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The Influence of the Adreno-Steroids, Desoxycorticosterone, Cortisone and Acth on the Deposition of Ascorbic Acid in the Adrenalectomized Rat

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By

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BIOGRAPHICAL SKETCH

Mildren Melvin Montgomery was born on January 27, 1929 to Mr. and Mrs. Lewis W. Montgomery of Henderson, Texas. He attended elementary school in Henderson, and was graduated from Antioch High School, Henderson, Texas, in May 1946. After attending Prairie View A & M College for four years, he received his B. S. degree in biology in May 1950. In September of the same year he enrolled in the graduate division of Prairie View A & M College but was called to the Armed Forces where he spent 24 months in and out of the continenal United Sates. Upon being separated from the Armed Forces, he re-entered Prairie View A & M College on a fellowship in biology. During this period, he completed all course requirements for the M. S. degree in biology and chemistry.

ACKNOWLEDGEMENT

The author wishes to express his appreciation to his advisor, Mr. C. H. Nicholos, Chairman of the Biology Department for his constructive criticism and for his assistance in obtaining the necessary drugs which made this experiment possible.

M. M. M.

DEDICATION

This thesis is dedicated to my dear family whose love, inspiration and guidance have inspired me thus far.

M. M. M.

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INTRODUCTION

The existence of a principle in extracts of the adrenal cortex, active in prolonging the lives of adrenalectomized animals, was firmly established in 1929 by the work of Steward, et.al. Many laboratories set out to isolate the pure crystalline substance from the adrenal cortex; however, it was not until 1936 that Wintersteiner and his co-workers isolated several different crystalline substances, most of which were shown to have twenty-one carbon atoms (23).

In 1937, Reichstein provided experimental proof that these compounds were adreno-steroids. By the end of 1937 the name "Cortin" was given to the four different but closely related compounds: corticosterone, 11-dehydrocorticosterone, 17-hydroxy-11-dehydrocorticosterone and 17-hydroxy-corticosterone. Within the next few years, two more compounds were added to the list as possessing "cortin activity"; these were 11-desoxycorticosterone and 11-desoxy-17-hydroxy-corticosterone. The main credit for the isolation of the adrenosteroids must be given to Reichstein and his colleagues for their brilliant research which provided the world with an extensive knowledge of the cortical steroids and with elegant methods for separation of the pure steroids and for the commerical preparation of these active substances (14, 15, 23).

Two of the most active steroids obtained from the adrenal gland are corticosterone which seems to be directly related to carbohydrate metabolism (1, 3, 11, 15, 24), and desoxycorticosterone which has been prepared synthetically and is widely used therapeutically. Although desoxycorticosterone does not have a favorable effect on carbohydrate metabolism, it is more powerful than corticosterone in its effect upon the electrolytic balance of sodium and potassium (1, 3, 5, 8, 11, 16, 24).

In 1885, Thomas Addison, an English physician, observed that patients with the following symptoms: (1) general languor and debility, (2) remarkable feebleness of the heart action, (3) irritability of the stomach, (4) peculiar changes of skin color, (5) low blood pressure, (6) lowered basal metabolic rate, (7) subnormal temperature, and (8) a disturbance in the water and electrolytic balance, had an atrophied adrenal. At the time of his discovery, very little was known of the adreno-steroids; however, since that time it has been found that Addison's disease results from an insufficency of the adreno-steroids. It has also been found since his time that the disturbance includes a loss of Na^+ and Cl^-, and an increase in K^+ ions, thereby affecting the kidney and resulting in urea retention (8, 16, 24, 32).

In order to correct the disturbance of the water and electrolytic imbalance, Loeb, et.al. (27) found that NaCl alone was of immense value to the sufferers of Addison's

disease; however, today the usual treatment consists of combining the salt with desoxycorticosterone. This treatment corrects the electrolytic and water imbalance by causing a retention of sodium and returning the blood serum level to normal (8, 24, 27, 32).

The adrenal cortex has a high concentration of both cholesterol and ascorbic acid. It is believed by a number of investigators (4, 18, 19, 24, 33, 35, 36) that cholesterol is a precursor of the steroid hormones elaborated by the adrenal cortex. In 1946, Sayers showed that when animals are injected with ACTH the adrenal cortex is stimulated, thereby causing a decrease of both cholesterol and ascorbic acid in the adrenal cortex, indicating that they are used up in the formation of the hormone or hormones. It is a well known fact that when the guinea pig has scurvy marked changes develop in the adrenals. The cortex of this gland is rich in hexuronic acid which was noted by Szent-Gyorgri (36); however, King (22) later showed that this substance was Vitamin C. This substance, now known as Vitamin C, ascorbic acid or cevitamic acid, was later synthesized by Haworth, Hist and Riechstein (14). Its absence in the body gives rise to a disease known as scurvy, a disease characterized by the following syndromes: anemia, pain in the joints, hemorrhages from the mucous membrane of the mouth and gastro-intestinal tract, skin, muscles, and a failure

in the formation and mainteance of intercellular material which in turn causes loosening of the teeth, poor wound healing and easy fracturability of the bone (14, 18, 25, 26). The biochemical function of Vitamin C is still unknown; a number of investigators have shown that a large quantity of Vitamin C is found in the adrenal cortex. Further, it has been shown that under the stimulation of ACTH Vitamin C is rapidly depleted. A similar depletion of Vitamin C is noted when experimental animals (guinea pigs) are injected with large quantities of diptheria toxins. In addition, increased losses of Vitamin C results in infection and fever. These observations suggest that the vitamin may play an important role in the reaction of the body to stress (13, 18, 27, 25, 26).

Shaffenburg, et.al. (32), have pointed out the interrelationship between ascorbic acid and cortisone, and between ascorbic acid and desoxycorticosterone in the disturbances of collagenous tissue. They have shown that when Vitamin C deficient guinea pigs were treated with ascorbic acid plus cortisone, the severity of scurvy was reduced; however, when Vitamin C deficient guinea pigs were treated with ascorbic acid plus desoxycorticosterone the severity of scurvy was increased in all respects. In addition, the workers have reported that adrenal tissue taken from animals on a cortisone-ascorbic acid regime contained greater quantities of ascorbic acid than adrenal tissue taken from

animals on an ascorbic acid-desoxycorticosterone regime. As a result of extensive research, it is believed by Shaffenburg that adrenal insufficiency and Vitamin C deficency are similar (32).

It is thought well worth while and valuable to make a study of the adreno-steroids and ACTH as they influence the deposition of ascorbic acid in the adrenalectomized animal for the following reasons: (1) the wide interest in the use of the adreno-steroids in the treatment of collagenous disease; (2) the belief that ascorbic acid is important in influencing the metabolism of the adreno-steroids, and (3) the belief that ascorbic acid is important in maintaining the integrity of collagenous tissue.

In recent years a great deal of work has been done in order to determine the effects of the adreno-steroids and ACTH upon the deposition of ascorbic acid in the normal animal; however, very little work has been done to determine the effects of the adreno-steroids and ACTH upon the deposition of ascorbic acid in the adrenalectomized animal. Hence, the author believes that such a study may contribute to a better understanding of the mechanism by which the adreno-steroids, ACTH and ascorbic acid; may be effective in the treatment of certain adrenal disorders and collagenous diseases.

