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The Influence Of The Adrenotrophic Hormone, Desoxycorticosteron, Cortisone And Adrenalin On The Cellular Elements Of The Blood And Adrenal Ascorbic Acid In The Albino Rat

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By

Frederick Douglas Todd, Sr.

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BIOGRAPHICAL SKETCH

Frederick Douglas Todd was born in Dallas, Texas on October 3, 1930. He attended the Phyllis Wheatley Elementary School of that city. In January of 1948, he was awarded the high school diploma from Lincoln High School, Dallas, Texas. Upon completion of his high school work he entered Prairie View Agricultural and Mechanical College from which he was awarded the Bachelor of Science degree in 1952 in the field of Biology with a minor in Chemistry.

After completion of his college work he was called to active duty as a commissioned officer in the United States Army. He served in Japan and Korea as well as in the United States. In July of 1955, he was separated from the service as a First Lieutenant.

He reentered Prairie View Agricultural and Mechanical College as a fellow in Biology and has completed the requirements for the Master of Science degree in Biology. He is now employed at the James Madison High School, Dallas, Texas as an instructor of Chemistry and Physics.

ACKNOWLEDGEMENT

The writer is indebted to Mr. Charles H. Nicholas for his guidance, interest, and criticism in the preparation of this paper.

F. D. T.

DEDICATION

To my deceased mother, Mrs. Adelaide Golden Todd, my father, Mr. Clifton Todd, and my loving wife, Mrs. Earle Nathan Todd, this study is dedicated.

F. D. T.

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CHAPTER I

INTRODUCTION

Clinical and experimental data reported in recent years have indicated that there is a relationship between the activity of the adrenal cortex and the hematologic picture of an individual. Recent studies by Crafts et al.(1941) clearly point out that there is a relationship between the endocrine system and the cellular elements of the blood. Craft noted the following in adrenalectomized animals: increase in reticulocytes, decrease hemoglobin percentage, increase of the color index, but a normal white cell count.

The abnormalities that arise in an individual's hemogram have been noted in many pathological conditions of the endocrine system as well as in experimental analyses involving the endocrines. White et al. (1944) proved that injection of ACTH decreased the lymphocyte count in an individual but increased the number of polymorphonuclear leucocytes. These investigators proved that lymphopenia is a specific response to ACTH because it does not occur in the adrenalectomized animals treated with ACTH. Therefore, these experiments by White and other related ones clearly point out that ACTH plays an important role in the normal function of the adrenal gland.

It is interesting to note that White (1944) observed two significant facts which were reported by Craft (1941). Both of these investigators observed that ACTH or adrenal cortical extract injections

resulted in a decrease in the number of red blood cells and a decrease in hemoglobin concentration.

According to Sayer et al. (1944) the content of ascorbic acid and cholesterol content of the adrenal gland is a good indication of the activity of ACTH. A single dose of this hormone diminishes both the ascorbic acid and cholesterol content of the adrenal gland. An experiment was performed by Long et al. (1945) in which epinephrine was used instead of ACTH. It was noted that injections of epinephrine caused a fall in both the ascorbic acid and cholesterol content of the adrenal gland which could be abolished upon hypophysectomy. From the results of Long's experiment it was not possible to obtain an explanation of this occurrence. Two theories which try to describe possible modes of action of epinephrine in the animal's body were proposed by Long. According to the first theory, the effect of epinephrine is due to the direct stimulation of the cells of the anterior pituitary which secretes the adrenotrophic hormone, but the second theory states that this occurrence may be due to changes in the composition of the blood acting agents.

The action of desoxycorticosterone acetate in the intact and hypophysectomized rat was observed by Greep et al. (1947). It was reported that daily desoxycorticosterone acetate injections completely inhibited ketosteroid production in the cells of the zona glomerulosa, a cellular layer of the adrenal cortex, in both the intact and hypophysectomized rats.

In recent years there have been numerous experiments on ascorbic metabolism and feeding. Li et al. (1949) observed that ascorbic

acid feeding produced polycythemia in both the male and female albino mice. This feeding also caused an increase in reticulocytes. Covian (1949) observed that after hypophysectomy there was a slight decrease in adrenal ascorbic acid at the end of an hour. The investigator believed this effect was due to the liberation of adrenocorticotropin during hypophyseal ablation. Thereafter, the ascorbic acid fell progressively while the adrenal gland gradually atrophied.

Many important facts have been established concerning changes which take place in the blood chemistry during ascorbic acid metabolism. Many studies dealing with ascorbic acid by Booker et al. (1951) show that ascorbic acid causes transitory changes in the electrolytes of the blood similar to changes seen in Addison's disease. The administration of ascorbic acid causes an increase in serum cholesterol which is greater in the presence of exogenous adrenocortical hormone. The adrenocortical hormones increase the deposition of ascorbic acid at the adrenal gland as reported by Booker (1951). As the ascorbic acid is increased cholesterol is decreased in the gland. According to Booker this evidence suggests that something of a blood cell barrier to ascorbic acid exists since the rise of cell ascorbic acid lags significantly behind the plasma ascorbic acid following the administration of ascorbic acid.

Bacchus (1951) reported the action of ascorbic acid on leukocyte response in the normal and adrenalectomized rat. The investigator noted that the eosinophils are the only leukocytes affected by the action of ascorbic acid. He further reported that ascorbic acid alters the eosinopenia of stress in the intact animal and delays the eosinopenia

characteristics of stress in the adrenalectomized rats.

It has been clearly established by Dury (1950) that there is statistically significant negative correlation of adrenal ascorbic acid content with the number of circulating neutrophiles in the normal rat. He reported further that the leucocytic picture was actively influenced by adrenalin injections which have been shown to be mediated via the adrenalcortical mechanism. Dury believes that this data suggest that the adrenal cortex is responsible to an, as yet, unknown extent in the regulation of the number of circulating neutrophiles in the normal rat.

There are numerous experiments which have shown the effect of ACTH and cortisone on the number of circulating neutrophiles in the blood. The results of the experiments of Best et al. (1950), Speirs et al. (1949), Upton et al. (1951), Scott et al. (1950), Ingbor et al. (1951), and Rosenthal et al. (1951) are so closely related that the results of this group of experiments may be summarized. Whereas injections of ACTH and cortisone in the normal rat caused an increase in eosinophils, it was noted that if the adrenal glands were removed, the injections of ACTH had no effect on the adrenalectomized animal.

A review of the literature has led the investigator to believe that there may be a functional relationship between the adrenal gland ascorbic acid content and the hematologic picture of an individual. ACTH plays an important role in normal functioning of the adrenal gland. Since it is an accepted fact that ACTH influence the normal functioning of the adrenal gland, it may be assumed that the lymphopenia produced in numerous experiments is a specific response to ACTH.

With the fore-mentioned facts in mind this research is designed to show the influence of the adrenotrophic hormone, cortisone, desoxycorticosterone and adrenalin on the cellular elements of the blood and the disposition of ascorbic acid in the plasma and adrenal gland. The general objectives are as follows:

1. To show the physiological changes that may occur in the blood when there is an excess of the adrenotrophic hormone and adrenosteroids.

2. To show a possible relationship between the amount of ascorbic acid in the plasma and the adrenal gland.

3. To show a possible relationship between the amount of ascorbic in the adrenal gland and the blood plasma with the hemological picture of the cellular elements of the blood.

CHAPTER II

MATERIALS AND METHODS

Albino rats from the Agricultural and Mechanical College of Texas strain, six to eight weeks of age, were used in this study.[1] The rats were fed Purina Chow and tap water ad lib., with lettuce being given once weekly. Each rat was then marked for the purpose of identification and its weight was recorded.

One hundred and twenty-eight rats were used in this experimental study. These animals were divided into five groups. The control group (Group A) consisted of five rats. Group B, C, D and E consisted of fifteen rats each. The rats in each group were designated by numbers for identification.

