EFFECTS OF DESOXYCORTICOSTERONE ACETATE ON SCORBUTIC GUINEA PIGS

by

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INTRODUCTION AND REVIEW OF LITERATURE

This study was undertaken to determine the effects of desoxycorticosterone acetate in the maintenance of animal tissues when the animals were kept on a scorbutic diet. That animals display histological changes when kept on a scorbutic diet was established as early as 1926 when Lindsay and Medes found there was degeneration of the seminal epithelium of guinea pig testes. Likewise, degeneration of the tubules of the testes was demonstrated. Walbach, et al. (1929) reported that supportive tissue such as dentin, bone matrix, and collagen are unable to produce and maintain intracellular substances. Degeneration of the pulp and odontoblasts (Zilva and Wells, 1919) and alveolar area of teeth (Harman, et al. 1938) have been associated with avitaminosis C. Other conditions associated with experimental scurvy have been described by Meer and McCormick (1928), Lindsay (1926), Hyman, et al. (1950), Schaffenburg, et al. (1950), Hughes, et al. (1952). Some of these conditions are: loss of weight, decreased activity, drowsiness, nervousness, pain in the joints, paralysis of the hind quarters, subcutaneous hemorrhages, fatty-degenerated liver, fatty-infiltrated adrenals, kidneys and lungs, disintegration of the epidermis and interstitial cells of the testes.

Bessesen (1923) reported adrenal weight in scorbutic animals was greatly increased in contrast to a general wasting of the animal as a whole. Morikawa (1920) found high lipoidal

depositions in the zona fasciculata of the adrenal cortex in animals maintained on a C-free diet.

Harman (1950) has shown that male guinea pigs maintained on a scorbutic diet become sexually inactive after three to four days. Further, this same worker and Warren (1951), have demonstrated that embryos from female guinea pigs kept on the vitamin C-free diet showed general retardation of development.

Siehrs and Miller (1933) and Giroud et al. (1938) have shown conclusively that the adrenal glands of animals maintained on a scorbutic diet contain lower than normal amounts of vitamin C. Since this organ normally contains high concentrations of ascorbic acid, the possibility of ascorbic acid utilization in the synthesis or metabolism of the adrenal cortical hormones has received much attention.

Harman and Bascom (1951) found that 25 to 50 dog units of cortin (Eschatin--Parke, Davis and Co.) per day did not alleviate the effects of scurvy in the guinea pig. Lockwood and Hartman (1933) and Lockwood, Swan, and Hartman (1936) found that a cortical extract prevented the onset of conditions associated with scurvy in guinea pigs. Herrick, et al. (1952) found that guinea pigs fed a scorbutic diet and receiving cortisone lived longer than those receiving no cortisone. Too, the extract delayed the onset of the symptoms of scurvy. Adrenocorticotrophic hormone was found to have no effects in alleviating the onset of scurvy according to Herrick et al. (1952).

Banerjee and Deb (1952), working with guinea pigs placed on a scorbutic diet, found that the blood sodium was decreased, blood potassium increased (less excreted in the urine), and that the normal uterine contraction was depressed. They suggest that the above results are related to a decrease in salt (NaCl) and carbohydrate regulating hormones. The decrease in these hormones is related to the lack of ascorbic acid and adrenal cholesterol. Inferences that these two substances are the precursors of the steroid hormones have been made by Stepto, et al. (1951) who found the cholesterol content of the adrenal glands decreased under scorbutic conditions.

Sayers, et al. (1946) and Long (1947) have shown that the corticotrophic hormone causes an abrupt drop in adrenal ascorbic acid and cholesterol (the latter responds much more slowly however). They associate adrenal cholesterol, vitamin C, and corticotrophin with the formation of adrenal cortical hormones.

Lowenstein and Zwemmer (1946) have isolated a fraction from the adrenal cortex which upon mild hydrolysis in the absence of air yields ascorbic acid. This has not been substantiated as yet, but it indicates an ascorbic acid relationship in the synthesis of the cortical hormones.

Studying the cytological distribution of ascorbic acid in the adrenal cortex of the rat, Deane and Morse (1948) found a relationship between the amount of ascorbic acid present and the ability of the cells to proliferate other cells. That ascorbic acid enters into enzyme or coenzyme functions in body

cells has been demonstrated by Sealock and Goodland (1951). They found vitamin C to be required in the first oxidation reaction in the metabolism of tyrosine in liver cells. Clark (1953) suggested that the primary role of the adrenal hormones is to regulate protein synthesis. This conclusion has a direct relationship to the work of Sealock and Goodland.

