EFFECTS OF ACTH ON CHOLESTEROL AND ASCORBIC ACID LEVELS IN THE BLOOD AND ADRENAL GLAND OF THE CHICK

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Bachelor of Arts

Oklahoma College for Women

Chickasha, Oklahoma

1954

Submitted to the Faculty of the Graduate School of
the Oklahoma Agricultural and Mechanical College
in partial fulfillment of the requirements
for the degree of
MASTER OF SCIENCE
May, 1956

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ACKNOWLEDGMENT

The author wishes to express her sincere appreciation to Dr. W. Stanley Newcomer, of the Department of Physiology, under whose guidance and with whose help this work was conducted.

The author also wishes to express appreciation to the Department of Physiology for the use of its laboratories and materials and to the Oklahoma A & M Research Foundation for financial support in this research.

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INTRODUCTION AND LITERATURE REVIEW

In 1924 Evans (20) reported that the injection of an extract of pig anterior pituitary into rats was followed by adrenocortical hypertrophy, and in 1927, Smith (63) demonstrated that hypophysectomy induced atrophy of the adrenal cortex of rats in 15-30 days. The substance of anterior pituitary origin responsible for these results was termed the adrenocorticotrophic hormone (ACTH) and was later separated from the other anterior pituitary hormones by Collip et al. (11) in 1933. By 1943 ACTH had been prepared in biologically pure form independently by Li et al. (39) from sheep pituitaries, and by Sayers et al. (60) from hog pituitaries. Li and his coworkers also characterized its physiological and chemical properties. The product from each laboratory produced identical effects in intact and hypophysectomized animals, but had no effect in adrenalectomized animals. Since this time, ACTH has become available in chemically pure form, extracted primarily from hog and beef pituitaries, and its biological actions have been studied extensively in the common laboratory animals.

In general, injection of ACTH into mammals caused an increased secretion of adrenocortical hormones, whose actions were indicated by many physiological criteria, for example, changes in blood electrolyte concentrations, carbohydrate metabolism, and blood cell proportions, as well as an hypertrophy of the gland (21). ACTH injected into hypophysectomized rats caused an increase of 50-300 % in weight of the right adrenal over that of the left, which had been previously removed as a control

measure (11). The zona reticularis and zona fasciculata of the mammalian adrenal cortex seemed to be more dependent on the hypophysis than was the outermost zona glomerulosa; these two zones underwent atrophy following hypophysectomy, while the zona glomerulosa increased in thickness due to a rearrangement in the cells (26).

Deane and Bergner (14) suggested that the fasciculata is the zone which secretes steroid hormones, on the basis of cytological evidence. The Golgi apparatus in this zone became shrunken, and the lipid droplets were diminished after hypophysectomy. In severe stress, the mitochondria also became swollen (26). Radioactively labelled ACTH was found to be localized mainly in the zona reticularis, and produced rapid depletion of the cholesterol from this zone (71). This evidence suggested to some investigators that the reticularis is the zone in which the cortical hormones are stored (17).

In 1951 two factors in ACTH were separated chemically by Dixon et al. (16) from pig pituitary. They termed these components the adrenal weight-increasing factor and the ascorbic acid-reducing factor, which may or may not also be the cholesterol-reducing factor. The effects of this first factor, that controlling adrenal weight, have been mentioned above. The second factor is concerned with the biochemical action of ACTH with respect to adrenal cortical ascorbic acid depletion. This action, as well as the effect on adrenal cortical cholesterol appear to be a direct effect of ACTH on the adrenal cortex, and not one due to increased secretion of the cortical hormones.

Ascorbic acid was first isolated as a crystalline compound from the adrenal gland by Szent-Gyorgi (29). It appears in both the adrenal cortex and medulla, but the amount in the cortex is greater. The quantity in the adrenals has been shown to vary markedly in different species; for example,

75 mg. % in the dog (29), 400 mg. % in the guinea pig, 133 mg. % in the human (1), 210 mg. % in the pig, 216 mg. % in the rabbit, 120 mg. % in the cat (18), and 400 mg. % in the rat. For most species the amount in the adrenal of the female is significantly greater than that in the male (29).

The content of ascorbic acid in the adrenal cortex has been shown to depend upon various factors (29). Its concentration was shown to be greatest in the fetus, showed a steady decline beginning at birth, and continued to decrease with age. Season and temperature were also factors in determining the amount of ascorbic acid present in the gland. Deggeller (15) observed a January-March minimum and a June-October maximum in humans; the same has also been observed in other mammals (29). Fatigue as well as a number of other stresses decreased the amount of ascorbic acid in the adrenal cortex (29).

One effect of ACTH on the adrenal cortex of the mammal was to deplete the gland of its ascorbic acid. Depletion in this respect refers to a reduction in concentration rather than a complete elimination of the substance from the gland. Bahn and Glick (3) reported that stress caused a decrease in the ascorbic acid level in the zona fasciculata and reticularis but not glomerulosa or medulla. This loss of ascorbic acid has been confirmed by means of $AgNO_3$ staining techniques following the administration of ACTH (17). It has been pointed out that the concentration of adrenal ascorbic acid was inversely proportional to the amount of ACTH released by the anterior pituitary (53). This ascorbic acid-depleting action of ACTH has been used in the evaluation of crude anterior pituitary extracts. In experiments by Sayers, Sayers, and Woodbury (59) on hypophysectomized rats weighing 120-160 grams, the depletion was expressed as the difference between the concentration of ascorbic acid in the left adrenal, removed immediately before injection, and that in the right adrenal one

hour after the intravenous administration of 48 µg. of pure ACTH per 100 gm. body weight. A rectilinear relation was found to exist between the depletion and the logarithm of the dose.

In the rat the fall in ascorbic acid concentration in the adrenal to 40 % of control levels began 10-15 minutes after intravenous injection of ACTH, and 1 hour after subcutaneous injection. The level usually remained low for 2 to 3 hours; the initial level was regained in 9 to 10 hours (57), and after 24 hours the level was slightly higher than that of controls (56). In the guinea pig, the adrenal ascorbic acid level fell to 50 % of normal in 3 hours, and rose to normal again in 24 hours (57).