The experimental animals received the following supplements intramuscularly at intervals of six hours for a period of twenty-four hours:

Group A	No supplement (Control Group)
Group B	2 mgs adrenotrophic hormone per 100 grams of body weight
Group C	2 mgs desoxycorticosterone per 100 grams of body weight
Group D	2 mgs cortisone per 100 grams of body weight

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[1]Donated by Dr. L. R. Richardson, Professor of Biochemistry, Texas Agricultural and Mechanical College, College Station, Texas.

Group E 0.1 cc of adrenalin per 100 grams of body weight

The ascorbic acid content of the adrenal gland was determined by the Bessy and King modification of the Tillman Method.[1] The glands from five specimens were removed and pooled, weighed on the precision balance (sensitivity: 0.00025 gm per division) and properly recorded. The weighed portion of tissue was ground into a thin paste with 25 cc of 8 per cent trichloroacetic acid and 2 per cent metaphosphoric acid solution. The mixture was then centrifuged and the clear liquid decanted. An aliquote portion of the mixture was then diluted and titrated with a standard solution of 2-6-dichloro-benzenoindo-phenol dye (1 ml equals 0.045 mg of ascorbic acid) to a permanent faint pink end-point. The calculations were made by the following equation:[2]

Titration blank x Ascorbic Acid equivalent (mg) per cc dye solution/gms sample in aliquot extract = mg Ascorbic Acid per gm sample

The blood ascorbic acid was determined by the Farmer and Abt Method. The equation for the calculations is as follows:[3]

Titration x Ascorbic Acid equivalents (mg) x $\frac{100}{0.2}$ per cc dye solution = mg Ascorbic Acid per 100 cc of plasma

The leucocyte and differential leucocyte determinations were made by standard procedures on blood obtained from the tails of the

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[1] P. B. Hawk, and O. Bergein, Practical Physiological Chemistry, 12th ed., The Blakiston Company, Philadelphia, Penn., 1951.

[2] Ibid.

[3] Ibid.

rats.[1] The differential leucocyte values were determined after counting at least one hundred white cells which had been prepared on a slide using Wright's stain. The hemoglobin determinations were made by the Homoglobin-Acid Hematin Method by using the Bausch and Lomb Spectronic 20 Colorimeter.[2]

In order to obtain a hemogram of the cellular elements and also the content of blood and adrenal ascorbic acid in the experimental animals all of the tests mentioned above were performed at intervals of three, six and nine hours after the last injection of the supplements. Each run consisted of five rats.

The entire experiment was performed twice in order to determine the validity of this work between two trials of the entire experiment.

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[1]F. E. D'Amour, and F. R. Blood, Manual for Laboratory Work in Mammalian Physiology. Chicago: University Press, 1953.

[2]Bausch and Lomb, Spectronic 20, Colorimeter, Methods and Calibrations, Catalog Number 30-29-40.

CHAPTER III

OBSERVATION AND RESULTS

This is an investigation to determine the effects ACTH and the adrenosteroids have upon the blood cellular elements and the adrenal gland and blood ascorbic acid. Of the total number of specimens (128) used in this research, three died as a result of an overdose of adrenalin.

The administration of the adrenosteroids and ACTH had varied effects upon the rats' hemogram and the blood and adrenal ascorbic acid. Data on the initial and final observations of each group of the experimental animals, before and after supplementation, are shown in Tables 1 to 4. Figures 1 to 8 show the effects the adrenosteroids and ACTH have on the total leucocyte count, differential count, and the blood and adrenal ascorbic acid.

Figures 1 and 2 represent the effects ACTH had upon the cellular elements and the blood and adrenal ascorbic acid. These figures show that animals treated with ACTH increased the total leucocyte count, neutrophile and monocyte percentages and blood ascorbic acid during the first three hours of the experimental period. Inspection of Figures 1 and 2 reveals that adrenal ascorbic acid and lymphocyte percentage decreased during the three hour period. The sixth hour is characterized by a gradual increase in the total number of leucocytes, lymphocytes, and monocytes but a decrease in the total number of

FIGURE 1

THE EFFECT OF ACTH ON TOTAL LEUCOCYTE COUNT, ADRENAL AND BLOOD ASCORBIC ACID

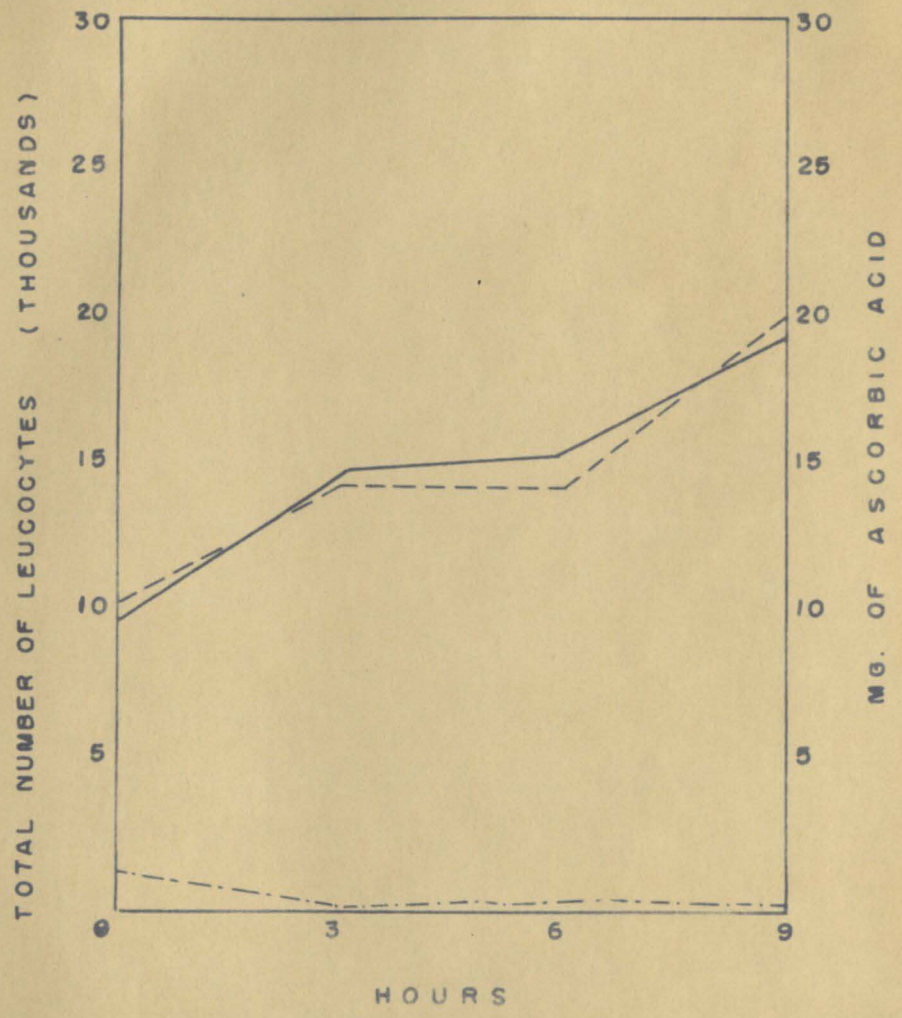
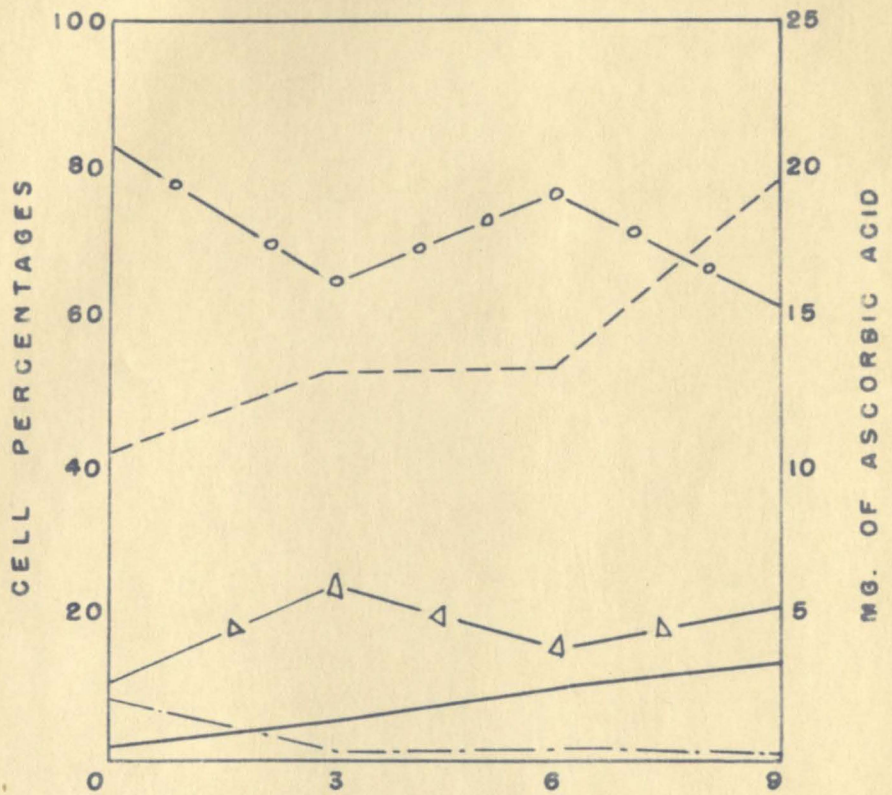


