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Title RESPONSE OF SCORBUTIC GUINEA PIGS TO ASCORBIC ACID AND ADRENOCORTICOTROPIC HORMONE (ACTH)

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Thirty-six female guinea pigs were placed on a scorbutogenic diet for 14 days and then divided into groups of four. Three groups received adrenocorticotropic hormone (ACTH) with each group given 10, 20, or 40 units. Three groups each received 50 mg of ascorbic acid and three groups received 50 mg of ascorbic acid plus ACTH at thelevel of either 10, 20, or 40 units.

Blood samples were drawn from all animals by heart puncture prior to treatment and one, two, and four hours after treatment. The plasma from these samples was then chemically analyzed for its ascorbic acid concentration by the 2, 4-dinitrophenylhydrazine micro-method of Lowry, Lopez, and Bessey (16).

The results of the chemical analyses showed that when ACTH is given alone there is no difference between the time intervals, but there is a significant difference ($p < .05$) between the levels given. The ascorbic acid alone showed a significant change with time

($p < .005$). A rapid increase in the plasma ascorbic acid concentration occurred at one hour, followed by a decline at two and four hours. When ascorbic acid plus ACTH is given there is a significant difference ($p < .005$) with time, in that the ascorbic acid plus ACTH showed a more rapid decline at two and four hours than ascorbic acid alone did. There is also a significant difference ($p < .005$) in the response with the different levels of ACTH. Forty units of ACTH showed a higher plasma ascorbic acid concentration at one hour than 20 units; however, the 10-unit level showed the highest concentration at one hour of all the levels tested. The 40 and 20 units appear to cause a pharmacological dose response, while 10 units may be nearer the physiological dose level.

These results are interpreted as being due to two possible body responses. One is that the ACTH increases the tissue ascorbic acid absorption. The other is that the ascorbic acid corrects the ascorbic acid:cholesterol ratio increasing the hormone production under the influence of ACTH, and increases the peripheral metabolism of these hormones.

It is felt that further work on the effects of ACTH on ascorbic acid should include studies on the urinary excretion values, tissue reabsorption times, and should also include the use of radioactive tracers.

RESPONSE OF SCORBUTIC GUINEA PIGS TO ASCORBIC ACID AND ADRENOCORTICOTROPIC HORMONE (ACTH)

by

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RESPONSE OF SCORBUTIC GUINEA PIGS TO ASCORBIC ACID AND ADRENOCORTICOTROPIC HORMONE (ACTH)

INTRODUCTION

The physiology and pathology of scurvy has been under investigation since the first true scientific report was published by James Lind in 1753 (17, p. 64-65); however the actual causative agent and pathological changes were only brought to light in the 1900's and the interest in the relationship of the adrenal glands with scurvy is even more recent.

The research in this area was first aided by the observation of Holst and Frölich in 1907 (17, p. 64-65) that guinea pigs were susceptible to scurvy. Prior to this it was believed that only man and other primates were susceptible. This work was followed by the report of McCorrison in 1919 (17, p. 88) that the adrenal glands were hypertrophied in scurvy. This observation went without much notice until Szent-Györgyi in 1928 (29, p. 1393-1398) isolated ascorbic acid from ox adrenals as hexuronic acid and showed the presence of a high concentration of this acid in the adrenal cortex. This work was improved upon by Harris and Ray in 1933 (11) when they demonstrated that scurvy in guinea pigs was accompanied by a depletion of adrenal ascorbic acid. Later the same year Harris and Ray (12) also demonstrated that the medulla of the adrenal had a high concentration of ascorbic acid. At this time they advanced the theory

that ascorbic acid was needed for protection of the normal functional activities of the adrenal gland.

The pituitary-adrenocortical axis mechanism came into the picture in 1948 when Sayers, et al. (23) found in both rats and guinea pigs that adrenotropic hormone injections caused a rapid fall in the adrenal ascorbic acid content, which was followed by a drop in the adrenal cholesterol content. Sayers et al. (23) believed this to be associated with the formation and release of cortical hormones. This hypothesis was first suggested by Giroud and co-workers in 1940, as cited by Meiklejohn (17, p. 80) and Pirani (19, p. 202-203); however Giroud's work was in question at the time of Sayers experiment.

