Effect of Cortisone and Adrenocorticotrophic Hormone (ACTH) on Experimental Scurvy in the Guinea Pig.* (18235)

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In recent clinical trials of adrenocorticotrophin (ACTH) in patients with purpura hemorrhagica, it has been found that the drug regularly brings about a temporary and almost complete remission of bleeding(1). Similar effects of the hyperadrenal state were seen whether the purpura was idiopathic or associated with aplastic anemia, leukemia, or lupus erythematosus disseminatus. Indeed, one of us (C.R.)† observed disappearance of the hemorrhagic manifestations in a scorbutic patient who was treated briefly with ACTH.

As a first approach to experimental analysis of the action of these hormones in purpura, a study of the influence of induced hyperadrenalism on scurvy in the guinea pig has been made. The disease is readily produced in this species and its manifestations are well known(2-5). Furthermore, a special interest in the relation of scurvy to the adrenal has already developed from the familiar facts that the gland contains extraordinary amounts of ascorbic acid(6) and that adrenal hypertrophy is a regular accompaniment of scurvy (7-11). Schaffenburg, *et al.*(12) have recently reported that cortisone exerts a protective action in guinea pigs placed on a scorbutic diet. Their studies were undertaken on the hypothesis that certain of the mesenchymal alterations of scurvy are the result of hyposecretion of cortisone-like steroids, which results from vitamin C deficiency (13,14). Studies of the effects of ACTH were not included in this report. In the present study it is shown that the effects of cortisone on the scorbutic guinea pig are also produced by ACTH. This finding lends support to the view that deficiency of ascorbic acid does not appreciably interfere with production of adrenal corticosteroids.

Methods. The guinea pigs studied were approximately two months old and weighed 300 to 400 g. Four were given a normal Rockland vitamin C-fortified diet(15) with added lettuce while 22 received an autoclaved, vitamin C-free diet(16) in the course of 2 experiments. In the first experiment,

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TABLE I. Summary of Data.

Treatment		Duration of scorbutic diet (days)	Duration of therapy (days)	Onset of wt loss after scorbutic diet (day)	Total max. wt loss (g)	Adrenal wt (mg)	Ratio: adrenal wt (mg) / body wt (g)	Adrenal ascorbic acid (μg/100 mg)
Normal—avg of 4		0	0	—	—	145	0.46	51.3
A. None	1	20	—	8	51	—	—	0
	2	21	—	8	158	—	—	0
	3	24	—	13	50	305	1.48	0
	7	20	—	8	65	300	0.98	2.2
	8	21	—	8	75	325	0.95	0
	15	23	—	10	170	300	1.16	2.1
	15A	20	—	6	135	300	1.22	—
	16	24	—	12	165	380	1.44	—
	17	24	—	10	145	300	1.41	2.3
Avg		21.9	0	9.2	113	315	1.2	—
B. Cortisone	4	25	15	8	85	120	.52	—
	5	26	16	19	90	—	—	—
	6	34	24	21	50	160	.58	0
	9	22	35	14	40	180	.46	0
	10	24	37	14	60	190	.52	—
	14	19	10	15	55	315	.9	4.3
	18	24	15	16	90	200	.65	3.9
	19	27	18	16	65	340	1.2	0
	24	30	21	16	200	300	1.13	0
Avg		26.5	22.3	15.4	80	225	.74	—
C. ACTH	20	30	21	16	155	600	2.22	0
	21	30	20	16	195	660	2.7	0
	22	29	19	16	180	430	1.72	0
	23	30	21	23	80	520	1.58	1.5
Avg		29.8	20.3	18	150	550	2.06	—

10 scorbutic animals were studied: 5 received cortisone‡ while 5 were not treated. Two animals received cortisone for 13 days before the scorbutic diet was started, and 3 received cortisone only after the diet had been given for 10 days. The second experiment involved 3 groups of 4 scorbutic animals. The first group was given cortisone after 10 days on the scorbutic diet. The second received ACTH,§ again after 10 days on the same regimen. The third group was not treated. The effects of the hormones in both experiments will be examined together. Cortisone was given once daily in a dose of 12.5 mg subcutaneously. ACTH was injected every 6 hours, 6.25 mg subcutaneously. In order to demonstrate glycogen and polysaccharide complexes, the periodic acid Schiff reaction and diastase method were employed. Toluidine blue was used in order to stain acid mucopolysaccharides. The ascorbic acid content of the adrenal gland and of the blood were determined by the method of Roe and Kuether(17). The important data are summarized in Table I.

Results. 1. *Scorbutic, untreated controls.* Group I, 9 animals. Two of the animals were female and 7 male. No significant differences related to sex were noted during the study. The average duration of life was 21.9 days with extremes of 20 to 24 days. Five animals were sacrificed after their weights had fallen to about 250 g from an average of 370 g. They then appeared moribund.