Experimental evidence agrees that rats are able to synthesize Vitamin C, whereas human beings are not (32). The question then arises, "do the adrenal glands play any signif-

icant role in the production of Vitamin C in the rat," if so, "to what extent would the deposition of ascorbic acid be changed in various body tissue when the glands are removed"? With the forestated thought in mind, this report is designed to describe:

1. the physiological and morphological changes following adrenalectomy.
2. the physiological, morphological and biochemical changes following the administration of the adreno-steroids and ACTH.
3. the deposition of ascorbic acid in various body tissues in the adrenalectomized rat following the injection of adreno-steroids and ACTH.
4. the most effective variation of the adreno-steroids and ACTH upon ascorbic acid deposition.

MATERIALS AND METHODS

Weanling albino rats of the Agricultural and Mechanical College of Texas strain[1], which were fed Purina Dog Chow, were employed in this study. At approximately six to seven weeks of age these rats were separated into groups and placed in metabolic cages for supplementation of 0.9% NaCl for three days. At the end of the three day period these rats were adrenalectomized bilaterally according to the method of D'Amour and Blood (7). After each rat had been marked for identification purposes, its weight was recorded. Seven days after adrenalectomy, each rat received per day 10 mgs. of ascorbic acid[2] per 100 grams of body weight for four days. The control group received ascorbic acid only as a supplement, and the experimental group received with ascorbic acid daily intramuscular injections of either 5 mgs. of DCA[3], 2 mgs. of cortisone[4], 5 DU of ACTH[5], or a variation of the three for four days. During the experimental period each rat was allowed food and water "ad lib".

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[1]Kindly donated by Dr. L. R. Richardson, Biochemistry Department, Agricultural and Mechanical College of Texas.

[2]Kindly donated by Dr. E. E. O'Banion, Chairman, Natural Science Department, Prairie View Agricultural and Mechanical College of Texas.

[3]The source of DCA was the Houston Prescription House, 809 Fannin St. Houston, Texas.

[4]Ibid.

[5]Ibid.

At the termination of the four day experimental period each rat was sacrificed by means of a cardiac puncture, then the blood, tissues and organs were removed and, after pooling each group, analyzed for the presence of ascorbic acid. The following tissues and organs were analyzed for ascorbic acid: blood, brain, liver, spleen, skeletal muscles and adipose tissue. Homogenates of the pooled tissues were prepared in 2% metaphosphoric acid-6% trichloroacetic acid mixture in a Waring blender, then centrifuged. The supinate was titrated with 2,6-dichloro-benzenoindophenol dye for tissue-ascorbic acid. Ascorbic acid in the plasma was determined by the method of Farmer and Abt (16).

This experiment, involving 96 rats was designed to describe the influence of the adreno-steroids, DCA, cortisone, and ACTH upon the deposition of ascorbic acid in the tissues of adrenalectomized rats.

OBSERVATION AND RESULTS

Out of a total of ninety-six rats used in this research problem fifteen died as a results of adrenal insufficency or operative technique. In the group which died the following symptoms were observed after adrenalectomy and during the post operative period: loss of appetite, diuresis, weakness, muscular paralyses, convulsions and death. The cause of these symptoms and death were traced to adrenal insufficency and operative technique. Post morten examinations of this group revealed a congestion of the gastro-intestinal tract, anemia, dehydration of the skin and visceral organs, and enlarged kidneys. On the other hand, the surviving animals showed marked variations in physiological and morphological changes after the administration of the adreno-steroids and ACTH (see Table I and figure 1).

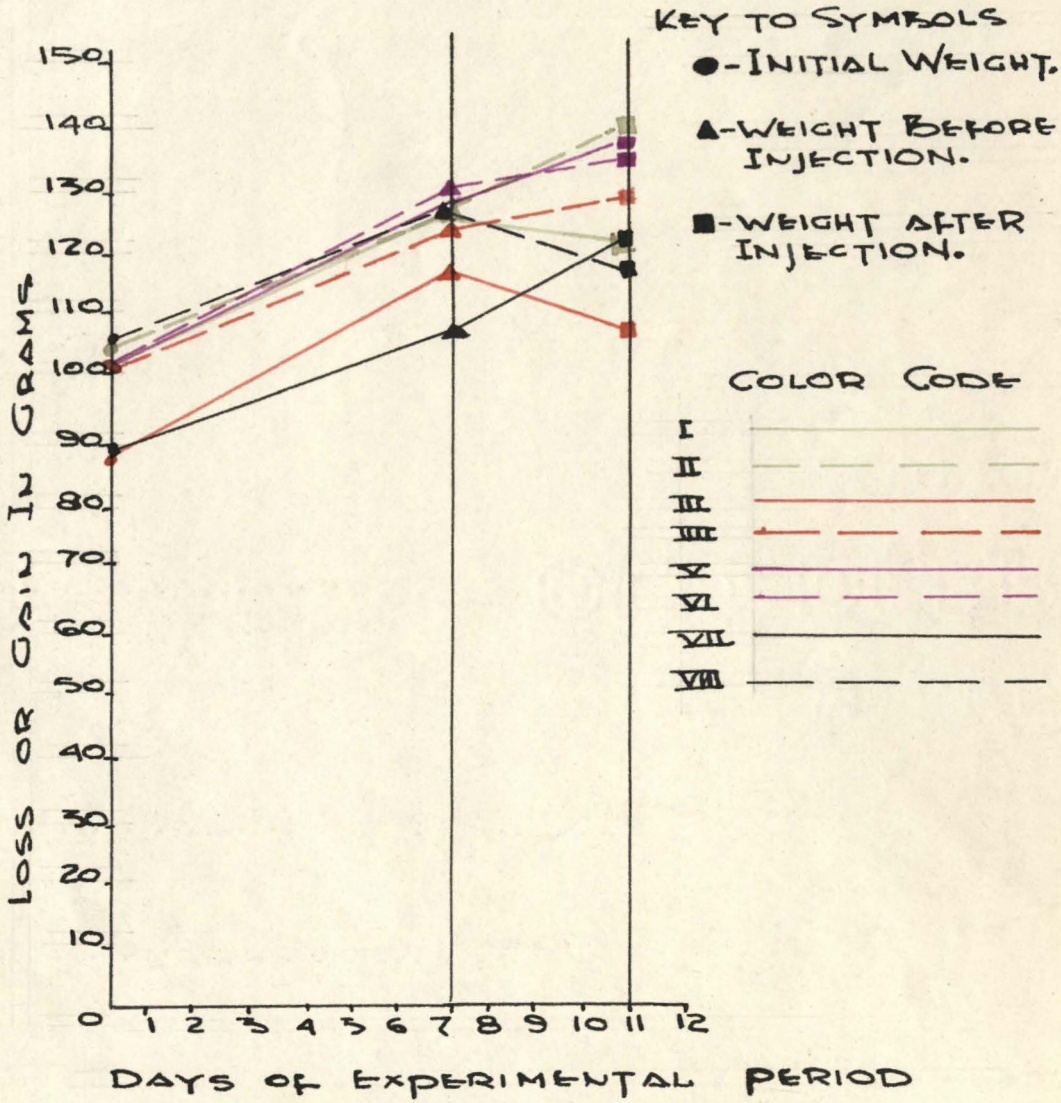
Table I presents data of the initial and final weight of each group before and after supplementation. Experimental evidence from this laboratory revealed that the animals treated with ascorbic acid only showed an average decrease of 2.2 grams; the group receiving ascorbic acid plus cortisone showed an average decrease of 11.3 grams; the group treated with ascorbic acid plus ACTH showed an average decrease of 6.5 grams over a four day injected period. The above supplementations seem to act antagonistically to a gain in body weight. However, it is of interest to note that the ascorbic acid plus DCA treated group showed a mark

Table I: Showing the Physiological and Morphological Changes as Influenced by Ascorbic Acid, Desoxycorticosterone Acetate, Cortisone Acetate and ACTH.

