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Vitamin B Complex Studies with Diets Differing in the Level of Protein.

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The level of dietary protein has been demonstrated by several investigators to be a factor in the metabolism of certain members of the vitamin B complex. The studies of Sarett and Perlzweig[1] and of Czaczkes and Guggenheim[2] have shown that on diets low in protein rats are incapable of retaining ingested riboflavin or maintaining tissue stores of this factor. The retention and storage of nicotinic acid similarly is influenced by the protein intake. Wright, Skeggs, and Sprague showed[3] that rats receiving liberal protein diets were less susceptible to the deficiency syndrome induced by feeding succinylsulfathiazole (SST) in *highly purified* diets. Such animals had higher hepatic stores of folic acid and pantothenic acid than control rats receiving the usual amount of protein in the diet. Nelson and Evans found[4] that pantothenic acid deficiency was less readily induced in rats receiving high protein diets than in those receiving diets of usual composition. The concentration of thiamine in the tissues of rats is not affected by the level of protein in the diet.[1] The requirement of rats for pyridoxine is increased when they are fed high protein diets.[5]

The studies reported in this paper were carried out for the purpose of obtaining additional information concerning the effect of the level of dietary protein on the metabolism of certain B vitamins in the rat. Diets used that were low in protein were, in consequence, deficient in essential amino acids. The coexistence of inadequate protein intakes and certain vitamin deficiencies frequently has been observed among various population groups.

Procedure. Three feeding experiments involving diets in which the level of protein was varied have been carried out. The low protein diet (5%) was compounded as follows: Casein, 50 g; sucrose, 748 g; salt mixture,[6] 40 g; cellus flour, 40 g; fat ('Primex'), 100 g; corn oil, 20 g; A-D-E concentrate,[3] 1 g; choline chloride, 1 g; vitamin K ('Menadione'), 10 mg; thiamine chloride, 8 mg; riboflavin, 16 mg; pyridoxine hydrochloride, 8 mg; nicotinic acid, 40 mg; calcium pantothenate, 44 mg; *p*-aminobenzoic acid, 40 mg; inositol, 216 mg. In the remaining diets, the protein level was increased, and succinylsulfathiazole was included at the expense of the sucrose. In the first experiment diets containing 5, 7, 10 and 18% casein and 18% casein plus 2% SST were fed *ad libitum* to male albino rats caged individually over wide mesh screening. Food intake determinations were carried out on representative rats throughout the course of the experiment. The second experiment was similar to the first except that controlled feeding was employed by arranging the rats in quintuplets and restricting the food intake of those receiving 7, 10 and 18% casein, and 18% casein plus 2% SST diets to that consumed by the animals receiving the 5% casein diet. The third experiment involved 3 groups of rats. The first received the 18% casein diet *ad libitum*. The second group was pair-fed with the first group and received the 30% casein diet. The third group received the 30% casein diet *ad libitum*. All animals were weighed at weekly intervals.

At intervals throughout the experiments 24-hour fecal collections were made. One-

1 Sarett, H. P., and Perlzweig, W. A., *J. Nutrition*, 1943, **25**, 173.

2 Czaczkes, J. W., and Guggenheim, K., *J. Biol. Chem.*, 1946, **162**, 267.

3 Wright, L. D., Skeggs, H. R., and Sprague, K. L., *J. Nutrition*, 1945, **29**, 431.

4 Nelson, M. M., and Evans, H. M., PROC. SOC. EXP. BIOL. AND MED., 1945, **60**, 319.

5 Cerecedo, L. R., and Foy, J. R., *Arch. Biochem.*, 1944, **5**, 207.

6 Hubbell, R. B., Mendel, L. B., and Wakeman, A. K., *J. Nutrition*, 1937, **14**, 273.

TABLE I.
Summary of Growth, Survival, and Food Intake Data.

Level of protein, %	Duration of exp., days	Change in wt, g	Survivors	Avg daily food intake, g
	Ad libitum feeding exper.			
5	76	−4	6/8	4.7 (50)*
7	76	25	7/8	5.7 (50)
10	76	83	8/8	9.3 (52)
18	76	173	8/8	12.1 (52)
18 + SST	76	89	2/8	8.4 (44)
	Controlled feeding exper.			
5	56	5	8/8	4.8 (448)
7	56	1	7/8	4.8
10	56	11	8/8	4.8
18	56	24	7/8	4 8
18 + SST	56	8	5/8	4.8
	High protein feeding exper.			
18 *ad lib.*	35	89	4/4	10.2 (140)
30 controlled	35	86	4/4	10.2
30 *ad lib.*	35	101	4/4	9.9 (44)

* Figures in parentheses represent number of determinations performed.

half of each sample was digested with takadiastase in pH 4.5 acetate buffer[7] prior to microbiological determination of folic acid, pantothenic acid, riboflavin, and nicotinic acid. The other half of each sample was autoclaved with 6N H_2SO_4 for one hour prior to microbiological assay for biotin. As an inhibitor of SST 10 mg of para aminobenzoic acid was added to each 100 ml of medium used for microbiological assay.