In 1950 both Sayers (22, p. 276-281) and Selye (24, p. 256-258) reported that various types of stress also deplete ascorbic acid from the adrenal cortex by stimulating the hypophysis to secrete adrenocorticotropic hormone (ACTH) which in turn stimulates the adrenal cortex.

It has also been reported by various workers as cited by Meiklejohn (17, p. 84-85) that prolonged ACTH treatments produce symptoms of scurvy but this effect could not be confirmed by others except under conditions of a low ascorbic acid intake.

Pirani (19, p. 205-222), in his review, states that he and

other workers have found that the administration of cortisone and ACTH in single, large doses causes an elevated plasma ascorbic acid content. The actual cause for this could not be explained.

With the great amount of conflicting, and in areas, incomplete information on the role of ascorbic acid and adrenocorticotropic hormones in the pituitary-adrenal axis, the present study was initiated to provide information on the relationships between ascorbic acid and adrenocorticosteroids and to check if there is a possible augmentation of ascorbic acid uptake with increased adrenal activity. This was done by using scorbutic guinea pigs into which simultaneous injections of ascorbic acid and ACTH singly, and in combination were given after which the ascorbic acid responses were determined.

REVIEW OF LITERATURE

Since the discovery of a relationship of ascorbic acid with the pituitary-adrenal axis and stress, there has been intensive work done in the field of ascorbic acid and the homeostasis of the adrenal gland. The actual work that has been done on the effects of simultaneous applications of adrenocorticotropins and ascorbic acid however, is small and largely contradictive.

The basic work on the relationship of adrenal activity and ascorbic acid was done by Sayers and his co-workers in the mid 1940's. Sayers et al. in 1946 (23) showed that when injections of purified adrenotropic hormones were given to rats and guinea pigs a prompt fall of ascorbic acid in the adrenal occurred followed by a slower decrease in cholesterol. No other tissues were affected. This depressed level of ascorbic acid in the adrenal was only seen for a short period (24 hours) in the rat. The guinea pig, however, showed a depression for longer periods of time and ascorbic acid levels had not reached normal after 24 hours. This response was believed to be associated with the formation and release of the cortical hormones.

Jailer and Boas (14) did a similar experiment on chicks. Despite prolonged administration of either epinephrine or ACTH,

they could not alter the adrenal ascorbic acid content. The adrenal gland itself showed stimulation, however, as it was hypertrophied.

Hyman, Ragan, and Turner (13), studying the effects of cortisone and ACTH on scurvy in the guinea pig, found that injections of both materials reduced the hemorrhagic symptoms and prolonged the life of scorbutic guinea pigs. These materials also caused a maintenance of glycogen stores in the liver, adrenal, and muscle which is connected with one of the major metabolic changes during scurvy. This was in agreement with the work by King (15) which showed that scorbutic guinea pigs had an increased oxygen consumption and a lowered capacity to metabolize glucose. King believed this was due to a reduced production of adrenal hormones. Eisentstein and Shank (10) found a similar response with ACTH but also noted that when the ACTH was administered there was a 50 percent reduction in circulating eosinophils, indicating that the adrenal gland was being stimulated to elaborate cortical hormones. Pirani, Stepto, and Sutherland (20) did work on the effect of cortisone on both normal and scorbutic guinea pigs. They found that cortisone treatment increased appetite and thus reduced loss due to scurvy due to inanition. There was also a partial prevention of adrenal hypertrophy and a reduction in some of the pathological changes but cortisone did not alter the course of the disease or prevent death.

The final conclusions reached by the latter three papers was that ascorbic acid did not appear to affect the production of adrenal corticoids. Pirani (19, p. 205), however, in his review states that even in severe scurvy all ascorbic acid is not removed from the tissues and there may only be small amounts required for certain hormone production. Stepto et al. (27) reported that the production of adrenal corticoids may be dependent on a ratio of cholesterol to ascorbic acid and, if this is not maintained, the biochemical and morphological data indicates a depletion of adrenal steroids. Booker et al. (7) found that ascorbic acid affects the serum cholesterol content which is in turn influenced by the addition of ACTH. They indicated that these three factors may be the basis of control and release of adrenal corticoids. Banerjee and Deb (3) found that scorbutic guinea pigs excreted significantly lower amounts of 17-Ketosteroids in the urine then normal. They suggest that the scorbutic guinea pig suffers from a hypofunction of the adrenal cortex, but that all function is not stopped.