Group	Number of Animals	Experimental Period	Average Initial Weight (gms)	Average Weight at Adrenalectomy	Final Weight	Average Weight gained or lost	Hair Coat
1. Control-							
Ascorbic 1	5	11 days	102.6	124.4	121.5	-2.9	Smooth
Acid(only) 2	5	" "	96.4	118.2	116.7	-1.5	Smooth
Average	5	" "	99.5	121.3	119.1	-2.2	Smooth
2. Ascorbic Ac.1	4	" "	102.4	12.0	137.7	15.7	Smooth
+ DCA 2	4	" "	93.3	12.3	140.3	17.2	Smooth
Average	4	" "	99.4	121.5	138.0	16.4	Smooth
3. Ascorbic.Ac.1	5	" "	103.0	130.0	120.4	-9.6	Rough
+ Cortisone 2	5	" "	68	98.5	85.6	-12.9	Rough
Average	5	" "	85.5	114.3	103.0	-11.9	Rough
4. Asc.Ac.,DCA 1	4	" "	109.7	134.0	139.0	5.0	Coarse
Cortisone 2	5	" "	86.2	107.0	112.6	5.6	Coarse
Average	4.5	" "	97.8	120.5	125.8	5.3	Coarse
5. Asc.Ac.,DCA,1	5	" "	114.0	137.0	149.0	12.0	Smooth
+Cort. 2	5	" "	84.0	106.0	117.6	11.6	Smooth
Average	5	" "	99.0	121.5	133.3	11.8	Smooth
6. Asc.Ac., Cort.1	5	" "	110.2	133.5	140.5	7.0	Rough
+ACTH 2	5	" "	91.2	116.6	129.2	10.6	Rough
Average	5	" "	100.7	125.0	134.8	8.8	Rough
7. Asc.Ac.,DCA 1	5	" "	97.0	107.0	122.4	14.4	Coarse
+ACTH 2	5	" "	76.3	103.7	120.0	16.2	Coarse
Average	5	" "	86.6	105.3	121.2	15.3	Coarse
8. Asc.Ad.,+ 1	5	" "	108.0	129.0	121.0	-8.0	Smooth
ACTH 2	5	" "	99.6	113.4	108.4	-5.0	Smooth
Average	5	" "	103.8	121.2	114.7	-6.5	Smooth

FIGURE:1 SHOWING THE INFLUENCE OF THE ADRENO-STEROIDS, DCA, CORTISONE AND ACTH UPON THE CHANGE IN BODY WEIGHT

I-ASCORBIC ACID (CONTROL)
II-ASCORBIC ACID + DCA
III-ASCORBIC ACID + CORTISONE
IIII-ASCORBIC ACID, DCA, CORT + ACTH
V-ASCORBIC ACID, DCA + CORTISONE
VI-ASCORBIC ACID, CORT + ACTH
VII-ASCORBIC ACID, DCA + ACTH
VIII-ASCORBIC ACID + ACTH



increase of 16.4 grams; while the ascorbic acid, DCA plus ACTH treated animals increased an average of 15.3 grams. Note that the animals treated with ascorbic acid, DCA, cortisone plus ACTH increased in body weight an average of 5.3 grams; while the animals treated with ascorbic acid, DCA plus cortisone increased in body weight an average of 11.8 grams, indicating that the above combinations of the basic supplement act synergically to increase body weight in the adrenalectomized rat.

Tissue distribution studies did not reveal any consistent trend regarding the influence of the adreno-steroids and ACTH on the deposition of ascorbic acid. Table II presents data on ascorbic acid deposition in various body tissues as influenced by the adreno-steroids and ACTH. The determination of ascorbic acid in all tissues are based on the basic supplementation of ascorbic acid plus variations of the adreno-steroids and ACTH. These variations and the results there-from are herewith reported for brain-ascorbic acid. Note that the group treated with ascorbic acid only had an average concentration of 12.48 mgs. of ascorbic acid per 100 grams of brain tissue. When DCA plus ascorbic acid were administered it reduced the ascorbic acid content by 67.5%. The above results seem to reveal that these variations of the basic supplement act antagonistically against ascorbic acid deposition in brain tissue.

Not all variations of the basic supplement which were

Table II: Showing the Influence of the Adreno-Steroids, Desoxycorticosterone, Cortisone and ACTH on the Deposition of Ascorbic Acid in the Adrenolectomized Rat.

Bio-Assay of Tissues		Group I Control Asc.Ac	Group II DCA Asc.Ac.	Group III Cort. Asc.Ac.	Group IV DCA, Cort. ACTH, Asc.Ac.	Group V DCA,Cort. Asc.Ac.	Group VI Cort, ACTH Asc.Ac	Group VII DCA, ACTH Asc.Ac	Group VIII ACTH Asc.Ac
	1.	14.74	10.45	4.40	3.85	17.60		36.85	28.05
BRAIN	2.	10.23	4.73	2.64	4.23	10.89	3.63	30.25	23.37
Ave. mgs		12.48	7.59	3.52	4.04	14.24	3.63	33.55	25.71
Ave. %		100	-39.2	-71.7	-67.5	+14.1	-70.9	+185.6	+106.0
	1.	6.60	1.65	1.10	7.70	3.30	2.20	21.45	5.50
SPLEEN	2.	2.09	1.26	.44	6.38	1.26	1.76	16.50	.88
Ave. mgs		4.25	1.45	.77	7.04	2.28	1.98	18.97	3.19
Ave. %		100	-65.6	-81.8	+65.6	-46.4	-53.4	+346.4	-24.9
	1.	2.20	8.80	7.70	2.20	3.30	9.90	45.10	14.30
LIVER	2.	1.70	6.38	6.60	2.03	2.63	5.39	40.45	11.00
Ave. mgs		1.95	7.95	7.15	2.11	2.96	7.64	42.77	12.65
Ave %		100	+307.7	+266.6	+8.2	+51.8	+291.8	+2,088	+543.6
	1.	0	0	0	0	0	2.20	6.60	5.50
ADIPOSE	2.	0	0	0	0	.22	.11	5.50	4.37
Ave. mgs		0	0	0	0	.11	1.15	6.05	4.95
Ave. %		0	0	0	0	+11	+115	+605	+495
	1.	4.40	2.20	1.10	3.30	2.20	4.40	20.95	16.50
KIDNEY	2.	2.75	1.05	.88	1.70	1.70	3.30	15.62	14.85
Ave. mgs		3.57	1.62	.99	2.00	1.95	3.85	18.28	15.67
Ave.%		100	-54.6	-75.0	-43.9	-45.4	+21.6	+412.0	+310.9
	1.	6.60	4.95	1.65	0	0	1.10	5.50	2.20
MUSCLE	2.	2.53	1.00	.44	.11	.71	.38	1.10	.99
Ave. mgs		4.26	2.97	1.04	.055	.355	.74	3.30	1.59
Ave.%		100	-30.3	-75.6	-98.7	-91.7	-82.6	-22.5	-62.7