At intervals throughout the first 2 experiments and at the conclusion of the 3 experiments total and differential white counts were made. Blood was obtained by clipping the tail except for that obtained at the conclusion of the experiments when it was obtained following decapitation of the rats. Separate experiments have demonstrated that white cell and differential counts obtained on blood by decapitation are in agreement with corresponding counts made on tail blood. Terminally red blood cell counts, hemoglobin, and hematocrit determinations were made.

Following decapitation of the rats the livers were dissected out and prepared for microbiological assay. One-half of each liver in a fragmented state was digested with takadiastase under water[8] prior to assay for folic acid, pantothenic acid, riboflavin and nicotinic acid. The other half of each sample was autoclaved with 6N H_2SO_4 for one hour[9] prior to microbiological assay for biotin.

Folic acid[10] and riboflavin[11] were determined microbiologically with *L. casei*. Biotin,[12] pantothenic acid,[13] and nicotinic acid[14] were determined with *L. arabinosus*.

Results. The growth, survival, and food intake data are summarized in Table I. In the *ad libitum* experiment alopecia, porphyrin-caked whiskers, and ophthalmitis were seen in most of the rats receiving the 5% casein and 18% casein plus SST diets. These deficiency signs were observed in a few of the rats receiving the 7% casein diet. The re-

7 Cheldelin, V. H., Eppright, M. A., Snell, E. E., and Guirard, B. M., *The University of Texas Publication*, 1942, No. 4237, 15.

8 Wright, L. D., Skeggs, H. R., and Welch, A. D., *Arch. Biochem.*, 1945, **6**, 15.

9 Wright, L. D., and Welch, A. D., *J. Nutrition*, 1944, **27**, 55.

10 Landy, M., and Dicken, D. M., *J. Lab. and Clin. Med.*, 1942, **27**, 1086.

11 Snell, E. E., and Strong, F. M., *Ind. Eng. Chem., Anal. Ed.*, 1939, **11**, 346.

12 Wright, L. D., and Skeggs, H. R., PROC. SOC. EXP. BIOL. AND MED., 1944, **56**, 95.

13 Skeggs, H. R., and Wright, L. D., *J. Biol. Chem.*, 1944, **156**, 21.

14 Snell, E. E., and Wright, L. D., *J. Biol. Chem.*, 1941, **139**, 675.

maining animals were normal in appearance. Except for the smaller size of the rats restricted in food intake (Experiment 2) the appearance of the animals of the various groups was quite similar to that observed in the *ad libitum* experiment. The animals of the third experiment were normal in appearance.

The fecal assay data (Table II) demonstrate that in *ad libitum* feeding the amount of folic acid and biotin synthesized in the intestine is a function of the level of dietary protein. Likewise the fecal elimination of pantothenic acid, riboflavin, and nicotinic acid, originating in part from the diet and in part from bacterial synthesis in the intestine, parallels the level of dietary protein. The data from the controlled feeding experiment demonstrate that the increased amounts of these factors eliminated on the higher levels of protein result only in part from increased food intakes accompanying the ingestion of diets more nearly adequate. With equal food intakes more biotin, pantothenic acid, and nicotinic acid are eliminated as the level of dietary protein is increased. The data with respect to folic acid and riboflavin were inconclusive. It should be pointed out that the fecal elimination of folic acid and biotin encountered in the first experiment in the 5% casein groups was as low as that found on the 18% casein plus SST diets. The daily elimination of pantothenic acid, riboflavin, and nicotinic acid was not significantly altered by the ingestion of SST.

The hepatic storage data, summarized in Table III, likewise demonstrate that in *ad libitum* feeding the storage of folic acid, biotin, pantothenic acid, riboflavin, and nicotinic acid, based on γ/g content of the liver, parallels the level of dietary protein. Here again when the food intakes were reduced to those consumed by the animals receiving the 5% casein diets the hepatic storage of the 5 factors paralleled the level of dietary protein. Similarly it should be pointed out that the hepatic stores of folic acid and biotin encountered in the 5% casein groups were as low as those found on the 18% casein plus SST diets and were characteristic of defi-

TABLE II. Summary of Fecal Assay Data.

