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SOME EFFECTS OF ACTH ON METABOLISM AND BLOOD CELLS IN THE CHICKEN

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SOME EFFECTS OF ACTH ON METABOLISM AND

BLOOD CELLS IN THE CHICKEN

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CHAPTER I

INTRODUCTION

Although a possible relationship between adrenal gland activity and certain aspects of metabolism in animals (pigeons) was reported by Meckel as early as 1820, subsequent reports failed to confirm this association (80) until Addison in 1855 demonstrated a definite relationship between adrenal gland deficiency and characteristic symptoms in man that usually terminated in death (106). The symptoms associated with adrenal cortical deficiency included muscular weakness, low blood pressure, impaired appetite and a general skin discoloration as well as a general apathy. Since Addison's description of the syndrome that now bears his name, a great amount of interest, as evidenced by the numerous physiological investigations, has been focused upon the responses of mammals to the hormones of the adrenal cortex with respect to their role in maintaining the life of mammals and to regulation of their secretion by adrenocorticotropic hormone (ACTH).

Mammals such as rats, guinea pigs, dogs and rabbits have been investigated much more extensively than have avian species, possibly because of their supposed closer relationships to humans and therefore easier extrapolation of findings that would be applicable in clinical experience. As a result, comparatively little is known about the role of this gland under normal conditions in birds. The translation of the interpretations of findings from mammalian investigations to avian species

is not wholly justified since even among the various species of mammals themselves incongruous results have been obtained; this lack of agreement has even been demonstrated between various tissues of the same animals.

The research reported herein is an attempt to determine some of the basic metabolic changes that occur in the bird due to increases in adrenal function as stimulated by exogenous ACTH at various time intervals prior to removal of tissues for analysis. Such information should be useful not only to augment the knowledge of the role of the adrenal cortex under normal conditions but also to supply information concerning the physiologic response of birds to stressors.

CHAPTER II

REVIEW OF LITERATURE

Carbohydrate Metabolism

The ability of the organism to metabolize carbohydrates is reflected by the blood level of glucose and its metabolites as well as the tissue concentrations of these compounds.

Blood Glucose - The observation that the pigeon adrenal gland was enlarged in some cases where hyperglycemia was noted, as well as the close anatomical association between the cortical and the medullary cells of this gland, has been taken to imply that the main function of the adrenal cortex was to regulate carbohydrate metabolism (80); later work in mammals, however, ruled out such a singular role for this part of the gland (18).

A reduction in blood sugar, probably due to a decreased supply from the liver which more than compensated for the decreased tissue uptake (98), was noted after adrenalectomy in cats and rats (18).

Adrenal cortical extracts (ACE) maintained the blood sugar of adrenalectomized mammals (17, 18) and birds (73, 97), and caused hyperglycemia in intact mammals. This increase in blood glucose was due to glucocorticoids (30, 95) although Brown (19) gave evidence that desoxycorticosterone acetate could inhibit the hypoglycemia that followed adrenalectomy.

Results similar to those found following ACE or glucocorticoid treatment in birds were obtained with adrenocorticotropic hormone (ACTH).

Howard and Constable (42) found a 25 percent increase in blood glucose following intra-muscular injections of 5 IU ACTH hourly for five hours.

In some conditions other hormones, in addition to ACTH and ACE, seem to be required to maintain or increase the blood sugar of mammals, for Constantides (23) found that ACTH failed to maintain blood sugar in hypophysectomized rats unless glucose infusions (by implication, a high dietary energy source) were given simultaneously. On the other hand, adrenal glucocorticoids alone increased the blood glucose level in dogs (98).

Blood Lactic Acid - There is little direct evidence indicating the effects of ACTH or adrenal steroids on the blood lactic acid levels in mammals aside from general changes that seem to parallel those of glucose (4, 77). The rapid removal of lactic acid by the livers of fasted normal rats was not altered by cortisone injections (41). Lactic acid levels were high in Cushing's Syndrome where a hyperfunction of the adrenal cortex occurred (83). Conversely, adrenalectomized rats were able to remove lactate from blood and convert it to glucose (111).