In the effects of stress, Selye (24, p. 256-258) emphasizes the importance of ascorbic acid to the adrenal cortex in its response to the General-Adaptive Syndrome (G. A. S.). He found that various stressors augment the ascorbic acid requirement of the body and that ascorbic acid tolerance may be increased or decreased during

stress. In experiments on rats involving various stresses, the response of the plasma ascorbic acid assumed a triphasic pattern with a marked decrease, followed by a rapid increase to above normal levels and in final stages a progressive decline. The rise of the ascorbic acid was found to be apparently related to severity of stress.

Bahn and Glick (2) found that stress conditions caused a decrease in the concentration of adrenal ascorbic acid. These losses were similar to those caused by treatment with ACTH. Injections of desoxycorticosterone acetate (DCA) and cortisone caused little change in the adrenal ascorbic acid content. However either DCA or cortisone in a single large dose prevented ascorbic acid loss in rats exposed to cold stress.

The histochemical work of this experiment showed that the changes in ascorbic acid occurred chiefly in the outer fasiculata or reticularis zone of the adrenal gland. Bacchus (1, p. 127-135) reports that under acute stress the adrenal cell is essentially normal but ascorbic acid granules assume a peripheral aggregation. Under more sustained stress there was cell enlargement with a marked depletion of ascorbic acid.

Pirani (19, p. 203) states that the fasicularis zone of the adrenal is the one involved with the production of corticoids thus

evidencing some change in secretory function of the cortical hormones.

Thus three views on the ascorbic acid and pituitary-adrenal axis have been developed. The first view that only ascorbic acid is involved with the release at formation of adrenalcortical hormones has largely been disproved. The second is that ascorbic acid is not essential for the formation of the adrenal cortical hormones. This view does not now appear to be any better than the first. The third view is that ascorbic acid has some effect on the release or production of cortical hormones. This is the view that most workers hold today. While most of the evidence points to this view, the actual mechanisms and proof of such have not been uncovered.

MATERIALS AND METHODS

Experimental Animals

Thirty-six young female guinea pigs between 400-500 grams in weight were divided into nine groups with four guinea pigs per group and then placed on a scorbutogenic diet consisting of 50 percent rolled barley, 25 percent oats, 20 percent mill run, and five percent soybean oil meal. This diet was felt to be adequate except for vitamins A and D. Adequate vitamins A and D (crushed pills) (Pioneer Drug Company) were provided in the drinking water. This diet was fed ad lib for 14 days. The 14-day period was decided on because the radioactive tracer work of Perić-Golia, Eik-nes, and Jones (18) showed that the most rapid loss of labeled hormone from the adrenal gland occurred at this time. Also, depletion of ascorbic acid from the animal's body is almost complete by 14 days.

Treatments and Sampling

After 14 days on the scorbutogenic diet three groups received ACTH with one group each given 10, 20, or 40 usp units of corticotropin-ACTH (ACTHAR-Armour) interperitoneally, three groups each received 50 mg of ascorbic acid (Cevalin-Lilly) intramuscularly, and three groups received a combination of ascorbic acid and ACTH with

each group receiving 50 mg ascorbic acid and either 10, 20, or 40 units ACTH.

Blood samples were drawn by heart puncture prior to treatment, and one hour, two hours, and four hours after treatment. The animals were under ether anesthesia for the heart puncture. The heart puncture was done with a 20 gauge needle and a five ml syringe which had been coated with a three percent solution of potassium oxalate. The animal was placed on its back and the needle inserted between the ribs just inside the left foreleg and into the heart. A 0.75 ml sample of blood was withdrawn.

Chemical Analysis

The determination of total ascorbic acid was done on blood plasma using the micro-method of Lowry, Lopez, and Bessey (16) which was modified from the 2, 4-dinitrophenylhydrazine method of Roe and Ruether (21). The readings were made on a Beckman model DU spectrophotometer and values taken from a standard curve.

