THE INFLUENCE OF CORTISONE AND ACTH ON THE GONADS OF SCORBUTIC GUINEA PIGS

by

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INTRODUCTION

This investigation was planned to determine if a relationship exists between the production of adrenal cortical hormone and degeneration of the testes in scorbutic guinea pigs. It has been well established by Harman (1950) that lack of vitamin C in the diet of guinea pigs results in changes of many organs and tissues, including degeneration of the testes.

Many investigators have found that cortisone has no effect upon the gonads in normal guinea pigs. It has been reported by Schaffenburg et al. (1950) and Hyman et al. (1950) that the administration of cortisone delays the onset of scurvy. However, the exact role of ascorbic acid in the secretion or production of the cortical hormone is not known. Hyman et al. (1950) reported that the effects of cortisone and adrenocorticotrophic hormone (ACTH) were similar in delaying the onset of the general manifestations of scurvy. Since there is a definite relationship between ascorbic acid and adrenal cortical function, it became of interest to determine if the administration of cortisone acetate or ACTH will prevent the degeneration of the testes in scorbutic guinea pigs.

REVIEW OF LITERATURE

As described by Hyman, Ragen and Turner (1950), some symptoms typical of avitaminosis C in guinea pigs are bloody diarrhea, hematuria, anoxeria, lethargy, hind leg paralysis, tachypnea and abdominal distention. They also noted marked hemorrhage in the subcutaneous tissues and some muscles, adrenal hypertrophy, and the characteristic lesions of scurvy. The average duration of life was approximately 22 days. This description was in agreement with the report made by Meyer and McCormick (1928). Harman and Miller (1939) stated that the degree of resistance to an attack of scurvy is an individual factor and that although there is a chance for recovery from a pronounced case of scurvy, there is greater probability of death.

As early as 1926, Lindsay and Medes described degenerative changes in the testes of guinea pigs that were kept on a diet deficient in vitamin C. Harman and Gillum (1937) observed that guinea pigs fed a vitamin C-free diet did not grow or reproduce, and soon died. Goettsch (1930) found that guinea pigs dying from scurvy contained motile sperms. Harman (1950) reported that in guinea pigs fed a vitamin C-free diet the testes left the scrotum and returned to the body cavity. Harman further stated that the entire walls of the tubules appeared to be in a process of degeneration, and that, in contrast to Goettsch's (1930) results, microscopic examination of the contents of the seminal vesicle did not show any spermatozoa.

There has been a special interest in the relation of scurvy to the adrenal gland, due to the fact that this gland contains extraordinary amounts of vitamin C (Turner, 1948, p. 226). Tuba, Hunter, and Osborne (1946) in histo-chemical preparations, stained guinea pig adrenals to determine if ascorbic acid were present. In the normal adrenal they found staining, which indicated the presence of ascorbic acid, in both cortex and medulla, with the greatest density in the zona fasiculata. In scorbutic adrenals they found only a few granules which indicated a low ascorbic acid content. Broch (1939) stated that the ascorbic acid content of the adrenals depended upon the amount ingested. Lauber and Rosenfeld (1938), Prina (1946), and Stepto, Pirani, and Consolazio (1950) observed that there was a marked reduction in adrenal ascorbic acid when the animals received no ascorbic acid in their diet. According to Mouriquand, Tete and Lavavd (1938) the amount of ascorbic acid in the adrenals of guinea pigs does not parallel the degree of scurvy which may be present. Deane and Morse (1948) stated that virtually all the cells in the rat's adrenal cortex normally possessed ascorbic acid, but that when they lose their capacity to synthesize steroid hormones they lose the vitamin C as well. According to Vogt (1948) loss of ascorbic acid from the adrenal cortex is associated with increased cortical secretion. Kuchel and Mitchell (1936) reported that stimulation of the adrenal glands of rats caused a fall in the average ascorbic acid content of these glands. As the result of their work, Giroud and Martinet

(1940) concluded that vitamin C is necessary for synthesis of cortico-sterone, and only when vitamin C value is normal does the adrenal gland function normally. The investigation of Harman and Bascom (1951) gave further evidence that there is a relationship between vitamin C in the diet and the functioning of the adrenals, but it failed to show just what that relationship was.