Table II-Continued

Bio-Assay of Tissues		Group I Control Asc. Ac.	Group II DCA Asc. Ac.	Group III Cort. Asc. Ac.	Group IV DCA, Cort. ACTH Asc. Ac.	Group V DCA. Cort Asc. Ac.	Group VI Cort, ACTH Asc. Ac.	Group VII DCA, ACTH Asc. Ac	Group VIII ACTH Asc. Ac.
	1.	1.10	1.10	0	1.10	1.10	3.30	3.30	1.10
PLASMA	2.	1.55	1.55	.60	1.10	.16	2.03	2.03	1.55
Ave. mgs		1.32	1.32	.30	1.10	.63	2.66	2.66	1.32
Ave. %		100	0	-77.3	-16.7	-52.3	+101.5	+101.5	0

Experiment 1 vs 2 As to total amount of vitamin C with eight (8) variables

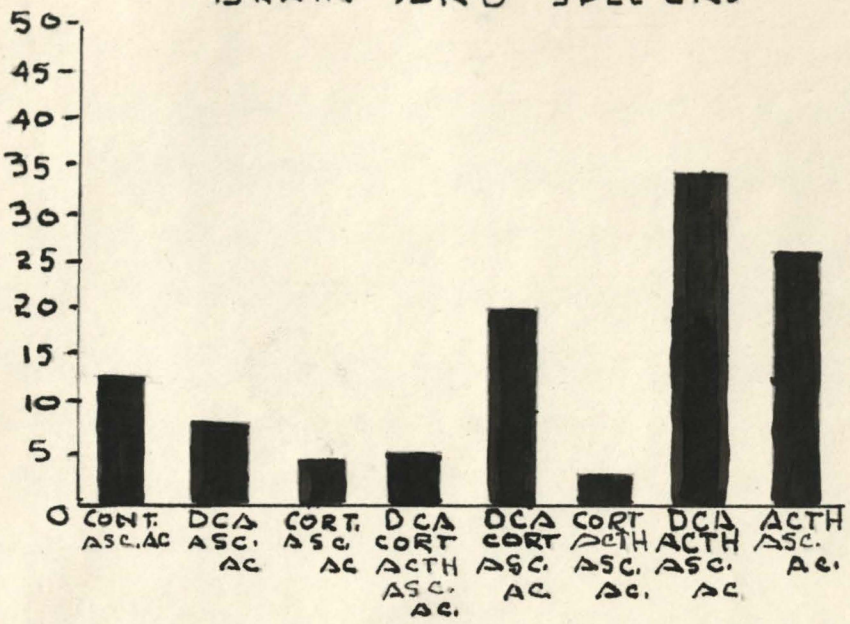
Formula: $$r = \frac{\Sigma xy - \frac{(\Sigma x)(\Sigma y)}{n}}{\sqrt{\Sigma x^2 - \frac{(\Sigma x)^2}{n} \times \Sigma y^2 - \frac{(\Sigma y)^2}{n}}}$$ =.96 which is significant $\frac{.05}{.707}$ $\frac{.01}{.834}$ *

* Thanks are given to Dr. J. Mitchem, Professor of Physical Education who made this calculation possible.

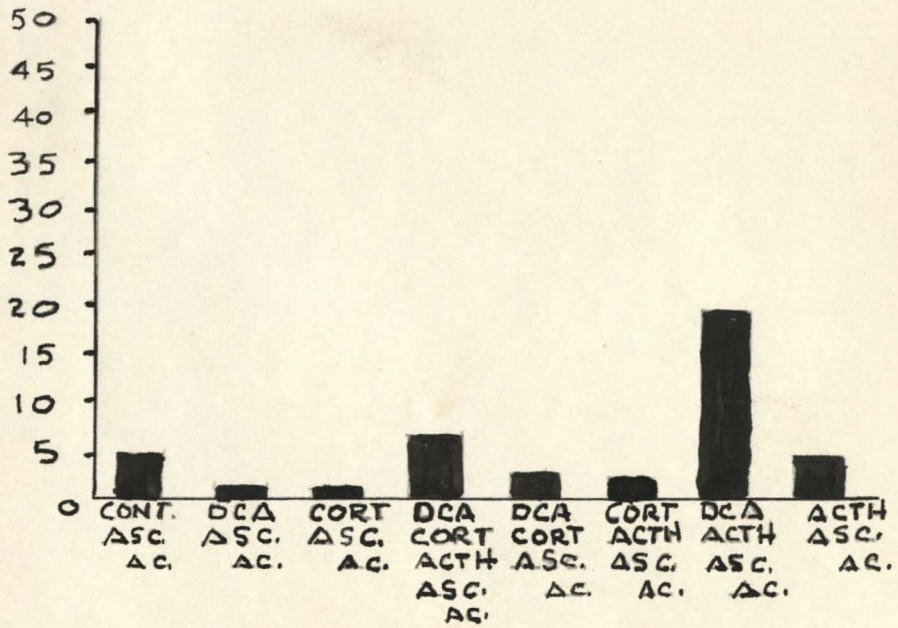
used in this experiment acted antagonistically against ascorbic acid storage in the brain tissue but the following combinations seemed to show that some of the adreno-steroids and ACTH act synergically to increase brain-ascorbic acid: the group treated with ascorbic acid, DCA plus cortisone increased the ascorbic acid content by 14.1%; ascorbic acid plus ACTH increased by 106%; while ascorbic acid, DCA and ACTH caused a further increased of 185.6% of ascorbic acid in the brain tissue (figure 2).

Table II and figure 2 presents data on the deposition of ascorbic acid in the spleen as influenced by the adreno-steroids and ACTH. The determination of ascorbic acid in the spleen revealed that the animals treated with ascorbic acid only had an average concentration of 4.25 mgs. of ascorbic acid per 100 grams of tissue. When ascorbic acid plus DCA were administered the ascorbic acid content was reduced by an average of 65.6%; ascorbic acid plus cortisone decreased the content by 81.8%; ascorbic acid, DCA plus cortisone by 46.4%; ascorbic acid, cortisone plus ACTH by 53.4% and ascorbic acid plus ACTH caused a 25% reduction of spleen-ascorbic acid. While the above variations seemed to act antagonistically against ascorbic storage in the spleen, the following combinations seem to act synergically to increase the ascorbic acid content in the spleen: ascorbic acid, DCA, cortisone plus ACTH increased the ascorbic acid content by 65.6%; ascorbic acid, DCA plus ACTH produced a marked average increase of 346.4%.

FIGURE:2 SHOWING THE INFLUENCE OF THE ADRENO-STEROID, DCA, CORTISONE AND ACTH ON THE DEPOSITION OF ASCORBIC ACID IN THE BRAIN AND SPLEEN.



