Circulating 17-Hydroxycorticosteroids in Ascorbic Acid-Deficient Guinea Pigs.* (20473)

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Since the demonstration by Szent-Györgyi (1) of high concentrations of ascorbic acid in the adrenal gland, numerous controversial reports concerning the relation of ascorbic acid to the pituitary-adrenal system and its secretions have appeared. These were recently reviewed by Pirani(2). The development of a technic for the quantitation of circulating 17-hydroxycorticosteroids(3) has permitted a more direct approach to this problem than had been previously available. The data presented in this report were obtained in preliminary studies of circulating 17-hydroxycorticosteroids in guinea pigs rendered ascorbic acid-deficient.

Methods. Male guinea pigs weighing 500 to 750 g were used in these experiments. Ascorbic acid deficiency was induced by a scorbutigenic diet described by Oesterling and Long(4). This diet was given *ad libitum* and the animals were weighed at regular intervals. In most instances a stabilization of weight curves occurred within a few days; rapid weight loss occurred after 14 to 18 days. Only those animals who exhibited a stabilization of weight for at least 10 days prior to the late weight loss and who gave no clinical indications of infection are included in this report. A group of animals who had exhibited a profound weight loss of 2 to 3 days' duration prior to the 17th day of scorbutigenic diet were treated with crystalline ascorbic acid, given orally in a dose of 25 mg per animal per day, from the 17th day until sacrifice on the 22nd day. Each of these animals responded with a moderate weight gain within one to 2 days. *Ad libitum* controls were maintained on a Purina pellet ration. All animals had free access to tap water. At the time of sacrifice, animals were rapidly anesthetized in an ethyl ether atmosphere and blood samples were obtained from the abdominal aorta into heparinized syringes. Immediately after exsanguination of the animal, adrenals were removed, cleaned, weighed and homogenized in 4% trichloroacetic acid. Plasma samples were separated by centrifugation and stored in deep-freeze until used. In some instances the plasmas of 2 to 3 guinea pigs were pooled to make 10 to 15 ml samples for 17-hydroxycorticosteroid determinations.

Circulating 17-hydroxycorticosteroid concentrations were determined by the plasma method of Nelson and Samuels(3). Adrenal ascorbic acid content was measured by the method of Roe and Kuether(5).

Observations. The duration of ascorbic acid-deficient intake and the severity of resultant weight loss are listed for each animal in Table I. As will be seen in this table, prolonged ascorbic acid deficiency resulted in the nearly complete depletion of adrenal ascorbic acid and a striking elevation of circulating 17-hydroxycorticosteroid concentrations. In the group of animals who developed severe ascorbic acid deficiency as evidenced by weight curves, but were treated with ascorbic acid for 5 days prior to sacrifice, the adrenal ascorbic acid content as well as plasma concentrations of 17-hydroxycorticosteroids were not significantly different from *ad libitum* controls (Table II).

Discussion. Exceedingly high plasma concentrations of 17-hydroxycorticosteroids were found in animals whose adrenal glands were almost completely devoid of ascorbic acid. This would suggest that if ascorbic acid is in any way essential to the formation of 17-hydroxycorticosteroids, it need be present only in minute amounts in the adrenal cortex. Concentrations of steroids reported here do not necessarily reflect an accelerated rate of adrenal cortical secretion, but may be the

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TABLE I. Plasma 17-Hydroxycorticosteroids in Guinea Pigs with Treated and Untreated Ascorbic Acid Deficiency.

	No. days on diet	Severity of scurvy*	Adrenal wt (mg/100 g body wt)	Adrenal ascorbic acid (mg/100 g adrenal)	Plasma 17-hydroxycorticosteroids (μg/100 ml)
Scorbutigenic diet	24	+	82	1.11	
	24	+	114	.84	152.5
	24	++	103	.90	
	22	++	108	1.23	380.0
	22	++	71	1.32	
	21	++	127	.84	478.0
	21	++	88	.99	153.0
	21	++	100	.72	124.0
	16	+++	115	.60	222.2
	22	+++	66	1.20	
	22	+++	151	.99	394.2
	22	+++	99		
	21	+++	101	.84	—
	22	+++	147	.51	526.0
	21	+++	98	1.08	—
	25	+++	101	.81	192.8
	24	+++	115	.57	—
	21	+++	105	.63	—
	23	++++	122	.84	282.1
	25	++++	142	.51	416.0
Mean	22		108 ± 5.1	.87 ± .018	301.9 ± 43.03
Scorbutigenic diet (ascorbic acid, 25 mg/day for last 5 days)	22	++	47	37.8	43.3
	22	++	84	39.0	65.4
	22	++	83	26.6	62.5
	22	++	65	42.0	30.5
	22	++++	119	33.7	141.8
Mean	22		80 ± 12.0	35.8 ± 2.66	68.7 ± 19.35
Ad libitum controls	Mean		47 ± 3.0	35.5 ± 3.86	32.9 ± 4.60
	No. animals		10	10	17