Lockwood and Hartman (1933) observed hypertrophy of the adrenal gland during vitamin C deficiencies, and they found that an extract of the adrenal gland contained some unidentified substances that aided in the utilization of vitamin C. In later investigations Lockwood et al. (1936) and Hartman (1941) found that an adrenal extract free from vitamin C retards the onset of the symptoms of avitaminosis C. Schaffenburg, Masson, and Corcoran (1950) and Hyman, Ragen, and Turner (1950) reported that cortisone exerts a protective action in guinea pigs placed on a scorbutic diet.

According to Corey and Britton (1931) there is an apparent interrelationship between the function of the adrenal cortex and the sex glands. Herrick and Trostveit (1938) demonstrated that adrenalectomy in fowls resulted in degeneration of the testes. Atwell (1932) lists clinico-pathological and experimental evidence for believing that the adrenals are related to sexual development and activity. Among the former are: (1) the relation of hypernephroma and sexual precocity, hirsuitism or virulism; (2) the fact that the cortex is larger in the female and that it

becomes hypertrophied during pregnancy; (3) the cortex is ill-developed in sexual deficiency; (4) changes occur in the cortex during the estrous cycle of many animals; (5) after castration the cortex is said to be hypertrophied. Experimental evidences are: (1) early opening of vagina in rats which survived multiple adrenal transplants; (2) induced precocious sexual maturity in the rat by injections of cortico-adrenal extracts; (3) degeneration of the testes following adrenalectomy in white rat, mature or immature; (4) adrenalectomy in female rats suppresses or makes irregular the oestrous cycle. Llusia (1949) states that the adrenal acts as a reserve gonad after the short life of the ovary or testes is expended and that it must be considered a third gonad. Llusia also concluded that the adrenal gives the individual the hormonal needs of the opposite sex. Lauber and Rosenfelt (1938) and Gersh and Grollman (1939) reported that the adrenal does not exert an androgenic function in the normal animal. Simpson, Kohn-Speyer and Korenchevsky (1933), Howard, and Grollman (1934), Gaunt and Parkins (1933), and Kinugasa (1930) observed that an adrenal extract did not alter the morphological character of the gonads when given to normal animals. In recent studies, Ingle (1950) found that the administration of five milligrams of cortisone acetate did not cause any significant change in the size of the testes or seminal vesicles of the adult rat. According to Ingle (1950), Winter, Silber and Stoerk did not note any changes in the reproductive organs of

male rats treated with cortisone acetate. Fitzhugh (1937), however, reported that there was marked atrophy of the reproductive system following adrenalectomy and that repair in both female and male systems followed treatment with corticoadrenal extract. The facts that lack of vitamin C causes degenerative changes in the testes, and that the adrenal cortical hormone delays the onset of scurvy appears to indicate that a direct relationship exists between the hypofunction of the adrenal gland in scurvy and the accompanying degeneration of the testes.

There have been several investigations to determine the role of ascorbic acid in the synthesis or release of the cortical hormone from the adrenal gland. Sayers, Sayers, Liang and Long (1945) suggested that it might be incorporated into the steroid structure of the hormones. The authors state that Lowenstein (in a personal communication) found that the gland contains a highly active water soluble steroid, to which ascorbic acid is attached. Sayers et al. (1945) suggested that the alterations in adrenal ascorbic acid is associated with the secretion of the cortical hormones and that it participates in the hormone formation.

Other investigators do not agree that ascorbic acid is necessary for the synthesis or release of the cortical hormone. They have studied the problem by using adrenocorticotrophic hormone to stimulate the adrenal cortex. Collip, Anderson and Thompson (1933), Dougherty and White (1944), Evans, Simpson and

Li (1948), Tyslowitz (1943), Bergner and Deane (1948) have confirmed that adrenocorticotrophic hormone (ACTH) from the pituitary gland stimulates the adrenal cortex and causes an increase in adrenal hormone production. Gordon (1950) reported that the response of the adrenal cortex to stimulation with ACTH may depend upon (1) the functional integrity of the gland, (2) the intensity of the stimulation, and (3) the duration of the stimulation. Gordon also stated that striking increases in adrenal cortical secretory activity have been produced by ACTH. Sayers, Sayers, Liang and Long (1946), Sayers, Sayers and Woodbury (1948), and Ducommun and Mach (1949) state that ACTH causes a sharp fall in adrenal ascorbic acid. It was suggested by Sayers et al. (1946) that these changes in adrenal ascorbic acid, under the influence of ACTH were associated with the formation and release of the adrenal cortical hormones. This was confirmed by Tepperman (1950) who reported that when purified ACTH was added in vitro to slices of dog adrenal cortex the oxygen consumption of the tissues is significantly increased and its ascorbic acid content significantly depressed. Sayers, Sayers, Lewis and Long (1944) reported that administration of ACTH caused the ascorbic acid of the adrenals to be diminished. These authors suggested that this process might be related to cortical hormone synthesis in or release from the adrenal gland.