‡ The cortisone used in these studies was purchased from Merck and Co., from funds provided by the U.S. Public Health Service.

§ The ACTH used in these studies was purchased from Armour and Co. Dosages are equivalent to Armour Standard LAIA.

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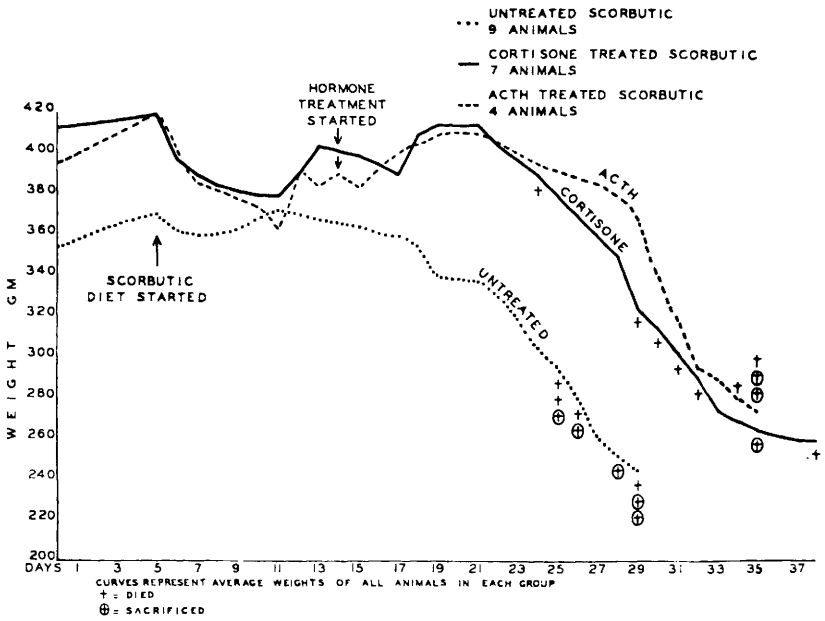

FIG. 1.
Effect of cortisone and ACTH on body weight and survival of scorbutic guinea pigs.

The clinical course was strikingly similar in all animals, resembling that described by Meyer(4). On the tenth to twelfth day, bloody diarrhea and hematuria developed in all animals, anorexia and lethargy was marked, while hind leg paralysis, tachypnea, and abdominal distention appeared in five. The weight curves in Fig. 1 reveal a failure to gain from the start of the diet, and a consistent loss of weight from about the ninth day. A very rapid fall occurred after the seventeenth day. Gross examination disclosed bladder and perivesical hemorrhage in all of the animals. Marked hemorrhage occurred in the subcutaneous tissues and muscles of the lower extremities, lungs, upper intestinal tract, cecum and spleen. There was considerable adrenal hypertrophy.

Microscopic examination of the viscera, bones and joints, muscle and subcutaneous tissue disclosed extensive, characteristic lesions of scurvy(5,10). Additional disorders present at death consisted of liver abscess in one animal and pneumonia in a second.

2. *Scorbutic, cortisone-treated.* Group II, 9 animals. Two male guinea pigs were treated with cortisone for 13 days before receiving the scorbutic diet. The hormone was then given until they died after 22 and 24 days on the diet. The course of these animals is not represented in Fig. 1. However, weight gain continued on cortisone until the scorbutic diet was instituted. The weight then levelled off for about 14 days, and finally dropped rapidly until death. In 3 male and 4 female pigs, cortisone therapy was started on the tenth day of the scorbutic diet. These animals lived for 19 to 34 days on the scorbutic diet, an average of 26.5 days. After 7 days of therapy, a relatively steep fall ensued and continued until death (Fig. 1). The onset of clinical scurvy was approximately one week later than in the scorbutic controls. In two animals death apparently resulted from inanition and pneumonia after 19 and 25 days of the diet. No gross hemorrhage was seen in either. The remaining 7 animals, however, did present hemorrhages, which seemed appreciably less than in the untreated group, although bloody diarrhea, hematuria and hind leg paralysis had appeared in most of them. One animal was sacrificed when moribund, the remainder dying without interference. Microscopic findings are reported