It has been demonstrated in rats (Roberts, 1953) that an accelerated mobilization of nitrogen in protein metabolism takes place upon injection of adrenocortical extracts. It was suggested that this labilized protein was thus translocated to those portions where the demand existed. Robertson and Schwartz (1953) were able to show that the ascorbic acid requirement increased as the formation of collagen by guinea pigs increased thus, it was assumed to be necessary for the formation of this substance.

Lockwood, Hartman, and Hartman (1933) have previously indicated the role of vitamin C and the adrenal hormones. They suggest that the adrenal glands act as an intermediary organ in the utilization of the vitamin.

Much work has been done concerning the relationship between vitamin C and cortisone. The classical experiments relating the two substances in treatment of rheumatoid arthritis have received much attention which appears to substantiate previous evidence cited that vitamin C has a definite relationship to cortisone (Hallberg, 1950 and Bacchus, 1952). This is not so true in the case of desoxycorticosterone. A search of the

literature has produced little conclusive evidence which would ascribe to desoxycorticosterone such a role as is the case with cortisone.

It has been known for years that desoxycorticosterone acetate (DOCA) has a striking influence upon water metabolism in the body. Birnie, et al. (1948), Hays and Mathieson (1945), and Green (1948) have shown that DOCA prevents the onset of water intoxication.

Hooker and Collings (1940), Emery and Greco (1940) have demonstrated some activity of DOCA with respect to the sex hormones. Hooker and Collings found DOCA to be 1/33 as active as androsterone. It was found to maintain the weight of the prostate gland and seminal vesicles, although it did not prevent histological changes. Emery and Greco demonstrated that DOCA and progesterone were effective in prolonging the life of adrenalectomized rats. However, it should be pointed out that progesterone alone was equally effective so more work would be necessary before concluding that the above is an activity of DOCA. Clausen (1942) failed to find any response on the organs of the castrate guinea pig treated with DOCA.

Using adrenalectomized rats, Segaloff (1946) was able to show that DOCA pellets made growth possible when the rats were on a carbohydrate free diet containing crude casein, fatty acids, minerals and synthetic vitamins. Growth and survival were diminished when purified casein was substituted or fatty acids removed from the diet. This would seem to add evidence

to the hypothesis of Clark (1953) that the primary role of the adrenal hormones is to regulate metabolism of proteins.

DOCA has been shown to increase the phosphotase of the epiphysis by Williams and Watson (1941).

Relationships between DOCA and vitamin C have received little attention to date. James, et al. (1950) found no beneficial results upon five rheumatoid arthritic patients treated with DOCA and ascorbic acid. Hughes, et al. (1952) found DOCA to promote rather than inhibit the onset of arthritic-like symptoms of guinea pigs on a scorbutic diet. Seneca, et al. (1950) demonstrated conclusively what could well be an important role of DOCA. Cultured adrenal, liver, testis, and kidney tissues were incubated with DOCA and then analyzed by paper chromatography for the presence of cortisone. These tissues contained insignificant amounts of this hormone prior to being incubated with DOCA. The relatively large quantities of cortisone found in adrenal tissue suggest that it was produced from DOCA by an oxidation-reduction action within the cells.

It is evident that the exact role of desoxycorticosterone is still without explanation. This study is designed primarily to study some of its effects upon animal tissues fed a scorbutogenic diet.

MATERIALS AND METHODS

This study was begun in October, 1952 and continued into July, 1953. Work was done in the laboratories of the Department

of Zoology of Kansas State College, Manhattan, Kansas. The experimental animals consisted of 19 sexually mature male guinea pigs obtained from the genetics laboratory of Dr. Herman L. Ibsen, Kansas State College, and ten similar ones from the Gopher State Caviary of St. Paul, Minnesota. The weights ranged from 360 grams to 722 grams. No attempt was made to maintain a constant temperature in the room where the animals were maintained. All animals were weighed to the nearest gram prior to beginning the experiment and every third day thereafter. All experimental guinea pigs were fed a scorbutic basal diet consisting of the following ingredients: twelve pounds of wheat bran, twelve pounds of rolled oats, three pounds of butter, twelve pounds of dried milk powder, three pounds of cod liver oil, and six and one-half ounces of salt. This basal diet was mixed by hand and was stored in a metal container until used. Vitamin A alcohol was added from time to time to maintain its concentration. Control animals received alfalfa pellets ad libitum in their diet as a source of vitamin C.