FIGURE 2

THE EFFECT OF ACTH ON THE CELLULAR ELEMENTS, ADRENAL AND BLOOD ASCORBIC ACID



neutrophiles. Adrenal and blood ascorbic acid remained constant during this period. At the end of the ninth hour the total number of leucocytes, neutrophiles, monocytes and blood ascorbic acid increased as adrenal ascorbic acid and lymphocyte values decreased. It is interesting to note that the blood ascorbic acid increased, leveled off, then increased again. The total leucocyte count and monocyte percentages followed the same general trend. The neutrophiles, however, showed an increase when the ACTH was administered during the three hour period, which was followed by a sharp decrease through the sixth hour, then showed an increase which paralleled that of the blood ascorbic acid. The adrenal ascorbic acid and lymphocyte percentage showed a general decrease throughout the experimental period.

Figures 3 and 4 present the results of injections of DCA. They show its effect on the cellular elements and the blood and adrenal ascorbic acid. DCA increased the total number of leucocytes, monocytes, neutrophiles and blood ascorbic acid and the lymphocyte percentages decreased. The total leucocyte count, neutrophil percentages and monocyte percentages gradually increased throughout the remainder of the experimental period while the total amount of lymphocytes continued to decrease. Adrenal and blood ascorbic acid values fluctuated during the remainder of the experimental period. Between the third and sixth hours, blood ascorbic acid decreased and adrenal ascorbic acid increased, but the reverse of this pattern was noted during the ninth hour. It is apparent, by the evidence presented thus far, that ACTH and DCA increase all cellular elements of the blood with the exception of the lymphocytes. The evidence seems apparent that these two

FIGURE 3

THE EFFECT OF DCA ON TOTAL LEUCOCYTE COUNT, ADRENAL AND BLOOD ASCORBIC ACID

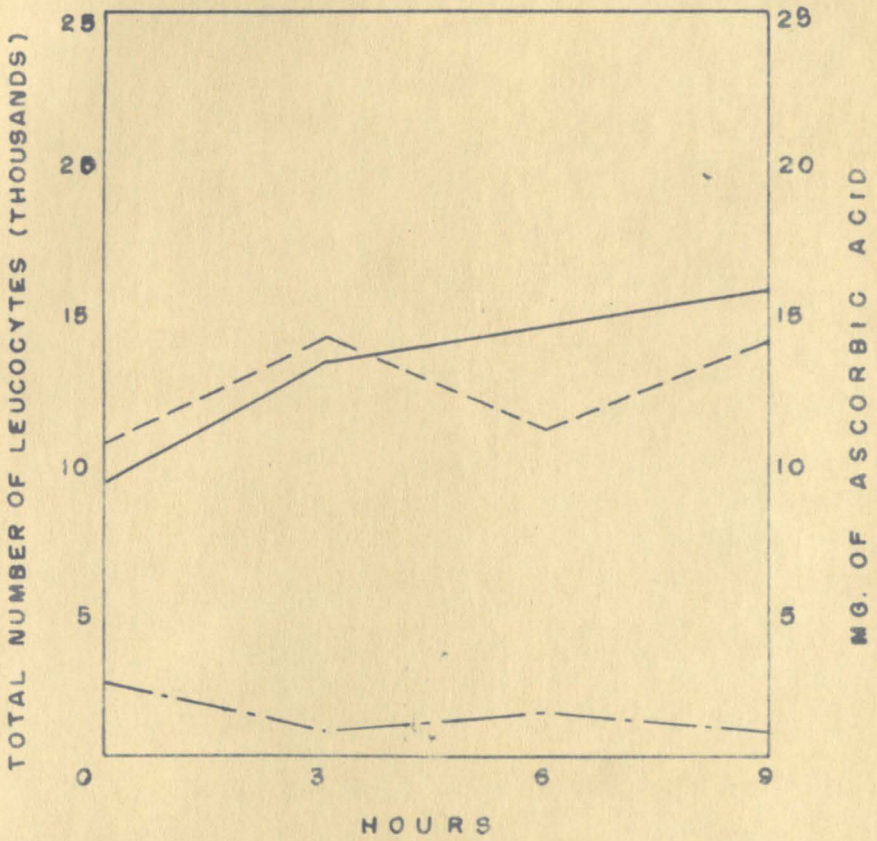
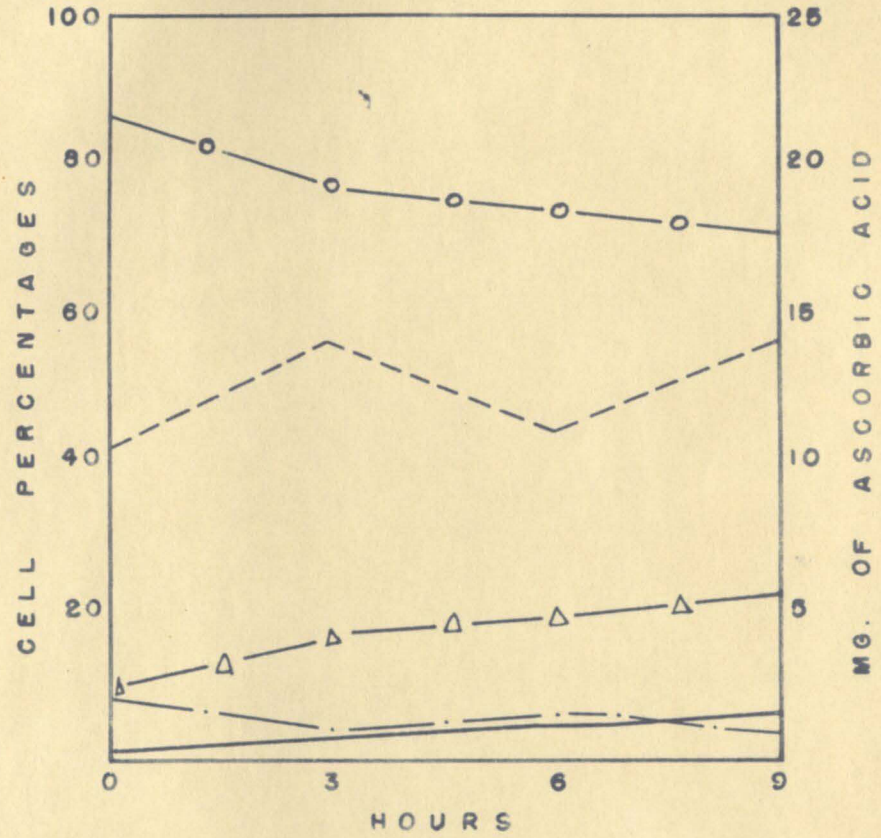


FIGURE 4

THE EFFECT OF DCA ON THE CELLULAR ELEMENTS OF ADRENAL AND BLOOD ASCORBIC ACID



——— MONOCYTE PERCENTAGES
—o— LYMPHOCYTE PERCENTAGES
—Δ— NEUTROPHILES PERCENTAGES
– – – MG. OF ADRENAL ASCORBIC ACID PER 100 ML. OF BLOOD
—·— MG. OF ADRENAL ASCORBIC ACID PER GRAM OF SAMPLE

hormones (ACTH and DCA) act similarly in the specimen's body, but vary in the degree of response.