Level of protein, %	No. of samples	Folic acid, γ/rat/day	Biotin, γ/rat/day	Pantothenic acid, γ/rat/day	Riboflavin, γ/rat/day	Nicotinic acid, γ/rat/day
Ad libitum feeding experiment.						
5	5	1.5 (1.1 -1.6)*	0.20 (0.15-0.25)	10 (6-12)	4.4 (4.0- 5.1)	11 (8-13)
7	5	2.0 (1.7 -2.4)	0.30 (0.21-0.33)	12 (11-13)	5.7 (4.2- 6.4)	12 (11-12)
10	5	3.5 (2.5 -4.8)	0.35 (0.28-0.41)	20 (15-27)	8.4 (5.2- 9.4)	21 (15-23)
18	5	6.0 (5.0 -7.1)	0.61 (0.38-0.75)	28 (22-40)	11.4 (7.3-14.1)	33 (25-46)
18 + SST	5	1.5 (1.2 -1.9)	0.29 (0.26-0.33)	25 (17-29)	13.1 (9.0-17.1)	40 (27-46)
Controlled feeding experiment.						
5	4	1.3 (1.1 -1.7)	0.13 (0.10-0.14)	7 (5- 9)	4.9 (3.0- 6.2)	8 (6-11)
7	4	1.2 (1.0 -1.4)	0.16 (0.15-0.20)	10 (6-17)	4.2 (2.8- 5.2)	8 (6-12)
10	4	1.3 (1.0 -1.6)	0.26 (0.22-0.30)	11 (7-17)	3.7 (3.1- 4.1)	9 (6-15)
18	4	1.4 (1.2 -1.6)	0.24 (0.21-0.30)	14 (12-16)	3.6 (3.0- 4.3)	12 (10-16)
18 + SST	3	0.53 (0.36-0.66)	0.18 (0.17-0.19)	11 (10-12)	4.0 (3.3- 4.6)	14 (12-16)
High protein feeding experiment.						
18 *ad. lib.*	3	4.0 (3.3 -4.5)	1.4 (0.85-2.3)	22 (18-26)	7.1 (6.0- 8.0)	27 (23-32)
30 controlled	3	3.8 (3.7 -3.9)	1.1 (0.92-1.4)	28 (24-32)	8.1 (6.9- 9.1)	32 (25-36)
30 *ad lib.*	3	4.6 (3.9 -5.4)	1.4 (0.93-2.3)	30 (22-36)	9.4 (7.8-10.4)	37 (28-44)

* Range in values.

TABLE III. Summary of Hepatic Storage Data.

Level of protein	No. of animals	Folic acid, γ/g	Biotin, γ/g	Pantothenic acid, γ/g	Riboflavin, γ/g	Nicotinic acid, γ/g
Ad libitum feeding experiment.						
5	6	0.31 (0.19-0.49)*	0.60 (0.40-0.76)	64 (57- 80)	17 (14-20)	102 (93-115)
7	6	0.34 (0.23-0.48)	0.47 (0.11-0.91)	69 (41- 95)	16 (12-20)	106 (88-119)
10	8	0.58 (0.23-1.4)	0.56 (0.20-1.2)	97 (74-111)	21 (16-29)	127 (108-150)
18	8	0.65 (0.32-0.99)	0.74 (0.36-1.3)	122 (74-164)	30 (24-34)	182 (154-230)
18 + SST	2	0.15 (0.10-0.21)	0.46 (0.31-0.61)	66 (58- 74)	29 (29-29)	143 (139-147)
Controlled feeding experiment.						
5	7	0.35 (0.13-0.67)	0.60 (0.40-0.88)	58 (38- 78)	20 (16-26)	109 (82-127)
7	6	0.55 (0.35-1.26)	0.89 (0.73-1.22)	88 (69-121)	28 (20-44)	134 (97-205)
10	8	0.48 (0.19-1.55)	0.93 (0.46-1.61)	94 (67-120)	32 (20-43)	156 (133-179)
18	7	1.23 (0.26-2.34)	1.22 (0.45-1.82)	125 (90-152)	48 (35-59)	204 (184-215)
18 + SST	2	0.15 (0.14-0.16)	0.68 (0.56-0.81)	61 (53- 69)	36 (31-41)	182 (175-189)
High protein feeding experiment.						
18 *ad lib.*	4	0.20 (0.11-0.32)	0.65 (0.57-0.78)	115 (98-141)	31 (24-38)	175 (139-193)
30 controlled	4	0.43 (0.36-0.55)	0.70 (0.64-0.80)	139 (120-151)	32 (31-35)	154 (148-164)
30 *ad lib.*	4	0.34 (0.19-0.44)	0.52 (0.44-0.56)	130 (105-167)	28 (24-31)	144 (137-154)