RESULTS

The results of the plasma ascorbic acid analysis, expressed as mg per 100 ml of blood plasma at definite intervals after treatment, are shown in Table 1. This includes individual results as well as the group means. The results taken from Table 1 show a low level of plasma ascorbic acid concentration prior to treatment in most animals. When ACTH alone was given, there did not appear to be a marked change in the plasma ascorbic acid concentration with time. The treatments of ascorbic acid and ascorbic acid plus ACTH, however, resulted in a marked increase in the plasma ascorbic acid concentration within one hour, after which there was a marked decline in the plasma ascorbic acid concentration in the animals receiving both ascorbic acid and ACTH, regardless of the level of ACTH given. The decline when ascorbic acid alone was given was not as marked. This can be seen more clearly in Figure 1 where the mean plasma ascorbic acid concentration of treatment groups is plotted against time. The data (Figure 1) also indicate a difference in the time response of ascorbic acid plus ACTH which is dependent upon the level of ACTH given as compared to the time response of ascorbic acid alone.

To determine whether the differences observed between treatments, levels, and times were significant, several analyses of variance were made on the effects of the level of ACTH given, the

Table 1. Results of blood analysis expressed in milligrams per 100 ml plasma.

Animal Number Within Group	50 mg Ascorbic Acid — Time of Sample Withdrawl in Hours				Amount of ACTH	No Ascorbic Acid — Time of Sample Withdrawl in Hours				Amount of ACTH	50 mg Ascorbic Acid — Time of Sample Withdrawl in Hours				
	Prior	1	2	4		Prior	1	2	4		Prior	1	2	4	
1	0.140	12.80	6.52	2.20	40 units	0.167	0.180	0.127	0.133	40 units	0.000	10.05	3.97	1.07	
2	0.167	12.68	6.73	1.81	40 units	0.167	0.150	0.160	0.133	40 units	0.100	11.15	6.45	1.85	
3	0.120	15.45	11.60	3.22	40 units	0.110	0.120	0.150	0.147	40 units	0.000	6.25	3.15	0.967	
4					40 units	0.187	0.217	0.153	0.120	40 units	0.000	13.03	3.16	1.13	
					(mean)	0.1578	0.1668	0.1475	0.1333	(mean)	0.025	10.120	4.183	1.254	
1	0.213	7.75	6.60	2.50	20 units	0.160	0.100	0.100	0.110	20 units	0.147	6.67	5.33	1.38	
2	0.153	7.80	6.75	1.52	20 units	0.100	0.125	0.113	0.113	20 units	0.177	10.90	4.88	1.38	
3	0.190	13.05	3.95	1.82	20 units	0.207	0.130	0.110	0.213	20 units	0.140	8.08	3.50	1.17	
4	0.150	4.47	5.17	5.10	20 units					20 units	0.550	10.23	3.12	0.903	
					(mean)	0.1557	0.1183	0.1077	0.1453	(mean)	0.2535	8.970	4.208	1.208	
1	0.113	9.30	9.37	4.32	10 units	0.100	0.000	0.000	0.000	10 units	0.140	10.58	5.20	1.43	
2	0.113	11.35	5.13	1.72	10 units	0.117	0.100	0.100	0.100	10 units	0.267	16.50	7.02	2.18	
3	0.120	11.87	7.03	2.55	10 units	0.000	0.000	0.000	0.000	10 units	0.140	10.18	4.98	1.67	
4	0.163	10.82	8.48	3.15	10 units	0.000	0.000	0.000	0.000	10 units	0.133	10.98	4.35	1.16	
(mean)	0.1493	10.667	7.030	2.719	(mean)	0.0543	0.025	0.025	0.025	(mean)	0.1650	12.06	5.388	1.610	

