stance (Fraction 1) was prepared by a procedure which avoided heating and use of alkalis and acids. The active principle was isolated in mixture with a non-dialyzable polynucleotide moiety. The organism of *L. icterohemorrhagiae* contained an appreciable amount of the polynucleotide substance and was composed of pentose—as well as desoxypentosenucleic acids. Crystalline ribo- and desoxyribonuclease altered the absorption spectrum of the nucleotide, but failed to inactivate the antigen. On the basis of the latter evidence it appeared likely that the polynucleotide was not the determinant of immunological reactivity. The active principle resisted the action of trypsin, hemicellulase and was not destroyed by heating for 1 hour at 100°C.

The author wishes to thank Dr. C. W. Hiatt for his guidance in this work and in the preparation of the manuscript. The author is also indebted to the Department of Veterinary Clinical Pathology for the performance of the complement-fixation tests and to the Department of Veterinary Bacteriology for supplying the leptospiral suspensions and hyperimmune serum. The technical assistance of Mr. N. Rothstein is gratefully acknowledged.

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Received March 18, 1953. P.S.E.B.M., 1953, v82.

Effect of Pantothenate Deficiency on Synthesis of Adrenal Cholesterol Following Stress.* (20210)

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The occurrence of pathological changes in the adrenal cortex of young rats maintained on diets deficient in pantothenic acid is well established(1-3). The functional capacity of the adrenal cortex is also impaired in this deficiency as indicated by the absence of a lymphopenia following swimming or ACTH (4,5), the absence of an eosinopenia following epinephrine or ACTH(5), and a decrease in glycogen deposition in the liver during fasting or anoxia(6,7). It has been inferred that this functional impairment may be related, at least in part, to low levels of adrenal steroids in the

* This investigation was aided by a grant from the Josiah Macy, Jr., Foundation.

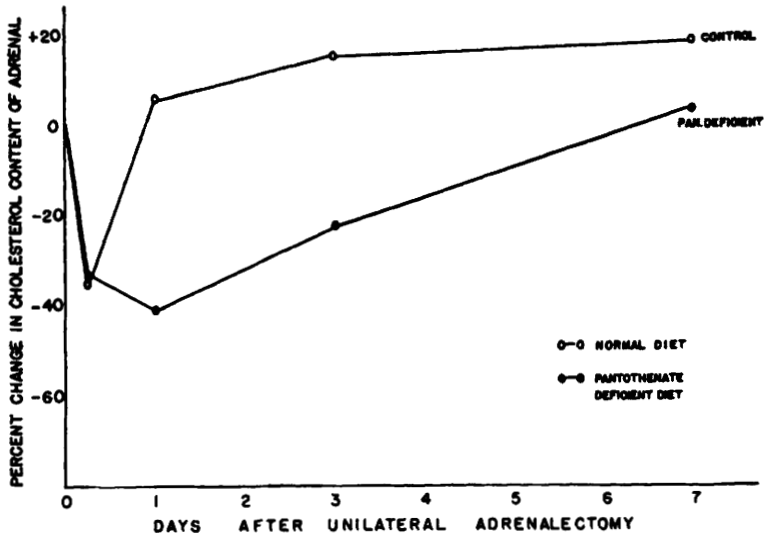

FIG. 1. Effect of Pantothenate Deficiency on Adrenal Cholesterol Determined at Intervals of 6 hr, and 1, 3 or 7 days after unilateral adrenalectomy.

pantothenate deficient rat(8-10). The data which we are presenting confirms the decreased adrenal cholesterol observed in pantothenate deficient rats(8,9) and, in addition, demonstrates that the ability of these animals to resynthesize adrenal cholesterol following stress is impaired.

Methods. Male rats, 40 days of age and weighing about 100 g, were placed on either a normal experimental diet providing 1 mg of calcium pantothenate per 100 g of diet, or on a diet deficient in pantothenic acid, but otherwise similar. After 30 days on the diets, the left adrenal of each rat was removed and analyzed for its cholesterol content by a modification of the Schoenheimer-Sperry method (11). The rats were continued on the preoperative diet after unilateral adrenalectomy. All animals received 1% NaCl as drinking water throughout the experiment. The right adrenal was removed at intervals of 6 or 24 hours or after 3 or 7 days and its cholesterol content determined.

Results. The data on the rats at the time of unilateral adrenalectomy are summarized in Table I. The body weight of the pantothenate deficient rats averaged 110 g compared with 197 g for the controls. The absolute adrenal weights were greater in the control than in the deficient rats, but when computed in terms of body weight, the adrenals of the deficient rats were relatively larger. Total adrenal weight was computed on the assumption that at the time of the operation the weights of the 2 adrenals were the same. Adrenal cholesterol was lower in the deficient rats either in terms of its concentration in the adrenal (2.29 compared to 4.40 g %) or in terms of the absolute content per adrenal (0.28 compared to 0.71 mg). Total adrenal cholesterol per 100 g body weight averaged 0.55 mg in the deficient and 0.75 mg in the control rats. The differences between the adrenal cholesterol values in the 2 dietary groups were significant as judged by the Fisher "t" test ($p < 0.01$) (12).

