when injected 15 minutes before or after the virus, less inhibition was observed. The fact that no inhibition of virus growth was demonstrable after 48 hours incubation further suggests that the polypeptides combine with the virus but active virus is subsequently released.

The inhibition of tobacco mosaic virus infectivity(2) and this inhibition of the production of influenza virus hemagglutinins by the lysine polypeptides suggest that these or similar synthetic polypeptides might be active against other viruses. The increased activities of the DL-lysine polypeptide and of the DL-lysine-DL-valine polypeptide suggest that the stereochemical structure as well as the amino acid composition of such synthetic polypeptides might be varied in order to produce greater antiviral activity.

Preliminary experiments failed to show a significant chemotherapeutic effect against influenza virus in mice when the polypeptides were administered as an aerosol.

Summary. Various synthetic high molecular weight lysine polypeptides were found to inhibit the growth of influenza virus in the chick embryo.

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Circulating Antibodies in Vitamin-Deficiency States. Pantothenic Acid and Pyridoxine Deficiencies.* (18589)

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A severe impairment of antibody response has been observed in pyridoxine(1,2) and pantothenic acid-deficient rats(1). Riboflavin, biotin, and thiamine deficiencies had considerably less effect(1,3,4). In our later experiments(5), particular emphasis was placed upon the use of "paired-weighed" in lieu of "paired-fed" rats as controls to the pantothenic acid-deficient animals. The evidence obtained proved conclusively that the absence of pantothenic acid *per se* and not the concomitant inanition was responsible for the decreased serum antibody titers. In addition, the pronounced effects of a relatively mild pantothenic acid deficiency were also demonstrated. It is generally recognized that a decreased antibody titer in serum is not unequivocal proof for the actual impairment of antibody synthesis. Other factors such as the effects of the vitamin deficiency upon the processes of antibody destruction and distribution must be evaluated before a definite relationship between vitamin and antibody synthesis can be established. Thus, a dietary inadequacy of pantothenic acid or pyridoxine may result in: (1) an actual impairment of antibody synthesis; (2) an accelerated rate of antibody destruction; or (3) a failure to release and distribute antibodies from their site of formation into the blood stream. Any one of these three mechanisms could produce a lowered serum antibody concentration.

The present experiments were, therefore, undertaken in an effort to shed some light on

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2. Stoerk, H. C., and Eisen, H. N., *Ibid.*, 1946, v62, 88.

3. Carter, B. B., and Axelrod, A. E., *Ibid.*, 1948, v67, 416.

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

5. Ludovici, P. P., Axelrod, A. E., and Carter, B.B., PROC. SOC. EXP. BIOL. AND MED., 1949, v72, 81.

TABLE I. Effect of Pantothenic Acid Deficiency Upon Serum Content of Preformed Antibody.*

Rat No.	Weeks on experiment after the initial bleeding						
	0	2	4	7	10	13	18
			Group I†				
1	1:640	1:640	1:2560	1:2560	1:2560	—	—
2	1:320	1:640	1:1280	1:2560	1:1280	1:2560	1:1280
3	1:320	1:320	1:2560	1:1280	1:1280	1:2560	1:1280
4	1:160	1:640	1:1280	1:2560	1:2560	1:640	1:2560
			Group II†				
5	1:640	1:640	1:2560	1:2560	1:2560	—	—
6	1:320	1:320	1:2560	1:2560	1:2560	1:2560	1:1280
7	1:160	1:640	1:2560	1:1280	1:1280	1:2560	1:640
8	1:320	1:320	1:640	1:2560	1:2560	1:2560	1:2560

* Individual hemagglutinin titers are presented.

† Group I, inanition control animals No. 1, 2, 3, and 4 were "paired-weighed" with Group II, pantothenic acid-deficient rats No. 5, 6, 7, and 8, respectively.

the mechanisms which may be operative in the impaired response of pantothenic acid and pyridoxine-deficient rats to the antigenic stimulus of human erythrocytes.

