Summary. 1. "Folic acid" deficiencies induced by aminopterin and by sulfa drugs in rats have been compared. 2. Aureomycin can counteract the toxic effects of 50, 75 and 100 μg of aminopterin/100 g of diet. The antibiotic is equally effective whether fed in the diet or given intraperitoneally. 3. Aureomycin is without effect on growth in rats made "folic acid deficient" on a sulfa diet. 4. Citrovorum factor concentrates are able to completely overcome the deficiency produced on either type of diet.

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Complement-Fixing Murine Typhus Antibodies in Vitamin Deficiency States.* (18499)

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The effect of malnutrition in epidemic typhus fever has been recognized for some time. Efforts have been made to find an explanation for the observation that increased mortality occurs in malnourished populations exposed to typhus fever. FitzPatrick(1) injected weanling male white rats, subjected to various vitamin and protein deficiencies, with viable rickettsiae (*Rickettsia typhi*) of murine typhus fever. It was demonstrated that the animals on a reduced vitamin B complex intake of 1 10th the optimal level were more susceptible than those receiving ample B vitamins. Thiamin, riboflavin, and pantothenic acid deficiencies studied separately, caused increasing susceptibility. It was also reported that animals on a natural diet were more resistant than those on a complete synthetic ration. The purpose of this investigation was to extend these observations. It was felt that the increase in susceptibility might in some manner be correlated with the inability of the animals to rapidly produce circulating antibodies when antigenic material was introduced.

The production of circulating antibodies in vitamin deficient states has been reported by a number of investigators. The antigenic materials employed for these studies have been of a variety of types. Axelrod, Carter, McCoy and Geisinger(2) demonstrated that pyridoxin, pantothenic acid deficiencies effected the antibody production by the rat in response to the injection of human red blood cells as antigenic stimulus. Carter and Axelrod(3) reported that the content of circulating antibodies in the thiamin and biotin deficient rats was less than that of the control rats. Ludovici, Axelrod, and Carter(4) observed a low production of circulating antibodies in the pantothenic acid deficiency state when rats were injected with human red blood cells. Stoerk, Eisen, and John(5) reported that pantothenic acid deficiency did not affect the level of circulating antibodies when the rats were immunized with sheep erythrocytes. In the present study 5 different diets were utilized. In the initial investigation (Table I), 5 rats were placed on an adequate diet and 5 were placed on a diet containing only one-tenth of the amount of vit. B complex considered to be the optimum. The second experiment employed 4 groups of animals; these were total vit. B deficiency,

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5. Stoerk, H. C., Eisen, H. N., and John, H. M., *J. Exp. Med.,* 1947, v85, 365.

TABLE I. C.F. Titers with Murine Typhus Rickettsiae and Rat Sera After Immunization with 4 cc Vaccine.

		C.F. titers following immunization		
	No.	1 wk	2 wk	3 wk
Normal control	1	1/640	1/640	1/320
Adequate B	2	1/320	1/320	*
Complex group	3	1/320	1/320	1/160
	4	1/320	1/320	1/160
	5	1/320	1/320	1/640
1/10th optimal	6	1/320	†	1/320
Level B complex	7	1/320	1/320	1/640
Group	8	1/640	†	1/640
	9	1/320	1/320	1/640
	10	1/320	1/320	1/640

All pre-sera were negative.
* Animal died following second bleeding.
† Insufficient serum.

pantothenic acid deficiency, thiamin deficiency, and an adequate vit. B complex control group. An effort was made to reproduce conditions comparable to those studied by FitzPatrick(1). The animals were subsequently immunized with washed, formalinized suspensions of *Rickettsia typhi* (murine typhus).† Following immunization, the rats were bled and the circulating antibodies were demonstrated by complement fixation(6-8).

Experimental. Ten male albino rats of the Sprague-Dawley strain, distributed into 2 groups as indicated in Table I, were housed individually in wide-meshed, screen-bottom cages and weighed daily. The basic diet was composed of 65.12% sucrose, 17.23% vitamin test casein, 9.55% vegetable oil hydrogenated, 3.83% General Biochemicals, Salt Mixture No. 2, 2% Squibb's Cod Liver Oil, 2% Mazola Corn Oil, 0.2% choline chloride, 0.03% i-inositol and 0.01% p-aminobenzoic acid. Rats on the adequate vit. B complex control group received their vitamins in the form of a daily pill, supplying the following vitamins: thiamin, 40 γ; pantothenic acid, 150 γ; pyridoxin, 50 γ; niacin, 100 γ; riboflavin, 60 γ(9). Rats on the one-tenth adequate B ration were given a pill daily which supplied one-tenth the amount of vitamin given to the normal control group. The basal diet was fed to the one-tenth adequate controls and the intake of each rat of the normal control group was restricted to that consumed during the previous day by the rats on the deficient diet.