Figures 5 and 6 show the effects of cortisone upon the cellular elements, blood ascorbic acid, and adrenal ascorbic acid. It can be observed that there were distinct variations between animals that were treated with cortisone and those treated with ACTH and DCA (Figures 5 and 6). A slight increase was noted in the total leucocyte count (Figure 5), while the neutrophile and monocyte percentages (Figure 6), were distinctively elevated during the three hour period. This period shows also that adrenal ascorbic acid decreased while blood ascorbic acid increased slightly. Lymphocyte percentage decreased through the six hour period, after which there was an increase (Figure 6) in these values. The total leucocyte count (Figure 5) and monocyte percentages (Figure 6) decreased while the neutrophiles increased; blood ascorbic acid decreased and adrenal ascorbic acid increased during the six hour. At the end of the experimental period, the total leucocyte count, neutrophile and monocyte percentages , and the adrenal ascorbic acid decreased as a result of cortisone injections, while ascorbic acid content of the blood remained relatively constant. It was observed that cortisone had the greatest influence on the percentages of neutrophils and monocytes during this experimental phase.

Figures 7 and 8 present the results of average values from this experiment which show the effects of adrenalin on the blood cellular elements, ascorbic acid content in the blood and adrenal gland. It was observed that during the first three hours of the experimental period all cellular elements of the blood increased, with the exception of

FIGURE 5

THE EFFECT OF CORTISONE ON TOTAL LEUCOCYTE COUNT, ADRENAL AND BLOOD ASCORBIC ACID

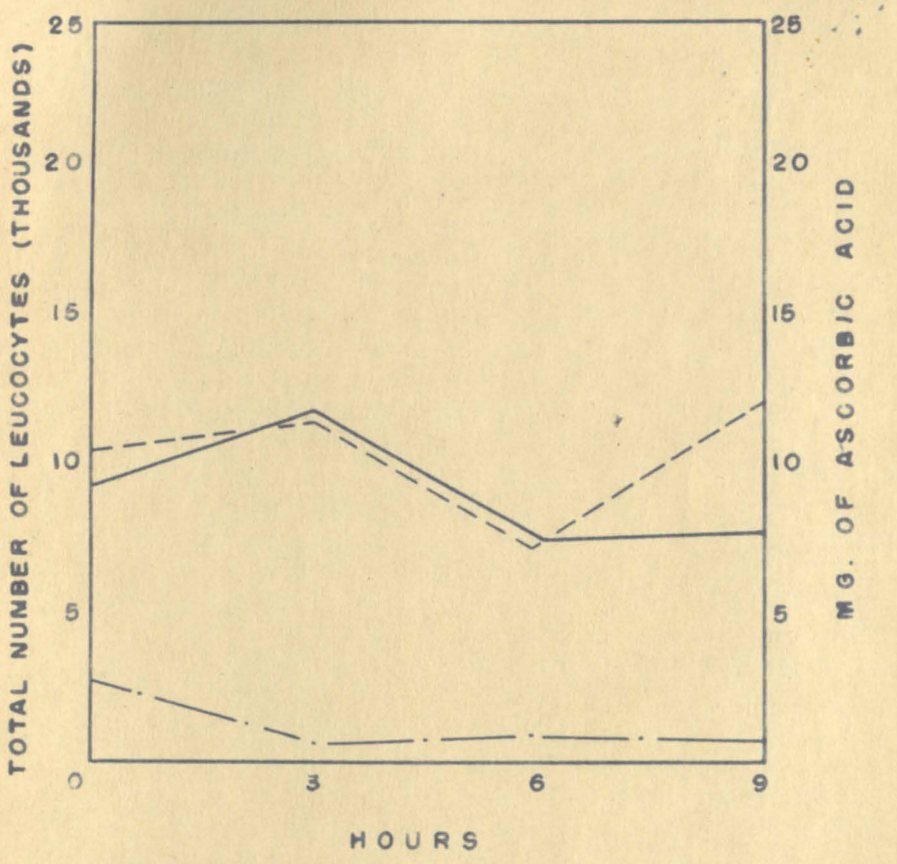
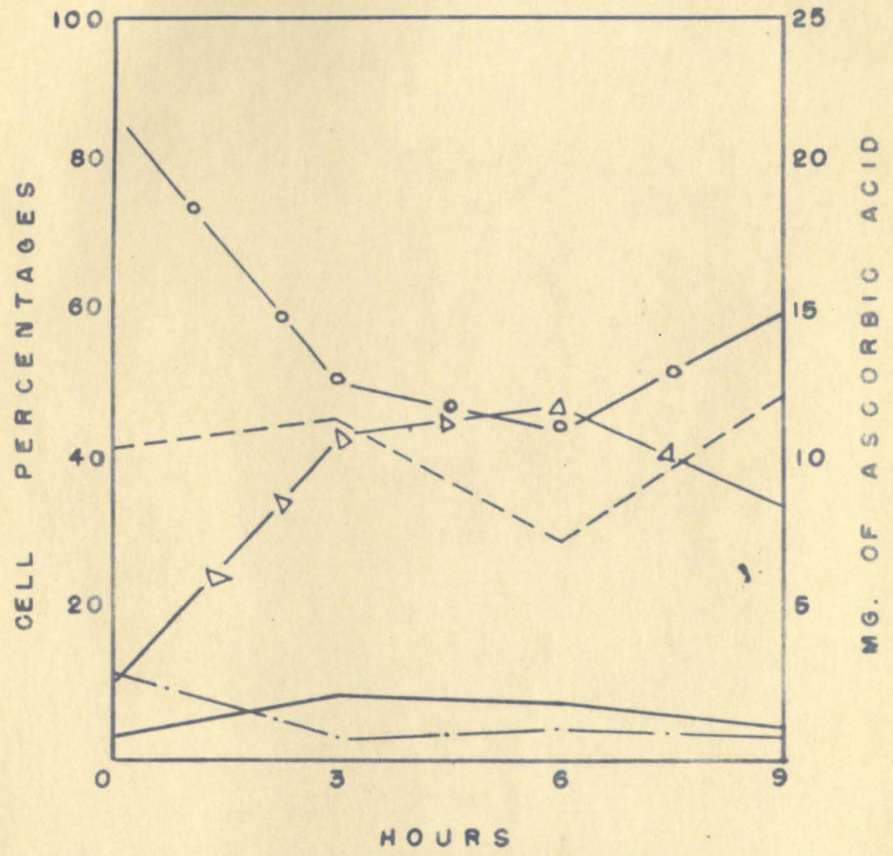


FIGURE 6

THE EFFECT OF CORTISONE ON THE CIRCULAR ELEMENTS, ADRENAL AND BLOOD ASCORBIC ACID



—— MONOCYTE PERCENTAGES
—o— LYMPHOCYTE PERCENTAGES
—Δ— NEUTROPHILES PERCENTAGES
– – – MG. OF ASCORBIC ACID PER 100 ML. OF BLOOD
—·— MG. OF ADRENAL ASCORBIC ACID PER GRAM SAMPLE

the lymphocytes. During this same period, as the blood ascorbic acid increased the adrenal ascorbic acid decreased (Figures 7 and 8). During the remainder of the experimental period all cellular elements, except the lymphocytes and the blood and adrenal ascorbic acid, decreased. The lymphocytes, during the ninth hour period, increased along with adrenal ascorbic acid as a result of adrenalin injections.