* Range in values.

ciencies of these factors.[9] Thus a folic acid deficiency was induced indirectly by a reduction in the level of dietary protein. In the *ad libitum* experiment, increasing the level of dietary protein increased the average hepatic storage of folic acid to levels previously established as borderline with respect to a folic acid deficiency.[9] In the second experiment hepatic stores of folic acid were encountered on the higher levels of casein in about half of the animals that were such that a folic acid deficiency should not have been expected.[9] The fecal elimination data demonstrated that these stores of folic acid probably did not come about from increased synthesis of folic acid in the intestine. It is reasonable to conclude that on higher levels of protein intake the folic acid available from bacterial synthesis in the intestine was utilized more efficiently or retained to a greater extent by the animals. It is suggested that a deficiency of certain amino acids or of total nitrogen was responsible for the leucopenia and agranulocytosis that was observed in certain of the animals with the higher hepatic stores of folic acid, although this is not established definitely by the data presented. The recent experiments of Kornberg demonstrating the importance of certain amino acids in blood cell production substantiate this hypothesis.[15]

Although not all animals encountered in the 5% casein or the 18% casein plus SST diets were found to have hepatic stores of pantothenic acid characteristic of a deficiency in this factor,[9] the values found were generally low and emphasize the importance of an adequate protein intake for the maintenance of a normal tissue concentration of this factor. The high renal clearance of administered calcium pantothenate and the normal occurrence of this factor in a combined state in blood and other tissues[16] suggest that in conditions of protein deprivation a substrate for pantothenic acid combination is not made available. The observations made here with respect to protein level

[15] Kornberg, A., *Fed. Proc.*, 1946, **5**, 142.

[16] Wright, L. D., Beyer, K. H., Skeggs, H. R., Russo, H. F., and Patch, E. A., *Am. J. Physiol.*, 1946, **145**, 633.

TABLE IV.
Summary of White Cell Data.