In 1954 Hamburger (27) demonstrated a result which was opposite to that obtained by Sayers. He injected 493 hypophysectomized rats with small doses of ACTH (1/40,000-1/25,000 unit per 100 gm. of body weight) and showed that these doses produced increased amounts of ascorbic acid in the adrenal gland.

A depletion of ascorbic acid following ACTH injection has been observed in two human patients (31). Patients who received ACTH therapy for rheumatoid arthritis suffered from a deficiency of vitamin C, and exhibited scurvy symptoms. This deficiency was remedied by the administration of vitamin C.

Not only did ACTH affect adrenal cortical ascorbic acid, but also it has been reported to affect blood levels of ascorbic acid. Plasma ascorbic acid of male rats given 12 mg. ACTH subcutaneously showed a slight increase (62). Stress (HCHO, 0.5 cc/100 gm. body weight) applied to rats increased blood ascorbic acid 102-106 % (69). Allison (2) also reported a 24 % increase in blood ascorbic acid of rats stressed with Nembutal and electrical stimulation of the cervical sympathetic trunk. Urinary ascorbic acid of the rat has also been shown to increase (6).

The above-reported adrenal cortical depletion of ascorbic acid in the rat, guinea pig, and human being has not been substantiated in other species. Elton and Zarrow (18) found no depletion in the pig, rabbit, cat, and dog following ACTH injection. A depletion in adrenal ascorbic acid failed to occur in those species that showed a normal concentration of 150-250 mg. %; species with a concentration of 400 mg. % or more showed a significant depletion.

The function of ascorbic acid in the adrenal gland has not been clearly elucidated, but it has been suggested that the correct functioning of the cortical cells demands a certain optimal level of the vitamin (23). It has been proposed by Harris and Ray (28) that the adrenals serve in the elaboration of vitamin C or in its utilization, or in maintaining adrenaline-like substances in a reduced condition. Cortical hormones may in some way influence the metabolism of ascorbic acid in the tissues and perhaps reduce the requirement of the organism for the vitamin (33).

Many other suggested explanations have been offered for the function of ascorbic acid in the mammalian adrenal gland. Lowenstein and Zwemer (41) suggested that the vitamin was incorporated directly into the cortical hormones. They have isolated an active compound from aqueous extracts of adrenal glands which was found to be a ketonic steroid. From this substance, ascorbic acid was isolated under conditions of mild hydrolysis in the absence of air. Vogt (70) has been unable to confirm this finding.

Even though ascorbic acid may not be incorporated into the cortical hormones, there seems to be a synergistic action between ACTH and ascorbic acid on adrenal weight (68). In hypophysectomized rats treated with ascorbic acid, the adrenal ascorbic acid proved to be decreased less than that of normal controls, when both groups of animals were subjected to stress (35). Other experiments showed that in advanced

scurvy in the guinea pig, there was a cortical insufficiency (65). Cortisone acetate suppressed the manifestations of vitamin C deficiency in these animals (32). There is some evidence that large doses of ascorbic acid improved the utilization of cortical hormones and prolonged their action by delaying their breakdown and excretion (19).

Another explanation of the function of ascorbic acid involved its necessity for liver and muscle glycogen production (45). The muscles of guinea pigs deprived of vitamin C contained more lactic acid than normal, and fatigued more readily. However, the administration of ascorbic acid to these animals caused an increase in liver and muscle glycogen (48). Ascorbic acid also increased the creatine phosphate content of guinea pig muscles (22).

Goldzieher et al. (24) suggested that glutathione and ascorbic acid form an oxidation-reduction system in the adrenal cortex, which is necessary for a group of enzyme systems which catalyze the formation of the cortical hormones. These enzymes of the adrenal are dependent on the SH groups of glutathione for their activity (13) and vitamin C possibly can protect the SH groups from oxidation. The fact that SH groups are necessary for the enzyme systems has been proven by the addition of several sulfhydryl binding agents to the systems; these agents markedly inhibited the rate of corticosteroid production (30). It is also possible that glutathione acts to keep ascorbic acid in the reduced state and that the effectiveness of the former is through ascorbic acid (8).

Another chemical substance in the adrenal whose metabolism is altered on administration of ACTH is cholesterol. Rogers and Williams (52) reported that the excretion of adrenal steroids was high during stress and the cholesterol content of the adrenal was low. It has been suggested that the adrenals are a source of cholesterol and that this gland controls the cholesterol content of the blood (9).

Sayers, Sayers, White, and Long (58) produced a distinct lowering of adrenal cholesterol levels 3 hours after the injection of 2 mg. ACTH into 24-day-old male rats. In another experiment, immature rats were injected with pure ACTH in aqueous solution, prepared from hog or beef pituitaries. A single dose diminished adrenal cholesterol from 1/2 to 2/3 of its normal level in 6-9 hours (55). All these workers reported that the levels returned to normal or above after 24 hours. In guinea pigs the adrenal cholesterol level fell to 80 % of control levels in 3 hours and to 70 % in 12-18 hours following ACTH injection. The level returned to 90 % of that of the controls in 24 hours (57).

According to Marino (43) cholesterol of the adrenals and blood occur in approximately constant proportion, that is, an increase in blood cholesterol is accompanied by an increase in the cholesterol of the adrenals. Increased production of adrenal steroids should result in a withdrawal of cholesterol from the plasma for this purpose. Pfeiffer (47) believes that destroyed erythrocytes are the source of cholesterol used for synthesizing the adrenal hormones. In one experiment, male and female rabbits injected with anterior pituitary extract produced hypercholesterolemia following both single and repeated doses (10). In another case, after one subcutaneous injection of anterior pituitary extract daily for 3 to 4 days, the total cholesterol and free cholesterol of the blood of adult male rabbits decreased 19 to 34 %. The cholesterol ester, however, increased 8 % (37). Mann and White (42) applied stress and injected ACTH into different dogs and demonstrated a decrease in esterified cholesterol of blood plasma. Mason et al. (44) demonstrated a similiar decrease in plasma cholesterol after administration of 25 to 100 mg. ACTH daily for 36 days.