It is evident, from the data presented here, that the adrenosteroids and ACTH generally increased the leucocyte counts, monocytes, neurtrophiles and blood ascorbic acid. Cortisone follows this general pattern with one exception. There was a decrease in the total leucocyte count below normal at the end of the experimental period, although there was an increase during the initial observations. Adrenosteroids and ACTH decreased the total number of lymphocytes and adrenal ascorbic acid throughout the entire experimental period. From the evidence presented, it is reasonable to assume that a reciprocal relationship exists between the adrenosteroids and ACTH and the total percentage of lymphocytes. There also appears to be a reciprocal relationship between blood ascorbic acid and adrenal ascorbic acid, since adrenal ascorbic acid declines as blood ascorbic acid increases. It was also observed that the adrenosteroids and ACTH influenced prominent changes in the cellular elements of the blood, but these observations do not clearly show their relationship or the mechanism by which the adrenosteroids and ACTH cause these occurrences.

From the data presented on Tables 1 to 4 it is apparent that the adrenosteroids and ACTH had no effect upon the basophiles and eosinophiles although a decrease in hemoglobin concentration was observed throughout

FIGURE 7

THE EFFECT OF ADRENALIN ON TOTAL LEUCOCYTE COUNT, ADRENAL AND BLOOD ASCORBIC ACID

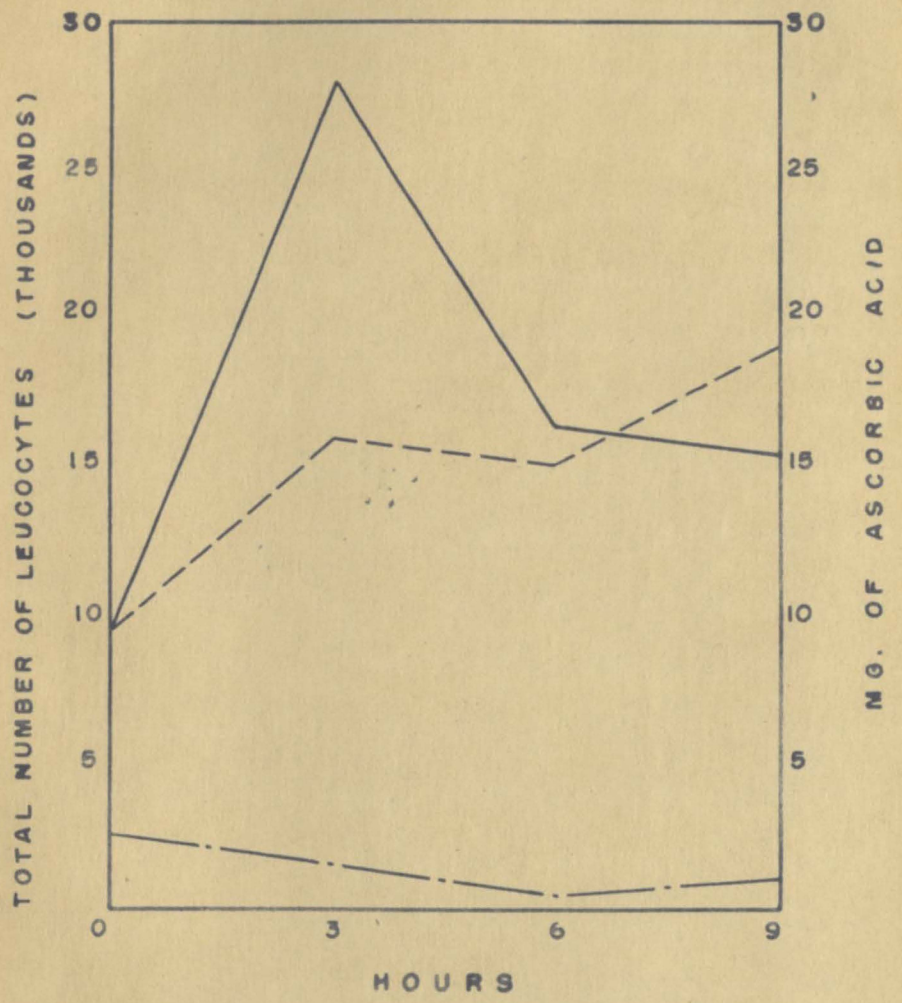
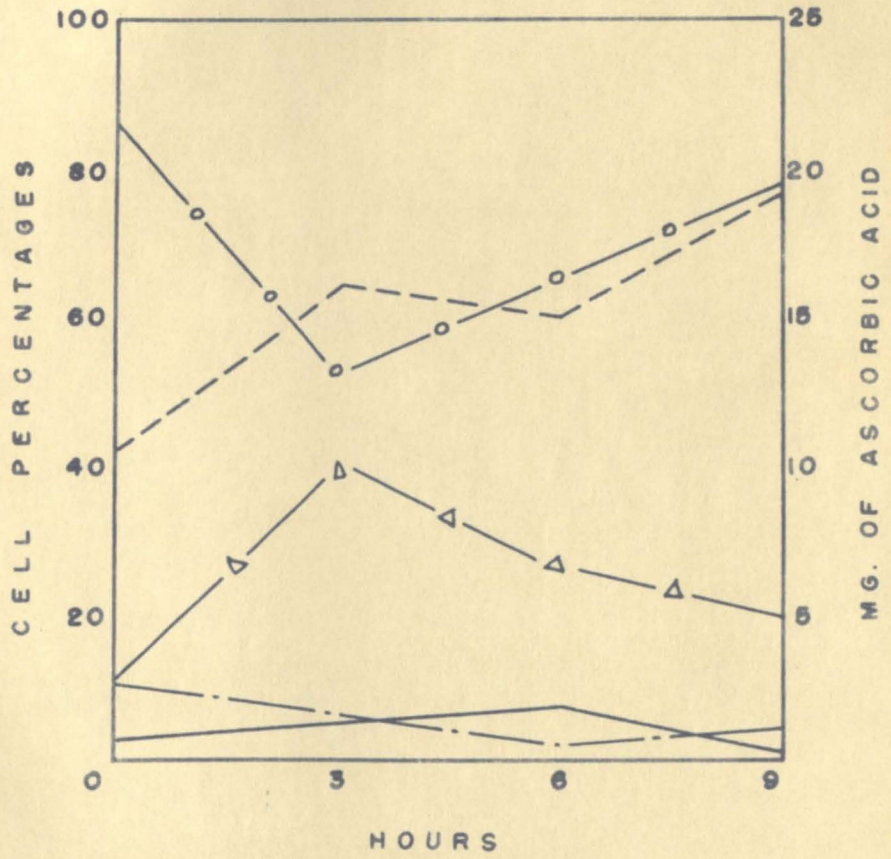


FIGURE 8

THE EFFECT OF ADRENALIN ON THE CELLULAR ELEMENTS, ADRENAL AND BLOOD ASCORBIC ACID



——— MONOCYTE PERCENTAGES
—o— LYMPHOCYTE PERCENTAGES
—△— NEUTROPHILES PERCENTAGES
– – – MG. OF ASCORBIC ACID PER 100ML. OF BLOOD
—·— MG. OF ADRENAL ASCORBIC ACID PER GRAM SAMPLE

the experiment.

