dogs. It is concluded that the presence of the pancreas is not essential for the aforementioned action of growth hormone. However, it remains to be established whether insulin is a necessary factor for the growth hormone induced hypoglycemia.

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Effect of Pantothenic Acid Deficiency upon Adrenal Cortex, Thymus, Spleen, and Circulating Lymphocytes in Mice.* (18378)

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During the course of an investigation on the distribution of pantothenic acid in the tissues of adult male mice of the CF No. 1 strain, it was possible to make observations on the effect of a deficiency of this dietary requirement on the white blood cell count as well as structural changes in the adrenal cortex, spleen, and thymus(1). Such a study is desirable because of the established functional interrelationship between the secretory activity of the adrenal cortex, lymphoid tissue, and alterations in the circulating lymphocytes (2).

Lippincott and Morris made histopathological studies on young and adult C3H strain mice maintained on a diet deficient in pantothenic acid until death intervened in 6 to 8 weeks and reported a hyperkeratotic, atrophic, and desquamative dermatosis as well as myelin degeneration in the sciatic nerves and in the spinal cord. These investigators also stated that other organs, including the adrenals, were essentially normal in the deficient animals(3).

Experimental. Five mature mice, approximately 3 months of age, were fed a pantothenic-acid deficient ration No. 80 as described in detail by Morris and Lippincott(4). Four control animals of the same age received the same ration except that it was supplemented with d-calcium pantothenate at the rate of 30 mg per kg. All animals were weighed every 3 days throughout the experimental period of 7 weeks. Total white cell counts and blood smears, stained with Wright's stain, were made at weekly intervals. Tail-vein blood was used. For a histochemical study of the effect of pantothenic acid deficiency on the adrenal gland, thymus, and spleen, a second group of 22 mature male mice was used. Seven control animals were placed on pantothenate-containing ration and 15 on pantothenate-deficient ration. Individuals were killed at various intervals during an experimental period of 7 weeks to furnish tissues. In addition, 6 male mice were placed on a pantothenate-containing ration for 2 weeks, after which they were allowed to starve. The average duration of life of these animals was 6.3 days. One adrenal of each pair was prepared for frozen sections by fixation in 10% neutral formalin while the other was prepared for paraffin sections by fixation in Zenker-formol followed by postchromation in 3% potassium dichromate for 3 days. Thymus gland was also fixed for frozen and paraffin sections. Spleen was fixed in Zenker-formol for routine examination. Frozen sections were cut at 10 μ and paraffin sections at 6μ. Lipids were characterized in frozen sections by Sudan IV, Sudan Black B, Oil Red O, Schiff's reagent, and 2-4 dinitrophenylhydrazine(5). Heidenhain's iron alum

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TABLE I. Effect of Pantothenic Acid Deficiency in Adult Male Mice on Body Wt, Total Leucocyte and Lymphocyte Counts.

Wk	Avg body wt in g		Total leucocyte, thousand per cmm			Total lymphocyte, thousand per cmm		
	Control	Deficient	Control	Deficient	Diff.	Control	Deficient	Diff.
0	25 ± 1*	25 ± 1	12181	12162	19	10584	10499	85
1	26 ± 1	25 ± 1						
2	26 ± 1	22 ± 1	13275	8675	4600†	11140	6794	4346‡
3	26 ± 1	22 ± 2	12581	10650	1931	10595	8455	2140
4	27 ± 1	21 ± 1	10025	6750	3275	8246	4186	4060†
5	26 ± 1	20 ± 1	14662	8750	5912	12490	4622	7868‡
6	27 ± 2	20 ± 1	14750	18138	3388	11450	8852	2598†
7	28 ± 1	20 ± 1	8688	18100	9412	6665	13097	6432

* Mean and stand. deviation $\sqrt{\frac{\Sigma d^2}{n-1}}$.

† Significant at P = .05.
‡ Significant at P = .01.

haematoxylin and eosin were used in the staining of tissues for routine study. Birefringence was determined on a polarizing microscope. Control sections for this method and the Schiff reaction were first extracted with acetone.

Results and discussion. Data relative to body weight changes and hematological findings are presented in Table I. It is to be noted that the average body weights of the animals on the pantothenate-free ration decreased, whereas the controls gained. In comparison with the controls the experimental animals developed a leucopenia, after which a significant leucocytosis appeared which was maintained until the seventh week. Test for significance was made according to Snedecor(6). These data also show a significant lymphopenia for 6 weeks followed by an insignificant lymphocytosis. Furthermore, an insignificant neutrophilia was observed throughout the deficiency period. It is suggested that the leucopenia observed for the first five weeks in the deficient animals was due to lymphopenia and the leucocytosis which occurred following the fifth week was probably the result of the neutrophilia. However, by the seventh week the leucocytosis may be attributed to the combined effect of the neutrophilia and lymphocytosis.

