid deficient group gained much less weight than their respective control group.

The table demonstrates the end-point titers obtained in the complement-fixation test using a suspension of washed murine typhus rickettsiae as the antigen. It appears that both riboflavin and folic acid deficiencies interfere with the production of circulating antibody when a relatively small amount of antigenic material was injected. None of the animals in these 2 groups demonstrated detectable amounts of complement-fixing antibodies. However, the sera of the inanition (paired-weighed) controls and the sera of the *ad libitum* controls demonstrated antibody formation in approximately 75% of the animals. The riboflavin deficient rats and the folic acid deficient rats both demonstrated antibody production after the introduction of more antigenic material (0.0365 mg N). However, the folic acid deficient rats produced less antibody than either the riboflavin deficient or control group. An average quantitative difference of approximately 3 two-fold dilutions existed between the folic acid deficient and folic acid control group even when relatively large amounts of murine rickettsiae were injected. There was an average difference of approximately one two-fold dilution between the riboflavin deficient and the riboflavin control group. It is evident that the reduced food intake alone did not appreciably influence antibody production as measured by complement-fixation.

Summary. 1. It appears as though the riboflavin requirement for antibody production is not as critical as the folic acid requirement. 2. This study indicates that both riboflavin and folic acid deficiencies impaired the production of circulating complement-fixing antibodies when relatively small amounts of antigen were injected. However, after more antigen was introduced, significant titers were obtained from the sera of riboflavin deficient rats but the folic acid deficient animals produced significantly less antibody. The inanition control (paired-weighed) and the normal control animals (fed *ad libitum*) demonstrated antibody production of approximately the same titers when injected with either 0.0073 mg N or 0.0365 mg N. Therefore, it appears as though the specific vitamin deficiency and not the inanition is the significant factor in the production of circulating complement-fixing antibody.

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Received May 12, 1952. P.S.E.B.M., 1952, v80.