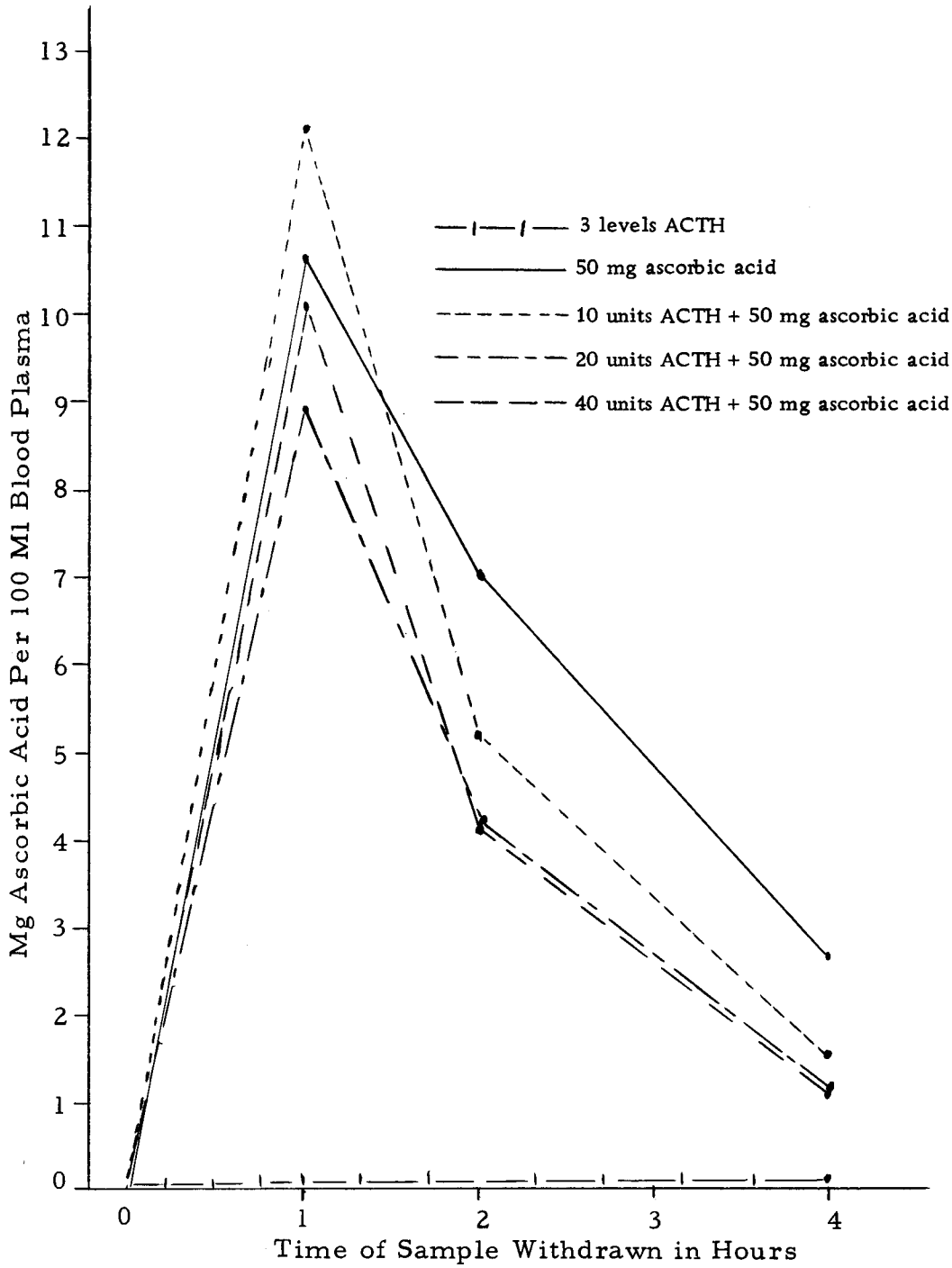

Figure 1. Mean changes in plasma ascorbic acid with time.

response with time, and how these differed with ascorbic acid alone versus ascorbic acid plus ACTH. However, preliminary analysis showed that the variance tended to be proportional to the mean, thus a square root transformation was made. The transformed values are shown in Table 2 and the analyses of variance are on the transformed data.

The initial analysis of variance on the transformed data showed a highly significant ($p < .005$) difference among all subgroups (Table 3). The analysis of variance was then run on each treatment group, ACTH alone, ascorbic acid alone, and ascorbic acid plus ACTH, to determine the effects of the level of treatment and the responses with time. The analysis of variance on ACTH alone (Table 4) showed a significant difference between the levels of ACTH ($p < .05$) but no significant change with time. The analysis of variance on ascorbic acid (Table 5) showed a highly significant change with time ($p < .005$). The analysis of variance of the ascorbic acid plus ACTH (Table 6) showed a highly significant change with time ($p < .005$) and a significant difference within levels ($p < .05$). The final analysis of variance was between ascorbic acid alone and ascorbic acid plus ACTH (Table 7). This analysis showed a highly significant difference ($p < .005$) between ascorbic acid alone and in conjunction with ACTH at various levels and between the time responses of the two treatments.

