a secondary effect of corticoid released under ACTH stimulation, since corticosterone significantly inhibited AIB uptake in adrenal and tended to lower AIB in diaphragm. Nor does it seem probable, in view of the results with corticosterone, that the effects of ACTH are due to stimulation of the release of endogenous insulin by corticoids released following ACTH administration. This is supported by the fact that ACTH given under similar conditions had no effect on D-xylose transfer into diaphragm muscle(1).

It should be noted that a single large intravenous dose of corticotropin A does not restore adrenal AIB distribution to that found in pituitary-intact rats. This might be due to the particular mode of administration of ACTH employed in this study, to a decreased responsivity of the adrenal to ACTH following hypophysectomy, or to lack of an additional factor, which could be either pituitary or extra-pituitary in origin. The latter possibility should be considered since it was found that when insulin was given together with ACTH to hypophysectomized rats, AIB distribution was raised to the pituitary-intact level.

The significance of studies with AIB is related to the assumption that the behavior of AIB serves as an index of the transport of natural amino acids, and further that a change in the availability of amino acid to the interior of cells may influence protein synthesis and/or amino acid metabolism. On this basis, the present studies suggest that ACTH increases the uptake of amino acids by adrenal cells, and that this effect may be important in the increased protein synthesis and growth caused by ACTH in adrenal cortex. Insulin also appears to be involved in amino acid transport in adrenal as well as muscle, thus providing additional evidence that this hormone may have a role in adrenal maintenance and growth.

Summary. Hypophysectomy causes a decline in accumulation of the amino acid analogue, α-aminoisobutyrate (AIB), in adrenal and diaphragm muscle of functionally nephrectomized rats. Either Corticotropin A or insulin increase AIB accumulation to an equivalent extent in adrenal of hypophysectomized rats; both hormones also increase AIB uptake in diaphragm muscle, but insulin is more effective in this tissue. The combined influence of these hormones on both tissues is greater than either alone. The significance of these observations for protein synthesis and growth in the adrenal are discussed.

1. Eichhorn, J., Halkerston, I. D. K., Feinstein, M., Hechter, O., PROC. SOC. EXP. BIOL. AND MED., 1960, v103, 515.

2. Levine, R., *Survey Biol. Progr.*, 1957, v3, 185.

3. Kipnis, D. M., Noall, M. W., *Biochim. et Biophys. Acta*, 1958, v28, 226.

4. Noall, M. W., Riggs, T. R., Walker, L. M., Christensen, H. N., *Science*, 1957, v126, 1002.

5. Salter, J. M., Davidson, I. W. F., Best, C. H., *Canad. J. Biochem. Physiol.*, 1957, v35, 913.

6. Miller, R. A., Riddle, O., PROC. SOC. EXP. BIOL. AND MED., 1941, v47, 449.

7. Kostyo, J. L., Hotchkiss, J., Knobil, E., *Science*, 1959, v130, 1653.

Received November 9, 1960. P.S.E.B.M., 1961, v106.

Observations on Effect of 3-Methylcholanthrene in Scorbutic Guinea Pigs. (26269)

G. R. MARTIN,* HAROLD M. FULLMER AND J. J. BURNS†

National Institute of Dental Research and National Heart Institute, N.I.H., P.H.S., U. S. Dept. of H.E.W., Bethesda, Md., and N. Y. University Research Service, Goldwater Memorial Hosp., N. Y.

Previous studies have demonstrated a marked increase in synthesis of L-ascorbic acid following administration of 3-methylcholanthrene (3-MC) to rats(1,2). Since guinea pigs lack the ability to synthesize L-

* Research Associate, Am. Dental Assn.

† Present address: Wellcome Research Laboratory, 1 Searsdale Road, Tuckahoe, N. Y.

ascorbic acid, it was thought of interest to test the response of scorbutic guinea pigs to this compound. Observations presented here indicate that administration of 3-MC reverses some of the signs of scurvy in guinea pigs without inducing any detectable L-ascorbic acid formation.

