plasma cholesterol rose even higher (122 mg per 100 cc) in those rats tested at the end of 144 hours. This increase did not appear to be due to some *non-specific* reaction of the rat to the introduction of rabbit serum because the five rats given the same serum and in equal amount but placed upon a cholesterol free diet did not have a marked rise in their plasma cholesterol either at 24, 48 or 144 hours. Likewise, the normal, untreated rats placed upon the high cholesterol diet showed only a slight rise in plasma cholesterol.

Discussion. The induction of hypercholesteremia in the rat by substituting rabbit serum for its own plasma and then feeding a high cholesterol diet of course suggests that the hypercholesteremia induced by dietary means in the rabbit itself is also mediated in part at least by a peculiarity of this animal's plasma—a peculiarity which can be transferred to another species. In other words, the plasma phenomenon in respect to cholesterol induced in other animals by Triton or cholate administration may already be an intrinsic property of rabbit plasma. If this is so, the dietary induced hypercholesteremia occurring in the rabbit is of similar mechanism to that type of hypercholesteremia set in motion by the administration of these detergents. Studies concerning this point are now in progress.

Summary. Rats subjected to a procedure which substituted normal rabbit serum for their own plasma became rapidly hypercholesteremic when placed upon a high cholesterol diet.

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Complement-Fixing Murine Typhus Antibodies in Vitamin Deficiency States* IV. B_{12} Deficiency. (19887)

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The authors have presented data(1-3) concerning the effects of thiamine, pantothenic acid, pyridoxine, nicotinic acid, riboflavin and folic acid deficiencies upon complement-fixing antibody production by rats in response to a suspension of washed murine rickettsiae (*Rickettsia typhi*) as the antigenic stimulus. The present paper is concerned with the effects of vit. B_{12} deficiency upon the production of complement-fixing antibody under similar experimental conditions. The purpose of this investigation, and others in progress, is an attempt to explain the increased susceptibility and mortality that occurs in malnourished populations exposed to typhus fever(4) and other infections. Wertman and Sarandria(1) reported that pantothenic acid and thiamine deficiencies influenced the antibody response when a relatively small amount of immunizing material was injected into the rat. However, the influence was not as apparent when a relatively large amount of the same antigenic material was employed. In addition, they also reported(2) a severe impairment of antibody response in pyridoxine deficient rats when a rickettsial antigen (*Rickettsia typhi*) containing 0.0073 mg N was injected. Rats deficient in nicotinic acid did respond and produce complement-fixing antibodies when stimulated with the same amount of antigen. These same investigators also reported(3) that the riboflavin requirement for antibody production was not as critical as the folic acid requirement. The inanition control (paired-weighed) and the normal control animals (fed

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ad libitum) demonstrated antibody production of comparable titers when injected with either 0.0073 mg N or 0.0365 mg N. It appeared as though the specific vitamin deficiency and not the inanition was the significant factor. Ludovici and Axelrod(5) reported no decrease in antibody production by vit. B_{12} deficient rats. A suspension of washed group O, Rh positive human erythrocytes in physiological saline was employed for both the production of circulation antibody and the hemagglutination determinations. This report was based on 7 experimental animals and only one amount of antigenic stimulation for antibody production.