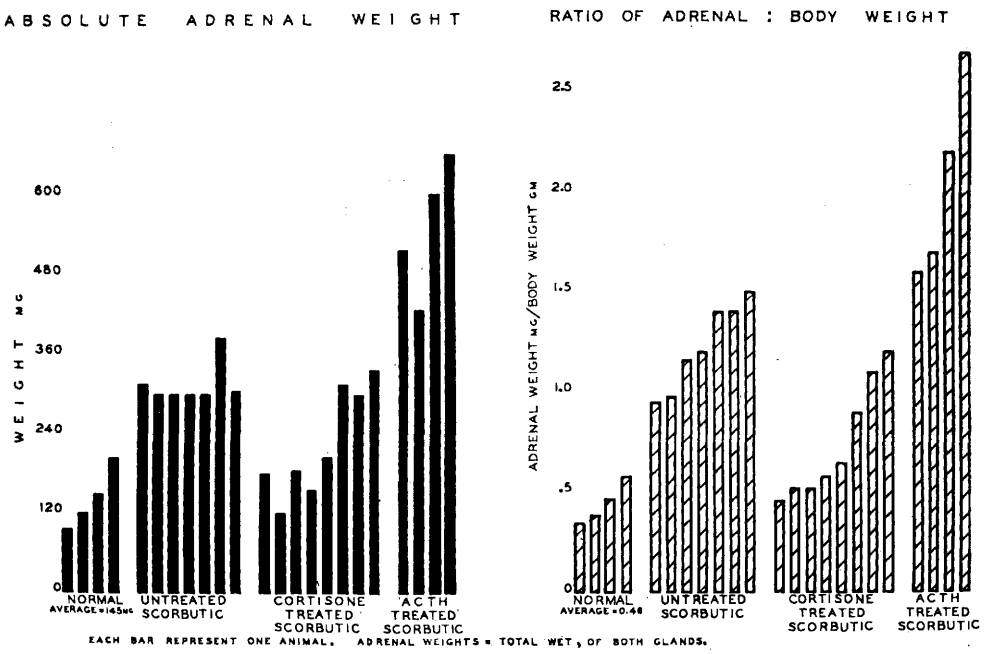

FIG. 2.
Effect of cortisone and ACTH on adrenal size.

with the ACTH-treated group.

3. *Scorbutic, ACTH-treated.* Group III, 4 animals. Four female guinea pigs were started on ACTH on the tenth day of the scorbutic diet. The course of ACTH was continued for 19 to 21 days, with three animals living 30 days, and one 29 days, an average of 29.8 days. In all cases a modest weight loss after initiation of the diet was followed by a plateau or slight rise (Fig. 1), but this was followed by a progressively rapid fall until death. Two moribund animals were sacrificed. In the other two, marked pulmonary edema was found at autopsy. Bloody diarrhea, hematuria and hind leg paralysis were observed terminally in three cases, but these signs developed about eight days later than in the controls of Group I. The hemorrhagic manifestations were roughly comparable to those occurring in the cortisone-treated group, and again seemed rather less than those of the untreated scorbutic controls. Microscopic examination generally confirmed the gross impression of amelioration of some of the scorbutic manifestations in the treated animals. The untreated scorbutic animals showed marked subcutaneous, intramuscular, periosteal, and epiphyseal hemorrhages. In the ACTH- and cortisone-treated animals, these regions showed hemorrhage but it was less extensive. All of the scorbutic animals had varying degrees of degeneration of muscle with no discernible differences between the treated and untreated. The histochemical technics employed failed to disclose any abnormalities of basement membranes or of perivascular tissues. Whereas glycogen was histochemically not demonstrable in the liver, adrenal or muscle sections of the untreated, scorbutic guinea pigs, small amounts of glycogen were present in each of these tissues in the animals that received either ACTH or cortisone,¶ even at the time of death from scurvy.

Studies of the adrenal glands. The data on adrenal size are shown graphically in Fig. 2. The four normal animals weighing 270 to 340 g had adrenal-pair weights averaging 145 mg $\pm$ 50 mg. The average ratio of adrenal weight in milligrams to body weight in grams—an index of relative adrenal

¶ Acknowledgement is gratefully made for the cooperation and assistance of Drs. J. W. Blunt and R. Lattes in these studies.

size—was 0.46 ± 0.12. The ascorbic acid content varied from 28.6 to 70.8 μg/100 mg of gland, averaging 51.3 μg. The adrenal glands of the untreated scorbutic animals were considerably hypertrophied, as has been previously described(7), but were otherwise grossly normal in appearance. The adrenal-pair weights were rather uniform, being 300 to 325 mg in 6 of 7 instances, and averaged 315 mg, about twice that of the normals. The gland/body ratio averaged 1.2 ± 0.28, which approximates the relative 250% increase for scurvy described by LaMer(7). The ascorbic acid content of these glands in every case was too low to be measurable.

In the cortisone-treated group, the adrenal gland size approached that of the normals except in 2 instances in which the weight was 300 and 340 mg, respectively. The average gland/body ratio was 0.74 ± 0.25, intermediate between the normal and the scorbutic controls. The adrenal ascorbic acid values were determined for six animals and found to be less than 2.5 μg/100 mg of fresh gland in four, *i.e.*, too low to be measurable. The other two had values of 3.9 and 4.3 μg/100 mg. In the ACTH group remarkable hypertrophy occurred, with adrenal pair weights varying from 430 to 660 mg, and averaging 550 mg. The gland/body ratio averaged 2.06 ± 0.54, the highest ratio of the experiment. The adrenal ascorbic acid content was under 2.5 μg/100 mg, again too low for measurement.