Methods and materials. The modified curative bioassay for Vit. C activity employed here was similar to that used previously(3). One hundred and thirty male guinea pigs weighing about 200 g were given a Vit. C free diet‡ for 21 days. After 14 days on the diet they were divided into the following groups and received the indicated treatment for the 7 remaining days: Group 1 was given 1.0 mg of L-ascorbic acid orally per day; Group 2, 1.0 mg of 3-MC in 0.5 ml corn oil per day by i.p. injection; Group 3, 3.0 mg of 3-MC in 0.5 ml corn oil per day by i.p. injection; Group 4, 5 mg of 3-MC in 0.5 ml corn oil by i.p. injection; Group 5, 10 mg of 3-MC in 0.5 ml corn oil per day by i.p. injection, and Group 6, 0.5 ml of corn oil per day by i.p. injection (negative controls). The animals were weighed daily and were sacrificed on the 21st day and the knee joints examined for gross signs of hemorrhage. The lower jaws from 7 or more animals in each group were immediately fixed in Lillie's(5) aqueous neutral calcium acetate 10% formalin solution for 24 hours, decalcified in 5% formic acid, dehydrated, embedded in paraffin and sectioned mesiodistally at 6 μ. Sections were stained with hematoxylin and eosin, the Rinehart stain(6), azure A (0.5% in aqueous phosphate buffer pH 4, 10 minutes, 25°C), and the alloxan-Schiff stain (7).

The tissue concentration of L-ascorbic acid was determined by the method of Roe and Kuether(8). The possibility that 3-MC induced synthesis of L-ascorbic acid was investigated by an isotopic procedure used previously(9,10). Ten milligrams of D-galactose-1-C^{14} with a specific activity of 4.72 μc/mg was administered on the 20th day of experiment to each of 2 guinea pigs receiving 10 mg of 3-MC (from Group 5). Twenty-four hours later the animals were sacrificed and liver, spleen, testes, kidneys and adrenals from each animal were pooled and homogenized in 4 times their weight of 5% trichloroacetic acid. L-Ascorbic acid was isolated from the extract after addition of 200 mg of carrier by ion exchange chromatography, converted to its 2,4-dinitrophenylosazone derivative and recrystallized successively from an acetone-alcohol mixture. Radioactivity in the derivative was assayed in a gas flow counter. The C^{14} in carbon 6 of L-ascorbic acid was determined by periodic acid cleavage of the primary alcohol group to give formaldehyde which was recovered and counted as its dimedon derivative.

‡ The vit. C free diet, prepared as described by Woodruff *et al.*(4), was purchased from Nutritional Biochemical Co., Cleveland, O.

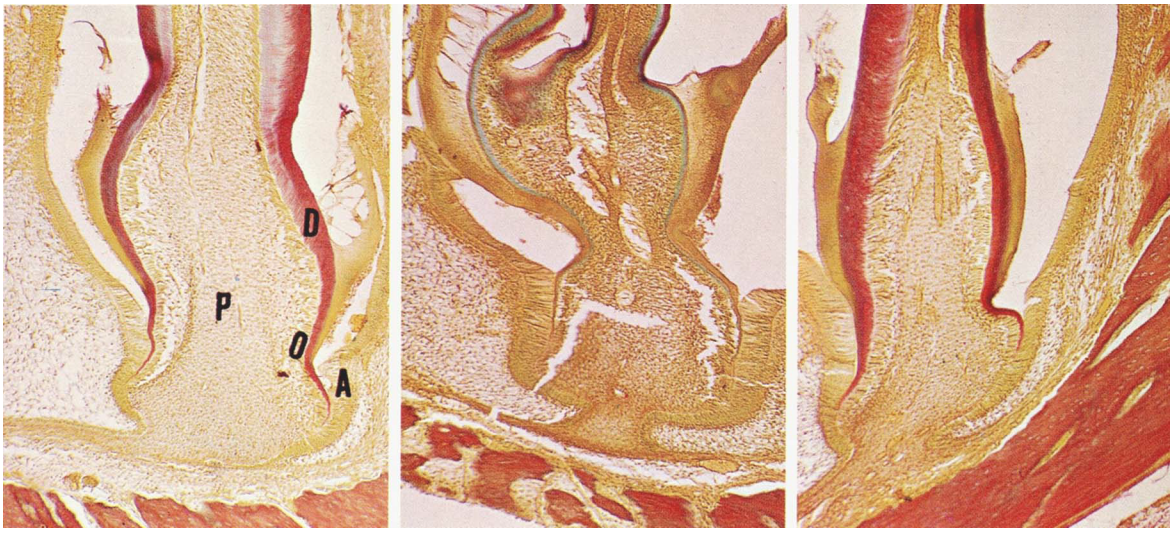
TABLE I. Incidence and Extent of Hind Knee Hemorrhages in Guinea Pigs Fed a Vit. C Free Diet with and without Supplements of L-Ascorbic Acid or 3-Methylcholanthrene.