Methods. Male weanling albino rats of the Sprague-Dawley strain were employed throughout this investigation. The animals were housed individually in wide-meshed, screen-bottom cages. All rats were fed a basal diet with the following percentage composition: sucrose, 56.76; Labco "vitamin free" casein, 25.00; salts(6), 4.00; cod-liver oil, 2.00; hydrogenated vegetable oil, 10.00; corn oil, 2.00; choline chloride, 0.20; *i*-inositol, 0.03; *p*-amino-benzoic acid, 0.01; *dl*-α-tocopherol acetate, 0.01; and 2-methyl-1,4-naphthoquinone, 0.001. All animals received additional vitamins in the form of a daily pill. Each of the pills fed to the control rats supplied the following vitamins: thiamin, 40 γ; riboflavin, 60 γ; pyridoxine, 5 or 50 γ; nicotinic acid, 100 γ; calcium pantothenate, 100 or 300 γ; biotin, 1 γ; and folic acid, 1 γ. The basal diet plus the complete vitamin pill will be referred to as the control diet in this paper. Unless otherwise specified, the complete vitamin pill contained 50 γ of pyridoxine and 300 γ of calcium pantothenate. For the pantothenic acid and pyridoxine-deficient animals, the appropriate vitamin was omitted from the pill. The procedure of "paired-weighing" consisted of restricting the amount of basal diet consumed by the control animal to a quantity sufficient to maintain its weight equal to that of its vitamin-deficient partner. Deficient rats were always fed *ad libitum*. Immunization was accomplished by injecting each rat intraperitoneally with a 10% suspension of Group O, Rh positive human erythrocytes in physiological saline. An initial dosage of 0.5 ml of the red blood cell suspension was followed by 2 one ml inoculations on alternate days. Five days after the final injection and at specified intervals thereafter the animals were bled from the heart under nembutal anesthesia.‡ The sera were tested for agglutinin titer utilizing serial two-fold dilutions as previously described(1). End point titers as observed in this laboratory are subject to an error of one dilution in either direction.

Experimental. A. *Effect of pantothenic acid or pyridoxine deficiency upon serum content of preformed antibody.* In these experiments, we investigated the possibility that the impairment of antibody response observed in pantothenic acid and pyridoxine deficiencies might be due to an increased rate of antibody destruction.

1. *Pantothenic acid deficiency.* Eight weanling rats were fed the control diet containing 100 γ of pantothenic acid *ad libitum* for 23 days. Immunization was instituted on the 14th day. After the initial antibody determinations, the results of which are shown in Column 2, Table I, the animals were paired

o. Jones, J. H., and Foster, C. J., *J. Nutrition*, 1942, v24, 245.

‡ Three mg per 100 g body weight were injected intraperitoneally.

according to titer into two equal groups. One group received the control diet minus pantothenic acid, while the other received the control diet containing 300 γ of pantothenic acid daily. Members of the latter group were "paired-weighed" with those in the former group. Serum antibody titers were determined periodically until the deficient rats succumbed. Following transfer to the deficient diet, the animals (average weight at the initial bleeding = 185 g) gained weight slowly until a maximum average weight of 300 g was attained after approximately 12 weeks on this regimen. Thereafter, the rats gradually lost weight until the experiment was terminated at 18 weeks, (final average weight = 240 g). One deficient rat succumbed during the 12th week while the remaining three were moribund at the final bleeding. Typical pantothenic acid deficiency symptoms were observed in these animals approximately one month prior to death. The "paired-weighed" controls, although severely starved, survived the experimental period. It may be seen in Table I that during the course of the experiment there was no significant difference between the antibody titers of the pantothenic acid-deficient rats and those of their inanition controls. It is of interest to note that relatively high antibody titers were demonstrable 18 weeks after immunization. We have similarly observed that normal rats fed the control diet *ad libitum* exhibited high antibody levels seven months after immunization (unpublished observations). It can be further seen in Table I that the initial titers were relatively low increasing to normal levels 4 weeks after the initial bleeding. A similar influence of age upon antibody formation in *young* normal rats has been observed frequently in this laboratory. Freund(7) has made comparable observations in rabbits.

2. *Pyridoxine deficiency.* A similar experiment to determine the effect of a pyridoxine deficiency upon preformed antibody was conducted. Ten weanling rats were fed the control diet containing 5 γ of pyridoxine *ad libitum* for 31 days. Immunization was instituted on the 22nd day of the experiment.