Several studies have been made to ascertain what effect ACTH has on scorbutic animals. The issue is highly controversial

and reports in the literature do not agree. Velasco (1946) found that an extract of the anterior pituitary had no beneficial effect on recovery from scurvy. In contrast, however, Hyman, Ragen, and Turner (1950) reported that the effects of cortisone on scorbutic guinea pigs were also produced by ACTH. This finding supported their view that deficiency of ascorbic acid does not appreciably interfere with production of adrenal cortico-steroids. Treager, Gabuzda, Zamiheck and Davidson (1950) also found normal adrenal cortical activity in patients with typical scurvy, but Stefanini and Rosenthal (1950) found that patients developed hemorrhagic manifestations presumably due to ascorbic acid deficiency while receiving large doses of ACTH.

MATERIALS AND METHODS

The experimental animals used in this study were sexually mature, male guinea pigs with an average body weight of 279 grams and were obtained from The Gopher State Caviary, St. Paul, Minnesota. Three animals were put in each cage and kept a temperature of 68-73° F. The guinea pigs were divided into four groups. All animals were fed the same basal vitamin C-free diet. The following ingredients composed the basal ration.

Hulled oats	75 lbs.
Soybean meal	25 lbs.
Lederle Fortifeed Supplement	½ lb.

APF which contained 2.5 grams of B_{12} per lb.	$\frac{1}{4}$ lb.
Alfalfa pellets	6-8 per animal per day
Vitamin A	56,000 IU
Vitamin D	adequate

The guinea pigs in Group I were given the basal diet alone and were used as scorbutic controls. There were eight animals in this group, and most of them were kept as long as they would survive. Because it was desirable to obtain the testes, which were to be used for microscopic study, as soon after death as possible, moribund animals were killed.

In Group II treatment with cortisone was started after the animals had been on the basal diet for five days. They were given five milligrams of cortisone acetate daily by a subcutaneous injection. The hormone used was cortisone acetate (11-dehydro-17-hydroxycorticosterone-21-acetate) which was prepared and donated by Merck and Company, Inc., Rahway, New Jersey. This product is sold under the trade name "Cortone." Three of the animals in this group were sacrificed when the number of days they were on the vitamin C-free diet corresponded to the survival time of the scorbutic controls. The other five animals in this group were kept as long as possible in order to obtain data on the length of survival of the cortisone treated animals. It was also desirable to compare the testes of these animals to those in Group I, as well as to the other animals in this group.

The guinea pigs in Group III were given injections containing two milligrams of adrenocorticotrophic hormone (ACTH) at

twelve hour intervals. This material which was a product of Lederle Laboratories Division of the American Cyanamid Company, New York, New York, was in a crystalline form when purchased. The crystals were pulverized and dissolved in physiological saline, to which one drop of HCl was added. This concentrated solution was then diluted until it contained four milligrams of ACTH per milliliter. This solution was put into bottles and frozen until used. The hormone was administered subcutaneously, and treatment was started after the guinea pigs had been on the basal diet for five days. This treatment was continued until death of the animals.

The animals in Group IV were given adequate vitamin C in the form of ascorbic acid in their diet, and in addition three drops of ascorbic acid solution was given orally every other day. This solution contained ten milligrams of ascorbic acid per cubic centimeter. These guinea pigs were the normal controls.

Examinations were made on all animals as soon as possible after death. The desired tissues were dissected from the bodies of the animals and immediately immersed in Bouin's fixative. The following procedure was used to prepare the tissue for sectioning.

