Interrelationships of Desoxycorticosterone, Cortisone and Vitamin C in the Genesis of Mesenchymal Lesions.* (17907)

CARL SCHAFFENBURG, G. M. C. MASSON, AND A. C. CORCORAN

From the Research Division of the Cleveland Clinic Foundation, and the Frank E. Bunts Educational Institute, Cleveland, O.

Degenerative and atrophic changes in the collagen of the heart and joints resembling those of rheumatic fever and rheumatoid arthritis have been experimentally elicited by vitamin C deficiency(1,2,3) and by treatment with desoxycorticosterone acetate (DCA)(4). On the other hand, it has been shown that adrenocorticotrophin (ACTH) and cortisone can prevent or cure lesions of experimental(5) or clinical(6,7) collagenous diseases. Vitamin C is known to be directly implicated in the activity of the adrenal cortex(8,9,10,11) so that it seemed possible that some of the mesenchymal alterations in scurvy were not due to deficiency of vitamin C as such, but resulted from hyposecretion of cortisone-like steroids(12). The experiments here reported were designed to test this hypothesis on animals susceptible to vitamin C deficiency and to add to what is known of the contrasting functions of DC-like and cortisone-like steroids.

Experimental. Twenty-six male guinea pigs weighing between 280 and 300 g were divided into 4 groups and placed on a scorbutogenic diet as used by Schultz(1). There were 6 animals in each of the first 3 groups and 8 in the last one. The guinea pigs of Group I received a daily dose of 5 mg of DCA; those of Group II received a daily dose of 5 mg of cortisone acetate (Cortone acetate, Merck and Co.) which was increased to 7.5 mg on the 11th day and to 10 mg on the 19th day of the experiment; those of Group III were given *per os* 5 cc of orange juice daily and kept as normal controls; those of Group IV consisted of untreated scorbutic animals. The steroids in the form of aqueous suspension were administered subcutaneously, the daily dose being given in one injection. The animals were housed in cages of 3 and given 1% saline to drink as a sensitizing agent to the effects of DCA. Since it has been shown previously(1) that alterations in connective tissue are more manifest in chronic scurvy than in the acute disease, animals of Groups I, II and IV were given 1 cc of orange juice on the 4th, 8th and 11th day of the experiment. The course of the scurvy was evaluated from 4 criteria: general habitus, body growth, onset of arthritis and subcutaneous hemorrhages. The joint examined was the one formed by the ulna, radius and carpal bones. This area was epilated first with barium sulfide; then starting on the 10th day, measurements were taken at regular intervals with a caliper. On the 14th and 17th day X-ray pictures of the joints

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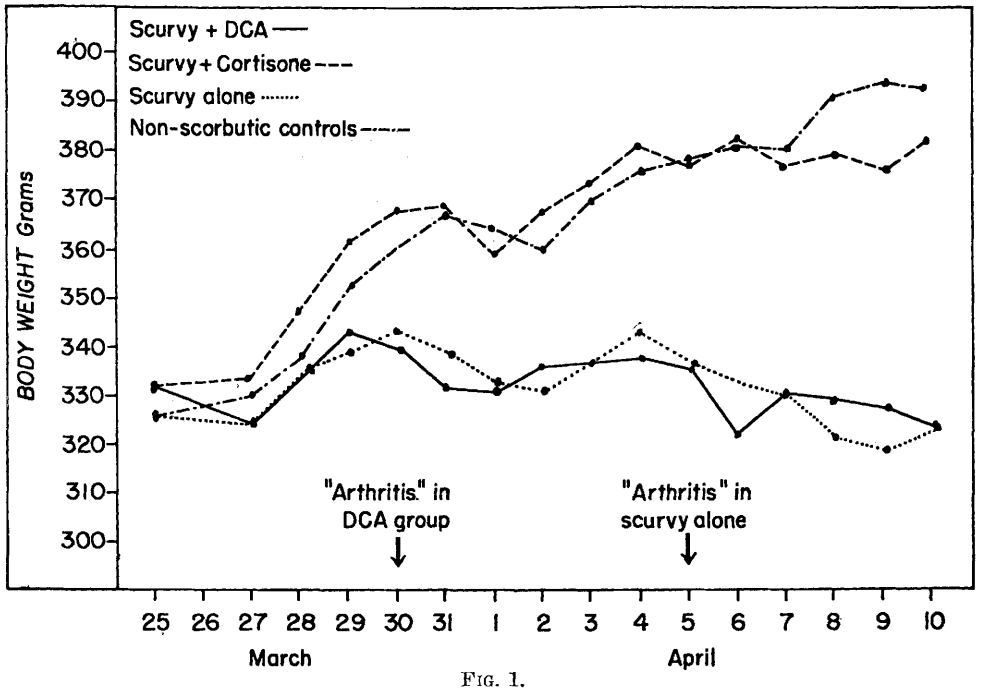

