myeloblastosis and of these cells in tissue culture. Examinations were made, also, of tissues of spleen, bone marrow and liver of the diseased birds. The results showed that intracellular virus of typical appearance was very rarely seen in myeloblasts of the circulation and only very infrequently in the cells after culture *in vitro*. This was the remarkable feature of the findings, since the cells liberate virus at a high rate for long periods in tissue culture, and large amounts of *extracellular* virus were associated with the cultured cells. In contrast, affected cells not myeloblasts in spleen, bone marrow and liver contained intracytoplasmic virus in high concentrations, most of it in inclusion-like bodies. The significance of the results was discussed in relation to similar findings with the Rous sarcoma and Murray-Begg endothelioma and to exploratory studies for the demonstration of virus in tumors of presently unknown etiology.

1. Burmester, B. R., In: Eckert, E. A., Beard, D., Beard, J. W., *J. Nat. Can. Inst.*, 1953, v13, 1167.

2. Eckert, E. A., Sharp, D. G., Mommaerts, E. B., Reeves, R. H., Beard, D., Beard, J. W., *ibid.*, 1954, v14, 1039.

3. Beaudreau, G. S., Becker, C., Sharp, D. G., Painter, J. C., Beard, J. W., *ibid.*, 1957, v20, 351.

4. Beard, J. W., *Ann. N. Y. Acad. Sci.*, 1957, v68, 473.

5. Beaudreau, G. S., Becker, C., *J. Nat. Can. Inst.*, 1957, v20, 339.

6. Palade, G. E., *J. Exp. Med.*, 1952, v95, 285.

7. Dmochowski, L., Grey, C. E., *Texas Rep. Biol. and Med.*, 1957, v15, 704.

8. ———, *Blood*, 1957,

9. Benedetti, E. L., Bernhard, W., Oberling, Ch., *Compt. rend. des seances de l'Acad. des Sci.*, 1956, v242, 2891.

10. Bonar, R. A., Beaudreau, G. S., Sharp, D. G., Beard, D., Beard, J. W., *J. Nat. Can. Inst.*, 1957, v19, 909.

11. Bernhard, W., Bonar, R. A., Beard, D., Beard, J. W., PROC. SOC. EXP. BIOL. AND MED., 1958, v97, 48.

12. Gaylord, W. H., Jr., *Cancer Res.*, 1955, v15, 80.

13. Bernhard, W., Oberling, Ch., Vigier, Ph., *Bull. du Cancer*, 1956, v43, 407.

14. Rouiller, C., Haguenau, F., Golde, A., Lacour, F., *ibid.*, 1956, v43, 10.

15. Bernhard, W., Bauer, A., Guerin, M., Oberling, Ch., *ibid.*, 1955, v42, 163.

16. Noyes, W. F., Mellors, R. C., *J. Exp. Med.*, 1957, v106, 555.

Received January 28, 1958. P.S.E.B.M., 1958, v97.

Excretion of 17-Hydroxycorticosteroid Hormones by Pantothenic Acid Deficient Guinea Pigs.* (23896)

ALBERT B. EISENSTEIN AND ALVIN S. WENNEKER (Introduced by R. E. Shank)
(With technical assistance of Benta Jensen)
Nutrition Research Lab., Dept. of Preventive Medicine, Washington University School of Medicine, St. Louis, Mo.

Although recent investigations have clearly established the important relationship of pantothenic acid to adrenal steroid secretion in the rat(1-5), the role of this vitamin in adrenocortical function in other animals has not been studied. The purpose of this report is to describe investigations of secretion of adrenal steroid hormones by guinea pigs made deficient in pantothenic acid. The results demonstrate a diminished excretion of 17-hydroxycorticosteroids in response to ACTH administration.