Liver and Muscle Glycogen - Carbohydrates are stored in high concentration in the liver (2 percent) and to a lesser extent in muscle (0.5 percent) and other tissues. The response of these tissues to ACTH, via the steroid hormones of the adrenal cortex, are quite similar although muscle glycogen responses seem to vary somewhat.

The loss of muscle glycogen following epinephrine injection was greater in adrenalectomized rats than in normal rats but there was no simultaneous increase in liver glycogen in the adrenalectomized rats (110). Balance studies have shown that adrenalectomy not only reduced the release of glucose from the liver to the plasma but also diminished the rate of

glucose uptake by the tissues (98). The overall effect of the liver and muscle glycogen changes may or may not result in a hypoglycemia.

Adrenal cortical extracts increased liver glycogen in mammals (17, 53) and in birds (31) as well as muscle glycogen in mammals (53). Golden and Long (31) did not find an increase in the muscle glycogen levels folfowing the injections of ACE in chicks, however.

The complexity of the regulation of muscle carbohydrate is indicated by the fact that cortisone did not completely inhibit the glycogen depletion in adrenalectomized rats following epinephrine injections (110) but cortisone, and to a lesser extent, DOCA inhibited the depletion of liver and muscle glycogen in hypophysectomized rats and chickens (19, 53). Information obtained from the measurement of responses in one muscle does not mean that another muscle in a different anatomical location will respond in the same way; differences in the response of various skeletal muscles to adrenal steroids have been reported (53). Rat uterine glycogen levels were found to increase more following DOCA injections than did those from uteri from rats receiving cortisone and cortisol (48), while cortisone and cortisol, but not DOCA, increased skeletal muscle glycogen.

Gratten and Jensen (36) demonstrated that only those adrenal hormones possessing a keto or hydroxy group at C^{11} are able to influence carbohydrate metabolism to any great extent. Reineke and Kendall (78) further defined the structural requirements for glucocorticoid activity by showing that the presence of a ketone group on C^{3} is also necessary for adrenal hormones to increase liver glycogen deposition. ACTH presumably exerts its influence on carbohydrates by way of its ability to stimulate the release of glucocorticoids from the adrenal cortex since ACTH injections have resulted in the same carbohydrate metabolic responses

as are seen in animals injected with cortisone. It is possible, however, that ACTH may have some direct influence on carbohydrate metabolism since the results of hypophysectomy were much more rapid than were those following adrenalectomy (10).

Influence of Glucocorticoids on the Enzymatic Mechanism of Carbohydrate Metabolism - Glucocorticoids, and ACTH via these hormones, increased liver and muscle carbohydrate stores by increasing their rate of formation while at the same time decreasing their utilization rate (109). ACTH injections, as well as glucocortiocoids, caused an increased protein catabolism as indicated by an increased nitrogen excretion (19, 20) and concommitant gluconeogenesis (83, 84). This increased protein catabolism might be one reason for the increased serum protein levels following injections of ACE or following stress in fowl (92, 104); however, it is generally believed that those amino acids that are destined for gluconeogenesis are transported as such rather than in peptide or protein forms.

The influence of ACTH and ACE on certain metabolic pathways and enzymatic activities helps to explain the increased storage and decreased utilization of carbohydrates although the use of different tissues as well as the age of the animals used, sex, season, etc. has resulted in some disagreement in the interpretation of the responses. Long (56) in one of the earliest experiments investigating the effects of adrenal hormones on glucose utilization reported a decreased rate of glucose oxidation in rats receiving adrenal cortical or anterior pituitary extracts. LaCroix and Leuson (51), on the other hand, found an increase in O_2 uptake in the diaphragms of male rats injected with cortisone but could not detect any change in the diaphragms of female rats similarly treated. Isolated uteri from rats injected with ACTH 45 minutes prior to excision of the organ did

not demonstrate an increased oxygen uptake over that of uteri from control rats (61). This may be due to the intrinsic nature of the particular organ used.