Group*	Supplement	Extent of hemorrhage: None	Slight	Moderate	Severe
1	1 mg L-ascorbic acid	21	1	1	
2	1 mg 3-MC	12	3	3	1
3	3 " "	4	2	1	
5	10 " "	18	2		
6	None	1	5	12	14

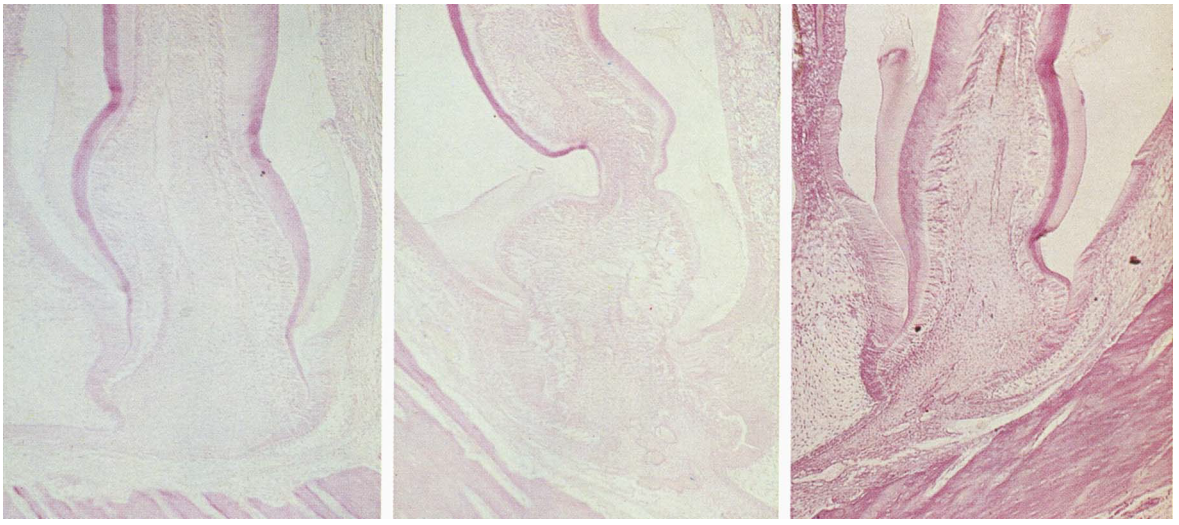
* Description of the various groups is contained in section on methods.

Results. Gross observations. The animals initially gained weight until about the 11th day on the diet when their weight began to decline. On the 15th day the animals were treated as described in the methods section. Animals in Group 1 gained an average of 10 g from the 15th to the 22nd day. During this period the animals in the negative control group as well as those receiving 3-MC lost about 20 g. Survival of animals in the various groups was essentially the same.

Incidence of gross hemorrhages of the knee joints in the various groups is listed in Table I. Administration of 3-MC to scorbutic guinea pigs markedly reduced incidence and extent of hemorrhages. This was particularly evident in those animals receiving the 10 mg dosage. *Histochemical observations.* In Plate 1A and 1D are shown photomicrographs of sections of molar teeth apices procured from the animals treated with L-ascorbic acid



(Left to right) *a.* Apex of a lower molar from a guinea pig treated with L-ascorbic acid (Group 1). Rinehart stain. Dentin at apex and that nearest pulp was produced last. Red stain indicates collagen, dentin or bone. Blue stain indicates mucopolysaccharide. D, dentin; P, pulp; O, odontoblasts; A, ameloblasts. × 50. *b.* Apex of a lower molar from a guinea pig in negative control group (Group 6). Rinehart stain. Note that very little dentin was produced during scorbutic state; and the strong reactivity of stain for mucopolysaccharide in abnormal osteodentin and connective tissue generally. × 50. *c.* Apex of a lower molar from a guinea pig treated with 10 mg of 3-MC daily for one week (Group 5). Rinehart stain. Note restoration of normal odontogenesis and lack of hemorrhages in the pulp. × 50.