Materials and methods. Young, male guinea pigs (Hartley Strain) weighing 150 to 250 g were used. Animals were housed individually in metabolism cages, weighed frequently, and 24-hour urine samples collected. Each urine specimen was diluted to 100 ml with distilled water and a 5 ml aliquot used for determination of 17-hydroxycorticosteroid hormone content by the method of Silber and Porter(6). To obtain biochemical evidence of pantothenic acid deficiency, coenzyme A analyses

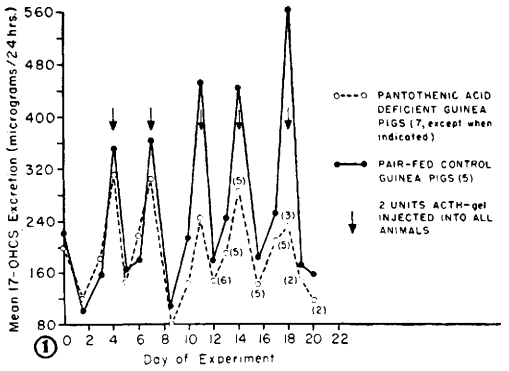
* This investigation was supported by grants from Williams-Waterman Fund and National Vitamin Fn.

were performed on hepatic tissue from each animal by the arsenolysis method(7). In one experiment, concentration of coenzyme A was also determined in adrenal tissue. In preliminary studies, an attempt was made to induce pantothenic acid deficiency by feeding a pantothenate deficient diet previously described by Reid(8). Manifestations of deficiency appeared slowly and animals gained weight for 4 weeks or longer after being placed on the diet. It was also observed that hepatic coenzyme A content of guinea pigs fed this diet for one month was only slightly less than that of control animals, thus demonstrating that severe pantothenate deficiency had not been induced. For this reason, omega-methyl pantothenic acid, a pantothenate antagonist, was incorporated in the diet fed to induce this deficiency in guinea pigs. *Exp. 1*: One group was fed synthetic, pantothenic acid deficient diet(8) containing 0.1% omega-methyl pantothenate. Controls were pair-fed a similar diet to which pantothenic acid was added (40 mg/kg). These animals were divided into 2 subgroups, one of which was fed the control diet without added omega-methyl pantothenate, and the other consumed the same diet containing this antimetabolite. Urinary excretion of adrenocortical hormones by each guinea pig was determined daily except for weekends when determinations were made on a 48-hour sample. Several times during the experiment, ACTH-gel (2 units) was administered subcutaneously to all animals. Guinea pigs of the deficient group were sacrificed when severely ill. When only a few animals survived, those remaining and all guinea pigs in control groups were sacrificed by decapitation. Immediately thereafter, a portion of liver tissue was removed, weighed, homogenized in distilled water, and prepared for subsequent coenzyme A analysis. Tissue homogenates were immediately frozen and kept at −20°C until these analyses were made. *Exp. 2*: One group consumed the pantothenic acid deficient diet containing 0.1% omega-methyl pantothenate while another consumed a similar diet deficient in ascorbic acid which contained no vitamin antagonist. Twenty-four hour urine collections for measurement of 17-hydroxycorticoid content were obtained 4 to 5 times/week and ACTH-gel (2 units) was administered several times. Guinea pigs of both groups were sacrificed when severe manifestations of vitamin deficiencies appeared and hepatic and adrenal tissue were removed for determination of coenzyme A content.

Results. Exp. 1: Guinea pigs fed the pantothenate deficient diet containing omega-methyl pantothenic acid gained weight slowly and seemed in good health for first 10 to 12 days. Subsequently, the animals moved about only when prodded, fur became ruffled, and weight gain ceased. Few guinea pigs survived longer than 3 weeks on this diet. In contrast, pair-fed control animals gained weight and were active throughout the period of observation, regardless of whether or not the diet contained omega-methyl pantothenic acid.

Liver Coenzyme A concentrations were significantly ($P = < 0.01$) less in deficient guinea pigs than in control animals which did not consume omega-methyl pantothenate, being 44.0 ± 3.3 and 82.9 ± 5.3 units/g of wet tissue respectively. Since pantothenic acid occurs in the body as integral part of coenzyme A, it is evident that guinea pigs consuming the deficient diet containing omega-methyl pantothenic acid were severely depleted of this vitamin. In control animals feeding of omega-methyl pantothenate did not significantly alter the liver content of coenzyme A.