Apparently adrenal cortical hormones had no effect on the rate of glucose absorption from the digestive tract of rats (56) and cortisol had no apparent influence, as measured by A-V differences, on the rate of glucose uptake by muscle (63). This agrees with enzymatic work conducted by Colowick, et al. (22) who found no change in the hexokinase activity of rat muscle incubated with ACE or with ACTH. Anterior pituitary extracts with ACE did inhibit the activity however.

There are reports of certain enzymes in metabolic pathways whose activity is altered in such a way as to account for the increased glycogen storage. Phosphoglyceromutase activity was inhibited by cortisone and ACTH injections in rats (50). This enzyme catalyzed the conversion of phosphoglyceric acid to pyruvate, hence a decreased activity of this enzyme reduced the utilization of glucose via the Embden-Myerhof pathway as well as by way of the hexose monophosphate shunt.

Not only does ACE decrease carbohydrate utilization, but there is also an increased formation and storage as glycogen, the latter originating mainly from protein sources. Work by Rosen et al. (83, 84) has demonstrated that glucocorticoids increased directly the rate of conversion of proteins to pyruvate by increasing the activity of liver glutamic-pyruvate transaminase (GPT) and to a lesser extent glutamic-oxalacetic transaminase (GOT). These two enzymes are associated with the conversion of aspartic and glutamic acids and alanine to pyruvic and oxalacetic acids respectively. Desoxycorticosterone reduced the activity of GPT by 50 percent. It is not certain whether the increased effect was due to an increased activation or whether there was an actual increase of the enzyme. Since the

liver protein concentrations increased following ACTH or cortisone injections (33) it is probable that the increased enzyme activity was due to an increase in the total enzyme concentration. The conversion of pyruvate to glucose via a reverse Embden-Myerhof pathway is enhanced by injection of cortisone into rats. Mokrasch et al. (65) observed an increased fructose - 1,6 - diphosphatase activity in the livers of rats so treated. The extent of alteration was presumably sufficient to account for the rate of liver glycogen storage following injections of these hormones.

Sources such as lipids must be sought as a possible origin of some of the increased glycogen synthesis that occurs following ACTH stimulation as the increased nitrogen excretion at this same time does not represent sufficient protein catabolism to account for all of it. Engel (30) indicated that both anterior pituitary and adrenal cortical extracts were capable of increasing the rate of lipid metabolism which in turn may ultimately increase pyruvate and glucose formation. As will be seen later, the blood lipids do increase following adrenocortical stimulation. Finally, glycogen storage was enhanced by a retardation in phosphorylase activity by both ACTH and cortisone (46). This enzyme is responsible for both the synthesis and hydrolysis of glycogen but the equilibrium favors the former.

Lipid Metabolism

The lipids offer organisms a very efficient means of energy storage and transfer. Most of the lipids are concentrated in adipose tissues but there seems to be a high turn-over rate of these stores. They are transported from one location to another in the circulatory system bound almost entirely to plasma proteins (24) and red cells. In man about one-

half of these lipids can be classified as phospholipids, one-fourth as cholesterol ester, one-fifth as tri-glycerides (25) and 5 percent as non-esterified fatty acids (NEFA) (24) which although comprising only a small portion of the total circulating lipids is probably the most important from the standpoint of energy transfer. The ability of the blood to take up lipids from the tissues seems to be dependent upon the available albumin present as a carrier (58). The storage and metabolism of lipids is under the control of various hormones, among which are those from the adrenal gland, although the exact roles of these hormones is not well established.