Morphology. Adrenal hypertrophy has been known to occur in scurvy since LaMer's description in 1920. In the untreated scorbutic animals (Group I), microscopic examination of the adrenals disclosed increased cell size, enlarged vesicular nuclei, and an increased number of cytoplasmic granules. The adrenals of the ACTH-treated guinea pigs showed the same changes but to a more marked degree, with actual mitotic figures occurring not infrequently, and greatly increased granule formation in the cells. The glands of the cortisone-treated group, by contrast, revealed small cells with few, if any, granules, and shrunken dark nuclei; a picture of involution.‖

Discussion. It would appear that the administration of ACTH or of cortisone moderates the course of scurvy in the guinea pig. Animals receiving the hormones were slower to lose weight and to show overt signs of the disease than were controls. Moreover, they lived appreciably longer. Post-mortem examination disclosed, both macroscopically and microscopically, that the treated animals presented relatively less extreme manifestations of scurvy. The finding of Schaffenburg, *et al.*(12), that cortisone exerts a protective action in guinea pigs rendered scorbutic is thus confirmed. However, the observation that a similar action is produced by ACTH makes it necessary to reconsider certain of the inferences that have hitherto been drawn in respect to the relation of ascorbic acid to adrenal function.

Because of the fall in adrenal ascorbic acid after the administration of ACTH(18-21), it has been assumed by many that ascorbic acid is necessary for, and is actually utilized in the production of the corticosteroids. In an unconfirmed report(22) an actual corticosteroid-ascorbate adrenal product has been described. As mentioned previously, Schaffenburg, *et al.*(12), reported on the possibility 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." They further postulated that compensatory ACTH overproduction occurred in consequence of insufficiency of carbohydrate-active corticosteroids secondary to vitamin C deficiency.

With this viewpoint, the mitigating effect of cortisone on the lesions of scurvy might be explained in terms of sparing ascorbic acid. One might suppose that the decreased activity of the adrenal cortex which follows upon cortisone administration is accompanied by

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decreased utilization of ascorbic acid. This, in turn, would release a certain amount of the vitamin which could serve to prolong the life of the animal on a scorbutic regimen.

However, an inference of the same hypothesis would be that ACTH should exert an opposite effect. For, by stimulating the adrenal, ACTH should accelerate the utilization of ascorbic acid and perhaps hasten the death of the animal rendered scorbutic. In fact, however, the influence of ACTH in scurvy appears to parallel that of cortisone. The intense hypertrophy and increase in the secreting granules of the adrenals indicate almost beyond doubt that corticosteroids were produced in large quantities, and this in spite of virtual absence of adrenal ascorbic acid on assay. The production of corticosteroids in the absence of vit. C had been previously suggested on other grounds, both for the guinea pig(20) and the chick(23). From the clinical side, additional evidence is derived from the observation that rheumatoid arthritis in one patient responded favorably to the administration of ACTH despite the coexistence of clinical scurvy. It is difficult to reconcile these findings with the concept that ascorbic acid constitutes a limiting factor in the synthesis of corticosteroids by the adrenal cortex.

The relation between vit. C and the activities of the adrenal cortex thus remains undefined. It may well be that the corticosteroids somehow influence the metabolism of ascorbic acid in many tissues and perhaps reduce the requirement of the organism for this vitamin.

As for the relation of the experimental findings to the clinically observed remissions of various types of purpura under the influence of ACTH, there is little that can be said without further study. It does appear that by the administration of cortisone or ACTH the hemorrhagic manifestations of scurvy may be made appreciably less severe in the guinea pig as well as in man.

Summary. 1. Cortisone and ACTH prolong life and reduce the hemorrhagic manifestations of scurvy in guinea pigs. The effects of the two hormones are similar. 2. The effect of these two hormones is also parallel in the direction of maintenance of glycogen storage in liver, adrenal, and muscle in the scorbutic guinea pig. 3. The moderating effect of ACTH on scurvy makes it seem probable that vitamin C is not needed for the production of the corticosteroids similar to cortisone. This idea receives further support from other observations, including the demonstration of increased adrenal activity induced by ACTH in the absence of demonstrable adrenal ascorbic acid. 4. The locus of action of adrenal corticosteroids in the amelioration of certain hemorrhagic phenomena is not clear, but may be on the blood vessels or perivascular tissues.

Addendum. Further experience has shown that it is possible to produce severe toxic effects in the guinea pigs with these relatively large doses of cortisone and ACTH so that the protective effect of these hormones then may not occur. Hyperadrenalism, however, is manifested in all the treated scorbutic animals.

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