BRAIN



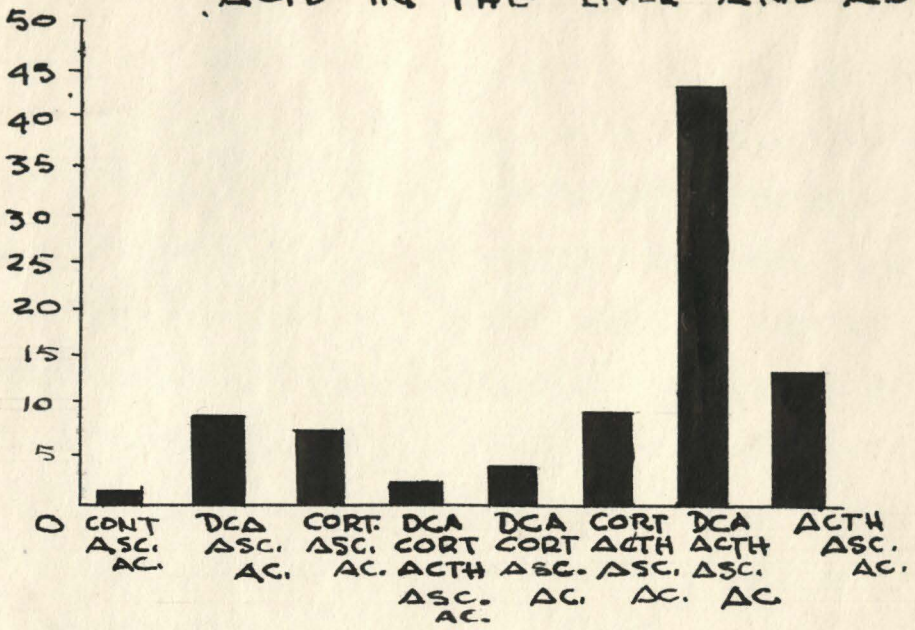
SPLEEN

Table II and figure 3 presents data on the deposition of ascorbic acid in the liver. The biological assay of ascorbic acid in the liver of the animals receiving the basic supplement (ascorbic acid only), showed an average concentration of 1.95 mgs. of ascorbic acid per 100 grams of tissue. All of the variations increased the ascorbic acid content in the liver: the group of animals treated with ascorbic acid plus DCA increased by 307.7%, ascorbic acid plus cortisone by 266.7%; ascorbic acid, DCA, cortisone plus ACTH by 8.2%; ascorbic acid, DCA plus cortisone by 51.8%; ascorbic acid, cortisone plus ACTH treated group by 291.8%; ascorbic acid plus ACTH by 543.6% and the ascorbic acid, DCA plus ACTH treated group showed a marked increase of 2,088 % (figure 3).

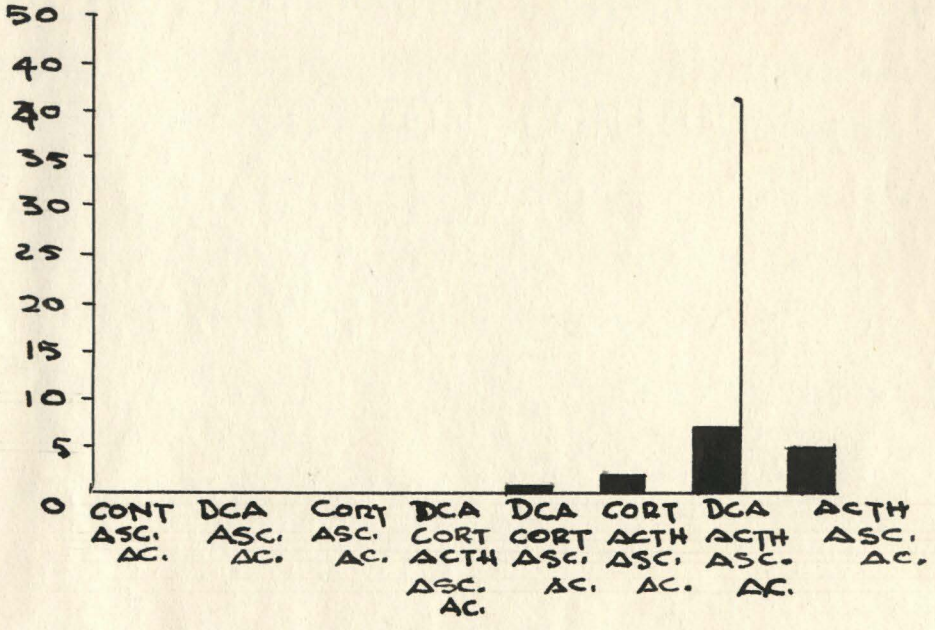
Table II and figure 4 presents data from the biological assay of ascorbic acid in adipose tissue. Note that the group receiving ascorbic acid only showed no ascorbic storage. Only the following variations increased the ascorbic acid content in the adipose tissue: the group receiving ascorbic acid, DCA plus cortisone increased the Vitamin C content by 11%; ascorbic acid, cortisone and ACTH by 115%; ascorbic acid plus ACTH increased the ascorbic acid content by 495% while the ascorbic acid DCA plus ACTH treated group increased the Vitamin C content by 605% in the adipose tissue.

Table II and figure 4 presents data on the deposition of ascorbic in the kidney as influenced by the adreno-

FIGURE 3 SHOWING THE INFLUENCES OF THE ADRENO-STEROIDS DCA, CORTISONE AND ACTH ON THE DEPOSITION OF ASCORBIC ACID IN THE LIVER AND ADIPOSE