Lipid Mobilization - Fasting adrenalectomized rats mobilize increased amounts of fat from their tissues. White and Engel (108) reported that ACTH markedly decreased the fat content in the epidimymal fat pads while hydrocortisone reduced it only slightly. Adrenalectomized rats treated with ACTH have also been reported to take up less H^3 into carcass fats than did non-injected controls (107). ACTH seems to be especially important in releasing those lipids that are destined for immediate metabolism. The NEFA of the serum are believed to be the main component for this purpose since they varied inversely with the available carbohydrates (24, 34, 79), and various conditions have been observed where some tissues depend entirely upon fatty acids for their energy requirements (16, 67). Laurell and Christiansen (52) reported that no changes occurred in plasma NEFA following ACTH injections but inspection of their results shows a small, if insignificant, increase. Others have found that ACTH caused lypolysis in adipose tissues to form increased amounts of NEFA in the epididymal fat bodies (90) and their release into the circulatory system as long as albumin was available (58). The reduction in the lipid levels in this area

resulted from some types of trauma such as scalding (64) and laparotomy (54). Stoerch and Porter (103) found that cortisone, but not DOCA, also inhibited loss of fat from adipose tissues of adrenalectomized rats even though the weight of the animal did not increase as much as the non-injected adrenalectomized rats. This latter work is more in agreement with the experimental work of Li, Simpson and Evans (55) who found that the carcass fat of ACTH injected hypophysectomized rats increased over that of non-injected hypophysectomized rats. Apparently some of the losses from these adipose depots reported by other workers may have been due to the influence of other anterior pituitary hormones.

In comparing the effects of ACTH and some glucocorticoids in lypolysis, evidence has been found that ACTH facilitated lipid depletion in the tissues while cortisone inhibited this depletion (108). Dissimilar effects of ACTH and hydrocortisone have also been observed in the liver. ACTH injected into adrenalectomized rats increased the liver lipid content (108). Simultaneous injection of hydrocortisone partially inhibited this increase; hydrocortisone injected alone was followed by a decrease in the liver fats. These results are not in complete agreement with Welt and Wilhelmi (107) who found a decreased uptake of H^3 into liver lipids of ACTH injected adrenalectomized rats. These results can be interpreted somewhat differently however, since in the former experiment the increased liver lipids were presumed to have their origin in the tissue lipid stores while in the report by Welt and Wilhelmi the amount of H^3 taken into the formation of new lipids from body water was measured. Also the latter workers had maintained their rats on a high carbohydrate diet which decreased the possibility that this exogenous energy source might also be used to maintain glycogen stores. If ACTH could increase the capacity

for liver and muscle to store glycogen there would be a corresponding decrease in carbohydrate available for lipid formation.

Duhlin (27) has demonstrated a marked increase in liver fat concentration in birds injected with cortisone and hydrocortisone. This discrepant result may have been due to the fact that he used repeated injections (10 days) of the steroids. An increased feed intake during this period may also have contributed to the lipid accumulation.

The reported actions of ACTH and of adrenal steroids on the liver and muscle lipids seem to indicate that ACTH may have some lipogenic activity that is not mediated through adrenal steroid hormones, some of which diminish lipogenesis (37).

Serum phospholipids gradually increased following injections of ACTH into humans over a period of 7-15 days (2). On the other hand adrenalectomy in dogs resulted in a gradual reduction of serum phospholipids and of cholesterol; these reductions accounted for most of the decrease in the total plasma lipids (76). From these results it may be postulated that ACTH increases the plasma lipid levels. It should be remembered that these levels are not regulated by ACTH or adrenal hormones alone however, since growth hormone (55) and insulin (37) also exert very strong lipolytic and lipogenic actions respectively.

Cholesterol - Plasma cholesterol was increased in birds on a high fat diet unless special efforts were made to maintain a simultaneous high protein intake (47). Birds that were caged seemed to be subject to higher plasma cholesterol values than those that were given freedom of movement. Variations in plasma cholesterol have been used as an indication of lipid metabolism. Goldziehier (32) first stressed the need of looking at this aspect of adrenal action in 1927.

Repeated ACTH injections (up to 106 days) were accompanied by an increased plasma cholesterol level in humans with collagen disease (1, 2). However, this same hormone had an opposite effect on patients who had an initial hypercholesteremia.

Using single injections of ACTH, Slover (93) did not observe any alteration in the plasma cholesterol level except in male birds 24 hours after injection where an hypercholestrolemia was observed. No changes were noticed in rats following injections of 1-4 mg. of ACTH (57).