The mean daily urinary excretion of 17-hydroxycorticosteroids by deficient and control animals is shown in Fig. 1. It is apparent that there was little difference in hormone excretion by the 2 groups on days when ACTH was not administered, although in most instances the value for control guinea pigs was higher than the deficient group. The first 2 times ACTH-gel was given, guinea pigs on pantothenic acid deficient diet exhibited no manifestations of deficiency and steroid excretion by this group was only slightly less than that of controls. As animals on the deficient diet developed signs of depletion, excretion of adrenocortical hormones remained relatively constant while control guinea pigs excreted larger amounts of corticosteroids with each injection of ACTH. Progressive increase in steroid excretion by controls in response to



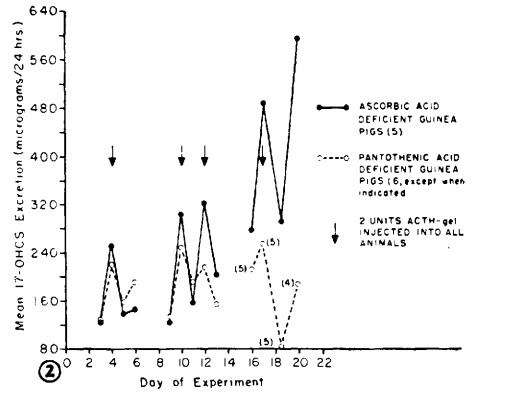


FIG. 1. Mean daily excretion of 17-hydroxycorticosteroid hormones by pantothenate deficient and control guinea pigs.

FIG. 2. Mean daily excretion of adrenocortical hormones by pantothenate deficient and ascorbic acid deficient guinea pigs.

ACTH was statistically significant ($P =< 0.05$) using linear regression and analysis of variance. In the same analysis, the difference between the mean 17-hydroxycorticoid excretion in response to ACTH of deficient and control groups was also statistically significant ($P =< 0.005$).

Exp. 2: Manifestations of pantothenic acid deficiency were similar in all respects to those mentioned in the first study. Animals consuming Vit. C-free diet gained weight slowly for about 13 days following which weight loss and other manifestations of deficiency appeared. Both deficiency states became severe almost simultaneously so that all animals were sacrificed between days 21 and 24 of experiment.

Severe depletion of pantothenic acid occurred in animals fed the diet deficient in this vitamin. Mean liver coenzyme A concentration in pantothenate depleted guinea pigs was 42.8 $\pm$ 2.0 units/g wet tissue compared to 97.8 $\pm$ 4.0 u/g in scorbutic animals. This is a significant difference ($P =< 0.01$). Mean adrenal coenzyme A levels were also significantly different ($P =< 0.05$), values for pantothenic acid deficient and scorbutic guinea pigs being 52.6 $\pm$ 4.8 u/g and 70.9 $\pm$ 4.0 u/g respectively. Liver coenzyme A content was somewhat higher in scorbutic guinea pigs than in controls of previous experiment, but this difference was not statistically significant.

Adrenocortical hormone excretion by the 2 groups is presented in Fig. 2. Pantothenate-deficient guinea pigs showed only a slight increase in excretion of these hormones when given ACTH-gel, as observed in previous experiment. Scorbutic animals, however, showed a striking rise in 17-hydroxycorticosteroid excretion following corticotrophin administration. The difference in mean steroid excretion by the 2 groups in response to ACTH was statistically significant, $P =< 0.005$. Excretion of adrenal steroids by scorbutic guinea pigs showed a progressive rise as they became depleted of Vit. C. On the final day when these animals were severely scorbutic, excretion of adrenocortical hormones was greater than at any other time, even though ACTH was not administered. The difference in mean steroid excretion by scorbutic and pantothenate deficient animals on this day was statistically significant, $P =< 0.001$.

Discussion. Excretion of 17-hydroxycorticosteroids in response to ACTH was reduced in guinea pigs fed a pantothenic acid deficient diet containing a pantothenate antagonist. Coenzyme A analyses performed on hepatic tissue from these animals revealed that severe pantothenate deficiency had been induced. Similar results have been observed in pantothenic acid depleted rats(4,5). One aspect which must be considered, however, is that pantothenate deficient guinea pigs were in poor general condition during the latter part of the study while control animals remained well. Thus, during the last few days excretion of adrenocortical hormones by sick animals was compared with that of healthy, though partially starved, guinea pigs and it seemed possible that decreased adrenal ster-

oid secretion might have occurred as a manifestation of illness rather than as a specific result of pantothenate depletion. Although in many nutritional experiments metabolic processes occurring in deficient and, often, sick animals are compared with those in animals that are well, it is desirable to control the factor of illness if possible. For this reason, it was decided to compare the excretion of adrenocortical hormones by pantothenate deficient guinea pigs with that of guinea pigs depleted of another vitamin.