FIG. 1.
Effect of DCA and cortisone on body weight in the scorbutic guinea pig.

were taken on all the animals. The experiment was terminated on the 21st day.

The guinea pigs were anesthetized with nembutal and blood was drawn from the carotid artery for chemical studies. At autopsy adrenals, heart, kidney, pituitary, sternum and front paws were removed, fixed in 10% formalin or Zenker's fluid, weighed and sectioned. Histological data will form the subject of a subsequent report.

Results. 1. *General habitus.* Only animals of Group I (DCA) and Group IV (scorbutic controls) presented symptoms of scurvy; these appeared first and were more severe in the DCA group. On the 10th day, these animals began to show a decreased muscular activity, became apathetic and their fur dull and dirty; by the 15th day, most of them were prostrate and dyspneic. Two guinea pigs of Group IV died on the 15th and 17th day. The condition of the others was deteriorating rapidly, so that the experiment had to be terminated on the 21st day. The cortisone-treated guinea pigs did not differ in behavior from the non-scorbutic controls.

2. *Body Weight.* As can be seen from Fig. 1, animals of Groups I (DCA) and IV (scorbutic controls) started to lose weight at the same rate on the 10th day of the experiment, and continued to do so until the end. On the other hand, animals of Groups II (cortisone) and III (normal controls) gained weight in a parallel fashion, except at the end when the cortisone group barely maintained its gain.

3. *Joint lesions.* The effects of DCA and cortisone on the development of articular

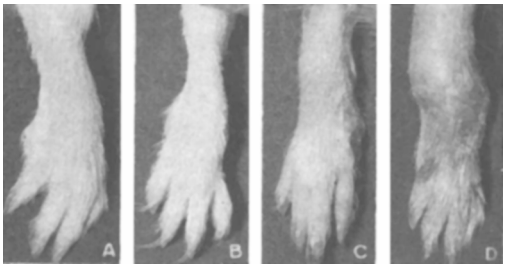

FIG. 2.
Right front paws taken on the 16th day of the experiment. A—Normal control. B—Cortisone-treated scorbutic guinea pig. C—Untreated scorbutic guinea pig. D—DCA-treated scorbutic guinea pig.

TABLE I.
Morphologic Changes Produced by DCA and Cortisone in Scorbutic Guinea Pigs.

Treatment	Micrometer reading right front paw, mm Day			Wt		
	10th	13th	15th	Adrenals, mg ± S.E.D.	Heart, g ± S.E.D.	Kidney, g (range)
I. Scurvy + DCA	7.58 ±.38	8.11 ±.25	8.51 ±.22	210 ±16.9	1.026 ±.043	1.666 (1.40-1.94)
II. Scurvy + cortisone	6.68 ±.23	6.56 ±.10	6.44 ±.02	136 ± 9.7	1.447 ±.015	1.922 (1.64-2.14)
III. Normal controls	6.76 ±.07	6.82 ±.06	6.91 ±.08	158 ±16.9	1.310 ±.120	1.830 (1.52-2.05)
IV. Scorbutic controls	6.71 ±.21	7.11 ±.28	7.30 ±.28	236 ±26.5	1.032 ±.165	1.713 (1.32-2.00)
P values*		I-III .01	I-III .01	III-IV .05		
		I-IV .02	I-IV .01	I-IV .4		
				II-IV .01	II-IV .05	