Glucocorticoids increase the plasma levels of cholesterol. Cortisone was the most active in man whereas hydrocortisone was more active in birds in bringing about this increase (27, 95).

Adrenal cholesterol was decreased by ACTH injections into rats (14, 57, 87), mice (57, 100), rabbits (26) and guinea pigs (57) as well as in chickens (42, 43, 44, 93). The maximum decrease in mammals usually occurs three to six hours after the injection while in birds the onset of the reduction may occur between six and twelve hours for no differences were found at the three and twenty-four hour intervals. Adrenalectomized dogs maintained with DOCA were not able to maintain normal plasma cholesterol levels (76).

Miller and Riddle (62) studied the adrenal cholesterol changes following ACTH injections in pigeons by histological techniques. Cholesterol was normally found evenly distributed throughout the cortical cells. Only the peripheral portion of the gland lost cholesterol under conditions of weak stimulation while the decrease was general following larger doses of ACTH. Also, the adrenals of adult pigeons were more active as measured by rate of depletion than were those from young birds.

Cholesterol is now generally agreed to be a precursor for steroidogene-

sis (89). Depletion of adrenal cholesterol has been correlated with evidence of adrenal cortical activity such as increased liver glycogen deposition. The increased plasma cholesterol values that have been observed may possibly serve as an increased pool for synthesis of adrenal steroids.

Other Metabolic Effects

Adrenal Ascorbic Acid - Although vitamins other than vitamin C may modify the actions of ACTH and adrenal hormones (77) none of them has been implicated with adrenal function as closely as has ascorbic acid. Along with cholesterol, this compound is in higher concentration in the adrenal gland than in any other organ with the possible exception of the corpus luteum (57).

The depletion of adrenal ascorbic acid in rats has been employed as a method of assaying for ACTH (88) as well as for the ability of certain hypothalamic compounds to stimulate ACTH release (21). Depletions have also been observed in old and young rats following the stress of laparotomy (82). Monkeys lost more of the acid when exposed to the stress of hypoxia and hypothermia than they did following injections of ACTH (6). An ascorbic acid reduction in the adrenals of guinea pigs also occurs following ACTH injections. An adrenal ascorbic acid depletion however has not been found in the frog, toad, pig, rabbit, cat and dog as well as the chicken and possibly the opossum following ACTH injections (29).

It has been supposed that a normal ascorbic acid content of about 400 mg percent is generally required before a depletion response to ACTH could be demonstrated, notwithstanding the fact that guinea pig adrenals (130-160 mg. percent) are depleted of ascorbic acid following the injection of the hormone. This latter effect may have been due to the inability of this animal to synthesize the vitamin as can the rat (44).

Several researchers failed to find an adrenal ascorbic acid depletion following injections of ACTH in chickens (29, 39, 43, 44), quail (7, 112) and ducks (113). Similarly, neither hypophysectomy (70) nor pituitary stimulants such as histamine diphosphate, insulin, saline or small doses of sodium salicylate caused an adrenal response in birds (42). Larger doses (up to 50 mg/100 gm.)of salicylate did decrease the acid level but this may or may not have been associated with an ACTH release. Since the chicken adrenal ascorbic acid content is low (generally said to range from 90-200 mg. percent although Perek and Eckstein (74) have recently reported values to 330 mg. percent) in comparison to that of the rat (400 mg. percent) it was assumed either that this was the reason for the refractoriness or that the response of birds to ACTH is not comparable to that of mammals.

Slover (93) found a decrease adrenal ascorbic acid level in female chicks 12 hours after ACTH injections but not in the male chicks. Other evidence that an acid depletion may occur was reported by Baldini and Zarrow (7) who observed that the quail adrenal gland lost ascorbic acid following exposure to cold. Recently Perek et al. (74) have demonstrated that the failure of the chicken adrenal to become depleted of ascorbic acid was not so much a matter of the adrenal content as it was the age of the bird. Three month old pullets having a normal adrenal ascorbic acid content of 335 mg. percent were not depleted of this acid following the injection of ACTH as were year old laying hens having an initial level of but 101 mg. percent. Molting, they found, was also associated with a decreased adrenal ascorbic acid level.