LIVER

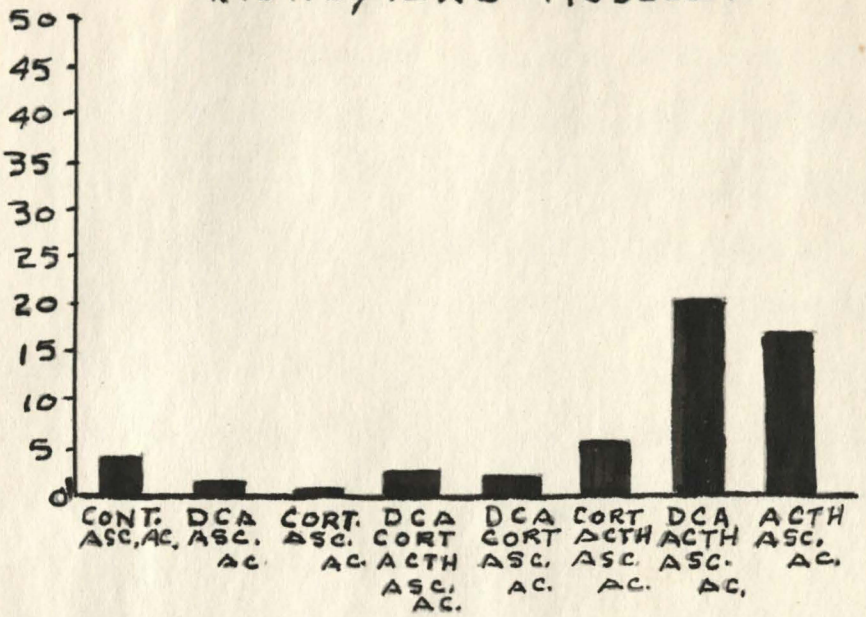


ADIPOSE

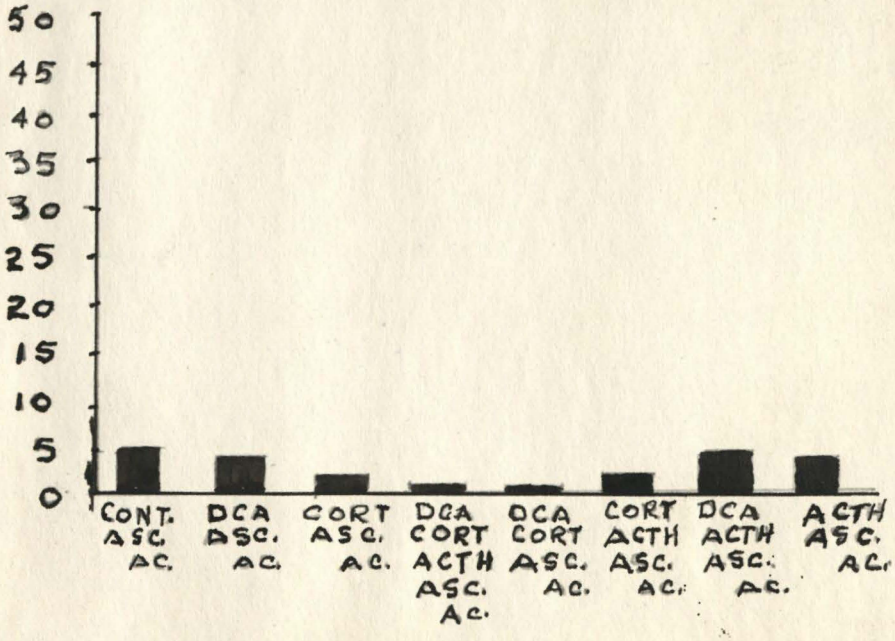
steroids and ACTH. The determination of ascorbic acid as revealed from the biological assay showed that the animals treated with ascorbic acid only had an average concentration of 3.57 mgs. per 100 grams of tissue. The experimental results obtained from the variations of the adreno-steroids and ACTH showed that the following combinations decreased ascorbic acid deposition in the kidney: the group treated with ascorbic acid plus DCA decreased the kidney content by 54.6%; ascorbic acid plus cortisone by 75.0%; ascorbic acid, DCA, cortisone plus ACTH by 44%; and the group treated with ascorbic acid, DCA plus cortisone caused a decrease of 45.4%. Whereas, the proceeding combinations of the basic supplement acted antagonistically against ascorbic acid storage; the following variations seem to act synergically to increase the kidney-ascorbic acid content: the group treated with ascorbic acid, cortisone plus ACTH increased the kidney-ascorbic acid content by 78.4%; ascorbic acid plus ACTH by 310.9%; while the group treated with ascorbic acid, DCA plus ACTH showed the greatest ascorbic acid content with an increase of 412.0%.

Table II and figure 4 present data on ascorbic acid in the skeletal muscles following four days administration of the adreno-steroids and ACTH. It is of interest to note that the adreno-steroids and ACTH used separately or as a group decreased the ascorbic acid content in the muscle tissue below that of the ascorbic acid treated

FIGURE: 4 SHOWING THE INFLUENCE OF THE ADRENO-STEROID, DCA, CORTISONE, AND ACTH ON THE DEPOSITION OF ASCORBIC ACID IN THE KIDNEY AND MUSCLE.



KIDNEY

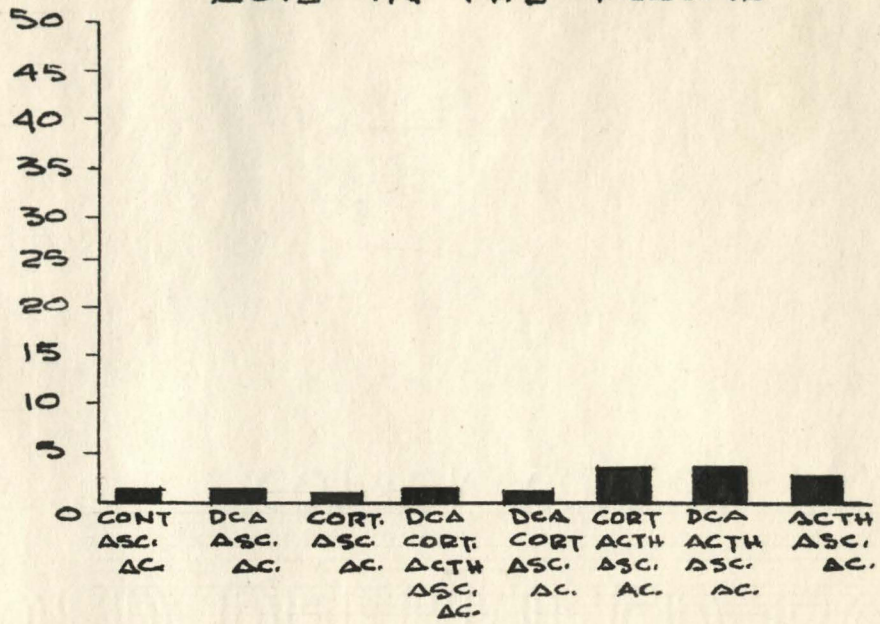


MUSCLE

group in the following order: the group treated with ascorbic acid, DCA, cortisone plus ACTH decreased the muscle-ascorbic acid by 98.7%; ascorbic acid, DCA plus cortisone treated group by 91.7%; ascorbic acid, cortisone plus ACTH group by 82.6%; ascorbic acid plus cortisone treated group by 75.6%; ascorbic acid plus DCA decreased the ascorbic acid content by 30.3%; while the ascorbic acid, DCA plus ACTH treated group showed an average decrease of 22.5%; ACTH plus ascorbic acid decreased the muscle-ascorbic acid content by 62.7%.

The plasma-ascorbic acid content as shown in Table II and figure 5 reveals that the group treated with ascorbic acid only had an average concentration of 1.32 mgs. of ascorbic acid per 100 cc of plasma. Here, the ascorbic acid plus DCA treated group and ascorbic acid plus ACTH treated group showed no change in the deposition of plasma-ascorbic acid. This indicates that neither of the above combinations act synergically nor antagonistically to ascorbic acid storage in the plasma. The following variations reduced the plasma-ascorbic acid content: the ascorbic acid plus cortisone treated group showed the greatest decrease of 77.3%; the ascorbic acid, DCA plus cortisone treated group decreased by 52.3%; while ascorbic acid, DCA, cortisone and ACTH treated group showed the least decrease (16.7%). Not all combinations seemed to act antagonistically against ascorbic acid deposition in the plasma. Note

FIGURE: 5 SHOWING THE INFLUNCES OF THE ADRENO-STEROIOS DCA, CORTISONE AND ACTH ON THE DEPOSITION OF ASCORBIC ACID IN THE PLASMA



PLASMA

that when ascorbic acid, cortisone plus ACTH and ascorbic acid, DCA plus ACTH were administered the plasma-ascorbic acid content was increased by 101.5% respectively.

DISCUSSION

The foregoing experiments are supported by Hartman and Thorn (16) in showing that the growth pattern as in Table I and figure 1, in the adrenal insufficent animals is due entirely to a secondary change, principally the reduction of food intake. The cessation of growth observed in adrenal insufficent animals in this experiment showed that the food and water intake were much less than the experimental animals on the adreno-steroid and ACTH regime. In the presence of adreno-steroids and ACTH the animals resumed growth, which is in accordance with Hartman, et. al., who have shown that the administration of adreno-cortical extracts caused a decrease in non-protein-nitrogen. This, according to these workers caused an increase in the weight of the rats which received adreno-cortical extracts whereas the adrealectomized animals which did not receive the hormone showed an increase in non-protein-nitrogen with a loss in body weight.