It is possible that the variations in the cholesterol content of

the adrenal and blood reflect changes in the concentration of steroid hormones (58). Levin (38) has interpreted the loss in cholesterol from the adrenal as being due to an increased rate of conversion to the active cortical hormones. Evidence proposed by Sayers et al. (57) showed glycogen deposition in the guinea pig to be greatest at the time when the cholesterol content of the gland was at a minimum. Following the return of cholesterol to normal levels in the adrenal gland, glycogen returned to its normal level. This evidence suggests that the depletion of cholesterol in the adrenal is associated with an increased secretion of those steroids of the corticosterone type that are known to increase liver glycogen. Long (40) stated that the fall in cholesterol was roughly proportional to the quantity of ACTH administered. Since it was the ester cholesterol that was depleted, this is probably the fraction that is the precursor (12).

In order to study the conversion of cholesterol to the cortical hormones, several perfusion experiments have been performed. Beef adrenals perfused with radioactive cholesterol and acetate released radioactive 17-hydroxy-corticosterone and corticosterone into the perfusate (72). Several pathways have been suggested for this conversion. One mechanism of the synthesis of corticosteroids has been postulated in which progesterone is a key intermediate. As proof of this hypothesis, deuterio-cholesterol was taken by a woman in her 8th month of pregnancy. Recovery of the isotope showed clearly that pregnanediol, a product of progesterone metabolism had been formed from cholesterol (7). It has now been established that cholesterol transformation to progesterone proceeds exclusively via pregnenolone (66). In other experiments, progesterone has been shown to be converted into cortical hormones through a series of 17-desoxy and 17-hydroxy steroids. Seventeen-hydroxy steroids

were hydroxylated stepwise first at the C_{21} and then at the C_{11} position to form corticosterone and hydrocortisone respectively (30). A dual pathway may have been involved, or at some point in the synthesis an unknown intermediate may have been formed.

In contrast to the many studies concerning the effects of ACTH in the mammal, little work has been done on the effects of ACTH on the chick adrenal. Although structural differences between the bird and mammalian adrenal exist, the bird adrenal has been shown to hypertrophy after the injection of ACTH. In the bird adrenal there is no true cortex as in the mammal, but chromaffin tissue is intermingled with interrenal, or cortical tissue. This interrenal tissue is not zonated as in the mammal (67). The interrenal cells are arranged in cords and strands, and gather around the periphery in masses resembling a mammalian glomerular zone (29). The cytoplasm of the cells contains mitochondria (more numerous in young cells) and lipid droplets. The interrenal cells are thought to originate in the periphery and migrate inward where they die (29). Interrenal tissue of male chickens comprises 40 % of the adrenal, and in the female 71 % (67).

Bates, Riddle, and Miller (5) found that the adrenal weight of 2-day-old chicks increased in proportion to the dosage of an anterior pituitary extract. However, so-called "alarm" agents such as cold and fatigue failed to stimulate the adrenal, and it was suggested that the hypophysis of the chick has little power to cause the adrenals to enlarge. Jailor and Boas (34) injected chicks at various ages with 25 mg. ACTH per bird and showed that although the adrenals hypertrophied, the ascorbic acid content was not altered in 1-4 hours, in comparison with controls. Only in one experiment was there a significant fall in ascorbic acid, which averaged 17 %. These workers suggested that the

release of corticosteroids from the chick adrenal is not dependent upon a fall in adrenal ascorbic acid.

Baldini and Zarrow (4) applied stress of cold for 1-3 hours and injected 4 mg. ACTH into separate groups of bobwhite quails, and reported the following results on adrenal ascorbic acid determinations:

Treatment	Controls, mg. % Ascorbic Acid	Experimentals mg. % Ascorbic Acid
Cold, 1 hour	148	158
2 hours		160
3 hours		174
ACTH, 1 hour	164	158

In later experiments the same workers produced hypertrophy of the adrenals by prolonged treatment (7 and 14 days) with cold stress (2°-4°C) and with injections of 4 and 10 mg. ACTH, adrenaline, and stilbesterol (73).

Zarrow and Zarrow (74) used cold stress (3°-5°C) and epinephrine in experiments with 18 to 23-day-old ducks, and also obtained no depletion of adrenal ascorbic acid.

Cold, 3°-5°C	mg. % Adrenal Ascorbic Acid
1 hour	123
2 hours	115
3 hours	138
Controls	125

Other evidence that the chick may not behave like the mammal lies in an experiment done by Seneca et al. (61) on adrenal slices from cats, dogs, rats, guinea pigs, and chickens incubated in flasks containing vitamin C. Cortisone was found in all flasks but that containing the chicken adrenal. It is possible that although ascorbic acid is necessary for the formation of the cortical hormones in mammals, it has no such function in the bird.

Elton and Zarrow (18) found no significant depletion in the cholesterol or ascorbic acid content of the adrenals of 10-day-old chicks injected with 20 mg. ACTH, after 1-4 hours.

From the above review, it is evident that there is a lack of agreement on the effect of ACTH injection on adrenal cholesterol and ascorbic acid in the bird, and no information on the blood levels of these two substances under the same experimental conditions. The following experiments were designed to obtain information concerning the effects of ACTH on ascorbic acid and cholesterol levels in the blood and adrenal gland of the chick.

METHOD

For the entire series of experiments, White Leghorn cockerels and pullets were obtained from the Stillwater Hatchery on the day of hatching, and were maintained on Purina chick starter in brooder batteries at 95°F for 4-10 days until autopsied. At this time the chicks weighed 50-70 gm. The food was removed about 12 hours before autopsy of the chicks, irrespective of the time of injection of ACTH.