1.	Tissue in Bouin's fixative	24 hours
2.	Wash in 70 percent alcohol	time varies
3.	Wash in 70 percent alcohol	time varies
4.	60 percent isopropyl alcohol	1 hour
5.	99 percent isopropyl alcohol	1 hour

6.	99 percent isopropyl alcohol	2 hours
7.	99 percent isopropyl alcohol, place in oven (40-45° C.)	3 hours
8.	Paraffin I	1 hour
9.	Paraffin II	overnight
10.	Imbed	

The imbedded testes were placed on blocks and sectioned at six micra. Delafield's haematoxylin with eosin as a counter-stain was used. The procedure followed for staining was as follows:

1.	Remove paraffin with xylol	5 minutes
2.	Place in xylol	5 minutes
3.	Place in absolute alcohol	)
4.	Place in 95 percent alcohol	)
5.	Place in 85 percent alcohol	)
6.	Place in 70 percent alcohol	) each 1 minute
7.	Place in 50 percent alcohol	)
8.	Place in 35 percent alcohol	)
9.	Place in distilled water	5 minutes
10.	Stain in haematoxylin	2 minutes
11.	Rinse in tap water until dark blue	time varies
12.	Destain in 2 percent solution of HCl in water	time varies
13.	Rinse in tap water until blue again	time varies
14.	Rinse in distilled water	)
15.	Rinse in 35 percent alcohol	)
16.	Rinse in 50 percent alcohol	) each 1 minute
17.	Rinse in 70 percent alcohol	)

18.	Counterstain in eosin until cytoplasm is faintly pink	time varies
19.	Rinse in 95 percent alcohol	)
20.	Rinse in 95 percent alcohol	) each 1 minute
21.	Rinse in absolute alcohol	)
22.	Rinse in carbol xylol	2 minutes
23.	Rinse in xylol	5 minutes
24.	Cover with a number one cover slip using Piccolite and allow to dry	

The slides were then ready for examination under a binocular compound microscope. They were examined under low power, and the measurements of the seminiferous tubules were made under high power with a calibrated ocular micrometer.

RESULTS

The scorbutic controls (Group I) exhibited the typical symptoms of scurvy after they had been on the vitamin C-free diet for eleven days. Signs of soreness were apparent in one animal after it had been deprived of vitamin C for seven days. The average duration of life was 16.2 days, with extremes of 12 to 19 days. When these guinea pigs were started on the vitamin C-free diet their average body weight was 280 grams. The animals which were given cortisone (Group II) lived for 37 to 50 days on the scorbutic diet, an average of 46 days. They continued to gain weight for approximately 23 days. Following this was a rapid loss of body weight which lasted until the

animals died. The average body weight of these guinea pigs at death was 256 grams. The manifestations of scurvy evident in the scorbutic controls were also pronounced in the animals that received ACTH therapy (Group III). Two of these animals lived for 9 and 10 days, respectively, but the other one survived for 27 days. The average body weight of this group which was 262 grams when they were put on the vitamin C-free diet, fell to 211 grams at death. The normal controls (Group IV) remained in good health throughout the experiment. Their average body weight at the beginning of the observation was 267 grams. At the termination of the experiment, they weighed an average of 319 grams, which showed a gain of 52 grams during the experiment (Table 1).

As a means of evaluating the degree of development and maintenance of the testes, measurements were made of the total diameter of the seminiferous tubules, diameter of the lumina and thickness of the wall. These are listed in Table 2. Normal tubules were found to be the largest, and those of the scorbutic controls were the smallest. In the testes of the cortisone-treated animals, which were on the diet for an average of 21 days tubules were almost as large as those of the normal animals. The tubules of the testes of the cortisone-treated guinea pigs that were on the diet for 46 days were still larger than those of the untreated scorbutic controls. In the ACTH-treated animals, the tubules of the testes were approximately the same size as those of the cortisone-treated animals. However, the ACTH-

Table 1. Body weights and longevity of experimental guinea pigs which were deprived of vitamin C and treated with cortisone or ACTH.

Animal	Weight at start of diet, grams	Maximum weight, grams	Weight at death	Longevity days
		Group I, scorbutic controls		
14	285	same as at start	225	19
15	245		185	19
17	225		188	15
18	375		250	19
38	292		175	14
41	299		182	12
42	277		202	16
45	245		195	16
Average	280		200	16
		Group II, cortisone-treated		
21	245	306	206	37
23	285	380	261	43
24	235	377	252	45
54	381	420	273	50
57	241	306	257	50
58	200	232	278	50
Average	264	337	256	46
		Group II, cortisone-treated (killed)		
19	280	365	365	26
20	250	294	206	17
22	325	386	386	17
Average	285	320	319	21
		Group III, ACTH-treated		
53	266	-	209	9
55	300	348	244	27
63	220	220	181	10
Average	262	291	211	15
		Group IV, normal controls		
31	315	478		
32	270	305	280	
34	246	367	367	
35	265	330	310	
36	240	245		
60	268	516		
Average	267	374	319	

treated animals were on the diet only 15 days. The mean total diameters of the seminiferous tubules in these groups were: normal controls, 154.8 micra; scorbutic controls, 100.0 micra; cortisone-treated (21 days on diet) 140.7 micra; cortisone-treated (on diet for 47 days) 120.1 micra; ACTH-treated 144.0 micra.