It is of interest to note that not all variations of the adreno-steroids and ACTH caused an increase in body weight. This is in accordance with Ingle et.al. (20). They have shown that the relationship of the cortical hormones to growth may be unspecific. Further, the inhibitory effect of these hormones to body growth may be due to an over-dosage of the adrenal cortical hormones such as the eleven oxygenated compounds which represent speci-

fic growth inhibitors that are normally balanced against the growth hormone of the anterior pituitary (20).

Table I and figure 1 present data showing the antagonism of cortisone and ACTH when administered with ascorbic acid. The influence of cortisone upon the retardation of body growth is in agreement with Ingle, et.al. (21), who found that when normal rats are given cortisone acetate they develop a negative nitrogen balance which results in weight loss. In view of the evidence presented by these investigators, the decrease in body weight may be attributed to the following: (1) an imbalance of ACTH and cortisone and, (2) a negative nitrogen balance produced by cortisone acetate.

The decrease in body weight of the group receiving the basic supplement (ascorbic acid only) is in agreement with Lockwood, et.al. (27), who found that experimental guinea pigs on a control diet consistently lost weight over a twenty-eight day period after adrenalectomy. According to Grollman, et.al. (11), the loss in body weight is due to an imbalance of sodium and potassium, and to improper carbohydrate and protein metabolism. In view of the established fact that adrenalectomized animals, when on a deficient adreno-cortical hormone regime, lose weight, the author believes that the loss of weight is due mainly to adrenal insufficiency.

When DCA was administered with ascorbic acid there was an increase in body weight which is in agreement

with Massen, et.al. (29). They have shown that DCA inhibits body and tail growth of normal rats, but it failed to inhibit the increase in body weight. Best and Taylor (1) in a study of the effects of DCA upon salt and water metabolism found that DCA increases the plasma volume and concentration of sodium in body fluids, but that DCA reduces the concentration of potassium. Additional experiments reported by these authors stated that DCA reduces the intercellular concentration of potassium and increases that of sodium, thus causing a shift in the electrolytic balance. Further, DCA appears to influence membrane permeability of the tissues, thereby causing a retention of sodium and water which may be followed by edema. Gordon (11) agrees that in the regulation of electrolytes and water metabolism, DCA has been shown to be the most potent of all the adrenosteroids. On the basis of findings of Best and Taylor (1) and Gordon (12) the author feels that the gain in body weight of the group receiving DCA and various combinations of DCA may be attributed to a retention of sodium in the body tissue, thereby causing edema. In the case of DCA plus cortisone, the increase in weight is due to a normal electrolyte balance of sodium and potassium causing a normal growth pattern. According to Roemmelt, et.al., these animals exhibited a reduced capacity to eliminate sodium under high salt load, analogous to their reduced capacity to eliminate water under high water load. The reduction

in sodium and water excretion under this condition is due to an increase tubular reabsorption rather than a reduced glomerular filtration (32). Further, they have stated that the low volume and high salt concentration of the urine and the very considerable osmotic work performed by the kidney of the adrenalectomized animal under salt load suggest an over production or decreased destruction of, or, hypersensitivity to antidiuretic hormones. Such a view is consistent with the known susceptibility of the adrenalectomized animal in water intoxication. When relatively small quantities of pitressin are injected into normal animals, a negative sodium and chlorine balances were observed as in the untreated adrenalectomized animals. Roemmelt is of the opinion that the retention of potassium is a manifestation of specifically increased ionic reabsorption and not directly related to diminished sodium absorption (32).

In agreement with Thorne, et.al. (38), Table I presents data pertaining to the effects of the adreno-steroids on the hair and skin appearance. Note that the experimental animals receiving cortisone and various combinations of cortisone showed a change in hair appearance from a normal to a rough coat. According to Thorn, cortisone is a potent hormonal substance which produces definitely physiological effects. Further, these investigators report that with high or prolonged dosage, and in some cases the recommended dosage, undesirable effects on skin and hair

appearance are produced. The experimental animals which did not receive cortisone acetate showed no change in hair appearance which is in agreement with Thorn, et. al. (38), who have shown that the other adreno-cortical hormones produce no physiological effects on hair appearance, and that after cortisone administration is discontinued the effects are reversible.

Table II and figures II through IX show that tissue distribution studies did not reveal any consistent trend regarding the influence of adreno-steroids and ACTH on the deposition of ascorbic acid in the adrenalectomized rat. The data presented in these tables are in agreement with similar work done by Booker, et. al. (4). Note that when DCA, cortisone, and a combination of DCA, cortisone plus ACTH were administered, the ascorbic acid content was reduced below the control group in the following tissues: brain, kidney, plasma and adipose tissue. The above results reveal that these variations are antagonistic to ascorbic acid deposition in these tissues. The exact mechanism between the adreno-steroids and ACTH cannot be adequately stated according to Booker (2). In experimental studies of normal rats, it has been reported that when adreno-cortical extracts are administered along with ascorbic acid, they facilitate the storage of ascorbic acid at the adrenal gland. The increase of ascorbic acid in the adrenal gland in the presence of exogenous adreno-cortical hormones is suggestive evidence that the hor-

mones may be acting to increase the storage of ascorbic acid in the adrenal glands (2). The question arises as to the significance of the exogenous adreno-steroids upon ascorbic acid deposition in other body tissue after bilateral adrenalectomy. Booker (2) has reported that with an increase of ascorbic acid in the adrenal glands, there was a decrease in cholesterol indicating that cholesterol may be a precursor of the adreno-steroids; further, Sayers has presented evidence which supports this point of view. The present data suggest that the exogenous adreno-steroids are not enough to maintain normal metabolic processes in the absence of the adrenal glands as indicated by ascorbic acid storage in body tissues.

Tissue distribution studies of the effect of DCA, cortisone and ACTH plus cortisone are in accordance with Collins (6), who has reported that these substances decreased the ascorbic acid content in the brain tissue of more than 100 per cent below the control group receiving ascorbic acid only. It is of interest to note that DCA plus ACTH, and ACTH plus ascorbic acid increased the ascorbic acid content of the brain by 185.6 and 106 per cent respectively, above that of the animals receiving ascorbic acid only. On the other hand, Collins (6) reports that the above variations decreased the ascorbic acid content more than 100 per cent. The regulatory mechanism for this difference cannot be definitely stated; however, it is logical to assume that the adrenals are the determing factor.