Level of protein, %	Duration of feeding, days	Total count, thous. per mm³	Neutrophils, thous. per mm³
		Ad libitum feeding experiment.	
5	11	13.6 (7.8-23.9)* (8)†	2.3 (1.2 -5.8) (8)
5	28	12.5 (8.3-21.3) (8)	1.6 (0.5 -8.9) (8)
5	43	13.3 (5.9-26.7) (7)	1.3 (0.4 -2.4) (7)
5	56	11.5 (6.9-21.3) (7)	1.2 (0.2 -2.5) (7)
5	70	7.7 (4.4-12.3) (7)	0.6 (0.2 -1.1) (7)
5	76	4.8 (3.0- 6.0) (3)	0.3 (0.2 -0.5) (3)
5	82	5.0 (2.6- 6.6) (3)	0.6 (0.2 -0.9) (3)
7	11	17.2 (13.3-21.8) (8)	2.3 (0.7 -4.3) (8)
7	28	14.2 (9.8-22.5) (8)	1.4 (0.4 -3.5) (8)
7	43	13.4 (8.0-18.2) (8)	1.5 (0.3 -4.3) (8)
7	56	10.5 (6.3-15.9) (7)	1.4 (0.2 -3.1) (7)
7	70	10.3 (6.1-18.8) (7)	1.0 (0.2 -2.7) (7)
7	76	6.5 (3.7-11.8) (4)	1.0 (0.1 -2.9) (4)
7	82	6.7 (5.6- 7.7) (2)	0.8 (0.2 -1.4) (2)
10	11	18.0 (13.5-27.8) (8)	2.3 (1.3 -3.1) (8)
10	28	16.8 (9.7-23.7) (8)	1.8 (0.7 -2.9) (8)
10	43	16.9 (12.9-25.2) (7)	2.1 (1.3 -3.2) (7)
10	56	15.9 (12.5-20.3) (8)	2.4 (1.5 -3.4) (8)
10	70	13.0 (7.2-20.1) (8)	1.7 (0.3 -2.9) (8)
10	76	8.7 (4.9-11.6) (4)	1.1 (0.2 -2.1) (4)
10	82	8.3 (7.1-11.3) (4)	0.7 (0.3 -1.3) (4)
18	11	16.2 (8.6-20.9) (7)	2.2 (1.4 -2.8) (7)
18	28	19.7 (14.1-29.5) (8)	1.9 (0.8 -3.2) (8)
18	43	16.2 (13.4-20.9) (8)	1.8 (0.7 -2.3) (8)
18	56	18.9 (14.6-25.5) (8)	2.1 (1.3 -2.9) (8)
18	70	18.1 (16.0-27.8) (8)	2.7 (1.1 -3.8) (8)
18	76	12.0 (10.6-13.1) (3)	1.5 (1.2 -1.9) (3)
18	82	9.7 (7.8-12.7) (5)	1.2 (0.7 -2.0) (5)
18 + SST	11	11.1 (7.3-15.2) (8)	1.1 (0.4 -2.3) (8)
18 + SST	28	11.5 (6.5-17.1) (8)	1.4 (0.1 -2.7) (8)
18 + SST	43	8.4 (5.7-11.0) (7)	0.8 (0.2 -1.8) (7)
18 + SST	56	5.9 (1.7-10.8) (7)	0.4 (0.02-0.7) (7)
18 + SST	70	6.5 (4.2- 8.6) (3)	0.8 (0.3 -1.7) (3)
18 + SST	76	3.7 (3.4- 4.1) (2)	0.2 (0.2 -0.2) (2)
		Controlled feeding experiment.	
5	31	14.3 (6.9-22.2) (8)	1.3 (0.5 -3.0) (8)
5	56	7.4 (3.6- 9.6) (4)	0.5 (0.3 -0.9) (4)
5	67	4.9 (3.4- 6.1) (3)	0.4 (0.03-0.6) (3)
7	31	16.6 (11.5-25.5) (8)	1.4 (0.9 -1.7) (8)
7	56	6.6 (5.9- 7.8) (3)	0.7 (0.6 -0.9) (3)
7	67	5.8 (3.6- 7.7) (3)	0.4 (0.3 -0.6) (3)
10	31	17.0 (12.1-23.4) (8)	1.8 (0.3 -5.3) (8)
10	56	5.3 (4.5- 6.5) (4)	0.7 (0.3 -1.1) (4)
10	67	4.4 (2.7- 6.2) (4)	0.2 (0.04-0.4) (4)
18	31	13.6 (4.7-19.7) (8)	1.0 (0.5 -1.6) (8)
18	56	4.6 (4.0- 5.6) (3)	0.4 (0.4 -0.5) (3)
18	67	5.4 (3.9- 8.0) (4)	0.4 (0.03-0.8) (4)
18 + SST	31	6.2 (4.4- 7.3) (7)	0.5 (0.04-0.9) (7)
18 + SST	56	2.4 (1.9- 2.9) (2)	0.1 (0.1 -0.2) (2)
		High protein feeding experiment.	
18 *ad lib.*	35	7.3 (5.3- 8.4) (4)	0.7 (0.4 -1.0) (4)
30 controlled	35	7.2 (4.3-11.1) (4)	0.5 (0.2 -1.0) (4)
30 *ad lib.*	35	9.5 (6.7-13.1) (4)	0.9 (0.7 -1.3) (4)

* Range in values.
† No. of determinations.

TABLE V.
Summary of Red Cell Data.

Level of protein %	No. of animals	Hematocrit, % avg	Hemoglobin, g/100 ml avg	RBC, million/mm³ avg
		Ad libitum feeding exper.		
5	6	42	14.2	8.6
7	6	45	16.4	9.3
10	8	45	16.4	9.0
18	8	47	18.0	8.9
18 + SST	2	43	15.2	7.6
		Controlled feeding exper.		
5	7	36	12.9	7.4
7	6	43	15.5	8.6
10	8	44	15.9	8.3
18	7	47	17.5	8.8
18 + SST	2	41	16.4	6.7
		High protein feeding exper.		
18 *ad lib.*	4	44	15.9	9.5
30 controlled	4	46	16.5	8.4
30 *ad lib.*	4	45	17.3	8.7

and tissue storage of pantothenic acid confirm similar observations made earlier[3] and offer a possible explanation for the increased resistance of rats receiving high protein diets to induced pantothenic acid deficiency as recently observed by Nelson and Evans.[4]