In order to determine the relationship between the two runs of this experiment the following formula was used:[1]

$$r = \frac{\sum XY - \left(\frac{\Sigma X}{N} \cdot \frac{\Sigma Y}{N}\right)}{\sqrt{\Sigma X^2 - \left(\frac{X}{N}\right)^2} \cdot \sqrt{\Sigma Y^2 - \left(\frac{\Sigma Y}{N}\right)^2}}$$

x Values in first experiment

y Values in second experiment

n Number of variables

The average correlation for this group of experiments was 0.914.

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[1]John F. Bovard, Frederick W. Cozens, and E. Patricia Hagman, Test and Measurements in Physical Education, Third Edition, W. B. Saunders Company, Philadelphia and London, 1949, Chapter XIII.

TABLE 1

EFFECT OF ACTH ON THE CELLULAR ELEMENTS OF THE BLOOD, BLOOD ASCORBIC ACID AND ADRENAL ASCORBIC ACID

Interval	Runs	Leucocyte Determinations						Number of Cells Counted	Homo-globin g/100 ml	Blood Ascorbic Acid mg/100 ml	Adrenal Ascorbic Acid mg/g Sample
		Total Count	Neutro-phile %	Lympho-cyte %	Eosino-phile %	Baso-phile %	Mono-cyte %				
0 hrs.	1	8,300	13	85	0	1	1	500	16.80	9.40	4.33
	2	10,150	7	88.8	0	1	3.2	500	15.32	11.70	1.38
	Ave.	9,225	10	86.9	0	1	2.1	500	16.01	10.55	2.86
3 hrs.	1	10,120	23.2	66.4	0	0.6	9.8	500	13.58	11.82	0.234
	2	18,270	25.0	72.4	0	0.6	2.0	500	13.82	15.30	0.445
	Ave.	14,195	24.1	69.4	0	0.6	5.9	500	13.70	13.50	0.339
6 hrs.	1	14,970	17.4	72.2	0	0	10.4	500	16.30	10.80	0.514
	2	15,330	14.6	75.8	0	0	9.6	500	14.50	16.20	0.680
	Ave.	15,150	16.0	74.0	0	0	10.0	500	15.40	13.50	0.592
9 hrs.	1	19,110	19.40	58.2	0	0	22.4	500	13.72	22.27	0.398
	2	18,438	23.50	72.75	0	0	3.75	400	13.50	15.00	0.442
	Ave.	18,774	21.45	65.475	0	0	13.075	450	13.61	19.24	0.420

TABLE 2

EFFECTS OF DCA ON THE CELLULAR ELEMENTS OF THE BLOOD, BLOOD ASCORBIC ACID AND ADRENAL ASCORBIC ACID

Interval	Runs	Leucocyte Determinations						Number of Cells Counted	Homo-globin c/100 ml	Blood Ascorbic Acid mg/100 ml	Adrenal Ascorbic Acid mg/g Sample
		Total Count	Neutro-phile %	Lympho-cyte %	Eosino-phile %	Baso-phile %	Mono-cyte %				
0 hrs.	1	8,300	13	85	0	1	1	500	16.80	9.40	4.33
	2	10,150	7	88.8	0	1	3.2	500	15.32	11.70	1.38
	Ave.	9,225	10	86.9	0	1	2.1	500	16.01	10.55	2.86
3 hrs.	1	8,800	15.4	77.4	0.8	3.2	3.2	500	16.84	13.71	1.112
	2	18,130	19.4	77.0	0	0.4	3.2	500	15.72	14.59	1.05
	Ave.	13,465	17.4	77.2	0.4	1.8	3.2	500	16.28	14.15	1.081
6 hrs.	1	12,510	14.2	79.0	0.2	0.6	5.0	500	15.18	11.49	1.342
	2	16,520	22.8	70.8	0	0.2	6.2	500	17.46	12.35	1.500
	Ave.	14,515	18.5	74.9	0.1	0.4	5.6	500	16.32	11.92	1.421
9 hrs.	1	12,280	20.4	73.4	0	0.6	5.6	500	13.38	13.50	0.910
	2	19,000	23.5	70.0	0	0	6.5	400	15.37	14.35	1.202
	Ave.	15,640	21.95	71.7	0	0.3	6.05	450	14.37	13.92	1.056

TABLE 3

EFFECTS OF CORTISONE ON THE CELLULAR ELEMENTS OF THE BLOOD, BLOOD ASCORBIC ACID AND ADRENAL ASCORBIC ACID

Interval	Runs	Leucocyte Determinations						Number of Cells Counted	Homo-globin c/100 ml	Blood Ascorbic Acid mg/100 ml	Adrenal Ascorbic Acid mg/g Sample
		Total Count	Neutro-phile %	Lympho-cyte %	Eosino-phile %	Baso-phile %	Mono-cyte %				
0 hrs.	1	8,300	13	85	0	1	1	500	16.80	9.40	4.33
	2	10,150	7	88.8	0	1	3.2	500	15.32	11.70	1.38
	Ave.	9,225	10	86.9	0	1	2.1	500	16.01	10.55	2.86
3 hrs.	1	13,410	48.2	42.6	0	0.2	9	500	13.48	9.00	0.72
	2	10,130	41.0	59.4	0	0	8.6	500	16.60	13.02	0.99
	Ave.	11,770	44.6	51.0	0	0.1	8.8	500	15.04	11.01	0.86
6 hrs.	1	7,820	48.2	43.6	0	0.2	8	500	12.24	6.76	0.91
	2	7,360	45.2	45.4	0	0.6	7.8	500	12.96	8.55	0.92
	Ave.	7,590	46.7	44.5	0	0.4	7.9	500	12.60	7.65	0.915
9 hrs.	1	8,040	35.6	57.6	0	0.8	6	500	13.96	15.25	0.78
	2	7,530	33	63.2	0.4	0	3.4	500	16.66	9.10	1.03
	Ave.	7,785	34.3	60.4	0.2	0.4	4.7	500	15.31	12.18	0.91

TABLE 4

EFFECTS OF ADRENALIN ON THE CELLULAR ELEMENTS OF THE BLOOD, BLOOD ASCORBIC ACID AND ADRENAL ASCORBIC ACID

Interval	Runs	Leucocyte Determinations						Number of Cells Counted	Homo-globin c/100 ml	Blood Ascorbic Acid mg/100 ml	Adrenal Ascorbic Acid mg/g Sample
		Total Count	Neutro-phile %	Lympho-cyte %	Eosino-phile %	Baso-phile %	Mono-cyte %				
0 hrs.	1	8,300	13	85	0	1	1	500	16.80	9.40	4.33
	2	10,150	7	88.8	0	1	3.2	500	15.32	11.70	1.379
	Ave.	9,225	10	86.9	0	1	2.1	500	16.01	10.55	2.86
3 hrs.	1	28,713	39.5	55.5	0	0	5.0	400	14.38	16.20	2.9
	2	27,960	41.0	50.4	0	0	8.6	500	15.10	15.97	1.170
	Ave.	28,337	40.25	52.95	0	0	6.80	450	16.24	16.09	1.68
6 hrs.	1	16,325	9.8	85	0	0	5.2	400	12.35	13.95	1.12
	2	17,340	45.2	46.4	0	0.6	7.8	500	11.94	17.31	0.100
	Ave.	16,833	27.0	65.7	0	0.3	7.0	450	12.50	15.63	0.61
9 hrs.	1	13,575	8.0	90.75	0	0	1.25	400	11.80	16.49	1.04
	2	17,480	33.0	63.2	0.4	0	3.4	500	10.54	22.00	1.370
	Ave.	15,528	20.5	76.975	0.2	0	2.325	450	11.17	19.25	1.21

CHAPTER IV

DISCUSSION

The results of the foregoing experiments show that the ACTH and adrenosteroids have a definite influence on the cellular elements of the blood and generally increase blood ascorbic acid while decreasing adrenal ascorbic acid. The evidence for these influences are supported by many related experiments that point out the effects which the ACTH and adrenosteroids have upon the hemogram, adrenal ascorbic acid and blood ascorbic acid. Reinhart et al. (1944), Germzell (1952), Bacchus et al. (1944), Dury (1950), Dougherty et al. (1944), Booker et al. (1950) and Soylemezoglu et al. (1951) are investigators whose experiments support these findings.