Plasma Ascorbic Acid - Plasma ascorbic acid has been noted to increase in man following injections of ACTH, and this rise has been demonstrated to be a more reliable, but not as sensitive, indicator of some types of stress

than is eosinopenia (3).

Slover (93) failed to observe any change in the plasma ascorbic acid of chicks following single injections of ACTH, but Howard and Constable (42) found a decrease in the plasma ascorbic acid level when ACTH was injected over a period of one week. On the other hand they observed an increased level in the plasma following a large injection of sodium salicylate, but this increase may have been due to the transient drop in adrenal ascorbic acid that occurred at the same time.

Possible Function of Ascorbic Acid - Sayers, et al. (87) suggested that ascorbic acid, under the influence of ACTH, is associated with the formation and release of adrenal cortical hormones. Lowenstein and Zwemmer (60) visualized the adrenal secretion to be an actual complex of the steroids and ascorbic acid. Jailor and Boaz (44) have demonstrated that an adrenal ascorbic acid depletion is not required for steroid output. They observed a reduced Sudan IV stainable material in the adrenal, indicating an increased steroid output, without a concommitant ascorbic acid depletion.

Another possible area of relationship between ascorbic acid and adrenal steroids is suggested in certain literature reports the data from which imply that the action is peripheral rather than in the gland itself. Ascorbic acid may combine with the adrenal steroids in the tissues and the resulting complex be the active form, or it is possible that the acid prolongs the activity of the steroids by maintaining their level in the tissues, i.e. decreasing their rate of excretion, especially if the steroid level is low. Cortisone injected into mice prevented an adrenal ascorbic acid decrease following stress or ACTH injections (14). In rats, an inverse relationship between the adrenal ascorbic acid depletion and the size of the dose of corticosterone and desoxycorticosterone injected was observed

(86). Conversely, ascorbic acid increased the ability of cortisone to increase the survival time of both intact and adrenalectomized mice subjected to cold stress (13). This result was not confirmed in man (77) but here the criterion was the effect of the acid in causing the blood glucose and lactic acid to return to normal after an individual was exposed to cold stress. This criterion however may not be a valid estimate of the extent of the effects of stress since carbohydrate changes are only a portion of the total picture of stress.

Ascorbic acid was found to decrease the excretion of 17-ketosteroids in adrenalectomized rats injected with cortisone (5). This observation can be used to explain earlier reports that ascorbic acid prolonged the hematologic effects of ACTH in intact rats. Further evidence that ascorbic acid aids the peripheral action of these hormones was given by Peric-Golia et al. (75). They observed a decreased loss of labeled cortisol-4-C^{14} from the adrenal gland of normal guinea pigs when compared to ascorbic acid deficient animals following stress.

Protein Metabolism - The increase in liver and muscle glycogen following ACTH and glucocorticoid injections is due not only to a decrease in carbohydrate utilization but also to an increased formation. This increased storage is mainly acquired through gluconeogenesis from protein sources. An increase in protein metabolism results in an increased nitrogen loss from the body which is measured as urea or uric acid, the latter being the main form in birds. Both ACTH and adrenal hormone injections were followed by not only a hyperglycemia, and often a glycosuria, but also by a concomitant increase in nitrogen excretion (11, 19, 81). In birds the increased nitrogen excretion seems to be in the non-uric acid portion (19).

There is not only a conversion of protein to carbohydrates and/or fats, but also a transfer of protein from one organ to another. Injections of cortisone, but presumably also of ACTH, resulted in a mobilization of protein from the muscles of rats to the liver and other viscera (33, 85). The rate of protein synthesis in the liver and viscera following these injections was more dependent on the energy content than on its protein content. The catabolic effect in the muscles did not seem to be affected by the protein or energy level in the diet; high glucose levels however are reported to stimulate amino acid uptake in muscles.