The study was conducted in a series of three groups with the weight of the animal being the primary criterion for placing it in its proper group. The average weight of animals placed in Group I was 651 grams; those of Group II were 448 grams, and those in Group III were 401.6 grams. Each group of animals was divided as follows: Group I, consisting of seven guinea pigs, was divided to have two on a vitamin C-free diet and five which were placed on a C-free diet plus a daily subcutaneous

injection of two mg. desoxycorticosterone acetate. Group II, consisting of the ten animals from the St. Paul Caviary, had four guinea pigs receiving desoxycorticosterone acetate in addition to the basal diet, three on the basal diet, and three animals received normal diets. In Group III, four animals were placed on the basal diet and eight received the basal diet with a daily injection of desoxycorticosterone acetate. Animals within each group were separated on the basis of treatment received.

Guinea pigs in Group I were placed on the scorbutogenic diet and injections were begun three days afterward. Injections were made daily until the animal was killed or until it died. Desoxycorticosterone acetate was dissolved in Mazola oil (4mg/1cc) and kept in air tight dispensing bottles until used. The desoxycorticosterone was supplied by the Schering Corporation, Bloomfield, New Jersey.

The animals of Group I were killed or died within 28 days after beginning the C-free diet. The guinea pigs were allowed to live as long as possible or were killed if it were evident that they would die by the next day. Each guinea pig was opened as soon after death as possible to remove the sterna, adrenal glands, and testes which were placed in Bouin's picro-formol fixing fluid. Within twenty-four hours after being placed in the fixing solution, the tissues were placed in two washes of 70 percent isopropyl alcohol and kept therein until embedding in paraffin. The adrenal glands and testes were embedded in

paraffin by dioxane embedding technique. The dioxane method consisted of the following: Tissue from 70 percent isopropyl alcohol to dioxane-alcohol (50-50) for one hour; dioxane I for two hours, dioxane II for four hours, melted Tissuemat (melting point of 50-52°C) for four hours to overnight, Tissuemat (melting point of 54-56°C) three to five hours, embedding and blocking. The adrenal and testicular tissues were sectioned at six micra.

Bone tissue was embedded after the celloidin method and the technique consisted of the following: From the 70 percent isopropyl alcohol to a solution of 90 percent water and 10 percent concentrated nitric acid for 24 hours, this length of time was sufficient for complete decalcification. The bones were then allowed to wash for 24 to 48 hours in running water to wash out the excess acid. They were then placed in 95 percent isopropyl alcohol for 24 hours (with two changes), absolute isopropyl alcohol for 24 hours (with two changes), absolute isopropyl alcohol and ether (equal parts) for 24 hours, thin celloidin (made by dissolving Paraloidin nitrocellulose in two volumes of absolute alcohol and ether (50-50) for 24 hours to one week, thick celloidin (made by dissolving Paraloidin nitrocellulose with equal volumes of (50-50) absolute alcohol and ether) for 24 hours to the time of mounting. Bone tissues were mounted on hard wood and after allowing them to air dry for several minutes they were placed in a sealed jar containing 80 percent isopropyl alcohol until sectioning. Sections were

made at 20 micra.

The sections of the adrenal gland and testes were mounted on slides by use of egg albumin and were stained with Mallory's Triple Stain as follows:

Remove paraffin with paraffin-xylol	5 minutes
Place in xylol	10 minutes
Place in absolute isopropyl alcohol	2 "
Place in 95 percent isopropyl alcohol	" "
Place in 80 percent isopropyl alcohol	" "
Place in 70 percent isopropyl alcohol	" "
Place in 50 percent isopropyl alcohol	" "
Place in 35 percent isopropyl alcohol	" "
Place in distilled water	
Place in Mallory's #1	3 "
Wash in water for a few seconds	
Place in Mallory's #2	7 "
Wash in clean water for a few seconds	
Place in 95 percent isopropyl alcohol until color of blue desired	
Place in absolute isopropyl alcohol	1 minute
Place in carbol-xylol	5 minutes
Place in xylol	" "

The sections were mounted in Piccolite, covered with number one coverslips and allowed to dry. The sections were then observed microscopically.

The staining process for the bone tissue was essentially the same as for the adrenal and testes tissues except that the

tissues were passed directly from absolute isopropyl alcohol to xylol leaving out the carbol-xylol bath.

Groups II and III were treated in the same manner as Group I.

EXPERIMENTAL RESULTS

All experimental animals of Groups I, II, and III which were maintained on a vitamin C-free diet died or were sacrificed within 33 days. The range in life, while on the diet, was from 11 to 33 days with an average of 25 days. All displayed typical scorbutic conditions such as loss of hair, sore feet, stiffness of the hind quarters, and loss of appetite by the end of the tenth day. With the exception of one animal, number 11, all of these animals lost weight immediately and steadily throughout the extent of their life. Animals in Group I, receiving the C-free diet, showed a greater loss of weight than those of Group II or Group III. The average individual loss of weight for these animals was 180 grams for the larger animals of Group I, 144.3 gm. for animals of Group II, and 145 grams for those of Group III (Fig. 1). The maximum weight for the guinea pigs on the basal ration was, with the exception of 2 animals, the initial weight (Table 1).