7. Freund, J., *J. Immunol.*, 1930, v18, 315.

TABLE II. Effect of Pyridoxine Deficiency Upon the Serum Content of Preformed Antibody.*

Rat No.	Weeks on experiment† 0	4	8	12
	Group I‡			
1	1:640	1:2560	1:2560	1:2560
2	1:2560	1:2560	1:2560	1:2560
3	1:2560	1:2560	1:2560	1:2560
4	1:2560	1:2560	1:2560	1:2560
5	1:2560	1:2560	1:1280	1:1280
	Group II‡			
6	1:2560	1:2560	1:2560	1:1280
7	1:2560	1:2560	1:1280	1:640
8	1:2560	1:2560	1:1280	1:640
9	1:2560	1:2560	1:2560	1:2560
10	1:2560	1:2560	1:2560	1:1280

* Individual hemagglutinin titers are presented.
† Weeks after the initial bleeding.
‡ Group I, inanition control animals No. 1, 2, 3, 4, and 5 were "paired-weighed" with Group II, pyridoxine-deficient rats No. 6, 7, 8, 9, and 10, respectively.

Following the initial bleeding, the animals were paired according to weight into two equal groups. "Paired-weighed" controls received the control diet containing 50 γ of pyridoxine daily, whereas pyridoxine was omitted from the diet of the remaining animals. Blood samples for antibody titration were taken periodically until the experiment was terminated.

Following the removal of pyridoxine from the diet, the rats (average weight at the initial bleeding = 145 g) continued to gain weight slowly until a maximum average weight of 200 g was attained after approximately 10 weeks. Thereafter, the animals gradually lost weight until the experiment was terminated at 12 weeks, (final average weight = 190 g). Incipient symptoms of pyridoxine deficiency became apparent during the 9th week and were well developed by the 12th. All deficient rats as well as their inanition controls survived the experimental period. It may be seen in Table II that there was no significant difference between the antibody titers of the pyridoxine-deficient rats and those of their inanition controls during the three month period. These results were comparable to those observed in the previous experiment where pantothenic acid was removed from the diet (Table I).

B. *Effect of adrenal cortical extract or*

vitamin therapy upon serum antibody levels of vitamin-deficient rats. These experiments were concerned with the possible role of pantothenic acid or pyridoxine in the release and distribution of antibodies from their site of formation into the blood stream.

1. *Effect of aqueous adrenal cortical extract§ (A.C.E.) upon serum antibody titers of pantothenic acid-deficient rats.* Pathological changes in the adrenal cortex, as well as a probable depletion of adrenal cortical hormones, have been demonstrated in pantothenic acid-deficient rats(8-11). Since adrenal cortical activity may be concerned with the liberation of antibodies from their site of formation(12), it is conceivable that the lowered serum antibody titers of pantothenic acid-deficient rats could be attributed to a decreased activity of the adrenal cortex. This hypothesis was tested as follows: Seven weanling rats were fed *ad libitum* the control diet minus pantothenic acid. "Paired-weighed" partners of each of these rats received the control diet containing 300 γ of pantothenic acid daily throughout the experiment. Immunization was instituted in all animals after 6 weeks. Four of the 7 animals in each group were injected subcutaneously with one ml of A.C.E. immediately following each injection of the antigen. It is evident from the results presented in Table III that A.C.E. had no effect upon the antibody titers of either inanition controls or pantothenic acid-deficient rats. No influence of the extract upon the growth of pantothenic acid-deficient animals was observed. In a similar experiment except that A.C.E. was administered in a single 2 ml dose three weeks *following* immunization, the extract was again ineffective in elevating the antibody titers of 4 "paired-weighed" controls and 4 pantothenic acid-deficient animals. In this case antibody titers were determined 5 and 18 hours following A.C.E. injection. These results, therefore, furnish no support for the suggestion that the activity of pantothenic acid in antibody fabrication is mediated through the adrenal cortex.