It was noted that when the experimental animals received injections of ACTH there was an increase in the blood ascorbic acid but a decrease in the adrenal ascorbic acid. This reciprocal relationship (Figure 2) appears to be of great significance.

Throughout this investigation, regardless of the supplement used, it was observed that as the adrenal ascorbic acid decreased, the blood ascorbic acid increased. Chambers (1947) reported that the ACTH and adrenosteroids increased plasma ascorbic acid. This fact is supported by Collins (1953) who states that DCA, ACTH and cortisone increase the amount of ascorbic acid present in the plasma. Chambers attributes this observation to a decrease in capillary permeability there-

by preventing the ascorbic acid from passing freely into the tissue.

The exact role which the adrenosteroids and ACTH play in the depletion of ascorbic acid from the adrenal gland is difficult to assign. It is believed that ACTH plays a role in the elaboration of the cortical hormones. Long (1947) believes that while the specific role of ACTH in the depletion of adrenal ascorbic acid is not understood, it has been generally accepted that the loss of ascorbic acid is associated with the release of the cortical steroids. Long postulated that these changes in the concentration of ascorbic acid in the adrenal cortex may be used as an index of adrenal cortical activity.

Data presented showing the effects DCA and cortisone have on adrenal ascorbic acid deposition may be observed in Figures 4 and 6. These figures reveal that DCA and cortisone injections resulted in a decrease in the adrenal ascorbic acid. A similar investigation which supports the findings of this research problem was performed by Sayer et al. (1945) who reported that if rats were bled, the adrenal ascorbic acid decreased and was accompanied by an increase in plasma ascorbic acid.

Sayer's observation that adrenal ascorbic acid decreased as a result of hemorrhages leads us to ask what mechanism are these results accomplished. Sayer (1945) believes the adrenal cortex is stimulated to increase its activity when the organism is subjected to stress of this type. The progressive change in ascorbic acid content of the adrenal gland which occurs following a non-fatal hemorrhage parallels in degree and in time those which occur following ACTH injections. Furthermore, removal of the pituitary leaves the ascorbic acid content of

the adrenal gland inert when the animal has suffered a non-fatal hemorrhage. This evidence leaves little doubt that it is the secretion of the anterior pituitary which stimulates the adrenal cortex to activity when the animal is exposed to stress.

The question now arises as to what part the adrenal medulla plays since adrenalin caused a reduction of adrenal ascorbic acid (Figure 8). Dury (1948) observed injections of adrenalin decreased adrenal ascorbic acid. Dury believes that the change in adrenal ascorbic acid would indicate that the adrenal cortex has been involved. However, since the role and relationship of adrenal ascorbic acid content to its endocrine function are not known, this may indicate a vitamin-humoral regulation of a physiological process. The mechanisms, whether humoral, vitamin or nervous, which during stress stimulates the anterior pituitary are unknown but it is believed that adrenalin plays a significant role in specimens under stress or animals injected with adrenalin.

Along with the influence on ascorbic acid deposition in the blood and adrenal gland, the adrenosteroids and ACTH caused significant changes in the cellular elements. This investigator noted that ACTH, DCA and adrenalin increased the total leucocyte count and the neutrophil and monocyte values (Figures 1 through 8). The numerical limits between blood cell fluctuations, in the normal, suggest the existence of regulatory processes. The mechanism may be concerned with: first, the production of the blood cells, and second, the disposition of these cells. The dynamic balance which is obtained at any particular time between these mechanisms determines the nature of the

cellular picture of the blood.

Dougherty and White (1944) present evidence which establishes the pituitary adrenotrophic hormone as a prime factory in the regulation of blood lymphocyte level. This regulatory influence is extended by the trophic action of this hormone on the adrenal cortex. The conclusion that the pituitary adrenotrophic hormone is concerned with blood lymphocyte level is based upon the findings of Dougherty and White (1944). These investigators observed that injections of the adrenotrophic hormone or the products of its stimulatory action, i. e., adrenal cortical substances (cortisone and DCA) into a variety of normal animal species produced absolute lymphopenia. Identical results are observed in the hypophysectomized rat which has been given the adrenotrophic hormone and in adrenalectomized mice which have been treated with the adrenal cortical extracts.

The effects of DCA on the cellular elements paralleled that of ACTH. While the total leucocyte count decreased, an increase in the neutrophil and monocyte values were observed (Figure 1, 2, 3, and 4). Although only a very slight decrease was noted in the lymphocytes, these findings are in agreement with Dougherty and White (1944). The reduction in lymphocytes, resulting from DCA injections, may be due to increased dilution of the blood cells rather than a decrease in the total number of lymphocytes, for it was reported by Collins (1953) that injections of DCA increases the plasma volume. He believes that this is accomplished by the effect DCA exhibits over renal function and by the transfer of water and electrolytes to the blood stream from tissues. Injections of DCA increase reabsorption of water by the kidney.

Cortisone exhibited the same general pattern as ACTH and DCA, the only exception being in the total leucocyte count. Prior to the injection of cortisone it was established that the normal total leucocyte count was 9,500 cells per cu. mm. When the cortisone was administered to the experimental animals, it was observed that at the end of the third hour the total leucocyte count rose approximately 2,200 cells per cu. mm., but at the end of the ninth hour the count was approximately 1900 cells per cu. mm. below normal. This observation is similar to the findings of Danford and Danford (1951). Although Danford and Danford did not suggest a mode of action for this occurrence when cortisone is administered, Long (1944) reports that Wells and Kendell noted that concurrently with this blood picture there is an involution of the thymus gland following the administration of cortisone. The latter workers feel that the thymus may play a role in the responses noted.

Data on the effects of adrenalin on the cellular elements and blood and adrenal ascorbic acid are presented in Table 4 and Figures 7 and 8. This table and these figures show that both the adrenal and blood ascorbic acid values as well as the cellular elements, with the exception of the lymphocytes, increased as a result adrenalin injections. Dury (1948) observed that injections of adrenalin actively influenced the leucocyte picture which was shown to be mediated via the adrenal-cortical mechanism. It was further observed by this investigator that adrenalin reduced the amount of adrenal ascorbic acid three hours after the final injection. Although there appears to be a relationship between the leucocyte picture and the decrease in adrenal ascorbic acid, Dury (1948) concludes that there is not a correlation be-

tween the lymphocyte and neutrophile values, and the decrease in adrenal cortex is responsible to an unknown extent in the regulation of circulation neutrophiles in the normal rat. Long and Fry (1945) state that at the present time, it is not possible to decide whether the effect of epinephrine is due to a direct stimulation of the cells of the anterior pituitary that secrete the adrenotrophic hormone or whether it is due to changes in the blood acting agents.

Dury (1948) believes that the changes in the cellular elements and the decrease in adrenal ascorbic acid content following adrenalin injections would indicate that the adrenal cortex has been involved. However, he feels that since the role and relationship of ascorbic acid content of the adrenal to its endocrine function is not known, this may indicate a vitamin-humoral regulation of a physiological process.

The relationship between the cellular elements as affected by the administration of the adrenosteroids and ACTH appears to be a reciprocal one because with an increase in the neutrophiles and monocyte values, as well as an increase in blood ascorbic acid, there is a tendency for the lymphocytes values and adrenal ascorbic acid values to decrease (Figures 2, 4, 6, and 8).

Hemoglobin concentration decreased, generally, throughout the experiment (Tables 1 through 4). The results of this portion of the experiment is supported by the observations of Dougherty et. al. (1944) who observed that ACTH caused a decrease in the red blood cell count along with a fall in hemoglobin concentration. These observations are explained by the generally accepted fact that the adrenosteroids do effect pituitary secretions.