The thickness of the wall of the tubules is the most accurate measurement of the degree of degeneration. As the testes degenerate the walls become thin due to a sloughing off of the epithelial cells of the tubule. It was found that in the scorbutic animals the walls of the testis tubules were only a little thinner than the normal, but those of the ACTH-treated animals were considerably thinner. The average thickness of the walls of the tubules for the different groups were: normal controls, 55.2 micra; scorbutic controls, 24.3 micra; cortisone-treated (21 days on diet), 49.1 micra; cortisone-treated (47 days on diet), 31.1 micra; and ACTH-treated, 35.5 micra.

The diameter of the lumina is not indicative of the degree of degeneration of the testes as it varies with the condition of the walls as well as the total diameter of the tubules. The mean measurements of the lumina were: normal controls, 50.4 micra; scorbutic controls, 50.2 micra; cortisone-treated (21 days on diet), 44.0 micra, (47 days on diet) 59.0 micra; ACTH-treated 59.4 micra.

Table 2. Weights and measurements of testes from guinea pigs deprived of vitamin C and treated with cortisone or ACTH.

Animal	Weight of testes (milligrams)	Measurements of tubules (micra)		
		Total diameter	Diameter of lumina	Thickness of walls
Group I, scorbutic controls				
14	330	63.0	29.4	17.8
15	285	97.4	61.6	19.7
17	325	129.1	60.7	34.8
18	239	147.0	49.9	47.0
38	217	95.5	67.0	11.6
41	355	77.9	41.5	13.6
42	430	94.4	49.7	24.0
45	329	95.5	41.4	25.6
Average	315	100.0	50.2	24.3
Group II, cortisone-treated				
21	477	128.2	54.2	35.0
23	950	125.6	52.5	32.8
24	1224	196.6	73.9	65.2
54	155	66.5	37.7	13.5
57	555	80.5	49.0	16.3
58	610	123.4	86.8	23.8
Average	662	120.1	59.0	31.1
Group II, cortisone-treated (killed)				
19	829	110.9	43.7	34.7
20	225	145.2	42.4	50.9
22	710	171.1	45.8	61.8
Average	588	140.7	44.0	49.1
Group III, ACTH-treated				
53	370	156.3	60.0	44.4
55	301	131.8	58.8	26.6
63	-	-	-	-
Average	336	144.0	59.4	35.5
Group IV, normal controls				
32	987	162.3	57.0	60.0
34	950	158.4	48.8	54.1
35	943	143.7	45.3	51.6
Average	954	154.8	50.4	55.2

In a statistical analysis, the measurements of the scorbutic controls and the cortisone-treated animals were compared. It was found that the measurements of the total diameter of the tubule and the thickness of the walls were highly significantly different (P .00001 for both total diameter and thickness of walls). The measurements of the lumina were not significantly different (P = .40). A more complete report of the statistical analysis is given in Table 3.

The testes of the normal animals averaged 954 milligrams in weight. In the scorbutic, untreated animals the average weight was 314 milligrams, which is about one-third as large as those in the normal guinea pigs. In cortisone-treated guinea pigs, the testes averaged 662 milligrams in weight, nearly twice as large as those of the untreated animals.

Microscopic examination of the testes of the scorbutic animals showed degeneration of the entire wall of the tubules, and cells were lying free in the lumina. There were no sperm flagella visible in the lumina of the tubules or any indication of spermatid transformation. The condition of these testes was similar to that described by Harman in 1950 (Plate I, Fig. 1). The testes of the cortisone-treated animals showed little histological degeneration. The lumina of the tubules were small and flagella of sperm cells could be seen in them. The walls of the tubules were intact, some of the nuclei of the cells appeared to be undergoing mitosis, spermatids were present in the epithelial lining of the tubules, and in some areas

there were indications of maturation (Plate I, Fig. 2). The condition of the testes from the ACTH-treated animals closely resembled that found in the scorbutic animals (Plate II, Fig. 1). The testes of normal controls were intact, and active spermatogenesis seemed to be taking place. The lumina were small, many sperm flagella could be seen in them, and spermatids were present in the epithelial lining of the tubules. The cells in the walls of the tubules appeared to be undergoing mitotic division (Plate II, Fig. 2).