TABLE III. Influence of Aqueous Adrenal Cortical Extract Upon Serum Antibody Titers.*

Group I† Inanition controls	Group II† Inanition controls + A.C.E.‡	Group III Pantothenic acid-deficient	Group IV Pantothenic acid-deficient + A.C.E.‡
1:2560	1:1280	1:80	1:20
1:2560	1:2560	1:80	1:20
1:1280	1:1280	1:80	1:80
	1:5120		0

* Titers for individual rats are presented.
† Inanition controls received 300 γ of pantothenic acid daily. Rats of Groups I and II were "paired-weighed" with those of Groups III and IV, respectively, throughout experiment.
‡ A.C.E. = Aqueous adrenal cortical extract.

2. *Pantothenic acid therapy.* Seven weanling rats fed the pantothenic acid-deficient diet for 6 weeks were immunized and their serum antibody titers determined. As indicated in the 2nd column of Table IV detectable titers were observed in only 2 animals. Four days later the deficient rats were injected intraperitoneally with 10 mg of calcium pantothenate on 2 successive days. In addition, these animals received 300 γ of pantothenic acid daily *per os* for the remainder of the experiment. Blood samples for antibody determinations were taken periodically as indicated in Table IV. It is evident that the effect of pantothenic acid upon serum antibody levels is very gradual, becoming perceptible only after approximately 23 days of therapy. It is of interest that, although the antibody response to pantothenic acid was delayed, the growth stimulating effects of this vitamin were apparent immediately. Thus, the average daily weight gain during the 9 days following pantothenic acid injection was 7 g. Five normal controls maintained a constant antibody titer of 1:2560 throughout the investigation. An identical experiment employing 18 rats yielded the same results.

3. *Pyridoxine therapy.* In this study a procedure similar to the one just described

§ Parke, Davis and Co.

8. Daft, F. S., and Sebrell, W. H., *Pub. Health Rep.*, 1939, v54, 2247.
9. Daft, F. S., and Sebrell, W. H., *Ibid.*, 1940, v55, 1333.
10. Ashburn, L. L., *Ibid.*, 1940, v55, 1337.
11. Deanne, H. W., and McKibbin, J. M., *Endocrinology*, 1946, v38, 385.
12. Chase, J. H., White, A., and Dougherty, T. F., *J. Immunol.*, 1946, v52, 101.

TABLE IV. Effect of Pantothenic Acid Therapy Upon Antibody Titers of Rats Previously Immunized While in a Pantothenic Acid Deficiency State.*

Rat No.	Initial titer‡	Days on experiment†					
		1	9	23	37	51	65
1	0	0	0	1:40	1:160	1:320	1:320
2	0	0	0	1:40	1:320	1:640	1:640
3	0	0	0	1:40	1:640	1:640	1:640
4	1:40	0	0	0	1:40	1:160	1:160
5	0	0	1:20	1:20	1:1280	1:2560	1:2560
6	0	0	0	1:40	1:640	1:640	1:640
7	1:80	1:40	1:40	1:80	1:1280	1:2560	1:2560

* Individual serum antibody titers are presented.
† Days after the second vitamin injection.
‡ Initial titers were determined 4 days prior to pantothenic acid administration.

TABLE V. Effect of Pyridoxine Therapy Upon Antibody Titers of Rats Previously Immunized While in a Pyridoxine Deficiency State.*

Rat No.	Initial titer‡	Days on experiment†					
		3	11	32	46	60	82
1	0	0	0	1:160	1:160	1:640	1:640
2	1:10	1:20	1:20	1:160	1:320	1:640	1:640
3	1:40	1:40	1:80	1:640	1:640	1:640	1:640
4	1:40	1:40	1:160	1:1280	1:1280	1:2560	1:2560
5	1:80	1:80	1:160	1:640	1:640	1:1280	1:2560
6	1:20	1:80	1:80	1:1280	1:2560	1:2560	1:2560
7	1:80	1:80	1:160	1:320	1:640	1:640	1:1280

* Individual serum antibody titers are presented.
† Days after the pyridoxine injection.
‡ Initial titers were determined 2 days prior to pyridoxine administration.