* Severity of scurvy was classified on the basis of weight response curves (extent and rapidity of late weight loss) without regard to laboratory data. In the ascorbic acid treated group the classification was based on the status of the animals before treatment was started.

result of interference with the utilization, conjugation or excretion of 17-hydroxycorticosteroids. Ascorbic acid deficiency of the severity produced in these experiments undoubtedly constitutes a chronic stressful situation which may account, wholly or in part, for the excessive amounts of circulating 17-hydroxycorticosteroids observed. However, irrespective of the initiating circumstances, it would appear that these steroids can be produced in the virtual absence of ascorbic acid and that adrenal cortical insufficiency, at least from the standpoint of the ability to produce 17-hydroxycorticosteroids, does not result from a depletion of adrenal ascorbic acid. Whatever the nature of the abnormality responsible for the excessively high plasma concentrations of 17-hydroxycorticosteroids noted, it is largely corrected by the administration of excessive amounts of ascorbic acid over a brief period.

TABLE II. Probability Values, Plasma 17-hydroxycorticosteroids in Guinea Pigs with Treated and Untreated Ascorbic Acid Deficiency.

Scorbutigenic diet	vs. *ad libitum* controls	$p < .01$
	vs. scorbutigenic diet + ascorbic acid	$p < .01$
Scorbutigenic diet + ascorbic acid	vs. *ad libitum* controls	p .09

In an attempt to evaluate the role of various factors involved, further experiments are in progress utilizing pair-fed controls, adrenalectomized controls, varying schedules of ascorbic acid administration, and less severe ascorbic acid deficiency; these include urinary excretion of these steroids.

Summary. 1. Quantitative determinations of plasma 17-hydroxycorticosteroids in guinea

pigs with severe ascorbic acid deficiency were made. 2. Plasma concentrations of these steroids were found to be approximately 10 times those found in normal guinea pigs despite the virtual absence of adrenal ascorbic acid. 3. Scorbutic animals treated with ascorbic acid for 5 days prior to sacrifice had circulating 17-hydroxycorticosteroids in amounts not significantly higher than those of control animals.

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Effects of Chloramphenicol on Incorporation of Glycine-2-C^{14} into Mammalian Tumor Cell Proteins and Purines.* (20474)

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It has been demonstrated by Wisseman *et al.*(1) that the antibiotic d(—) *threo* chloramphenicol is effective in inhibiting protein synthesis in susceptible microorganisms such as *E. coli*. They have further demonstrated that higher concentrations of l(+) *threo* chloramphenicol do not give this effect, and that concentrations of the d(—) threo isomer which effectively stop protein synthesis (50 μg/ml) permit nucleic acid purine and ribose synthesis to proceed for a significant time. Since viable suspensions of mammalian tumor cells were available and had been shown to carry on incorporation of glycine-2-C^{14} into purines and proteins(2), it was of some interest to test the effects of chloramphenicol isomers on this mammalian cell system.

Methods. The Ehrlich carcinoma cells were obtained by intraperitoneal injection of 6-10-week-old Rockland strain white mice with approximately 10^7 cells per mouse. The cells were withdrawn from mice after 5-7 days, under light ether anesthesia with a hypodermic syringe and No. 18 needle. At this time the suspension was approximately 90% tumor cells. The Gardner lymphosarcoma (6C3HED) cells were obtained in similar fashion from C3H mice 7-10 days after injection, at which time the suspensions were approximately 96% tumor cells. The cells were washed 3-4 times by suspending in isotonic saline and centrifuging. Warburg respirometer vessels of 60 ml capacity were used as reaction vessels. Incubation was for one hour at 38°C. Each vessel contained a total of 12 ml of medium, consisting of Robinson's medium with glucose and bicarbonate(3), 150 μg glycine-2-C^{14}† and cells equivalent to 30 mg dry weight. The chloramphenicols‡ were weighed dry into each such flask and dissolved in the medium. The cells were added just prior to incubation, except where otherwise indicated. Purines and proteins were isolated and counted for radioactivity as described earlier(2). Paper spots as counted contained 200-300 μg of purine. Counting was continued until statistically significant to within 5%.

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† Specific activity 20,600,000 counts/min/mg; obtained from Tracerlab, Inc., on allocation by the U. S. Atomic Energy Commission.

‡ The d(-) threo chloramphenicol and l(+) threo chloramphenicol were obtained through the courtesy of Parke-Davis and Co. and Major Charles L. Wisseman, Jr., respectively.