Table 2. Square-root transformation of data in Table 1.

Animal Number Within Group	50 mg Ascorbic Acid — Time of Sample Withdrawl in Hours				Amount of ACTH	No Ascorbic Acid — Time of Sample Withdrawl in Hours				Amount of ACTH	50 mg Ascorbic Acid — Time of Sample Withdrawl in Hours			
	Prior	1	2	4		Prior	1	2	4		Prior	1	2	4
1	0.37417	3.57771	2.55343	1.48324	40 units	0.40866	0.42426	0.35637	0.36469	40 units	0.000	3.17018	1.99249	1.03441
2	0.40866	3.56090	2.59422	1.34536	40 units	0.40866	0.38730	0.40000	0.36469	40 units	0.31623	3.33917	2.53969	1.36015
3	0.34641	3.93070	3.40591	1.79444	40 units	0.33166	0.34641	0.38730	0.38341	40 units	0.000	2.50000	1.77482	0.98336
4					40 units	0.43244	0.46583	0.39115	0.34641	40 units	0.000	3.60976	1.77764	1.06302
					(mean)	0.39536	0.40595	0.38371	0.36480	(mean)	0.07906	3.15478	2.02116	1.11024
1	0.46152	2.78388	2.56905	1.58114	20 units	0.40000	0.31623	0.31623	0.33166	20 units	0.38341	2.58263	2.30868	1.17473
2	0.39115	2.79285	2.59808	1.23288	20 units	0.31623	0.35355	0.33616	0.33616	20 units	0.42071	3.30152	2.20907	1.17473
3	0.43589	3.61254	1.98946	1.34907	20 units	0.45497	0.36056	0.33166	0.46152	20 units	0.37417	2.84253	1.87083	1.08167
4	0.38730	2.11424	2.27376	2.25832	20 units					20 units	0.74162	3.19850	2.03873	0.95026
					(mean)	0.39040	0.34345	0.32768	0.31623	(mean)	0.47998	2.98130	2.03873	1.09535
1	0.33616	3.04959	3.06105	2.07846	10 units	0.31623	0.000	0.000	0.000	10 units	0.37417	3.25272	2.28035	1.19583
2	0.33616	3.36898	2.26495	1.31149	10 units	0.34205	0.31623	0.31623	0.31623	10 units	0.51672	4.06272	2.64953	1.47643
3	0.34641	3.44538	2.65142	1.59687	10 units	0.000	0.000	0.000	0.000	10 units	0.37417	3.19064	2.23159	1.29229
4	0.40373	3.28943	2.91204	1.77482	10 units	0.000	0.000	0.000	0.000	10 units	0.33616	3.31363	2.08567	1.07703
(mean)	0.38432	3.22966	2.62467	1.61874	(mean)	0.16457	0.07906	0.07906	0.07906	(mean)	0.40031	3.45493	2.31179	1.26040

Table 3. Analysis of variance of subgroups.

Source	d.f.	Sum of Squares	Mean Squares	f
Total	135	188.24582	1.39441	
Subgroup	27	178.39799	6.60733	72.46185 p <.005
Error	108	9.84783	0.091184	

Table 4. Analysis of variance - ACTH alone.

Source	d.f.	Sum of Squares	Mean Squares	f*
Total	32	0.90497	0.02828	
Subgroup	8	0.65751	0.082189	0.90136
Between levels	2	0.65040	0.32520	3.56644 p <.05
Between times	2	0.00032	0.00016	0.00174
Interaction	4	0.00679	0.00170	0.01862

*Error from subgroup analysis 0.091184 with 108 d.f.

Table 5. Analysis of variance - ascorbic acid alone.

Source	d.f.	Sum of Squares	Mean Squares	f*
Total	32	19.81113	0.61910	
Between times	2	14.56766	7.28383	79.88091 p <.005
Error	30	5.24347	0.17478	

*Error from subgroup analysis 0.091184 with 108 d.f.

Table 6. Analysis of variance - ascorbic acid plus ACTH.