Table I and figure 3 are partly in accordance with Collins (6) whose work shows that tissue distribution studies did not reveal a consistent trend regarding the influence of the adreno-steroids and ACTH on spleen ascorbic-acid. Note that only DCA, cortisone plus ACTH, and DCA plus ACTH caused an increase of 65.6 and 346.4 per cent respectively, above the group of adrenalectomized animals receiving the basic supplement. In a similar experiment with normal rats, Collins (6) has reported that only DCA plus ascorbic acid increased the spleen ascorbic acid content, a fact which is an indication that the other variation acts antagonistically to spleen ascorbic acid storage. When the adreno-steroids and ACTH were administered with ascorbic acid in the adrenalectomized rat, all combinations decreased the muscle-ascorbic acid content which is in accordance with Collins (6) who in a similar experiment with normal rats reported that all variations except DCA decreased the muscle-ascorbic acid content. Consistent points of difference are observed when normal and adrenalectomized animals are compared under similar experimental conditions. (1) In all tissues examined DCA plus ascorbic acid increased the ascorbic acid storage in the normal rat above the control group as reported by Collins (6), whereas, in the adrenalectomized rat, DCA plus ascorbic acid decreased the tissue ascorbic acid content in the following tissues: brain, spleen, kidney, and muscles. The ascorbic acid content in adipose

tissue and plasma did not show any change. (2) In the liver, all variations of the adreno-steroids and ACTH increased the ascorbic acid content; however, Collins (6) reports that only DCA increased the liver-ascorbic acid. (3) In the adipose tissue of the adrenalectomized rats, DCA plus cortisone, cortisone plus ACTH, ACTH plus ascorbic acid, and DCA plus ACTH increased the ascorbic acid content by 11, 115, 493 and 605 per cent respectively, showing no change when DCA plus ascorbic acid, cortisone plus ascorbic or a combination of both hormones and ACTH were administered with ascorbic acid. Here, Collins reports that only DCA plus ascorbic acid and DCA, ACTH plus ascorbic acid increased the ascorbic acid content of the adipose tissue (6). (4) The kidney ascorbic acid content of the adrenalectomized rat shows that only cortisone plus ACTH, DCA plus ACTH and ACTH plus ascorbic acid caused an increase ascorbic acid content by 78.4, 412, and 310.9 per cent respectively; whereas, the normal rats receiving the same variations showed a decrease in kidney-ascorbic acid content with a slight increase when DCA plus ascorbic acid was administered. (5) The plasma-ascorbic acid content as revealed from the biological assay of the adrenalectomized rats shows that only cortisone plus ACTH and DCA plus ACTH increased the plasma-ascorbic acid content by 101.5 per cent respectively, whereas, DCA plus ascorbic acid and ACTH plus ascorbic acid showed no significant change in the plasma ascorbic acid content. In the normal rat, Collins (6) reports that all com-

binations of the adreno-steroids increased the deposition of plasma-ascorbic acid while DCA plus ACTH showed the greatest increase of all variations used in this investigation.

In view of the above differences, it is of interest to note that DCA was more effective than cortisone in increasing ascorbic acid in all tissues analyzed which is in accordance with Booker, et.al. (3) and Collins (6) who in their studies found no consistent trend in the deposition of ascorbic acid in normal rats treated with the adreno-steroids. Further, they have shown that the adreno-steroids as a group exert roughly 60 to 70 per cent reduction of Vitamin C in the urine and tissues as compared with the ascorbic acid treated normal rats.

In agreement with Booker, et.al. (5), DCA and ACTH are more effective in increasing plasma ascorbic acid than either DCA or cortisone. A number of investigators (7, 10, 28) have shown that the adreno-cortical hormones may increase the circulation of plasma volume in adrenal insufficency by (1) the control of renal function and (2) the transfer of water and electrolytes to the blood stream from the tissues. Furthermore, according to Swingle (34), in adreno-cortical insufficency an increase in permeability is indicated by a pathological disturbance of fluid exchange in favor of outward filtration and the production of a marked hemo-concentration.

Due to the similarity in structural formulae of glucose and ascorbic acid, a number of investigators beleive that

their intermediate metabolic processes are related. Umbreit (38) has presented evidence that D-amino-acid oxidase is under the control of cortisone in the liver, but not in the kidney; whereas, DCA was not effective in maintaining or restoring the proline system. Van Arman has presented evidence that amino acids are precursors of epinephrine by injecting large quanities of insulin followed by glucose and noticed that the epinephrine content was reduced by 39 per cent. The findings that no recovery took place with glucose alone probable means that the body cannot utilize its own nitrogenous compounds to synthesize epinephrine at an appreciable rate. Under this condition it appears that the dietary precursor is an amino-acid (38).

In the adrenalectomized rat where the adreno-cortical hormones and the epinephrine content is "nil", the limited exogenous cortical hormones must serve to maintain both inorganic and organic metabolic processes, it is logical to assume that the absence of the adrenal cortex and medulla are involved in this process.

SUMMARY AND CONCLUSION

This experiment has been described comparing the effects of adreno-corticotrophic (ACTH), desoxycorticosterone (DCA), cortisone and different variations of the above on the deposition of ascorbic acid in the adrenalectomized rat. The data shows that all variations reduced the brain-ascorbic acid content except DCA plus cortisone, ACTH plus ascorbic acid and DCA plus ACTH, while the latter group showed the greatest percentage increase.

In the spleen, DCA, cortisone plus ACTH, and DCA plus ACTH increased the spleen-ascorbic acid concentration while all other combinations seem to act antagonistically to ascorbic acid deposition in the spleen.

All variations of the basic supplement increased ascorbic acid storage in the liver above the control group receiving only ascorbic acid. Here, DCA plus ACTH was most effective.

In the adipose tissue where the ascorbic acid content was "nil" in the control group; DCA plus cortisone, cortisone plus ACTH, ACTH plus ascorbic acid, and DCA plus ACTH showind the greatest concentration.

The data further shows that in respect to kidney-ascorbic acid concentration; cortisone plus ACTH, DCA plus ACTH, and ascorbic acid plus ACTH increased the ascorbic acid content.

The plasma-ascorbic acid concentration was increased only when cortisone plus ACTH, and DCA plus ACTH were administered. DCA plus ascorbic acid, and ACTH plus ascorbic acid had no effect in increasing or decreasing the ascorbic acid concentration while the remaining varables decrease the plasma ascorbic acid content.

With respect to tissue ascorbic acid it has been shown that DCA plus ACTH is most effective in all tissues analyzed, and DCA is more effective than cortisone in maintaining ascorbic acid levels in the tissues of the adrenalectomized rat. The regulatory mechanism for this action is not known but in view of the evidence presented the absence of the adrenal cortex and medulla seem to be the determing factor.

This experiment also described the effects of adrenosteroids and ACTH upon the changes in body weight and hair appearance. DCA caused the greatest increase in body weight of all the steroids administered either together or separately; DCA plus ACTH caused the next highest gain, while DCA plus cortisone, ACTH plus cortisone produced weight gains proportionally. The animals receiving cortisone plus ascorbic acid showed the greatest weight loss; while ACTH plus ascorbic acid showed the next highest loss in body weight. The group of animals receiving only ascorbic acid showed the least weight loss than either of the above combinations.

In respect to hair appearance, all groups receiving a combination of cortisone showed marked changes in hair coat,

while the groups receiving combinations of DCA showed no change in hair appearance.

The present data suggest that the gain in body weight of the group receiving DCA and combinations of DCA may be attributed to an electrolytic imbalance of sodium and potassium, thus causing a retention of sodium and water in the body tissues producing an edema effect. The groups receiving cortisone perhaps lost weight due to a negative nitrogen balance and a loss of sodium and water from the body tissues with a likewise increase in potassium ions. The changes in hair appearance of the animals receiving cortisone is attributed to the potency of cortisone acetate which produces marked physiological appearences when administered.

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