All control animals in Group II that received an adequate diet had a steady increase in weight over the time of the experiment. No scorbutic conditions were observed and all were

in good health when sacrificed. The weight gained ranged from 110 grams to 200 grams with an average of 153.3 grams (Table 2).

Those animals, within the three groups, which received daily injections of desoxycorticosterone acetate had a life span of 14 to 36 days after being placed on the deficient diet. The average longevity was 21.6 days. All displayed conditions associated with scurvy within ten days. These animals, with the exception of numbers 18 and 0 (both of Group II), gained weight for 9 to 19 days before starting a drop in weight. The average length of weight gain was 14 days. Animal number 18 slowly lost weight throughout the experiment (Table 3). The animals in Group I had an average loss of weight over the experimentals of 120 grams, those of Group II, 104.5 grams, and those of Group III, 50.5 grams (Fig. 1). The percent of the initial body weight lost by guinea pigs of the three groups during the experiment is summarized in table 4.

Histological studies were made on tissues from all animals except numbers 18 (Group II) and 15 (Group III) both of which had been dead for a period of at least eight hours before opening. In each case, tissue deterioration was in progress. The histological study of the adrenal glands from normal animals revealed the following information. Zona glomerulosa: this zone contained relatively small columnar cells which are closely packed into ovoid groups. It varied in width from one to two of these ovoid groupings with the innermost packet of cells forming a "cap" over the outer cells of the zona

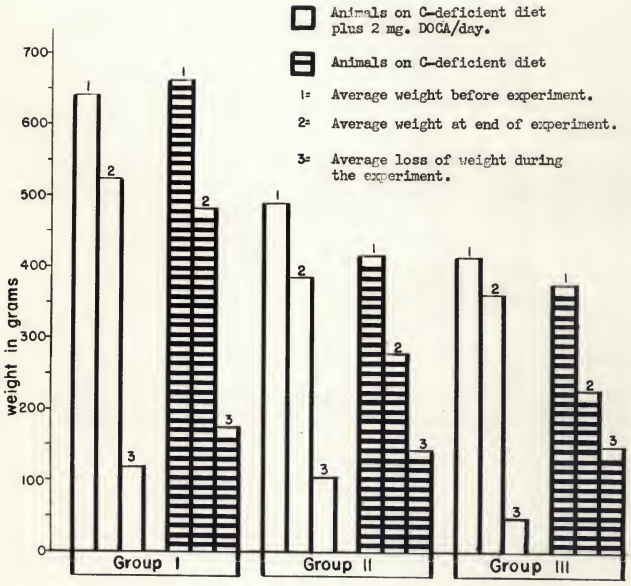

Figure 1. Average weights of guinea pigs before and after the experiment with the average weight loss.

Table 1. Body weights and longevity of experimental guinea pigs which were maintained on a vitamin C deficient diet.

Animal	Group	Weight in Grams: At Start of Diet	Weight in Grams: Maximum	Weight in Grams: At Death	Survival (in days)
4	I	650	650	448	26
6	I	673	678	520	24
8	II	400	400	276	23
11	II	416	450	280	22
6	II	440	440	252	33
3	III	400	400	240	25
4	III	360	360	180	11
33	III	380	380	234	31
77	III	360	360	206	30
Average		453.8	455.3	292.9	25

Table 2. Body weights and survival time of guinea pigs which were maintained on a diet containing adequate vitamin C.

Animal	Group	Weight in Grams: At Start of Diet	Weight in Grams: Maximum	Weight in Grams: At Death	Survival (in days)
14	II	450	560	560	all were sacrificed on 36th day on diet
16	II	420	570	570	
13	II	400	600	600	
Average		423.3	576.6	576.6	

Table 3. Body weights and longevity of experimental guinea pigs which were maintained on a vitamin C deficient diet and 2 mg. desoxycorticosterone acetate per day.

Animal	Group	Weight in Grams: At Start of Diet	Maximum	At Death	Survival (in days)
9	I	538	596	528	20
12	I	685	700	560	21
13	I	635	650	510	23
15	I	722	740	572	21
16	I	600	696	460	28
17	II	480	532	484	24
0	II	480	480	294	17
18	II	500	500	402	18
7	II	498	528	360	36
232	III	420	448	360	14
5	III	400	470	360	22
7	III	420	450	340	20
14	III	420	422	280	25
15	III	420	460	340	25
20	III	440	535	460	16
22	III	400	466	408	23
44	III	400	420	360	14
Average		500.4	529.	416.3	21.6

Table 4. Average percent of body weight lost for guinea pigs within each group during the experiment.