Experimental. Male weanling albino rats of the Sprague-Dawley strain were housed individually in wide-meshed, screen-bottom cages and weighed daily. The room temperature was maintained at 68°F. The rats were fed a basal diet and received controlled amounts of vitamins in the form of a daily pill. The *ad libitum* and inanition controls received, in addition, 0.2 γ of vit. B_{12} (Berubigen) per day which was administered by the subcutaneous route. The basal diet was as follows: defatted, whole ground yellow corn, 45.76%; soy flour (low fat), 45.75%; Nutritional Biochemicals, salt mixture No. 2, 2%; corn oil (Mazola), 5%; l-cystine, 0.3%; choline chloride, 0.10%; i-inositol, 0.02%; p-aminobenzoic acid, 0.01%; iodinated casein (Protomine), 0.05%; 2-methyl-1,4-naphthoquinone, 0.001%; alpha tocopherol, 0.001%; and sulfasuxidine, 1%. Each of the vitamin pills supplied the following: thiamine, 80 γ; riboflavin, 120 γ; pyridoxine, 100 γ; pantothenic acid, 600 γ; nicotinic acid, 300 γ; folic acid, 10 γ; and biotin, 10 γ. Two drops of Haliver oil (3,000 USP of vit. A and 24 USP of vit. D) were administered to each rat once a week. In addition to the *ad libitum* control animals that were given the complete basal diet plus the entire vit. B complex requirement, inanition control animals were included. This was accomplished in an attempt to determine the influence of a reduced food intake upon antibody production when all the necessary B vitamins were present. Nineteen control rats were paired with 19 rats in the vit. B_{12} deficient group. When the deficient animals began to plateau or lose weight, which was approximately 3 weeks after being placed on the deficient diet, each rat of all groups was injected intraperitoneally with 0.5 cc (0.0073 mg N) of a washed, formalized suspension 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). The animals were bled 7 days after the first inoculation and 7 days after receiving 2.5 cc of murine typhus antigen to determine the effect of different amounts of antigenic stimulus. Rats were bled prior to starting the experiment and all sera were found to be negative when tested with murine typhus complement-fixing antigen. The complement-fixation technic employed in this study was identical to that of Plotz(6), Plotz and Wertman(7), and Wertman(8). 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 to each tube and incubated for 30 minutes at 37°C in a water bath. The tests were read following secondary incubation and only 4+ and 3+ fixation were accepted as the end-points. The controls necessary to insure valid results and reproducibility were included in all titrations and serum dilution tests.

Results. The vit. B_{12} control rats at the end of the feeding period had all gained weight, appeared healthy and possessed smooth even coats. The vit. B_{12} deficient rats began to lose weight and showed symptoms of the deficiency during the third week of the experiment. The members of the inanition control group were maintained at approximately the same weights as their mates in the deficient group. The initial and final average weight in grams of each group are recorded in Table I.

The end-point titers obtained in the complement-fixation test, using a suspension of murine typhus rickettsiae as the complement-fixing antigen, are likewise recorded in Table I. It appeared that a B_{12} deficiency interfered with the production of circulating anti-

† Purified antigen supplied by Dr. Herald Cox, Lederle Laboratories, American Cyanamid Co., Pearl River, N. Y.

TABLE I. Complement-Fixation Titers and Body Weight Change of Rats in a Vitamin B_{12} Deficient State.

Diet and immunization	No. of rats	Complement-fixation titers 0	1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:1280	1:2560	Avg body wt, g Init.	Final
B_{12} deficient													
1 inj.	38	38	—	—	—	—	—	—	—	—	—	45	112
3 "	36*	—	—	—	—	8	12	15	1	—	—		
B_{12} control (inanition)													
1 inj.	19	2	8	9	—	—	—	—	—	—	—	45	115
3 "	17*	—	—	—	—	—	—	2	8	7	—		
B_{12} control *(ad lib.)*													
1 inj.	21	2	10	8	1	—	—	—	—	—	—	45	171
3 "	21	—	—	—	—	—	—	—	5	14	2		

* Two rats died.

bodies when either a relatively small or large amount of antigenic material was injected. When a relatively small amount (0.0073 mg N) of antigenic material was employed, no detectable complement-fixing antibody was present in the sera of the deficient animals. However, the sera of 90% of the inanition and *ad libitum* controls demonstrated complement-fixing antibody when the same amount of antigenic material was employed. When a relatively large amount (0.0365 mg N) of antigen was introduced, the sera of the deficient animals did not demonstrate antibody titers as high as either the inanition or *ad libitum* control animals.

Summary. This study indicates that a vit. B_{12} deficiency impaired the production of circulating complement-fixing antibody. The sera of the inanition control and the *ad libitum* control animals possessed antibody concentrations of approximately the same titers. This was evident when either 0.0073 mg N or 0.0365 mg N was employed as the antigen. Therefore, the specific vitamin deficiency and not inanition appears to be the significant factor affecting the production of circulating complement-fixing antibody under these experimental conditions.

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Protective Effect of Adrenal Steroid Administration on Irradiated Mice. (19888)

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Altering the resistance or radiosensitivity of animals to ionizing radiation and ascertaining the physiological processes behind such resistance or radiosensitivity have been the subjects of many investigations(1-4). The idea that the condition of stress set up in animals by radiation can be altered by the administration of certain hormones has also been stud-