Source	d. f.	Sum of Squares	Mean Square	f*
Total	35	28. 24136	0. 80690	
Subgroups	8	25. 77093	3. 22136	35. 32835 p <. 005
Between levels	2	0. 62641	0. 31320	3. 43486 p <. 05
Between times	2	25. 03246	12. 51623	137. 26402 p <. 005
Interaction	4	0. 00121	0. 00030	0. 00331

*Error from subgroup analysis 0. 091184 with 108 d. f.

Table 7. Analysis of variance between ascorbic acid alone and ascorbic acid plus ACTH.

Source	d. f.	Sum of Squares	Mean Square	f*
Subgroup	11	42. 23947	3. 83995	42. 11210
Between times	2	38. 82315	19. 41158	212. 88362 p <. 005
Between levels	3	2. 52738	0. 84246	9. 23912 p <. 005
Time by levels	6	0. 88894	0. 14816	1. 62485

*Error from subgroup analysis 0. 091184 with 108 d. f.

A summary of the results of the analyses of variance for the experiment shows that when scorbutic guinea pigs are treated with ACTH with and without ascorbic acid, there was a significant difference in response to the level of ACTH supplied. A highly significant difference was found in the response with time when ascorbic acid was supplied with either 0, 10, 20, or 40 units of ACTH. It was found that when ascorbic acid alone was given there was a rapid increase in the plasma ascorbic acid within one hour, followed by a decrease two and four hours after treatment. If ACTH was given with the ascorbic acid, regardless of the level of ACTH given, there was a similar rise in the plasma ascorbic acid within one hour, followed by a sharper decline at two and four hours than when ascorbic acid alone is given.

DISCUSSION

The difference between the response of animals receiving ascorbic acid alone and those receiving ascorbic acid plus ACTH, where the ascorbic acid shows a less rapid decline in the plasma ascorbic acid concentration than ascorbic acid plus ACTH - regardless of the level of ACTH - may be due to three main factors associated with the treatments and the scorbutic condition of the guinea pigs.

The first factor to be considered is the effect of ACTH on the renal clearance and urinary excretion of ascorbic acid. Beck, Browne, and MacKenzie (4, p. 1009-1019) report that the action of ACTH increases the glomerular filtration rate, renal plasma flow, and tubular reabsorption. They state that these are responses through the adrenal and not a direct effect of the ACTH.

In 1946, Dutcher and Guerrant (9, p. 281) reported that when large doses of ascorbic acid were given to humans deficient in ascorbic acid, as much as 1500 mg were given without significant urinary loss, but a great variability was observed among patients as to the renal threshold of ascorbic acid. Booker, Hayes, and Dent (5) in 1950 report that animals under severe stress will secrete more ascorbic acid through the urine than is taken in. This was interpreted as an inability to control administered ascorbic acid as well

as a loss of ability to hold stored ascorbic acid. When their animals were stressed and given ascorbic acid plus ACTH the urinary secretion was significantly less and plasma ascorbic acid values were observed to rise. It should be kept in mind that the animals were on adequate ascorbic acid intake. Beck, Browne, and MacKenzie (4, p. 1006-1022) working on patients with various diseases and exhibiting low ascorbic acid values, showed that ACTH alone could increase the urinary excretion of ascorbic acid when these patients were placed on a diet adequate in ascorbic acid.

It can be seen that ACTH appears to increase urinary excretion of ascorbic acid. However, with the effects of ascorbic acid deficiency and treatment with ascorbic acid, the urinary excretion values are changed and do not offer as clear a picture. This is further complicated by the finding of Burns, Dayton, and Schulenberg (8) in 1956, using uniformly labeled L-ascorbic acid, that the main route of metabolism involves complete oxidation of the entire chain to CO_2 which is eliminated in the respiratory gases; a smaller fraction is excreted in the urine as ascorbic acid, diketogulonic acid, and oxalic acid. This brings up the question of how reliable the urinary ascorbic acid values may be in indicating the ascorbic acid state of the body and the utilization that is occurring.

The second factor to consider is that tissue tolerance of ascorbic acid is increased with ACTH; thus tissues in a deficient animal

treated with ACTH could show a more rapid uptake of ascorbic acid. Booker et al. (7) states that ACTH increases the deposition of ascorbic acid in the adrenal gland and other tissues. Sluscher and Roberts (25) report that in severe stress, such as hemorrhage, ascorbic acid can be released from such high-content organs as the liver and spleen. This phenomenon can occur in the absence of the activation of the pituitary-adrenal axis.