	C-deficient Diet				C-deficient diet plus DOCA		
Group	Initial Weight	Death Weight	% wt. Lost	Group	Initial Weight	Death Weight	% wt. Lost
I	664 gm.	484 gm.	27.2	I	646 gm.	526 gm.	18.6
II	418.6	269.3	36.	II	489.5	385	21.4
III	375	215	42.6	III	415	363.5	12.4
Average	485.86	322.16	35.26		516.83	424.83	17.46

fasciculata. There were many large droplets of fat present in the cytoplasm with a few larger than the darkly stained nuclei.

Zona fasciculata: this zone was made up of cells of irregular shape which were arranged with each other to form "cords" leading from the inner edge of the zona glomerulosa to zona reticularis. The differentiation between the zona reticularis and zona fasciculata is not as definite as between the zona fasciculata and zona glomerulosa. The outer regions of this zone contain many fat droplets most of which are smaller than the nuclei. The cytoplasm appeared to be mere strands between the nuclei. The inner part of the zone had cells with many evenly distributed fat droplets usually much smaller than the centrally located nuclei. Mitotic figures were seen in the transition zone between the zona fasciculata and glomerulosa.

Zona reticularis: this, the inner zone of the adrenal cortex begins at the inner edge of the zona fasciculata where the cells show little histological difference from the latter zone. Deeper into the zone, the cells were more easily distinguished and were of two types, light and dark cells. The dark cells contained nuclei which stained more darkly and were smaller than the nuclei of the light cells. The cytoplasm of the dark cells contained minute fat droplets, but in no case as much as was found in the zona fasciculata.

Study of the adrenal glands of animals maintained on the

vitamin C-free, or basal diet, has shown the following results. Zona glomerulosa: this zone has cells which are larger than the control group and had enlarged nuclei. Fat droplets were present in each case but the size of the vacuoles varied from normal (animal 4, Group II) to large droplets as large as the nuclei. The cell shapes were polyhedral to columnar. Deterioration of this zone was evident in various stages ranging from no disintegration (number 4) to well advanced (number 6, Group I).

Zona fasciculata: this zone showed heavy vacuolation in the outer regions with relatively large vacuoles. The inner regions are vacuolated, but less so than control tissues. The cells were normal in size, much more compact and displayed disintegration in some instances.

Zona reticularis: the cells of this zone had less vacuolation than either of the outer zones or normal controls. There was disintegration of the inner most regions (animals number 8 and 11) in the area where the zona reticularis merges with the medulla.

The histological studies of animals maintained on the vitamin C-free diet and receiving daily injections of desoxycorticosterone acetate produced the following results. Zona glomerulosa: The nuclei of this zone were slightly larger than those of the normal cells. The cells were for the most part packed tightly together and appeared more cuboidal than columnar. Fat vacuoles were well distributed in the cytoplasm, but were much smaller than the nuclei. The width of the zone appeared

more narrow than the corresponding zone of normal adrenals. The adrenal from animal number 7 (Group II) showed some degeneration of the ovoid grouping of the cells which were in early stages of degeneration. Differentiation between this zone and the zona fasciculata was obscure.

There was heavy vacuolation of the outer regions of the zona fasciculata. The vacuoles ranged from smaller than the nuclei to some larger than the nuclei. Cells of the inner regions had minute vacuoles which were smaller than the nuclei. In all cases they were smaller than corresponding normal tissues. The cells were nearly normal in size. Deterioration was evident in animals number 7, 0, 9, and 16. There was no observable difference in mitotic activity between these tissues and normal tissues.

Zona reticularis: the light cells were larger than the dark cells, both of which were, by comparison, nearly as large to larger than the cells of the fascicular cells. They were about the same size as similar cells of the normal tissues with the exception that there was less vacuolation in the cytoplasm of these cells. Animal number 0 had heavy fat infiltration along with excessive disintegration of the innermost regions of the zone.

In summary, it may be stated that adreno-cortical tissue underwent profound changes both in the scorbutic animal and the scorbutic animals treated with desoxycorticosterone acetate. Increased fat deposition was evident in both groups of animals

with the scorbutic-untreated ones consistently possessing the greater amount. In scorbutic animals treated with DOCA, there were areas in the adrenal cortex that showed marked vacuolation with other areas apparently normal in all respects. Deterioration was evident in tissues from both types of animals. The degree to which deterioration had progressed varied, however, with the scorbutic untreated adrenals showing most deterioration. Some of the adrenal glands of the treated (DOCA) animals appeared normal. (Plate I).