Table 3. Statistical analysis of measurements of the seminiferous tubules of the testes from the experimental guinea pigs.

Group	Mean	Standard deviation	t	Approximate P
Diameter of lumen				
scorbutic	49.72	12.78	.88	.40
cortisone-treated	52.88	9.81		
Total diameter				
scorbutic	98.74	24.53	5.78	less than .00001
cortisone-treated	146.42	27.59		
Thickness of wall				
scorbutic	23.82	10.27	6.39	less than .00001
cortisone-treated	47.05	12.60		

For each of three comparisons, the variation was homogeneous; that is: 12.78 is not significantly different from 9.81
27.59 is not significantly different from 24.53
12.60 is not significantly different from 10.27

The means of the diameters of the lumina are not significantly different. The means of the total diameter of the tubules and the thickness of the walls are highly significantly different.

EXPLANATION OF PLATE I

Fig. 1. Photomicrograph of testes from scorbutic guinea pig showing degeneration of the entire wall of the tubule, sloughing epithelial cells, and no indication of spermatid transformation.

Fig. 2. Photomicrograph of testes from guinea pig deprived of vitamin C and treated with cortisone showing intact walls, flagella of maturing sperms in the lumina, and spermatids.

PLATE I

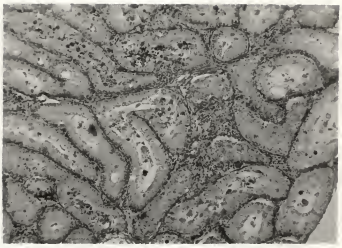

Fig. 1.

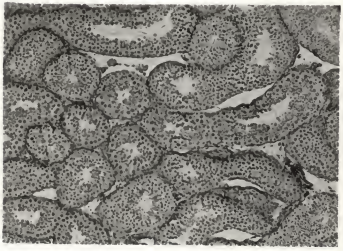

Fig. 2.

EXPLANATION OF PLATE II

Fig. 1. Photomicrograph of testes from guinea pig deprived of vitamin C and treated with ACTH showing degeneration of entire wall of the tubule, and sloughing epithelial cells.

Fig. 2. Photomicrograph of testes from a normal guinea pig showing spermatids in the epithelial lining of the seminiferous tubules and flagella of maturing sperms in the lumina.

PLATE II

Fig. 1.

Fig. 2.

CONCLUSIONS

This study confirms previous works that if guinea pigs are deprived of vitamin C the testes show marked degeneration. It is also in agreement with Schaffenburg, Masson and Corcoran (1950) and Hyman, Ragen and Turner (1950) who stated that cortisone delayed the onset of scurvy. This view is supported by the increased duration of life, decreased loss of body weight and the delay in the onset of the external symptoms of scurvy in the animals that received cortisone.

Due to the small number of animals in Group III (ACTH-treated), it was not possible to draw extensive conclusions, but the results of this brief work did not support the view that ACTH given to scorbutic animals has the same effect as cortisone. The data, however, indicated that ACTH did not prevent or delay the attack of scurvy as shown by external symptoms, longevity and degeneration of the testes, all of which were similar to the conditions found in the scorbutic controls.

This investigation has shown that when cortisone acetate is given to guinea pigs that are on a vitamin C-free diet, the testes do not degenerate. Microscopic study of the testes showed that they were nearly normal, and measurements of the total diameter of the tubules and the thickness of the walls showed there was no significant difference between the size of these tubules and those of normal testes. Gross weight of the testes from the cortisone-treated animals also presented

conclusive evidence that they were considerably larger than those from the scorbutic controls. From these data, it is concluded that cortisone prevents the degeneration of the testes in guinea pigs deprived of vitamin C.

SUMMARY

1. The loss of general body weight which results from the lack of vitamin C was decreased by the administration of five milligrams of cortisone acetate daily.

2. Longevity of the scorbutic animals was increased an average of 30 days by treatment with cortisone.

3. Lack of vitamin C in the diet of guinea pigs results in degeneration of the testes but this can be prevented by treatment with cortisone.

4. The results of this experiment indicated that the administration of ACTH to scorbutic guinea pigs did not increase the life span of the animals or prevent the degeneration of the testes.