The final factor under consideration is that an increased stimulation of the adrenal gland, in the presence of ascorbic acid, may cause an increased hormone production. This causes an increase in ascorbic acid utilization at the site of hormone production as well as in peripheral metabolism. Stepto et al. (27, p. 758-763) report that during scurvy, the depletion of cortical steroids is due to the depletion of ascorbic acid which upsets the ratio of cholesterol and ascorbic acid that is needed for normal steroid production in the adrenal cortex. Stewart, Horn, and Robson (28) found that ACTH alone produced no change in the total plasma ascorbic acid, but increased the reduced ascorbic acid level, thus reducing the dehydroascorbic acid level. This change was believed to be associated with the increased production of adrenal hormones caused by ACTH. Slusher and Roberts (26) report that this action of ACTH is an indirect measure of the corticosteroid secretion and is not an indirect action of ACTH. Booker, Hayes, and Dent (6) working on the blood

level of ascorbic acid in dogs, found that ACTH increased the plasma levels of ascorbic acid in dogs that received supplemental ascorbic acid.

The responses observed in the present experiment are believed to be a composite of the three factors mentioned. The animals used were deficient in ascorbic acid, yet the adrenals still appeared to be functional in regards to their response by stimulation of ACTH. It is regrettable that due to technical difficulties it was not possible to obtain samples of urine at the same time as blood samples were taken and thus narrow down the possibilities of action of the ascorbic acid plus ACTH.

It is felt that the response of ascorbic acid plus ACTH observed is due to two factors. The first is that the animal, being in a deficient state, absorbs into the tissues more ascorbic acid, when ACTH is present to augment this absorption, than when ascorbic acid alone is given. Thus, a sharper decline occurs in the plasma ascorbic acid when ACTH is present, regardless of the level, than when ascorbic acid alone is given. The second factor is that the deficient animal with an imbalance of the ascorbic acid:cholesterol ratio could, upon receiving ACTH in the presence of ascorbic acid, improve this balance and thus increase the adrenal corticoid production and as a result increase the utilization of the ascorbic acid both at the site of production and in the peripheral metabolism of these

hormones.

The one factor that appears to contradict this is that there is a different response within the levels of ACTH. The application of 40 and 20 units of ACTH with ascorbic acid appears to provide a logical response; with more response being observed with the former than the latter. However, the addition of ACTH at a level of 10 units shows a greater response at one hour with the same sharp decline observed, but not as great as either 40 or 20 units. This brings in the effect of physiological dosage versus pharmacological dosage. There is the possibility that the lower levels of ACTH, 10 units, is closer to the physiological level than either 40, or 20 units of ACTH; which may be a pharmacological level.

The response of ACTH alone varies as to the level applied, but there is no time response. This could be in response to the stimulation caused by ACTH on the adrenal gland. It is felt that a greater amount of study should be conducted on the problems of ascorbic acid and ACTH in both normal and deficient animals to determine their possible interrelationship. These studies should include the checking of the urinary excretion of ascorbic acid, the tissue absorption responses of ascorbic acid, and the use of radioactive tracer techniques to establish the exact physiological effects of ascorbic acid and ACTH in the body.

SUMMARY AND CONCLUSIONS

1. There was a significant difference ($p < .05$) observed in the plasma ascorbic acid concentration between 10, 20, and 40 units of ACTH when given alone which is related to the degree of stimulation of the adrenal gland.

2. There, also, was a significant difference ($p < .005$) between the response of ascorbic acid plus ACTH within one hour; the 40 units of ACTH response was greater than the 20 units of ACTH and the 10 units of ACTH response was greater than either 40 or 20 units of ACTH.

3. The responses of ascorbic acid plus ACTH, regardless of levels of ACTH, show a sharper decrease after one hour in the plasma ascorbic acid concentration than when ascorbic acid is given alone.

4. These responses cannot be interpreted completely without the added information that could be obtained from the urinary excretion values of ascorbic acid. However, it is believed that the differences may be due to a combination of the ACTH increasing tissue absorption plus the increase of adrenal hormone production due to the change in the ascorbic acid:cholesterol ratio and the effects of ascorbic acid on peripheral metabolism.

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