Microscopical studies of the testes revealed the following information. Scorbutic-untreated guinea pigs displayed general deterioration in all respects. The walls of the seminiferous tubules were in the process of degeneration, few lumina contained spermatozoa, and the lumina were often filled with cells sloughed off from the wall. (Plate II, Fig. 2). The testes of the normal controls were intact and active spermatogenisis was observed. In the lumina, there were many maturing spermatozoa (Plate III, Fig. 1). The study of testicular tissue from scorbutic guinea pigs treated with DOCA revealed the following: Animal number 14 had pronounced deterioration and vacuolation of the tubule walls. There was no resemblance of this tissue to tissues of other animals, normal or scorbutic. The testes of other animals receiving similar treatment showed little degeneration while others indicated more severe breakdown. (Plate III, Fig. 2). Evidence that cells were being sloughed off into the lumina was observed. Although there was

no active spermatogenisis, it was evident, however, that spermatogenisis had been prolonged due to the maturing spermatozoa in the lumina of a portion of the seminiferous tubules from each tissue indicating that cellular breakdown had been delayed. The thicknesses of the tubule walls were measured and used as a further correlation of the ability of DOCA to reduce cellular degeneration. The average thickness of the tubule walls of the scorbutic animals was 44.89 micra as compared to 59.71 micra for the scorbutic animals which received DOCA. Statistical analysis of the measurements of the tubule walls indicated that the difference between the means (14.82 micra) was highly significant. ($T = 4.66$).

The microscopical study of the sterna afforded the following evidence. The differentiation between bone tissue appeared in various degrees of degredation with the tissues of the scorbutic showing the greater amount of breakdown. In both the scorbutic and the treated, the breakdown occurred at the zone of calcification of cartilage. Little periosteal bone degeneration was observed.

DISCUSSION

The administration of desoxycorticosterone acetate promotes rather than inhibits the onset of scurvy. This is indicated by the reduction of the life span of the animal and more rapid onset of conditions associated with the disease.

Results obtained in this experiment are in agreement with those obtained by Meer and McCormick (1928), Lindsay and Medes (1926), Hyman, et al. (1950), Herrick, et al. (1952), Hughes, et al. (1952), and Morikawa (1920) who found increased lipoidal content in the adrenal glands and general disintegration of the testicular tissues. This work has indicated that DOCA does not have the same influence upon the maintenance of adrenal tissue as does cortisone. Herrick, et al. (1952) have demonstrated that cortisone injected into scorbutic guinea pigs causes the cells of the adrenal gland to atrophy.

Clark (1953) has suggested that the primary role of the adrenal-cortical hormones is to act in the regulation of protein metabolism. The present study appears to add evidence to this hypothesis in that histological studies of both the adrenal glands and testes of the scorbutic animals were in many cases similar to normal tissues. The fact that the administration of DOCA had a striking effect upon the ability of the animal to maintain body tissue even when subjected to a scorbutic diet may be taken as further evidence. The ability of these animals to maintain their weight over a longer period and even to increase their weight indicates possible activity of DOCA in regulating protein metabolism. Additional work using intact animals would be required to substantiate this.

That DOCA was not the only compound necessary for protein metabolism was evident from the fact that degeneration of tissues was observed in the microscopical examination of the

tissues. This would indicate that a relationship exists between this hormone and ascorbic acid. The work of Sealock and Goodland (1951) showing that ascorbic acid acts as a coenzyme in tyrosine metabolism can not be overlooked in this respect. Hypothetically, it could be that ascorbic acid reacts with certain amino acids in one phase, probably the first as Sealock and Goodland have proposed, and the hormone or hormones react on the resulting compound afterwards. This would seem to give partial explanation for the ability of the animals to increase in weight after being treated with DOCA wherein a more extensive utilization of the vitamin C present in the tissues after being placed on the C-free diet is made. Additional investigation must be made before any conclusive statement can be made.

SUMMARY

1. Daily administration of 2 mg. DOCA to scorbutic guinea pigs decreased their survival time an average of three days as compared to scorbutic-untreated animals.

2. Animals treated with DOCA lost less weight than those on the scorbutic diet only.

3. Adrenal glands of the scorbutic animals showed abnormally high vacuolation and cellular degeneration.

4. Adrenal glands of the scorbutic--DOCA animals showed increased vacuolation and less cellular degeneration than those

of the scorbutic animals.

5. Testicular tissues from animals that had received DOCA showed more degeneration than normal, but much less than was observed in the testes of scorbutic animals.