* P values of less than 0.05 are considered statistically significant.

lesions due to scurvy were very striking (Fig. 2). In Table I, we list measurements of the radial-carpal joint taken at various intervals. Guinea pigs in Groups I and IV developed typical swelling accompanied with redness and elevation of temperature; it became progressively more severe in the course of the experiment. While there was no obvious qualitative difference in the joint swelling of the DCA and the scorbutic animals, it must be pointed out that it appeared sooner in the DCA-treated (10th day) than in the scorbutic control (15th day) guinea pigs, and, that on the 15th day it was present in 5 out of 6 animals in the former group and only in 3 out of 8 in the latter. Furthermore, measurement values of the joints, summarized in Table I, show that the difference between the 2 groups became significant on the 13th day. Cortisone prevented these lesions throughout the experiment in all except one guinea pig. This animal developed pronounced and diffuse swelling in all four limbs, extending beyond the limits of the joints. However, it continued to gain weight and at autopsy the organs were found to be normal. Since these articular and periarticular lesions seemed to be of a different nature than those seen in the scorbutic controls, they were omitted from Table I.

4. *Hemorrhages.* With the exception of the normal controls, all other groups showed diffuse hemorrhages at autopsy. These were mainly in the subcutaneous tissue, the muscles and the joints. They were less severe in the cortisone group and more so in the DCA group. One guinea pig treated with cortisone did not show hemorrhages.

5. *Organ weights.* Weights of adrenals, heart and kidney are summarized in Table I. Scurvy caused a marked adrenal hypertrophy which was completely prevented by cortisone but only partially suppressed by DCA. The adrenals of the DCA-treated guinea pigs were somewhat smaller than those of the scorbutic controls and showed subcapsular and parenchymal hemorrhages. The weight of the heart tended to be less in the DCA and the scorbutic animals than in the cortisone and the normal control groups. These changes, however, were not significant. There were no obvious differences in kidney weight between the various groups. It should be noted, however, that the kidneys of the

DCA group presented patchy areas of pallor.

Determinations of arterial pressure, serum CO_2 content, chloride and alkaline phosphatase in some animals of each group did not indicate any striking difference. Total blood glutathione was kindly measured in all by Dr. A. Lazarow according to a method based on the formation of a complex glutathion-alloxan(13). It was decreased in all the experimental groups; this was most marked in the cortisone-treated animals. The data correspond with observations on normal rats similarly treated(14).

Discussion. It has been postulated(12) that most of the symptoms of experimental scurvy are secondary to adrenal insufficiency; this is based on certain similarities between the two conditions: (1) clinical symptoms (asthenia, adynamia, loss of weight) (2) disturbance in water (edema), salt (potassium retention), carbohydrate (decrease in liver glycogen and phosphocreatin) and protein metabolism (increase in blood urea nitrogen). Later it was found that the adrenal corticosteroid content is markedly diminished in vitamin C deficiency(15,16,17) and that symptoms of scurvy can be partially corrected with cortical extract(8,9) but not with desoxycorticosterone(18). The present experiments establish with crystalline cortisone the anti-scorbutic properties of carbohydrate active corticosteroids. The aggravation of the symptoms of scurvy under the influence of DCA emphasizes an antagonism between cortisone and DCA which has been demonstrated in other respects by previous investigators. Adreno-cortical hypertrophy during vitamin C depletion has been previously observed(19,20,21). This hypertrophy is presumably due to a compensatory ACTH overproduction resulting from a decrease in circulating adreno-cortical steroids. That cortisone was able completely to prevent this hypertrophy while DCA showed little effect, favors the view that the factors responsible for the secretion of these two types of cortical hormones are independent.