(Left to right) *d.* Apex of a lower molar from a guinea pig treated with L-ascorbic acid (Group 1). Ninhydrin-Schiff stain. Pink and red stain indicates reactive protein. × 50. *e.* Apex of a lower molar from a guinea pig in negative control group (Group 6). Ninhydrin-Schiff stain. Note lack of reactive protein in dentin produced during scorbutic state. × 50. *f.* Apex of a lower molar from a guinea pig treated with 10 mg of 3-MC daily for one week. Ninhydrin-Schiff stain. Note restoration of normal protein reactivity. × 50.

(Group 1). Note the preservation of the odontoblasts and ameloblasts, and the regularly oriented tubular dentin which stains red with the Rinehart stain (1A), and pink with the ninhydrin-Schiff method (1D). Animals in this group also demonstrated abundant acid mucopolysaccharide exhibited by its metachromasia with azure A in their gingivae.

The jaws of guinea pigs in the negative control group (Group 6) showed the hemorrhages, bone resorption and interruption of dentinogenesis which are characteristic of the disease (1B). Analysis of sections stained with the ninhydrin-Schiff method (1E) corroborated our previous observations(3) that dentin and bone, produced during the scorbutic state, are unreactive with the alloxan-Schiff method for proteins. The gingivae usually failed to induce metachromasia with azure A, but occasionally a very slight metachromasia was exhibited.

The periodontia of animals to which 10 mg of 3-MC had been administered (Group 5) usually did not manifest histological or histochemical evidence of scurvy. Administration of 10 mg 3-MC not only prevented the characteristic destruction of those odontoblasts situated at the onset of dentinogenesis, but preserved their integrity and metabolic function to the degree that apparently normal dentin was produced as evidenced by the normal reactions of the Rinehart (1C) and ninhydrin-Schiff (1F) stains. The widely dilated blood vessels and hemorrhages in the pulp tissues characteristic of scurvy were alleviated in the 10 mg 3-MC treated group to such a degree that normal or near-normal blood vessels without hemorrhage were usually observed (1C). Metachromasia frequently appeared to be fully restored in the gingivae of animals treated with 10 mg 3-MC, and bone resorption also appeared to be arrested. Animals that received lesser amounts of 3-MC (Groups 2, 3 and 4) manifested scurvy to some degree but in general the various pathoses were noticeably reversed.

Ascorbic acid synthesis and retention. Since 3-MC is known to increase the rate of L-ascorbic acid formation when administered to rats, the possibility that it induced synthesis of the vitamin in guinea pigs was investigated.

TABLE II. Conversion of D-Galactose-1-C^{14} to L-Ascorbic Acid-6-C^{14} in Rats and 3-MC Treated Guinea Pigs.

Animal	% conversion to tissue L-ascorbic acid*
Rat 1	.044
" 2	.066
Guinea pig 1	<.0004
" " 2	<.0003

* L-ascorbic acid was isolated by a carrier dilution technic(9) from a pooled sample of liver, spleen, kidney, adrenal and testes 24 hr after a dose of D-galactose-1-C^{14}. Guinea pigs used in this experiment had received 10 mg dosage of 3-MC (Group 5). Data obtained in rats have been published(10).

Levels of L-ascorbic acid in brain, liver, testis, eye, intestine, muscle and adrenal were measured in scorbutic animals (negative control group) and in animals receiving 10 mg of 3-MC per day (Group 5). The values were found to be low and to fall within the same range. Levels of L-ascorbic acid in brain and liver from animals receiving 1.0 mg L-ascorbic acid per day (Group 1) were found to be elevated above those in the scorbutic animals (Group 6) from 7.0 to 15.8 mg % in brain and from 1.0 to 3.0 mg % in liver. More definitive evidence against synthesis of L-ascorbic acid in 3-MC treated guinea pigs was obtained by introducing D-galactose-1-C^{14} into the carbohydrate pool from which L-ascorbic acid is readily synthesized in rats. Conversion of D-galactose-1-C^{14} to L-ascorbic acid-6-C^{14} in rats averaged 0.05% whereas less than one-hundredth this value was observed under the same conditions in 3-MC treated guinea pigs (Table II).

Discussion. Administration of 3-MC reduced markedly the incidence of joint hemorrhages associated with scurvy but loss of weight was not prevented. It is not known whether the weight loss was part of the scorbutic syndrome or due to toxicity of the 3-MC.¶ Histological data indicating that 3-MC reverses some of the pathoses of scurvy include restoration of mucopolysaccharides in

¶ The latter may be the case since administration of 1 mg of 3-MC per day for 4 days to normal guinea pigs was found to induce a 20 g weight loss.

the gingivae, preservation of the odontoblasts to a metabolic state whereby normal dentin was produced, and cessation of bone resorption. In most cases the jaws of guinea pigs receiving the 10 mg dosage of 3-MC were indistinguishable from those animals receiving the L-ascorbic acid supplement.