Post-operative survival was good in both groups of rats. After unilateral adrenalectomy the animals on the normal diets continued to gain, whereas the deficient animals tended to lose weight. At sacrifice the right adrenals of the deficient animals were found to be visibly hemorrhagic in 21 of 25 animals. No hemorrhagic adrenals were observed in the control rats. The cholesterol content of the remaining adrenal decreased in both groups of rats immediately after unilateral adrenalectomy and returned to its original level much more promptly in the normal than in the deficient rats. The per cent difference between the cholesterol content of the right and left

TABLE I. Effect of Pantothenate Deficiency on Adrenal Weight and Cholesterol Content.

	Control diet, mean ± S.D.	Pantothenate deficient diet, mean ± S.D.
No. of rats	30	25
Body wt (g)	197 ±36	110 ±25
Wt of left adrenal (mg)	16.1 ± 2.6	13.3 ± 3.7
Total adrenal wt (mg/100 g body wt)	16.9 ± 4.0	24.8 ± 7.1
Adrenal cholesterol (g %)	4.40 ± .74	2.29 ± .90
" " (mg/adrenal)	.708± .161	.277± .104
Total adrenal cholesterol (mg/100 g body wt)	.747± .231	.553± .230

adrenal was computed for each rat and the average difference at each time interval is shown in Fig. 1. Each point represents values on 5 or more rats. This method of presentation was selected because of the extreme changes in adrenal weight and cholesterol concentration which were associated with the development of hemorrhagic adrenals in the pantothenate deficient rats. As was shown in Table I, the initial adrenal cholesterol level was significantly lower in the deficient than in the control rats.

Six hours after unilateral adrenalectomy the cholesterol content of the remaining adrenal had decreased about 35% in the rats in both diet groups. No significant change in adrenal weight occurred during this period. Twenty-four hours after unilateral adrenalectomy, the cholesterol content of the remaining adrenal in the control rats had returned to approximately the level observed in the adrenal removed previously. An increase of approximately 10% in adrenal weight had occurred during this time. In the pantothenate deficient rats, however, the cholesterol content of the right adrenal was still decreased at 24 hours. This was associated with an average increase of about 10% in adrenal weight.

By 3 days after unilateral adrenalectomy the cholesterol content of the right adrenal of the control rats had increased about 15% above the pre-operative value and the adrenal weight had increased to the same extent. In the deficient rats, at 3 days, the cholesterol content of the right adrenal was greater than at 24 hours, but had not reached the pre-operative level, the concentration ranging from 0.5 to 3.5 g %. At this time the right adrenals of the deficient rats weighed 20 to 30% more than the adrenals previously removed, and with a single exception the right adrenals of the deficient rats were visibly hemorrhagic.

By 7 days the cholesterol content of the right adrenal of the control animals was about 20% above the pre-operative level, reflecting restoration of the original cholesterol concentration and moderate hypertrophy of the gland. In the deficient animals by 7 days, the original cholesterol content was restored in about half the group and was still depressed in the remainder, the concentrations varying from 3.5 to 0.4 g %. This was associated with very marked increase in adrenal weight in some animals, ranging up to almost 400% in rats with grossly hemorrhagic glands. Low cholesterol concentrations were found in the most markedly hemorrhagic adrenals.

The difference between the response of the adrenal cholesterol in the control and pantothenate deficient rats, illustrated in Fig. 1, is a significant one as evaluated by the Analysis of Covariance(12). In this analysis the total right adrenal cholesterol was the dependent variable and the total left adrenal cholesterol the independent variable. The analysis confirms the fact that a significantly different response occurred in the two dietary groups ($p < 0.01$).

Discussion. These results support the suggestion that adequate amounts of pantothenate are necessary for the synthesis of adrenal cholesterol(10). Our data on the effect of pantothenate deficiency on the cholesterol content of the adrenal confirm the observations of other observers(8,9). In addition, our observations indicate that the ability of the pantothenate deficient rat to resynthesize adrenal cholesterol after stress is decreased, as compared to the normal animal. Adrenal cholesterol is known to be synthesized from

acetate(13), and the role of pantothenate, as part of coenzyme A, in acetate metabolism is established by the work of many observers (14-16).

Both the initial increase in relative adrenal weight by the pantothenate deficient rats and the development of hemorrhagic adrenals by such rats after unilateral adrenalectomy tend to exclude the possibility of any associated pituitary defect under these circumstances. Hemorrhagic adrenals have been produced in rats on normal diets by injections of large amounts of ACTH(17). Adrenal cortical hypertrophy, an early sign of pantothenate deficiency(3), is also a typical response to the injection of ACTH(18) and can be prevented by cortisone(10).

These results, therefore, suggest that the decreased capacity to respond to stress which has been demonstrated in pantothenate deficient rats(4-7) is related to a decreased ability of such animals to synthesize the steroid hormones of the adrenal cortex.

Summary. The adrenal cholesterol of pantothenate deficient rats was significantly depressed compared to that of control animals on a normal diet. After the stress of unilateral adrenalectomy, the cholesterol content of the remaining adrenal decreased immediately in both diet groups. By 24 hours after unilateral adrenalectomy, the adrenal cholesterol of the normal rats had returned to its initial value; in the deficient rats adrenal cholesterol remained depressed up to 7 days after the stress. The data are interpreted as evidence that the synthesis of adrenal cholesterol is decreased in pantothenate deficiency.

We are indebted to Miss Elisabeth Street for the statistical analysis of the data.

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