Groups of chicks were injected 3, 6, 12, or 24 hours before autopsy, with 1 mg. of Armour ACTH in 0.1 ml. of solution. Normal control birds were treated under similiar donditions except for the ACTH injection.

At the time of autopsy, each chick was lightly anesthetized with ether, weighed, and a heparinized blood sample removed directly from the heart. The chick was then killed with ether and the adrenals were removed, weighed immediately on a torsion balance, and macerated in an appropriate solution. The blood and adrenals were then analyzed for either ascorbic acid or cholesterol. The blood and adrenal filtrates for the ascorbic acid determinations were made shortly after removal of the tissue from the bird and frozen to prevent destruction of the vitamin.

In determining blood and adrenal ascorbic acid levels, the method of Roe and Kuether (51) was used. The two adrenals from each chick were placed in a centrifuge tube containing sand and 7.5 ml. of 4 % trichloroacetic acid. They were then macerated with a stirring rod and 0.3 gm. of acid-washed Norit added. After stirring, the mixture was filtered. To 2 ml. of blood from each chick, 6 ml. of 6 % trichloroacetic acid

were added dropwise to precipitate the protein. After stirring with 0.3 gm Norit, this solution was filtered to obtain the clear supernatant liquid.

A 5 ml. aliquot of the adrenal and blood filtrates was used in determining total ascorbic acid. To each 5 ml. aliquot were added 1 drop of 5 % thiourea solution and 1 ml. of 2 % 2-4 dinitrophenylhydrazine reagent. This solution was incubated at 37°C in a water bath for 3 hours, after which the tubes were placed in ice water and 5 ml. of 85 % sulfuric acid were added. After warming to room temperature, the transmittance of the orange colored solution was read in a Bausch and Lomb spectrophotometer at 540 millimicrons, and the ascorbic acid content read from a previously prepared calibration chart.

If the 3 substances, ascorbic acid, 2-4 dinitrophenylhydrazine, and sulfuric acid are added in any sequence other than as stated above, the orange color is not obtained (50). The reaction producing the color is due to the action of sulfuric acid on the coupled 2-4 dinitrophenylhydrazine-dehydro ascorbic acid compound. It has been suggested that the mechanism is a dehydration. Interfering substances are usually not present in great enough quantity in the adrenals and blood to cause any interference. This method appears to be completely specific, however, the color may be changed by charred sugars or other organic matter if the sample tubes are not kept in ice water on addition of the sulfuric acid. Such compounds as pyruvic acid and acetoacetic acid readily couple with 2-4 dinitrophenylhydrazine, but their derivatives do not react with sulfuric acid, and sulfuric acid solutions of the derivatives do not absorb light in the 540 millimicron region of the spectrum (51). The orange color is stable and shows no change in 40 minutes.

Norit clarifies the solution and oxidizes ascorbic acid to dehydro

ascorbic acid. Ascorbic acid is not oxidized quantitatively by Norit unless the solution contains a reagent like acetic acid or trichloroacetic acid. Apparently the acetic acid is preferentially adsorbed on the Norit and active oxygen is eluted in quantities sufficient for rapid oxidation of the ascorbic acid. The Norit is washed with HCl prior to use to prevent interference by the ferric ion (51).

Blood cholesterol was determined by the method of Zlatkis, Zak, and Boyle (75), and adrenal cholesterol by the method of Knobil, Hagney, Wilder, and Briggs (36). The adrenals were macerated with sand in 5 ml. of glacial acetic acid and the solution filtered. The blood from the same chick was centrifuged, the plasma removed and diluted 10 times with distilled water. A 0.5 ml. aliquot of the adrenal filtrate and a 0.2 ml. aliquot of the diluted plasma respectively were used for each determination. These aliquots were diluted to 3.5 ml. with glacial acetic acid and 2.5 ml. cholesterol color reagent (1 ml. of a 10 % $FeCl_3$ solution, made up to 100 ml. with concentrated sulfuric acid) were added as the tube was rotated in a circular motion. Full color development required approximately 1 minute. The transmittance of this solution was read at 525 millimicrons in a Cenco photelometer and the amount in the sample read from a previously standardized curve.

The effect of proteins in the plasma is negligible. Bilirubin at 0.1-0.8 mg. % also has virtually no effect. The only impurity which might interfere is glyoxylic acid in the glacial acetic acid.

The data obtained were analyzed statistically by the Student "t" test (64). Results were considered significant if they exhibited a probability of 5 % or less.

RESULTS

The results of adrenal and blood cholesterol and ascorbic acid determinations 3, 6, 12, and 24 hours after the injection of 1 mg. ACTH are presented in Tables I-VIII. This treatment resulted in a statistically significant decrease of 19 % in the adrenal cholesterol of cockerels and of 14 % in pullets 6 hours after the injection, as well as a decrease of 16.7 % in 4-day-old cockerels 12 hours after injection. Eight-day-old cockerels also autopsied 12 hours after injection showed no such decrease. (Tables I and II)

Blood cholesterol levels in the same groups were not significantly changed by the ACTH treatment, except for an increase of 10 %, significant at the 5 % level, in the cockerels autopsied 24 hours after injection. Pullets from the comparable 24-hour group showed an 11 % increase which was not significant. (Tables III and IV)

The adrenal ascorbic acid of the cockerels was significantly decreased 16.5 % 12 hours after ACTH injection. (Table V) There were no other changes in adrenal ascorbic acid in either the cockerel or pullet groups, except for a 13 % increase in the ascorbic acid content of the pullets autopsied 3 hours after injection. (Table VI) No significant changes in blood ascorbic acid were obtained at any time for either sex. (Tables VII and VIII)

The above results are illustrated graphically in Figures 1-4. In Figure 1, data for the 8-day-old birds rather than that for 4-day-old birds were used for the 12 hour point.