Chemical analysis of L-ascorbic acid in various tissues from Groups 1, 5 and 6 indicated that 3-MC does not alter retention or synthesis of L-ascorbic acid. Experiments utilizing D-galactose-1-C^{14} virtually exclude the possibility of 3-MC induced L-ascorbic acid synthesis in scorbutic guinea pigs at least through known pathways(11).

The mechanism by which 3-MC reverses some of the signs of scurvy is not known. Other studies have shown that 3-MC exerts a stimulatory effect on protein synthesis and on the activity of certain enzyme systems(12, 13, 14, 15, 16). It is possible that some of the pathoses in scurvy result from impaired synthesis of certain proteins and/or a lessened activity of certain enzymes which are corrected in part by 3-MC.

Summary. 1. Some of the pathoses of scurvy in guinea pigs were reversed by administration of 3-methylcholanthrene. These included a marked reduction of incidence of hemorrhage, reduction of bone resorption, preservation of dentinogenesis and prevention of death of odontoblasts. 2. The effect 3-methylcholanthrene exerts upon scurvy does not appear to be mediated by induction of L-ascorbic acid synthesis nor by increased retention of the vitamin.

The authors are grateful to Miss C. Mecca for assistance in bioassay experiments and to Miss C. Evans for carrying out the radioactive experiments. Photomicrographs are the courtesy of Mr. John W. McGuire.

1. Conney, A. H., Burns, J. J., *Nature*, 1959, v184, 363.

2. Burns, J. J., Conney, A. H., Dayton, P. G., Evans, C., Martin, G. R., Teller, D., *J. Pharm. Exp. Therap.*, 1960, v129, 132.

3. Burns, J. J., Fullmer, H. M., Dayton, P. G., PROC. SOC. EXP. BIOL. AND MED., 1959, v101, 46.

4. Woodruff, C. W., Cherrington, M. E., Stockell, A. E., Darby, W. J., *J. Biol. Chem.*, 1949, v178, 861.

5. Lillie, R. D., *Histopathologic Technic and Practical Histochemistry*, 2nd ed., Blakiston Co., New York, N. Y., 1954.

6. Rinehart, J. F., Abul-Haj, S. K., *Arch. Path.*, 1951, v52, 189.

7. Yasuma, A., Ichikawa, T., *J. Lab. Clin. Med.*, 1953, v41, 296.

8. Roe, J. H., Kuether, C. A., *J. Biol. Chem.*, 1943, v147, 399.

9. Burns, J. J., Evans, C., *ibid.*, 1956, v223, 897.

10. Evans, C., Conney, A. H., Trousof, N., Burns, J. J., *Biochim. et Biophys. Acta*, 1960, v41, 9.

11. Burns, J. J., *Am. J. Med.*, 1959, v26, 740.

12. Conney, A. H., Miller, E. C., Miller, J. A., *Cancer Research*, 1956, v16, 450.

13. ———, *J. Biol. Chem.*, 1957, v228, 753.

14. Conney, A. H., Gillette, J. R., Inscoe, J. K., Trams, E. R., Posner, H. S., *Science*, 1959, v130, 1478.

15. Cramer, J. W., Miller, J. A., Miller, E. C., *J. Biol. Chem.*, 1960, v235, 250.

16. Conney, A. H., Davison, C., Gastel, R., Burns, J. J., *J. Pharm. Exp. Therap.*, in press.

Received July 11, 1960. P.S.E.B.M., 1961, v106.

Localization of Proelastase in the Goose-Fish (*Lophius piscatorius*).* (26270)

FRANCOIS LAMY AND ALBERT I. LANSING

Department of Anatomy, University of Pittsburgh School of Medicine, and Marine Biological Laboratory, Woods Hole, Mass.

It has been suggested(1,2) that elastase (3) is localized in the pancreatic islets of Langerhans. These observations were made before the existence of proelastase(4) had been recognized. As a consequence very little activity could be measured. In view of the possible role of elastase in the etiology of atherosclerosis(5) it was thought of interest

* This investigation was supported by research grants from Nat. Inst. Health, U.S.P.H.S.