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THE INFLUENCE OF CORTISONE AND ACTH ON THE GONADS OF SCORBUTIC GUINEA PIGS

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For a number of years it has been recognized that lack of vitamin C in the diet of guinea pigs results in changes of many organs and tissues, including the degeneration of the testes. Recent investigations have shown that the administration of cortisone delays the onset of scurvy. Several studies have been made to ascertain what effect adrenocorticotrophic hormone (ACTH) has on scorbutic animals. The issue is highly controversial and reports in the literature do not agree. Since there is a definite relationship between ascorbic acid and adrenal cortical function, it became of interest to determine if the administration of cortisone or ACTH will prevent the degeneration of the testes in guinea pigs deprived of vitamin C.

Guinea pigs were selected for sex, age, and weight and divided into groups as follows:

Group I - scorbutic controls. These animals received no vitamin C in their diet.

Group II - cortisone-treated. Five milligrams of cortisone acetate were given to the animals in this group each day by subcutaneous injections. These animals received a diet which contained no vitamin C.

Group III - ACTH-treated. These animals received two milligrams of ACTH, which was administered subcutaneously at twelve hour intervals. The animals in this group were also fed a vitamin C-free diet.

Group IV - normal controls. These animals received adequate vitamin C in their diet.

Soon after the guinea pigs died the desired tissues were dissected from the bodies and immediately immersed in Bouin's fixative. The isopropyl method was used to prepare them for imbedding. The tissues were then sectioned at six micra, mounted on slides, and stained with Delafeld's haematoxylin and eosin. The slides were examined under a binocular compound microscope and measurements of the seminiferous tubules were made under high power with a calibrated ocular micrometer. Table 1 shows a comparison of the measurements of the total diameter of the tubules, diameter of the lumina and thickness of the walls.

Table 1. Average weights and measurements of testes from guinea pigs deprived of vitamin C and treated with cortisone or ACTH.

Group	Weight of testes (milligrams)	Measurements of tubules (micra)		
		Total diameter	Diameter of lumina	Thickness of walls
I - scorbutic controls	315	100.0	50.2	24.3
II - cortisone-treated	662	120.1	59.0	31.1
II - cortisone treated, killed	588	140.7	44.0	49.1
III - ACTH-treated	336	144.0	59.4	35.5
IV - normal controls	954	154.8	50.4	55.2

Microscopic examination of the testes of the scorbutic animals showed degeneration of the entire wall of the tubules, and cells were lying free in the lumina. There were no sperm flagella visible in the lumina of the tubules or any indication of spermatid transformation. The condition of these testes was similar to that described by Harman in 1950. The testes of the cortisone-treated animals showed little histological degeneration. The lumina of the tubules were small and flagella of sperm cells could be seen in them. The walls of the tubules were intact, some of the nuclei of the cells appeared to be undergoing mitosis, spermatids were present in the epithelial lining of the tubules, and in some areas there were indications of maturation. The condition of the testes from the ACTH-treated animals closely resembled that found in the scorbutic animals. The testes of normal controls were intact, and active spermatogenesis seemed to be taking place. The lumina were small, many sperm flagella could be seen in them, spermatids were present in the epithelial lining of the tubules, and cells in the walls of the tubules appeared to be undergoing mitotic division.

Longevity of the guinea pigs deprived of vitamin C was increased and the loss of body weight was decreased by the administration of cortisone, but ACTH did not prevent or delay the onset of scurvy. Table 2 gives a summary of the longevity and body weights of the experimental animals.

Table 2. Average body weights and longevity of guinea pigs deprived of vitamin C and treated with cortisone or ACTH.

Group	Weight at start of diet, grams	Maximum weight grams	Weight at death grams	Longevity days
I - scorbutic controls	280	same as at start	200	16
II - cortisone-treated (on diet 46 days)	264	367	256	46
II - cortisone-treated (on diet 21 days)	285	320	319	21
III - ACTH-treated	262	291	211	15
IV - normal controls	267	374	319	

SUMMARY

1. The loss of general body weight which results from the lack of vitamin C was decreased by the administration of five milligrams of cortisone acetate daily.

2. Longevity of the scorbutic animals was increased an average of 30 days by treatment with cortisone.

3. Lack of vitamin C in the diet of guinea pigs results in degeneration of the testes but this can be prevented by treatment with cortisone acetate.

4. The results of this experiment indicated that the administration of ACTH to scorbutic guinea pigs did not increase the life span of the animals or prevent the degeneration of the testes.