By applying the histochemical methods previously described, it was possible to demonstrate that the lipids of the adrenal cortex of control mice showed reactions indicating the presence of ketosteroids. They gave a Schiff plasmal reaction, formed a yellow phenylhydrazone after the application of phenylhydrazine and revealed birefringence on the polarizing microscope. However, the adrenal cortex of the deficient animal showed a gradual, progressive depletion of sudanophilic and ketosteroid material from the zona fasciculata when compared to controls. This decrease was only slight at the end of the second week and was more pronounced by the end of the sixth week of the deficiency as shown in Fig. 1, 2, and 3. The zona glomerulosa remained unchanged.

Furthermore, by means of areal analysis of paraffin sections it was possible to demonstrate a gradual hypertrophy of the adrenal cortex during pantothenic acid deficiency. By the seventh week of the deficiency the adrenal cortex appeared to show signs of exhaustion of steroid material as evidenced by the reduction in birefringent material, particularly in the zona fasciculata. This probably is a contributing factor in the development of the lymphocytosis occurring at this time as shown in Table I. This cortical hypertrophy suggests an increased secretion of cortical hormone and its lipid depletion indicates an output below the physiological requirements of the organism. Deane and Greep have shown that the zona fasciculata secretes corticosterone-like hormones which govern gluconeogenesis whereas the zona glomerulosa is the site of hormones influencing electrolyte bal-