TABLE I. MALE ADRENAL CHOLESTEROL

Time	3 hour	Control	6 hour	Control	12 hour		Control		24 hour	Control
					(a)	(b)	(a)	(b)		
Mean ± S. D. gm./100 gm.	3.37 ±.53	3.58 ±.54	2.65 ±.45	3.28 ±.48	2.83 ±.39	3.71 ±.53	3.41 ±.78	3.80 ±.58	3.50 ±.84	3.92 ±.49
Number Birds	14	15	13	15	25	12	17	14	14	15
Age at Autopsy (Days)	7	7	7	7	4	8	4	8	7	7
t value and Probability	1.04	>10 %	3.57	< 1 %	3.13	.41	<1 %	>10 %	.785	>10 %
Experimental Mean / Control Mean X 100	94		81		83.3	98			89	

TABLE II. FEMALE ADRENAL CHOLESTEROL

Time	3 hour	Control	6 hour	Control	12 hour	Control	24 hour	Control
Mean ± S. D. gm./100 gm.	3.45 ±.49	3.31 ±.50	3.13 ±.27	3.65 ±.64	3.91 ±.64	3.57 ±.56	3.57 ±.71	3.57 ±.56
Number Birds	14	14	15	13	20	19	22	19
Age at Autopsy (Days)	5	5	7	7	8	9	7	8
t value and Probability	.736	>10 %	2.7	< 2 %	1.77	< 10 %	0	> 10 %
Experimental Mean / Control Mean X 100	104		86		109		100	

TABLE III. MALE BLOOD CHOLESTEROL

Time	3 hour	Control	6 hour	Control	12 hour		Control		24 hour	Control
					(a)	(b)	(a)	(b)		
Mean ± S. D. mg./100 ml.	167.6 ±29.3	184.8 ±21.6	144.1 ±56.2	167.3 ±33.9	299.3 ±86.7	192.9 ±29.4	351.9 ±129.0	190.7 ±19.5	215.0 ±47.7	184.8 ±21.6
Number Birds	14	15	13	15	23	12	11	14	16	15
Age at Autopsy (Days)	7	7	7	7	4	8	4	8	8	7
t value and Probability	1.82	< 10 %	1.43	> 10 %	1.55	.237	> 10 %	> 10 %	2.27	< 5 %
Experimental Mean / Control Mean X 100	91		87		85	101			110	

TABLE IV. FEMALE BLOOD CHOLESTEROL

Time	3 hour	Control	6 hour	Control	12 hour	Control	24 hour	Control
Mean ± S. D. mg./100 ml.	191.2 ±34.9	212.6 ±66.6	184.0 ±51.4	187.0 ±49.1	187.9 ±42.7	173.4 ±43.1	192.0 ±52.0	173.4 ±43.1
Number Birds	14	15	15	13	20	19	22	19
Age at Autopsy (Days)	5	5	7	7	8	9	7	8
t value and Probability	1.09	> 10 %	.263	> 10 %	1.07	> 10 %	1.25	> 10 %
Experimental Mean / Control Mean X 100	90		99		108		111	

TABLE V. MALE ADRENAL ASCORBIC ACID

Time	3 hour	Control	6 hour	Control	12 hour	Control	24 hour	Control
Mean ± S. D. mg./100 gm.	126.1 ±15.6	127.7 ±16.5	151.7 ±24.0	151.8 ±22.0	126.8 ±16.0	151.8 ±22.0	153.5 ±20.5	151.8 ±22.0
Number Birds	14	13	15	16	15	16	16	16
Age at Autopsy (Days)	7	7	7	7	8	7	8	7
t value and Probability	.251	>10 %	.011	>10 %	3.45	<1 %	.22	>10 %
$\frac{\text{Experimental Mean}}{\text{Control Mean}}$ X 100	99.5		100		83.5		101	

TABLE VI. FEMALE ADRENAL ASCORBIC ACID

Time	3 hour	Control	6 hour	Control	12 hour	Control	24 hour	Control
Mean ± S. D. mg./100 gm.	143.9 ±17.0	126.7 ±22.4	175.5 ±27.5	164.2 ±23.8	109.3 ±22.3	107.3 ±17.8	167.4 ±21.2	164.2 ±23.8
Number Birds	13	11	16	16	20	22	14	16
Age at Autopsy (Days)	7	7	7	7	9	10	8	7
t value and Probability	2.13	<5 %	1.24	>10 %	.327	>10 %	.385	>10 %
$\frac{\text{Experimental Mean}}{\text{Control Mean}}$ X 100	113		107		102		102	

TABLE VII. MALE BLOOD ASCORBIC ACID

Time	3 hour	Control	6 hour	Control	12 hour	Control	24 hour	Control
Mean ± S. D. mg./100 ml.	.762 ±.22	.797 ±.19	1.23 ±.24	1.31 ±.22	1.21 ±.25	1.31 ±.22	1.28 ±.22	1.31 ±.22
Number Birds	13	13	14	16	15	16	16	16
Age at Autopsy (Days)	7	7	7	7	8	7	8	7
t value and Probability	.433	>10 %	.958	>10 %	1.19	>10 %	.388	>10 %
$\frac{\text{Experimental Mean}}{\text{Control Mean}} \times 100$	91		94		92.5		98	

TABLE VIII. FEMALE BLOOD ASCORBIC ACID

Time	3 hour	Control	6 hour	Control	12 hour	Control	24 hour	Control
Mean ± S. D. mg./100 ml.	.775 ±.19	.766 ±.19	1.36 ±.28	1.31 ±.18	.854 ±.16	.838 ±.18	1.40 ±.22	1.31 ±.18
Number Birds	13	11	14	15	19	22	14	15
Age at Autopsy (Days)	7	7	8	7	9	10	8	7
t value and Probability	.0115	>10 %	.574	>10 %	.30	>10 %	1.21	>10 %
$\frac{\text{Experimental Mean}}{\text{Control Mean}} \times 100$	101		104		102		107	

Figures 1 and 2 show the similarity of the action of ACTH on adrenal cholesterol in the cockerel and pullet. Both curves show the greatest decrease at the 6 hour point, then a rise at 12 hours, and somewhat of a drop at 24 hours. This last drop is not significant however. The curve showing the blood levels of cholesterol for both sexes rises from a small initial drop to approximately 10 % above the control level at 24 hours, although only the increase for the male is significant.