Protein metabolism in the liver may be somewhat influenced by the rate in which amino acids can enter the liver cells. Hydrocortisone has been shown to be able to increase the rate in which the non-metabolizable amino acid ∝-amino isobutyric acid entered the liver, and to a lesser extent, the kidney cells (72). This occurred even when there was no concurrent serum increase of this acid. Interestingly enough, this increased uptake did not take place in the muscle cells. Glucocorticoids have also been shown to alter the rate at which amino acids were incorporated into proteins. Hess and Shaffran (41) fed C^{14} labeled alanine or lactic acid to rats that were injected with cortisone or to control rats. Cortisone increased the incorporation of C^{14} into liver proteins but did not affect entry into muscle protein.

Blood Cell Responses to ACTH

Hematocrit - Two investigators have reported a decreased hematocrit in the bird during conditions that may be a stress or stimulate the adrenal gland. Sturkie (105) found a decreased hematocrit following successive blood drawings but he attributed this to be mainly due to a hemodilution.

Newcomer (68) obtained similar results three to six hours following injections of ACTH in chickens although an increased red blood cell count was obtained in one of three experiments. An increase in the red blood cell count was also reported by Dougherty (26) for the rabbit.

Leucocytes - ACTH injections in birds resulted in an increased total leucocyte count up to 12 hours (69) while in rats, mice and rabbits a leucopenia was observed (26). A leucocytopenia was observed in rats, mice and rabbits following injections of ACTH and ACE (26). This decrease was due to a large reduction in the lymphocytes; the polymorphonuclear cells increased nearly two-fold. A leucocytophilia was observed in birds injected with ACTH, the increase being due mainly to a large increase in the acidophils or more specifically, to the heterophils (69). A lymphopenia however was not observed, in fact a slight increase was generally noted for 12 hours following ACTH injections. Here again, it is possible that ACTH had some direct function outside of its action through adrenal steroids as adrenal cortical extracts in birds caused a lymphopenia the first three hours following injections and they then returned to normal levels or above (92). It is possible that the leucopenia was a short term effect due to action of the cortical extracts on the lymphocytes directly and the latter increase was due to stimulation of the organs necessary for producing and/or releasing these cells. ACTH may be able to stimulate these latter target organs directly in the bird.

The basophils do not seem to be greatly influenced by ACTH injections. In rabbits a basophilic leucopenia was observed four hours following the injections for a period of from 3 to 20 hours thereafter (15). The response in birds was variable (69).

The eosinophils do not seem to be affected by ACTH in the chicken

(68, 96), but their counterpart in the acidophils, the neutrophils, responded very markedly to these injections and to stress (68, 92). This response was maximal at about six hours following single injections where a three-four fold increase was observed. Some stresses as anoxia and restraint also increased the acidophilia in birds while others such as small amounts of histamine or cold alone failed to do so (69).

CHAPTER III

MATERIALS AND METHODS

The following experimental procedures were designed to study some of the metabolic and blood cell changes that occur following subcutaneous injection of ACTH in chickens.

Experimental Chicks

Six-week old Silver-Oklabar chicks were used throughout these experiments. Since early work indicated that there was no apparent difference in the responses of male and female birds, cockerels were used exclusively with the exception of some early experiments and one case in which insufficient males were available.

The chicks were hatched and raised to six weeks of age by the Department of Poultry Science at Oklahoma State University and delivered to the Physiology Department two to seven days prior to the time that they were to be used. During this time the birds were fed ad lib. on the standard ration supplied by the Poultry Science Department until 6-18 hours prior to the time that they were to be sacrificed. They had continued, free access to water.

Experimental Procedure

Experiments were carried out in six series, each series consisting of two to five separate experiments. With the exception of series 6, a series was considered to comprise all of those experiments that were conducted on birds that were hatched the same day and autopsied within a

period of one week.

Series 6 was conducted on two separate hatches delivered two weeks apart. The 20 birds from the first hatch were sacrificed six hours after the injection of ACTH while the second hatch of 40 birds was sacrificed at 3 and 12 hours following the injections.