These experiments are of interest in the light of recent findings on the effect of cortisone and ACTH in collagenous diseases(6,7). The fact that most of the important evidences of scurvy were prevented by cortisone administration may elucidate the mode of action of this hormone in rheumatic conditions. Thus, in viewing scorbutic arthritis, vitamin C deficiency may be supposed to lead to insufficiency of carbohydrate-active corticosteroids; the lack of these may underlie the collagen degeneration which in turn results in arthritis. The deleterious effects of exogenous desoxycorticosterone is consistent with the view that the arthritis of untreated scorbutic animals may be due to cortical carbohydrate-active deficiency with preservation of production of desoxycorticosterone-like compounds, that is to a hormonal imbalance. The fact that the zona glomerulosa, where desoxycorticosterone is produced (22) is very poor in its content of vitamin C(23) while the fasiculata and reticularis are richer, would indicate that ascorbic acid may not be essential in the same degree for the production of these two types of cortical steroids.

Cortisone was unable to prevent some of the evidences of scurvy, notably the capillary hemorrhages. Very likely also it would not have indefinitely stayed the weight loss, at least at the dose employed in these experiments. Conceivably there may be in the

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adrenal cortex other steroids which may be more effectively anti-scorbutic than cortisone. More probably, some of the activities of vitamin C may be independent of the adrenal cortex.

The similarities between scurvy and rheumatic conditions are in many respects very striking. Consequently, the DCA-treated scorbutic guinea pig or the chronically scorbutic animal would seem to be excellent test subjects for the screening of compounds with anti-rheumatic activity. The advantage of such a test animal is the more evident as it now becomes apparent that adrenal glycogenic and anti-arthritic properties can be dissociated.

Summary. Cortisone inhibits many of the manifestations of scurvy in the guinea pig while desoxycorticosterone aggravates the condition. These activities of the hormones probably depend on their action on mesenchymal tissues. The scorbutic guinea pig, untreated or treated with DCA, may be a valuable test subject for the assay of anti-arthritic compounds.

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Effect of Tolerance on Inhibition of Respiration of Brain Homogenates by Thiopental. (17908)

THEODORE F. HUBBARD* AND LEO R. GOLDBAUM
(Introduced by Paul K. Smith)

From the Department of Basic Science and Department of Chemistry and Physics, Army Medical Department Research and Graduate School, Washington, D.C.

In a previous paper it was shown that mice become tolerant to the daily intraperitoneal injection of thiopental sodium, 45 mg per kg. This tolerance became maximal in 5 to 6 days and was indicated by shortened sleeping times and the ability of the experimental animals to awaken at higher brain levels of the barbiturate than controls(1). Since it has been proposed that barbiturates induce sedation by interference with the carbohydrate metabolism of the brain(2,3,4) it seemed important to study the inhibitory effects of barbiturates on the *in vitro* carbohydrate metabolism on the brains of control and tolerant mice.

Experimental. Male mice, weighing 23 to 25 g were made tolerant by the daily intraperitoneal injection of thiopental sodium, 45 mg per kg, for from 7 to 10 days; controls, kept under identical conditions, were given daily injections of saline. Records of the sleeping times of the experimental animals were kept, and the animals were used when their sleeping times were between 50 and 60% of their original sleeping times. For each experiment the whole brains from 5 control and 5 tolerant mice were rapidly removed and homogenized in cold distilled water in a glass homogenizer. No more than 15 minutes were allowed to elapse from the time the animals were killed until the homogenates were placed in the reaction vessels. The method of Reiner (5) was used in the study of glucose, pyruvate, and lactate oxidation. Since it seemed possible that the addition of the cofactors in these fortified homogenates might mask some difference in the metabolism of the control and tolerant mouse brains, isotonic homoge-

* Current address: Mayo Clinic, Rochester, Minn.

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