In contrast to the parallel changes in blood and adrenal cholesterol in males and females, Figures 3 and 4 show that only the male adrenal ascorbic acid level dropped at 12 hours, significant at the 1 % level. Female adrenal ascorbic acid levels rose at 3 hours, significant at the 5 % level, but fell to just above control levels again in 6-24 hours.

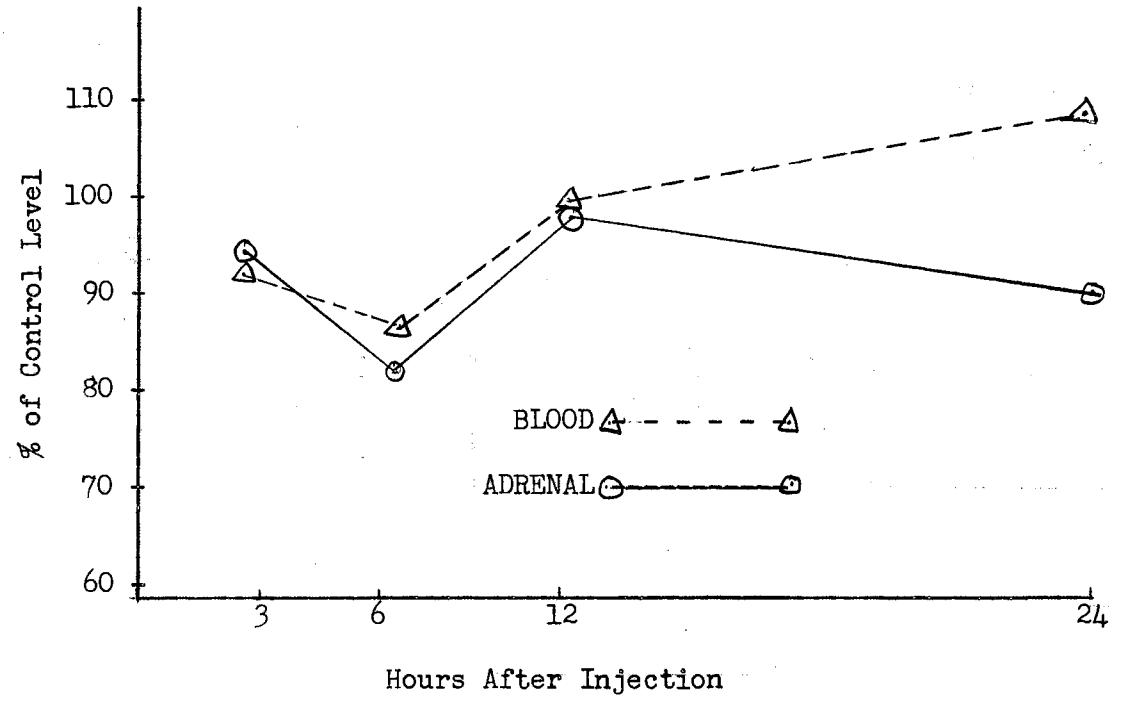

FIGURE 1. MALE BLOOD AND ADRENAL CHOLESTEROL

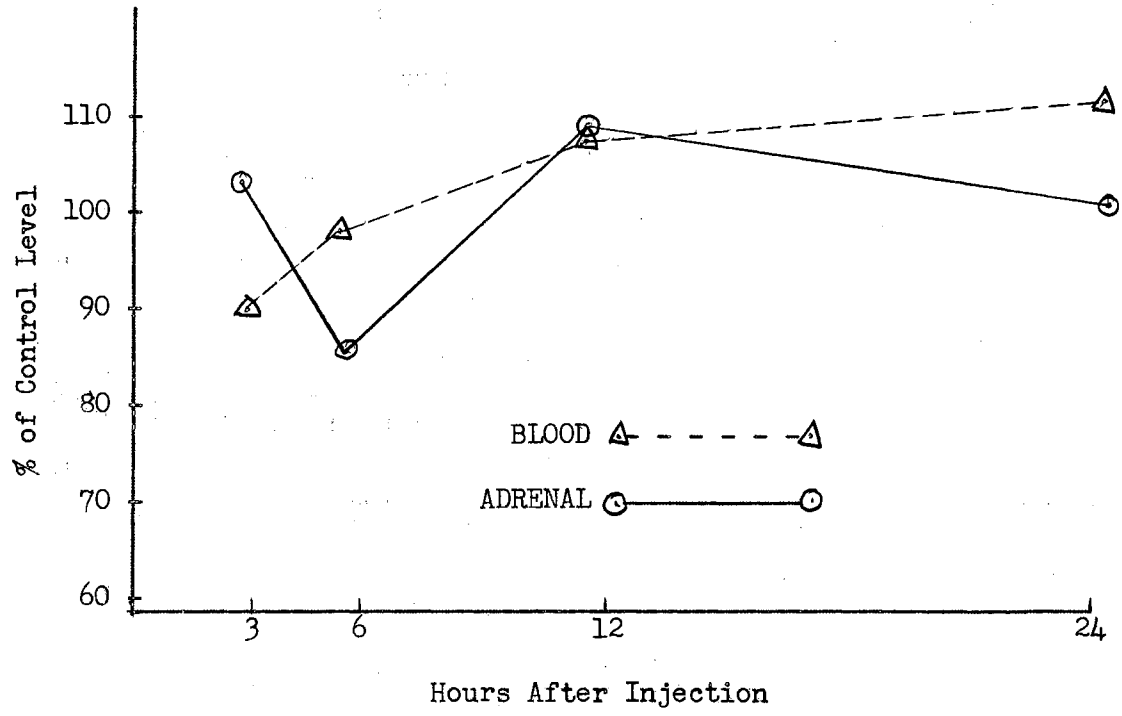

FIGURE 2. FEMALE BLOOD AND ADRENAL CHOLESTEROL

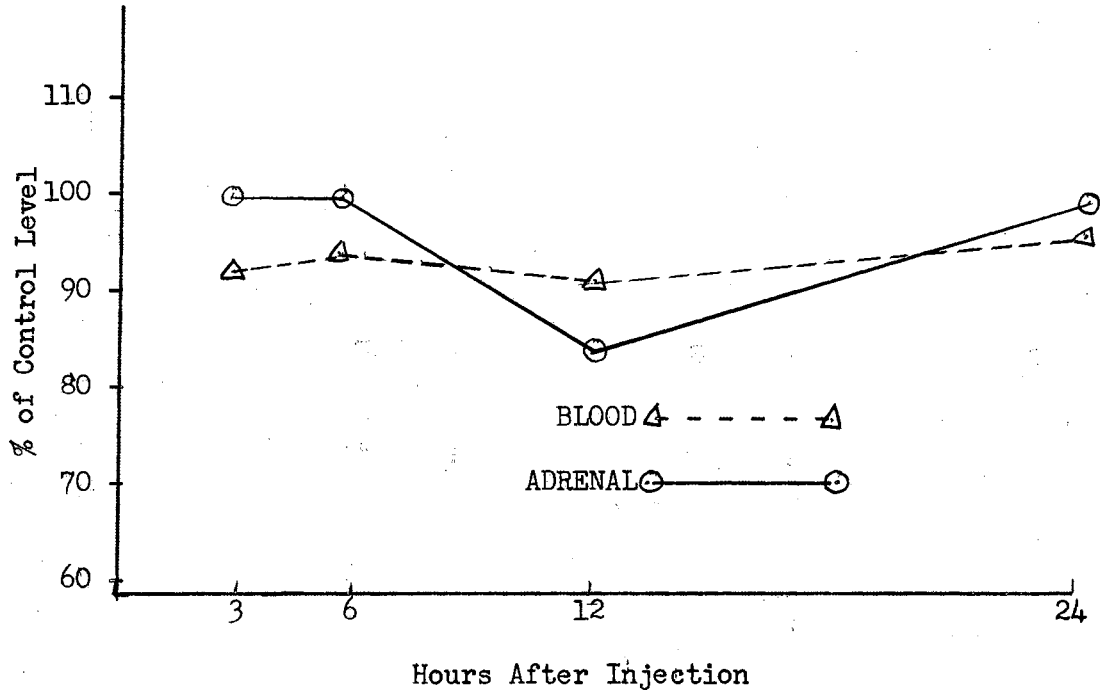

FIGURE 3. MALE BLOOD AND ADRENAL ASCORBIC ACID