CHAPTER V

SUMMARY AND CONCLUSIONS

This investigation has described the effects that ACTH, DCA, adrenalin and cortisone have upon the cellular elements of the blood, adrenal and blood ascorbic acid. The data show that the adrenosteroids and ACTH reduce the amount of adrenal ascorbic acid generally throughout the experimental periods. A reciprocal relationship was observed between blood ascorbic acid and adrenal ascorbic acid. As a result of the adrenosteroids and ACTH injections the data suggest that as adrenal ascorbic acid decreases blood ascorbic acid increased.

The data further show that in respect to the total leucocyte count the administration of all the adreno-steroids and ACTH increased the total number of leucocytes with the exception of cortisone. It was observed that adrenalin caused the greatest response in total leucocyte change.

The adrenosteroids and ACTH increased the neutrophile and monocyte values and caused the number of lymphocytes to decline. The degree of responses varied greatly between the supplements, adrenalin causing the greatest response and DCA the least. The basophiles and eosinophiles were not affected generally throughout the experimental period.

A relationship between the blood cellular elements and the adrenosteroids and ACTH was suggested by the data presented. This data

show that when either one of the adrenosteroids or ACTH is administered the lymphocyte values and adrenal ascorbic acid decrease, while the neutrophile and monocyte values increase with a rise in blood ascorbic acid. This data suggest that the changes in the cellular elements are paralleled by changes in ascorbic acid content of the blood and adrenal gland. It appears the controlling mechanism is a relationship between the pituitary-adrenal cortex and is effected by the injections of either one of the adrenosteroids or ACTH in the normal rat.

The adrenosteroids and ACTH decreased the hemoglobin concentration. The literature has attributed this occurrence to changes in the total red blood cell count, Dougherty et. al. (1944).

LITERATURE CITED

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Articles and Periodicals

Bacchus, Habeeb, Altszuler, Norman, and Heiffer, Melvin H. "Eosinophil Response to Stress in Ascorbic Acid Pretreated Mice," Endocrinology, 51: (1952 a), 1.

_____. "Decrease Excretion of Cortisone Metabolites Immediately Following Ascorbic Acid Treatment," Endocrinology, 51: (1952 b), 302.

Best, W., Samtet, Max, and Mark, Robert M. "Effects of Adrenocortical Stimulation on Eosinophiles of the Blood and Bone Marrow," Proc. Inst. Med. 18 (5): (1950), 120.

Booker, W. M., Dent, F. M., Hayes, R. L, Harris, W., and Green, S. "Comparison of Influence of ACTH, DCA, and Cortisone on Metabolism of Ascorbic Acid," Am. J. Physiol., 163: (1950), 700.

Booker, W. M., Hayes, R. L., Sewell, M. B., and Dent, F. M. "Experimental Studies on Ascorbic Acid Metabolism," Am. J. Physiol., 166: (1951), 374.

Chambers, R., and Zweifach, B. W. "Intercellular Cement and Capillary Permeability," Physiol. Revs., 81: (1947), 436.

Covian, M. R. "Variations de 'l Acide Ascorbique de la Surrenale Chez le Rat Hypophysoprive," Rend. Soc. Biol. 143: (1949), 1252.

Crafts, Roger. "The Effect of Endocrines on the Formed Elements of the Blood," Endocrinology, 29: (1941), 596.

Danford, H. G., and Danford, D. A. "Circulating Leukocytes in Sodium Chloride Deficient Rats and Their Response to Epinephrine, ACTH, Cortisone and Desoxycorticosterone Acetate," Am. J. Physiol., 166: (1951), 524.

Dougherty, Thomas F., and White, Abraham. "The Influence of Hormones on Lymphoid Tissue Structure and Function," Endocrinology, 35: (1944), 1.

Dury, Abraham. "The Correlation of the Circulating Polymorphonuclear Leucocytes (Neutrophiles) With the Adrenal Ascorbic Acid in the Rat," Endocrinology, 43: (1948), 336.

_____. "Changes in Circulating Eosinophiles and Adrenal Ascorbic Acid Concentration After Agents Altering Blood Sugar Levels and After Surgical Conditions," Am. J. Physiol., 163(1): (1950), 96.

Germzell, Carl A. "Decrease in the Formation of Adrenocorticotropin Following Administration of Epinephrine," Endocrinology, 50: (1952), 399.

Greep, Roy O., and Deane, Helen Wendler. "Cytochemical Evidence for the Cessation of Hormone Production in the Zona Glomerulosa of the Rats' Adrenal Cortex After Prolonged Treatment with Desoxycorticosterone Acetate," Endocrinology, 40: (1947), 417.

Ingbor, Sidney H., Otto, John F., and Koss, Edward H. "Effects of ACTH on Blood Glutathione and Sulfhydryl Content of Liver and Kidney in Rats," Proc. Soc. Exptl. Biol. and Med., 77(1): (1951), 20.

Li, C. W., Lu, C. C., Meng, C. C., and Shen, T. "Influences of l-Ascorbic Acid Upon the Erythropoieses in Albino Mice," Perking Nat. Hist. Bull., 18(2): (1949), 91.

Long, C. N., Long, H., and Fry, E. G. "Effects of Epinephrine on Adrenal Cholesterol and Ascorbic Acid," Proc. Soc. Exptl. Biol. and Med., 59: (1945), 67.

Reinhart, W. O., Aron, Henry, and Li, Cho Hoo. "Effects of Adrenotrophic Hormone on the Leucocyte Picture of Normal Rats and Dogs," Proc. Soc. Exptl. Biol. and Med., 57: (1944), 19.

Rosemberg, E., and Lewis, R. A. "Reduction in Eosinophil Level of Adrenalectomized Mice Following Injection of Adrenocorticotropin, 11-dehydro-17-hydroxy Corticosterone and Lipid Extracts of the Urine," J. Appl. Physiol., 44: (1950), 164.

Sayers, George, Sayers, Marion, Lewis, Helen, and Long, C. N. The Effects of Adrenotrophic Hormone on Ascorbic Acid and Cholesterol Content of the Adrenal," Endocrinology, 55: (1944), 238.

Sayers, George, Sayers, Marion A., and Tsan-Ying, C. N. "The Cholesterol and Ascorbic Acid Content of the Adrenal, Liver, Brain and Plasma Following Hemorrhage," Endocrinology, 37: (1945), 96.

Scott, J. "Differential Effect of Desoxycorticosterone and Socium Hormone on Eosinophil Count in the Mouse," Proc. Soc. Exptl. Biol. and Med., 75(2): (1950), 502.

Soylemezoglu, Burham, and Wells, J. A. "Comparison of Leukocyte Response to ACTH and Bacterial Pyrogen," Proc. Soc. Exptl. Biol. and Med., 77(1): (1951), 43.

Speirs, Robert S., and Meyer, Roland K. "The Effect of Stress, Adrenal and Adrenocorticotrophic Hormones on Circulating Eosinophils of Mice," Endocrinology, 45: (1949), 403.

Upton, Arthur C., and Coon, William. "Effects of Cortisone and Adrenocotricotropic Hormone on Wound Healing in Normal and Scorbutic Guinea Pigs," Proc. Soc. Exptl. Biol. and Med. 77(1): (1951), 153.

White, Abraham, and Dougherty, Thomas. "Influence of Pituitary Adrenotrophic Hormone on Lymphoid Tissue Structure to Serum Proteins," Proc. Soc. Exptl. Biol. and Med., 56: (1944), 26.

Zwemer, R. L., and Lyons, C. "Leucocyte Changes After Adrenal Removal," Am. J. Physiol., 86: (1928), 545.

Unpublished Material

Collins, L. C. "The Influence of the Adrenosteriods, DCA, Cortisone and ACTH on the Electrolytic Shift of Sodium and Potassium Upon the 24 Hour Urinary Excretion," Unpublished master's thesis, Department of Biology, Prairie View A. and M. College, 1953.