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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(Introduced by Ralph R. Mellon)

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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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methionine also possessed a marked pantothenic acid-sparing action. It, therefore, seemed of interest to determine whether DL-methionine could also spare the requirement of pantothenic acid for antibody production.

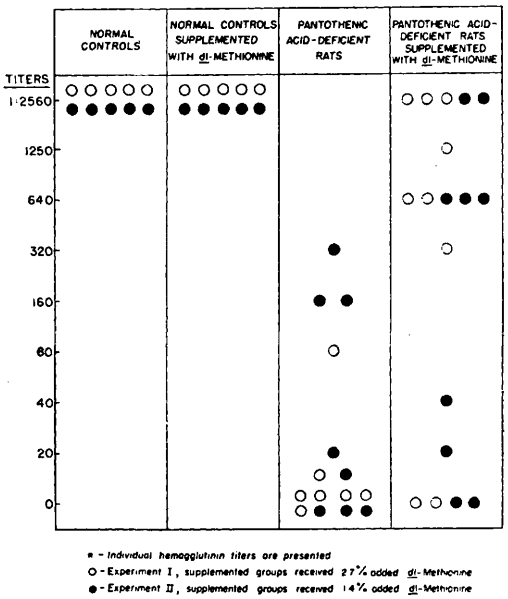
The effect of β-alanine upon antibody response in pantothenic acid-deficient rats has also been investigated.

Methods. The diets of the control and pantothenic acid-deficient animals were identical with those described in the preceding paper(6). The control diet (containing the complete vitamin supplement) furnished 300 γ of pantothenic acid and 50 γ of pyridoxine daily. DL-methionine was added to the diet at the expense of the sucrose, while the desired daily dosage of β-alanine was furnished in the vitamin pill. Male weanling albino rats were employed and, when indicated, their diets were supplemented with either DL-methionine or β-alanine from the onset of the experiment. The antigen, immunization procedure, and the determination of serum antibody titers were identical with those described in the preceding paper(6).

Experimental. A. *Pantothenic acid-sparing action of* DL-*methionine.* These studies were performed to determine the effect of DL-methionine upon the antibody response of pantothenic acid-deficient as well as normal rats. Two separate experiments, identical except for the DL-methionine content of the supplemented diets, were conducted. Levels of 2.7 and 1.4% DL-methionine were added to the diets of the DL-methionine supplemented groups of experiments I and II respectively. In each experiment the animals were distributed into 4 groups as indicated in Table I. All rats were fed their respective diets *ad libitum* throughout the investigation. The animals were immunized during the 7th week on experiment.

TABLE I

The Effect of dl-Methionine upon Serum Antibody Titers *



The individual hemagglutinin titers presented in Table I reveal that none of the unsupplemented pantothenic acid-deficient animals possessed serum antibody titers higher than 1:320. In contrast 60% of the pantothenic acid-deficient rats supplemented with DL-methionine exhibited titers of 1:640 or higher. It is also evident that additional DL-methionine had no effect upon the antibody titers of normal controls.

Supplementation with DL-methionine had no influence upon (1) the growth of either pantothenic acid-deficient rats or normal controls and (2) the severity of the pantothenic acid deficiency symptoms. Five of the 20 pantothenic acid-deficient rats and 2 of a comparable DL - methionine - supplemented group succumbed prior to immunization. Thus, the improved growth and survival observed by Nelson and Evans(5) were not demonstrable in our experiments. It should be noted, however, that Nelson and co-workers (3,4) reported that the pantothenic acid sparing effect of a high casein diet became more pronounced as the experimental period was extended beyond 60 days. Similar data on the time required for the pantothenic acid-

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TABLE II. Influence of β-alanine Upon Antibody Response and Growth of Pantothenic Acid-Deficient Rats.

Group	No. of rats	Body wt*		Antibody titers	
		Initial	Final†	Range	Avg
I. Normal control	5	42	223		1:2560‡
II. Pantothenic acid-deficient	10	44	89	1:5–1:320	1:148
III. Pantothenic acid-deficient + β-alanine	10	45	94	1:5–1:320	1:143

* Group avg in g.
† At the time of bleeding.
‡ All 5 rats had a titer of 1:2560.

sparing action of DL-methionine to become apparent have not been presented by these authors(5). Our failure to observe a significant improvement in growth and survival may be attributed to the shorter experimental period employed in the present experiments.

B. *Effect of β-alanine upon antibody response of pantothenic acid-deficient rats.* This experiment was conducted to determine whether β-alanine could substitute for pantothenic acid in the processes of antibody production. Twenty-five weanling rats were distributed into 3 groups as indicated in Table II. Each animal of Group III received 0.5 mg of β-alanine daily. The experimental diets were fed *ad libitum* to all rats and immunization was instituted during the 5th week.

It is evident from the results summarized in Table II that β-alanine cannot replace pantothenic acid for either antibody production or growth in the rat. The latter observation confirms the opinion of El-Sadr *et al.*(7) that the slight growth stimulation observed by Hoffer and Reichstein(8) was not significant.

Summary. 1. In albino rats DL-methionine possesses a significant sparing action upon the pantothenic acid requirement for antibody production. 2. β-alanine cannot replace pantothenic acid in the processes of antibody synthesis.

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Studies on β-Glucuronidase from *E. coli.* (18591)

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Although the preparation of β-glucuronidase from mammalian tissues has been studied by Masamune(1), Oshima(2), Fishman(3-6) and others(7-11), little attention has been

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