6. Bone tissues disclosed little change between the scorbutic-untreated and scorbutic-DOCA animals.

7. The results of this experiment indicate a relationship between vitamin C and DOCA, but the exact relationship is still obscure.

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APPENDIX

EXPLANATION OF PLATE I

Figure 1. Photomicrograph of adrenal gland from a scorbutic guinea pig showing deterioration and vacuolation.

Figure 2. Photomicrograph of adrenal gland from a scorbutic guinea pig which had received 2 mg. DOCA each day.

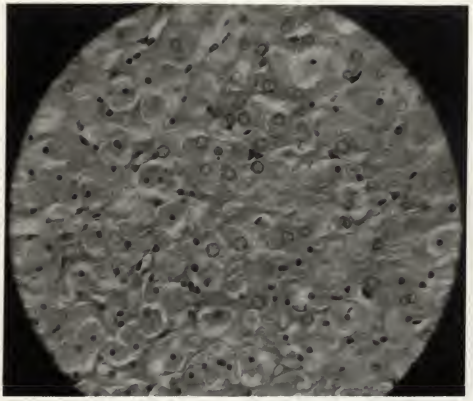

Figure 1

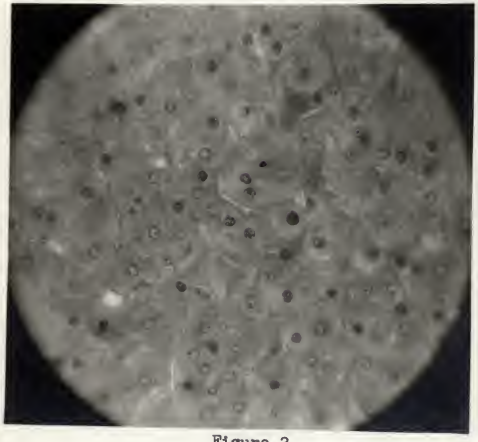

Figure 2

EXPLANATION OF PLATE II

Figure 1. Photomicrograph of adrenal gland taken from a normal guinea pig showing normal cells.

Figure 2. Photomicrograph of testis tissue from a scorbutic-untreated guinea pig showing degeneration.

PLATE II

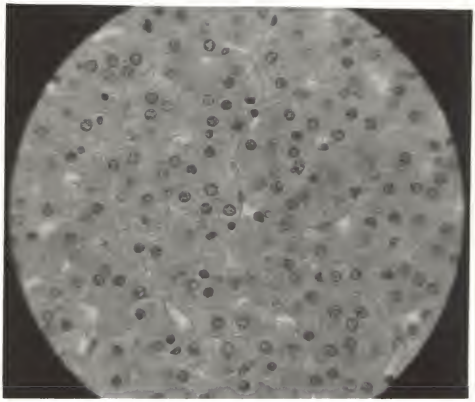

Figure 1

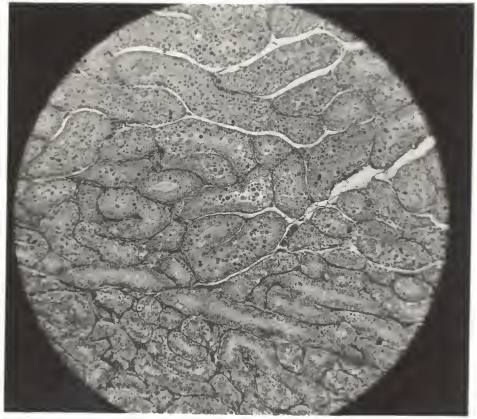

Figure 2

EXPLANATION OF PLATE III

Figure 1. Photomicrograph taken from testes of normal guinea pigs showing normal tubules and spermatogenisis.

Figure 2. Photomicrograph of testis tissue from a scorbutic-treated (2mg. DOCA/daily) guinea pig showing early stages of degeneration of tubule walls and some immature spermatozoa.

PLATE III

Figure 1

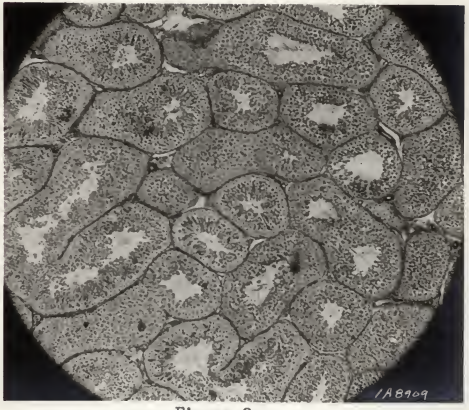

Figure 2

EFFECTS OF DESOXYCORTICOSTERONE ACETATE ON SCORBUTIC GUINEA PIGS

by

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B. A., Concordia College
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AN ABSTRACT

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MASTER OF SCIENCE

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KANSAS STATE COLLEGE
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It has been demonstrated that certain adreno-cortical extracts have a marked influence upon survival time and cellular protection. Cortisone both delays the onset of scorbutic conditions and increases the survival time of the scorbutic animal. In order to study further the relationships existing between extracts of the adrenal cortex and ascorbic acid, a study was made to determine some of the effects of desoxycorticosterone acetate (DOCA) when administered to scorbutic guinea pigs.