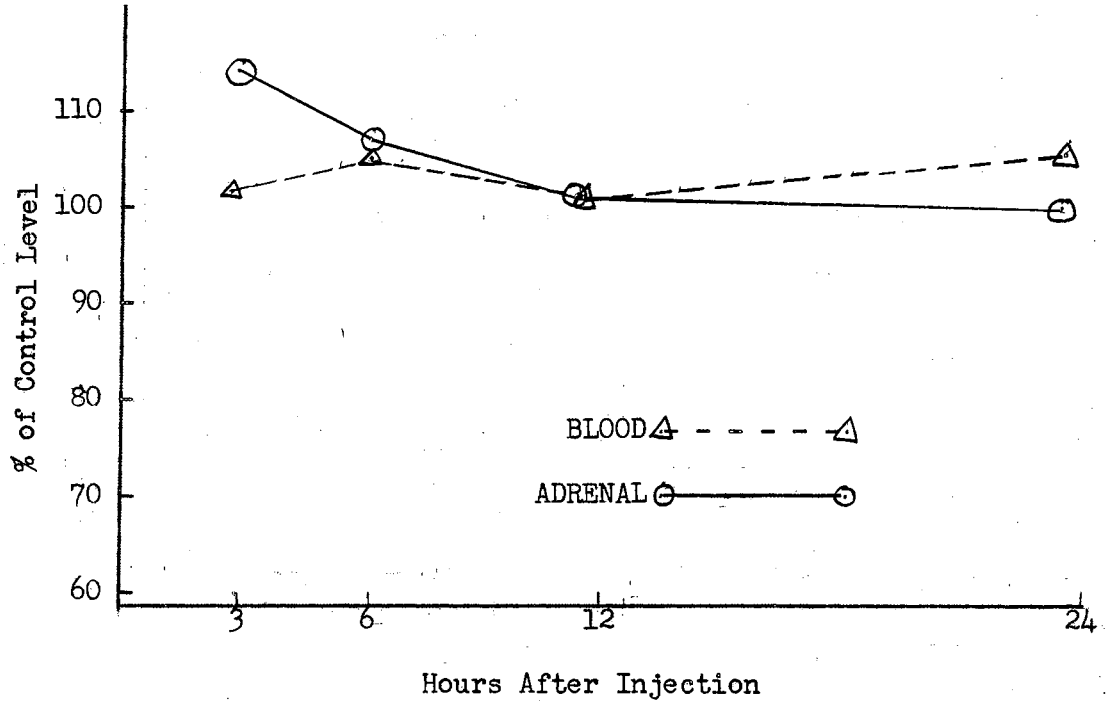

FIGURE 4. FEMALE BLOOD AND ADRENAL ASCORBIC ACID

DISCUSSION

Chicks autopsied 6 hours after injection with ACTH showed a drop in adrenal cholesterol, 19 % in the male and 14 % in the female. At the 12 hour period, 4-day-old birds showed a significant decrease of 16.7 % in adrenal cholesterol but 8-day-old birds autopsied at the same comparable time showed no similiar decrease. A possible reason for the decrease in adrenal cholesterol in the 4-day-old chicks, and not in the 8-day-old chicks is that the younger birds were more reactive to ACTH.