6. Snedecor, G. W., *Statistical Methods,* Ames, Iowa State College Press, 4th ed., 1946.

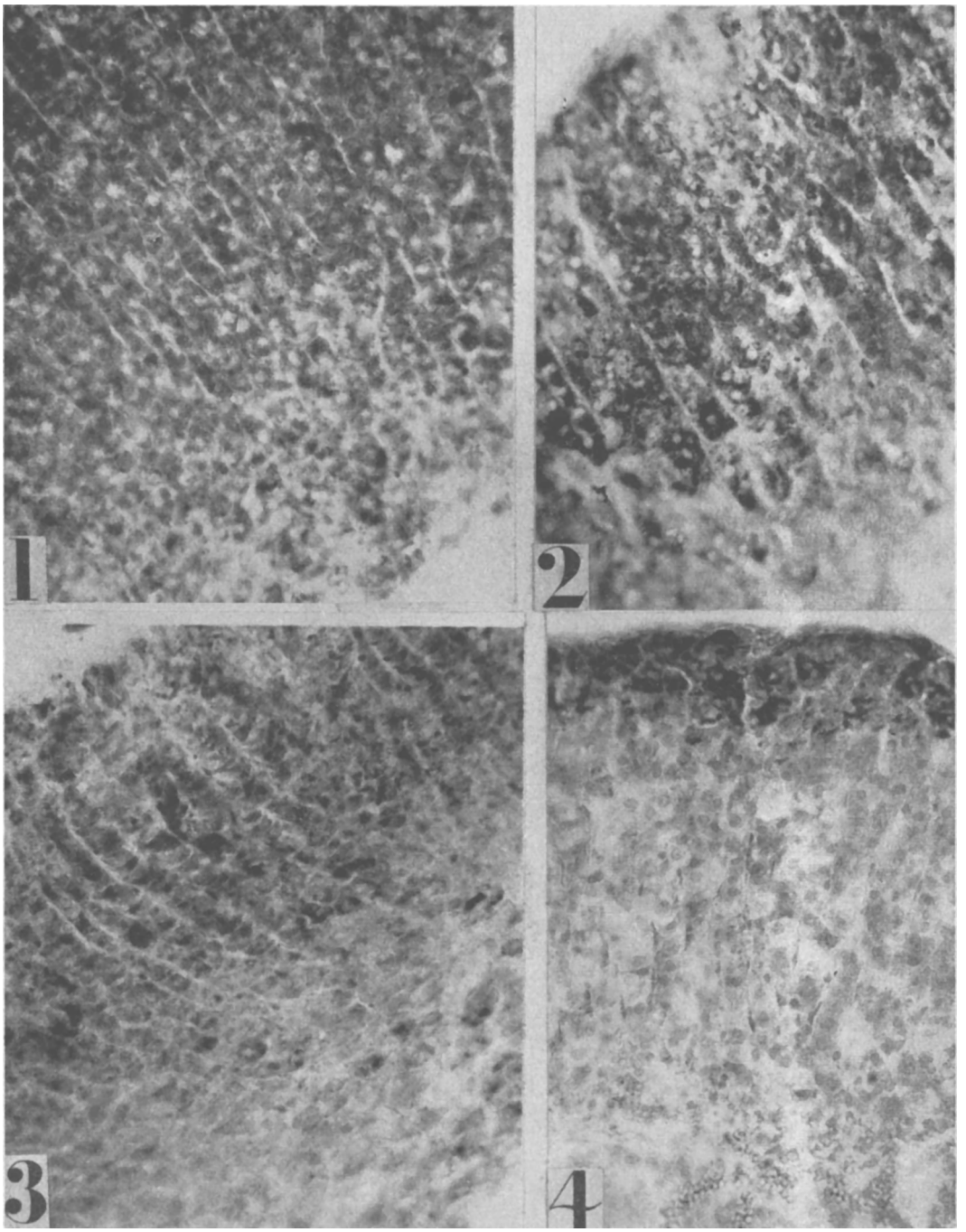

FIG. 1. Adrenal cortex from control animal stained with sudan black B and Mayer's carmalum. ×250.

FIG. 2. Adrenal cortex from animal on pantothenate-deficient ration 2 wk showing reduction of lipid in zona fasciculata. ×250.

FIG. 3. Adrenal cortex from animal on pantothenate-deficient ration 6 wk showing marked reduction of lipid from inner fasciculata. ×250.

FIG. 4. Adrenal cortex from starved animal showing total disappearance of lipid from zona fasciculata and presence of sudanophilic lipid in zona glomerulosa. ×250.

ance(7). There was no evidence of cortical necrosis or hemorrhage in the deficient mouse as has been reported for the rat. Furthermore, the changes occurring were more gradual in their appearance than in the case of the rat(8). Starvation caused complete exhaustion of sudanophilic and steroid materials from the zona fasciculata as shown in Fig. 4. In contrast, the zona glomerulosa of these animals retained some sudanophilic lipids but was practically depleted of ketosteroids. This would suggest that the physiological demands upon the adrenal cortex were much greater in starvation than in pantothenic acid deficiency.

The spleen weight of mice increased during the deficiency when compared with the controls but the amount of white pulp decreased significantly by the end of the sixth week as shown by areal analysis of sections. Histological examinations of spleen tissue from deficient mice indicated the following: (1) germ centers in the white pulp were rarely visible, (2) there were relatively few lymphocytes, and (3) lymphocytolysis was apparent as shown by the pycnotic nuclei. Furthermore, there was no indication of cell division. These changes may be correlated with the occurrence of the significant lymphopenia as shown in Table I. By the end of the seventh week the white pulp of the spleen was similar in appearance to that of the 6-week control in that the germ centers were active and the amount of white pulp had increased.

During pantothenic acid deficiency in the adult mouse, the thymus gland underwent involution and this was accompanied by an apparent increase in sudanophilic lipid in the interlobular and subcapsular connective tissues. By the seventh week there was a marked decrease in the number of lymphocytes in the thymus and as a result, the reticular network of the gland was much more conspicuous than in control animals. Dougherty and White have shown a direct relationship between the degree of involution of lymphoid tissue and the amount of corticosterone secreted(2).

Summary. Adult male mice placed on a pantothenate-deficient diet developed a lymphopenia followed by lymphocytosis when compared to control animals. The adrenal cortex showed hypertrophy and simultaneous, gradual depletion of lipid material, including ketosteroids, from the zona fasciculata. By the seventh week the zona fasciculata was nearly depleted of its hormone. In deficient mice, the thymus atrophied and there was a gradual enlargement of the spleen. Starvation caused a rapid depletion of the lipid, including the ketosteroids, from the zona fasciculata. The zona glomerulosa remained unchanged in pantothenic acid deficiency whereas in starvation it was depleted of steroid material but retained some sudanophilic lipids.

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8. Deane, H. W., and McKibbin, J. M., *Endocrinology*, 1946, v38, 385.

"Spontaneous" Leukemia Developing in C3H Mice Following Inoculation, In Infancy, with AK-Leukemic Extracts, or AK-Embryos.* (18379)

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The purpose of this study was to determine whether "spontaneous" leukemia could be prompted to develop in middle aged mice, of a line known to be essentially free from this disease, by inoculating such animals, in their early infancy, with extracts prepared from mouse leukemia, or with cell suspensions prepared from embryos removed from the uterus

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