At the end of the 6-week period, each rat was injected intraperitoneally with 1 cc of murine typhus vaccine. The inoculations were repeated at 7-day intervals until each rat had received a total of 4 cc. During this period, the animals were maintained on the same diet to that fed prior to immunization. The rats were bled 1, 2, and 3 weeks after the last inoculation of the rickettsial suspension. The sera were obtained under sterile conditions and stored at −20°C until tested. The complement-fixing antibodies produced by rats maintained on total vit. B, pantothenic acid, and thiamin deficiencies are indicated in Table II. These animals were bled 7 days after each inoculation of murine typhus vaccine to determine if the amount of antigenic stimulus played a role. These animals were maintained as were those recorded in Table I with the exception of their diets and of the time of bleeding. The complement fixation technic was employed for measuring the circulating antibody response. The technic employed in this study was identical to that of Plotz(6), Plotz and Wertman(7), and Plotz, Reagan, 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 then added and the tubes incubated for 30 minutes at 37°C in a waterbath. The tests were read following secondary incubation and only 4^+ and 3^+ fixation were accepted as end-points. The controls necessary to insure valid results were included in all the titrations and serum dilution tests.

Results. The normal control rats at the end of the 6-week feeding period, all had

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

6. Plotz, H., *Science,* 1943, v97, 20.

7. Plotz, H., and Wertman, K., *Science,* 1942, v95, 441.

8. Plotz, H., Reagan, R., and Wertman, K., PROC. SOC. EXP. BIOL. AND MED., 1944, v55, 173.

9. Griffith, J. Q., and Farris, E. J., The Rat in Laboratory Investigations, Philadelphia, J. B. Lippincott, 1949, 2nd Ed.

TABLE II. Complement-fixation Titers and Body Weight Change of Rats in Vitamin Deficiency States.

Diet	No.	Complement-fixation titers following 1 inj.	3 inj.	Body wt g Init. avg, g	Final avg, g
Adequate Vit. B	1	1:40	1:1280		
	2	1:80	1:2560		
	3	1:20	1:1280	74	118
	4	Neg.	1:2560		
	5	1:20	—		
Total vit. B deficient	11	Neg.	—		
	12	1:20	—		
	13	1:20	1:320		
	14	1:20	1:1280	70	58
	15	1:10	1:640		
	16	1:20	1:320		
	17	1:10	1:640		
	18	1:10	1:320		
Pantothenic acid deficient	21	Neg.	1:320		
	22	Neg.	1:640		
	23	Neg.	1:1280		
	24	Neg.	1:320	68	102
	25	Neg.	1:1280		
	26	1:20	1:2560		
	27	1:20	—		
	28	1:20	1:2560		
Thiamin deficient	31	1:20	—		
	32	Neg.	1:160		
	33	1:40	1:640		
	34	1:20	1:640	69	68
	35	Neg.	1:320		
	36	Neg.	1:160		
	37	1:80	1:160		
	38	Neg.	1:320		

— Animals died during investigation.

gained weight, appeared healthy and possessed smooth even coats. The rats fed a diet containing only one-tenth of the optimum B complex content appeared to be in relatively poor condition. The normal control rats at the end of the 6-week period showed an average gain in weight of 128 g but the rats on the deficient diet showed an average gain in weight of only 36 g.

The rats used in the second experiment showed the usual signs which might be expected to accompany their respective deficiency. The initial average and final average weight of each group are recorded in Table II. The total deficient and thiamin deficient animals either lost weight or gained very little and the normal control and pantothenic acid deficient rats all showed gains in weight.

Table I demonstrates the end-point titers obtained in the complement fixation test using a washed suspension of murine typhus rickettsiae as the antigen. It appears that the ration containing one-tenth of the optimal level of vit. B complex did not seriously interfere with circulating antibody production when a relatively large amount of antigenic material was injected over a period of 4 weeks.

Table II demonstrates the end-point titers obtained in the complement fixation test using a murine typhus rickettsial antigen. It appears that the animals maintained on a pantothenic acid and thiamin deficient diet did not produce antibodies to the same extent as did those on a totally vit. B deficient diet, or those of the normal control group after the injection of only 1 cc of murine typhus vaccine. However, the sera specimens obtained after the administration of 3 cc of vaccine did contain demonstrable concentrations of complement-fixing antibodies. The animals were bled prior to immunization and tested by complement fixation. All of these sera were found to be negative with the rickettsial antigen used in this investigation.

Summary. These studies indicate that rats on a diet containing what is considered to be an optimum vit. B complex content have the ability to produce circulating complement fixing antibodies when immunized with relatively large amounts of the rickettsiae of murine typhus fever. Likewise, rats maintained on a diet containing only one-tenth of the presumed optimum also have the ability to produce these same antibodies to approximately the same titers. Pantothenic acid and thiamin deficiencies influenced the antibody response when a relatively small amount of immunizing material was injected. The influence is not as apparent when relatively large amounts of the antigenic material was employed.

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