Adrenal ascorbic acid of the males was partially depleted at the 12 hour point; adrenals of the females showed no comparable decrease, but instead exhibited a rise of 13 % at the end of 3 hours following the injection of ACTH.

Both the depletions in ascorbic acid and cholesterol are contrary to the findings of other workers with the chick (34, 5, 18), duck (74), and quail (4). However, these workers autopsied their animals 1-4 hours after the injection of ACTH or application of stress; the changes in the present experiments occurred 6 hours after injection in the case of cholesterol and 12 hours after injection in the case of ascorbic acid.

These results, which differ from those of other workers, also differ somewhat from those known to occur in the mammal. In the rat and guinea pig the depletion of ascorbic acid appeared 1-3 hours after the injection of ACTH, and was to the extent of 40-50 % of control levels (54-57). In the same experiments, cholesterol was depleted 40-50 %; the greatest change came 3-6 hours after treatment. In the guinea pig, the drop of

30 % in adrenal cholesterol occurred 12-18 hours following ACTH treatment (57). It has been suggested that cholesterol is depleted from the adrenal as a result of its incorporation into adrenal cortical hormones, and that ascorbic acid is necessary for the formation of these hormones in the mammal (38, 24). The time lag between cholesterol depletion and ascorbic acid depletion in the chick indicates that ascorbic acid is perhaps not necessary to the release of corticosteroids as in the mammal.

This evidence suggests that the bird adrenal is not as reactive to mammalian ACTH in these respects as is that of the mammal. The difference does not lie in the dosage, for Sayers et al. (59) used 48 µg. per 100 gm. of body weight in rats and obtained the characteristic depletion in adrenal ascorbic acid. In other chick work, 4, 10, and 20 mg. ACTH have been used with no depletion being obtained. The smaller reductions in the adrenal concentrations of cholesterol and ascorbic acid, as well as the lag in depletion of these substances as compared with the situation in the rat may be due to a different type of cortical tissue possessed by the bird. The suggestion of Elton and Zarrow (18) that the adrenals of animals containing amounts of ascorbic acid less than 400 mg. % are not depleted of the substance on administration of ACTH, is not substantiated. It is possible that it takes longer for the ascorbic acid reduction to occur in these animals whose adrenals contain low levels of ascorbic acid, and since these workers autopsied 1-4 hours after the injection of ACTH, any reduction which might have occurred at 6-12 hours was not detected. Birds as well as other animals containing less adrenal ascorbic acid than the rat probably also lose less on stimulation with ACTH.

The blood cholesterol of the male and female chicks rose from an initial low point to about 10 % above the control levels in 24 hours.

This rise could indicate that adrenal cholesterol, when depleted from the adrenal cortex, contributes to the amount in the blood. However, this explanation would not be in line with the theory of the incorporation of cholesterol into the cortical hormones. The increase in blood cholesterol at 24 hours could also indicate that with elaboration of the cortical hormones by the gland, (blood and adrenal cholesterol being used for this) the formation of more cholesterol is stimulated in the liver or adrenal, and that this activity causes the rise in blood cholesterol. This increase in blood cholesterol is not specifically due to the cortical hormones, because they have been reported to cause a diminution of blood cholesterol (25). Since the decrease in blood cholesterol shown in Figure 1 follows the adrenal decrease, it is more likely that the depletion of cholesterol from the blood and adrenals is the stimulus for further cholesterol formation.

Blood changes might be expected in the ascorbic acid of the male chicks, since there was a change in adrenal ascorbic acid at 12 hours, and the rat has shown an increase in blood ascorbic acid under similiar circumstances (62, 69, 2). However, no such changes were found. This indicates that when ascorbic acid is depleted from the adrenal cortex, it does not cause a rise of ascorbic acid in the blood. Therefore, the ascorbic acid must be used up in some reaction in the adrenal. Another possibility is that the amount coming from the gland is too small to influence the concentration in the relatively large volume of blood.

In regard to ascorbic acid, there seems to be a difference between the male and female chicks in reaction to ACTH. No depletion in female ascorbic acid was obtained at any time and there was a rise in female blood ascorbic acid at the 3 hour point. These differences might be due to the different proportion of interrenal tissue of the sexes. The

interrenal tissue comprises 40 % of the adrenal weight in the male and 71 % in the female. It is possible that the sex hormones also play some part in the differences. A possible role of sex hormones in this respect could be tested by injecting ACTH into cockerels previously treated with female sex hormone and into pullets previously treated with male sex hormone.

The above experiment as well as others in which dosage or age could be varied could be performed to clarify this work, especially as to the role of ascorbic acid and its concentration in male and female blood and adrenal glands. Radioactive tracers would be useful in this type of work. It would be desirable to repeat experiments between 6 and 12 hours and analyze for ascorbic acid and cholesterol to determine the exact times when the changes occur.

A number of criteria or indices of adrenal cortical function have been used in the mammal (46, 2) but to date no satisfactory ones are available for the bird. The determinations made in this study of adrenal cholesterol depletions in both sexes 6 hours after the injection of ACTH, and ascorbic acid depletion in the male adrenal 12 hours after injection offer possibilities as criteria of adrenal cortical function in the bird. Further work must be done to show them to be repeatable and sensitive indicators.

CONCLUSIONS

One mg. of ACTH injected into 7 to 9-day-old White Leghorn cockerels and pullets resulted in a statistically significant decrease in adrenal cholesterol within 6 hours. A similiar dose of ACTH resulted in a statistically significant drop in adrenal ascorbic acid in 12 hours in the cockerels only. Slight rises in cockerel and pullet blood cholesterol were obtained 24 hours after ACTH injection, although the rise in the pullet level was not significant. No changes in male or female blood ascorbic acid occurred following ACTH treatment.

The evidence suggests that the chick adrenal is reactive to ACTH; however, this reaction is less in extent and requires a longer time to appear than that in the mammal. A possible sex difference is suggested in adrenal ascorbic acid levels following ACTH.

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