Three groups of guinea pigs were selected for age, sex, and weight and maintained on a basal ration which contained no vitamin C. All animals were weighed to the nearest gram prior to the experiment and every third day thereafter until they died or were sacrificed. Group I contained five animals which received, by subcutaneous injection, two mg. DOCA daily after the third day on the basal diet. Two animals were maintained on the basal diet to serve as scorbutic controls. The average weight of the animals of the group was 650 grams. Group II had four animals which received DOCA in addition to the basal diet, three animals which received the basal diet, and three guineapigs which received an adequate diet. The average weight of the guinea pigs in this group was 451 grams. Group III had eight guinea pigs which received DOCA in addition to the basal diet. The average weight of the guinea pigs in this group was 395 grams.

The adrenal glands, testes and a portion of the sternum

were removed as quickly as possible after the death of each guinea pig. Tissues were placed in Bouin's picro-formol fixing fluid. Soft tissues subsequently were washed in 70 percent isopropyl alcohol (24 hours later), embedded by the dioxane embedding technique, mounted and sectioned at six micra. Bone tissues were decalcified, embedded in celloidin, and sectioned at about 20 micra. All tissues were stained with Mallory's Triple Stain, mounted on slides, and studied under both low and high power magnification.

Survival time of scorbutic guinea pigs was decreased by the injection of DOCA. The onset of scorbutic conditions was aggravated by administration of DOCA to guinea pigs placed on a C-deficient diet. Guinea pigs, receiving the above treatment, showed weight gains for an average of 14 days followed by decreases and death.

Histological studies of the adrenal glands of both scorbutic guinea pigs and scorbutic treated guinea pigs showed increased vacuolation in the zona glomerulosa and fasciculata. In most cases, the tissues taken from scorbutic untreated guinea pigs possessed more vacuolation and cellular degeneration than those taken from scorbutic-DOCA animals. Normal tissues had healthy-secreting cells with fat vacuolation present in the outer regions of the zona fasciculata.

Microscopical examination of bone tissues revealed degeneration in both scorbutic-untreated and scorbutic-DOCA treated guinea pigs with differentiation between them being

obscure. Breakdown occurred primarily at the zone of calcification of cartilage to bone. Little periosteal changes were observed.

Examination of the testicular tissues revealed the following information: The seminiferous tubules of the scorbutic-untreated guinea pigs displayed generalized deterioration with few, if any, maturing spermatozoa present in the lumina. In many, the lumina were filled with cells which had been sloughed off from the tubule wall. Cell maturation was not observed in any case. The walls of the seminiferous tubules were measured, the average thickness being 44.89 micra. The testicular tissues taken from scorbutic guinea pigs receiving DOCA showed early stages of cellular and tubule degeneration. Maturation had apparently ceased, but in all tissues, except one, small numbers of maturing spermatozoa were observed indicating that spermatogenisis had been prolonged. Seminiferous tubules were measured in each tissue and the numerical mean was computed (59.71 micra). When subjected to a statistical analysis, the difference of the two means (14.82 micra) was shown to be highly significant ($T = 4.66$). The microscopical study of testes taken from normal guinea pigs revealed active maturation of the cells in the seminiferous tubules with no degeneration observed. In many cases the lumina were filled with maturing spermatozoa.

SUMMARY

1. Daily administration of two mg. DOCA to scorbutic guinea pigs decreased their survival time an average of three days as compared to scorbutic-untreated animals.

2. Animals treated with DOCA lost less weight than those on the scorbutic diet only.

3. Adrenal glands of the scorbutic--animals showed abnormally high vacuolation and cellular degeneration.

4. Adrenal glands of the scorbutic--DOCA animals showed increased vacuolation and less cellular degeneration than those of the scorbutic animals.

5. Testicular tissues from animals that had received DOCA showed more degeneration than normal, but much less than was observed in the testes of scorbutic animals.

6. Bone tissues disclosed little change between the scorbutic-untreated and scorbutic-DOCA animals.

7. The results of this experiment indicate a relationship between vitamin C and DOCA, but the exact relationship is still obscure.