was employed. Seven weanling rats fed the pyridoxine-deficient diet for 5 weeks were immunized and their antibody titers ascertained (column II, Table V). Two days later these deficient animals were injected intraperitoneally with 5 mg of pyridoxine and for the remainder of the experiment they received 50 γ of the vitamin daily *per os*. Periodic antibody determinations revealed (Table V) that the administration of pyridoxine resulted in a gradual elevation of antibody levels, the effect becoming pronounced in most animals only on the 32nd day. This response to pyridoxine was, therefore, comparable to that obtained with pantothenic acid therapy (Table IV). Pyridoxine administration also resulted in a marked growth stimulation in all deficient rats as evidenced by an average daily weight gain of 7 g during the first 11 days following the vitamin injection. Five normal control animals maintained a constant antibody titer of 1:2560 throughout the experiment.

Discussion. The data presented in this paper demonstrate that rats immunized while receiving a complete diet were able to maintain high serum antibody titers during a protracted period in which either pantothenic acid or pyridoxine was removed from the diet. This finding suggests that neither a pantothenic acid nor a pyridoxine deficiency results in an accelerated rate of antibody destruction. In view of the fact that pronounced deficiencies of these vitamins were produced during the experimental period, it does not seem likely that an accelerated rate of antibody destruction would be counterbalanced by a corresponding increase in the rate of antibody synthesis. Further information on this point could be gained by the utilization of "tagged" antibodies to compare the "turnover" rate of serum antibodies in normal and vitamin-deficient rats(13,14).

The previous observations that a suppression of circulating antibodies occurs in panto-

13. Schoenheimer, R., Ratner, S., Rittenberg, D., and Heidelberger, M., *J. Biol. Chem.*, 1942, v144, 545.

thenic acid(1,5) or pyridoxine-deficient(1,2) rats have been further verified in the present experiments. The lag in the increase of serum antibody titers following vitamin therapy in such rats appears to indicate that neither pantothenic acid nor pyridoxine is concerned with the release of preformed antibodies. In this connection Daft *et al.*(9) and Ashburn(10) have presented histological evidence that adrenal hemorrhage, atrophy, and necrosis in pantothenic acid-deficient rats can be repaired in 6 to 14 days by the daily administration of 100 γ of pantothenic acid. In the present experiments, although the pantothenic acid-deficient rats received 20 mg of pantothenic acid intraperitoneally and 300 γ of the vitamin daily *per os*, the serum antibody titers remained negligible until the 23rd day at which time they gradually began to increase reaching normal levels only after 51 days of vitamin therapy (Table IV). If pantothenic acid were concerned with a release or distribution mechanism functioning through the adrenal cortex a more immediate elevation of serum antibody titers to normal values would be expected. The gradual increase in serum antibody levels following pantothenic acid or pyridoxine therapy may be attributed to the corresponding slow rate of antibody synthesis catalyzed by the vitamin.

The evidence accumulated to date, albeit indirect in nature, suggests that both pantothenic acid and pyridoxine are concerned with the processes of antibody synthesis.

Summary. 1. Rats immunized while receiving a complete diet maintained high serum antibody titers during a protracted period of time in which either pantothenic acid or pyridoxine was removed from the diet. 2. Subcutaneous administration of aqueous adrenal cortical extract either immediately following each antigen injection or 3 weeks *after* immunization was ineffective in increasing the serum antibody titers of either pantothenic acid-deficient rats or their inanition controls. 3. Administration of massive doses of pantothenic acid or pyridoxine to rats immunized while in a corresponding vitamin deficiency state produced only a very gradual increase in serum antibody titers. Relatively normal levels were reached approximately two months after vitamin repletion. 4. These findings suggest that pantothenic acid and pyridoxine function in the processes of antibody synthesis.

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Circulating Antibodies in Vitamin Deficiency States. Pantothenic Acid-sparing Action of DL-Methionine. (18590)

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Previous experiments have shown that a dietary source of pantothenic acid is required for the production of circulating antibodies in the rat(1,2). Nelson and co-workers(3,4) have demonstrated the sparing action of high casein diets upon the pantothenic acid requirements of the rat for growth and survival. Recently, these authors(5) reported that DL-

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