In the second experiment, the 17-hydroxycorticoid excretion of pantothenate depleted animals was compared with that of Vit. C deficient guinea pigs. The decision to use ascorbic acid depleted animals as controls was based on 2 observations. First, other studies have shown that Vit. C deficiency can be induced in young guinea pigs in approximately 3 weeks, the same period required to induce severe pantothenate depletion. Secondly, previous investigation(9) demonstrated that ascorbic acid deficient guinea pigs excrete large quantities of adrenal steroid hormones, even when severe scurvy is present.

Since the purpose of this experiment was to learn whether pantothenate depletion *per se* or illness associated with deficiency resulted in diminished adrenal steroid secretion, it seemed appropriate to use as control nutritional deficiency one in which 17-hydroxycorticosteroid excretion is maintained at high levels. The ascorbic acid deficient group showed a progressive increase in adrenal steroid excretion, even though they demonstrated severe manifestations of vitamin depletion. In addition, a further striking rise in excretion of adrenal hormones occurred each time ACTH was administered. In contrast, excretion of adrenal steroids by the pantothenate deficient group remained relatively constant and was much less than that of the scorbutic group, especially during the latter part of the study when animals in both groups were ill. Coenzyme A content of liver and adrenal tissue confirmed the presence of marked pantothenic acid depletion in guinea pigs fed the diet deficient in this vitamin, but was normal in the scorbutic group.

The data show that illness occurring as a manifestation of nutritional deficiency does not necessarily result in decreased function of the adrenal cortex as measured by 17-hydroxycorticosteroid excretion. It is concluded that diminished adrenocortical hormone excretion which occurs in pantothenic acid deficient guinea pigs is a specific effect of vitamin deficiency and is probably due to diminished body content of coenzyme A, an essential cofactor in the synthesis of steroids, as well as other complex substances.

Summary. 1. Pantothenic acid deficiency was induced in guinea pigs by feeding deficient diet to which omega-methyl pantothenic acid, a vitamin antagonist, was added. 2. Urinary excretion of adrenocortical hormones by pantothenate depleted guinea pigs was compared with that of pair-fed controls and was significantly reduced. 3. Excretion of 17-hydroxycorticosteroids by pantothenic acid deficient guinea pigs was significantly decreased when compared with that of ascorbic acid depleted animals. 4. Diminished adrenocortical hormone secretion which occurred in these animals was a specific effect of pantothenic acid depletion and not a non-specific manifestation of nutritional deficiency.

Authors express sincere appreciation to Dr. Irby Bunding, Armour and Co., for gift of ACTH and to Dr. Arthur Kornberg for gift of *Clostridium kluyverii* cells. These bacteria served as the source of enzyme used in coenzyme A analyses.

1. Winters, R. W., Schultz, R. B., Krehl, W. A., *Endocrinology,* 1952, v50, 377.

2. Dumm, M. E., Gerschberg, H., Beck, E. M., Ralli, E. P., PROC. SOC. EXP. BIOL. AND MED., 1953, v82, 659.

3. Winters, R. W., Shultz, R. B., Krehl, W. A., *Endocrinology,* 1952, v50, 388.

4. Longwell, B. B., Reif, A. E., Hansbury, E., *Fed. Proc.,* 1956, v15, 303.

5. Eisenstein, A. B., *Endocrinology,* 1957, v60, 298.

6. Silber, R. H., Porter, C. C., *J. Biol. Chem.,* 1954, v210, 923.

7. Stadtman, E. R., Novelli, G. D., Lipmann, F., *ibid.,* 1951, v191, 365.

8. Reid, M. E., Briggs, G. M., *J. Nutrition,* 1953, v51, 341.

9. Nadel, E. M., Schneider, J. J., *Endocrinology,* 1952, v51, 5.

Received January 31, 1958. P.S.E.B.M., 1958, v97.