The low hepatic stores of riboflavin observed in rats receiving diets low in protein confirm the findings of Sarett and Perlzweig[1] and of Czaczkes and Guggenheim.[2] In the controlled feeding experiment the ingestion of the 18% casein diet in amounts resulting in severe restriction of growth was accompanied by hepatic stores of riboflavin in excess of that encountered in stock rats.[17]

The white cell data have been summarized in detail (Table IV) for the purpose of demonstrating the relative rate at which leucopenia and agranulocytosis were induced on the various rations. From the first experiment it may be seen that ingestion of the 5% casein diet induced low total and neutrophil counts at approximately the same rate as did ingestion of the SST-containing diet. As might have been expected from the fecal and hepatic assays, when diets of progressively increasing protein content were ingested, the development of leucopenia and agranulocytosis correspondingly was retarded, and at the conclusion of the experiment was less severe. In the second experiment leucopenia and agranulocytosis developed at approximately the same rate in all the groups except for the SST-fed rats where a combination of food restriction and sulfonamide ingestion resulted in a severe and rapid development of the abnormal blood condition.

The red cell data (Table V) demonstrate that the feeding of low protein or the SST-containing diet was accompanied by occasional low hematocrits, low hemoglobin values, and low red blood cell counts. In a deficiency of folic acid in the rat impairment of white cell formation rather than of red cell or hemoglobin production is the most characteristic blood dyscrasia observed.[18]

Summary. Experiments involving the feeding to rats of diets varying in the level of casein were carried out. The following facts were established:

1. In *ad libitum* feeding the fecal elimination of the B vitamins studied closely paralleled the level of dietary protein. In equalized feeding the fecal elimination of biotin, pantothenic acid, and nicotinic acid was directly proportional to the protein content of the diet.

2. The hepatic storage of the B vitamins studied was correlated directly with the pro-

[17] Wright, L. D., McMahan, J. R., Cheldelin, V. H., Taylor, A., Snell, E. E., and Williams, R. J., *The University of Texas Publication,* 1941, No. 4137, 38.

[18] Kornberg, A., Tabor, H., and Sebrell, W. H., *Am. J. Physiol.,* 1944, **142**, 604.

tein content of the diet. This relationship existed both in *ad libitum* and controlled feeding experiments. The hepatic stores of folic acid, biotin, pantothenic acid, and riboflavin found in rats fed the lower levels of protein were indicative of either frank or incipient deficiencies.

3. Leucopenia and agranulocytosis were induced readily by diets low in protein. Under these conditions the existence of the dyscrasia was correlated with the reduced fecal elimination and hepatic storage of folic acid. When diets more nearly optimal with respect to casein level, but in greatly restricted amounts, were fed, leucopenia and agranulocytosis were encountered despite the existence in many of the animals of adequate stores of folic acid. Reasons were presented for attributing the condition to the ingestion of insufficient protein and, more specifically, of certain essential amino acids.

4. Low hematocrits, hemoglobin values, and red cell counts were observed occasionally in rats ingesting low protein diets or *highly purified* diets containing succinylsulfathiazole (SST).

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pH Stability of St. Louis Encephalitis Virus.*

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Studies on several viruses have indicated that for each virus there is a range of hydrogen ion concentration over which the virus is stable and that at higher or lower pH values the virus activity is destroyed.[1-6] The observation[6] that Japanese B encephalitis virus is most stable at a hydrogen ion concentration near pH 8.5, while the other viruses so far studied were more stable in a less alkaline medium, suggested that the effect of hydrogen ion concentration on St. Louis encephalitis virus (S.L.E.) be studied to determine whether the pH at which the latter virus is stable is similar to that reported for the virus of Japanese B encephalitis. The purpose of the present communication is to report the experiments conducted to determine the hydrogen ion concentration at which St. Louis encephalitis virus is most stable.

The virus used was the Webster No. 3 strain of St. Louis encephalitis virus.† The suspension of virus used in the study was prepared as follows: Swiss mice (3-4 weeks old) were injected intracerebrally with 0.03 cc of a 10% saline suspension of mouse-brain virus. On the 3rd day following inoculation the mice showed signs characteristic for mice infected with S.L.E. The brains of 5 mice were removed and ground in a mortar with sand and sufficient saline to make a 10% suspension of infected mouse brains. Following centrifugation at 1,500 r.p.m. for 5 minutes, 10-fold serial dilutions of the supernatant fluid were made in saline containing 10% rabbit serum. The virus suspension was then titrated intracerebrally in

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† Kindly supplied by Dr. A. B. Sabin.