For each experiment the birds were divided into two groups of from 4-12 birds each. One group received subcutaneous injections of either 4, 6, or 8 IU of ACTH (Armour Laboratories' Adrenomone) in 0.1 or 0.2 ml. of a 16 percent gelatin solution - a commercial diluent also obtained from Armour Laboratories. The other group served as controls and received 0.1 or 0.2 ml. of the 16 percent gelatin carrier.

Series 1 - Five experiments were performed in which 4 IU ACTH was injected into the birds of the treated groups. In the first two experiments blood was withdrawn by cardiac puncture six hours after injection; the birds were then decapitated, the adrenals removed, weighed and placed in the deep freeze along with the plasma from the same birds until analyzed for cholesterol by a modification (93) of the method of Zlatkis et al. (114). Experiment one was composed entirely of pullets; the birds in experiment two were cockerels.

Electrophoretic analyses of pooled blood samples from each of the groups in these two experiments, as well as from birds autopsied at 12 hours in this same series, were carried out by Dr. C. D. Kochakian of the Oklahoma Medical Research Center, Oklahoma City. The quantities of the different fractions were measured with a Spinco Analytrol Model RA. The plasma protein curves for each experiment were compared and the results reported for the albumin, alpha$_1$ plus alpha$_2$ globulins, beta globulin, and the fibrinogen plus gamma globulin fractions. These results are

expressed as a percentage of the total protein on the strip.

In the next two experiments, the first with pullets and the second with cockerels, one ml. blood samples for hematocrit and acidophil counts were taken by heart puncture from each bird at 3, 6, and 12 hour periods following the injection of ACTH. The hematocrit was determined in Van Allen hematocrit tubes centrifuged 20 minutes at 2000 RPM in an International Type SB, Size 1, centrifuge. Acidophil counts were made by diluting whole blood 100 times with Wiseman's stain in a red cell diluting pipette. After allowing them to stand in the refrigerator for at least 24 hours, two separate counts of the acidophils were made on a Levy-Hauser counting chamber and the average count reported as acidophils per 0.009 $mm.^3$.

In the third experiment, 12 hours following injections of ACTH, the birds were decapitated after blood was withdrawn by cardiac puncture; the adrenal glands were removed, weighed and placed in a deep freeze for later analysis for ascorbic acid content by the method of Roe and Kuether (49).

Series 2 - This series consisted of three experiments, the first using cockerels, the second pullets and the third using birds of mixed sex. The birds were decapitated 12 hours after the injection of 4 IU ACTH; 750 - 1000 mg. samples of liver and breast muscle were immediately removed, weighed and placed in the deep freeze for later analysis for glycogen content.

The muscle glycogen was divided into two fractions (12): (1) glycogen that could be extracted from muscle homogenized in 10 percent trichloroacetic acid (TCA) and (2) glycogen remaining in the precipitate of this extraction which was released by digestion of the precipitate with boiling 30 percent potassium hydroxide (KOH). If separation of the

fractions were not desired, the total glycogen was obtained by digesting the whole muscle or liver sample in hot KOH. The glycogen that was brought into solution by either of these two methods was precipitated by ethanol, and the glycogen so obtained was hydrolyzed in hot N H_2SO_4 for 3 hours, neutralized with NaOH and after proper dilution the resulting glucose was determined by Nelson's (66) modification of Somogyi's method. The results are expressed as mg. glucose per 100 grams tissue.

Series 3 - This series consisted of five experiments using only male birds. The experimental birds received 6 IU ACTH 3, 6, or 12 hours prior to autopsy. The birds were anesthetized with sodium pentabarbital, the thoracic cavity immediately exposed by making a wide incision just behind the ribs and sternum, and blood withdrawn into a heparinized needle. One hundred to 200 mg. liver and muscle samples were then removed and treated as in series 2 for TCA and KOH muscle glycogen. A zinc hydroxide protein-free filtrate was made from the blood samples (38) and the glucose determined by Nelson's method.

Series 4 - This series consisted of three experiments using only male birds. The procedures in this series were the same as those described in series 3 except that no anticoagulant was used for the blood and a Folin-Wu blood filtrate for glucose determinations was prepared from the blood immediately after withdrawal of the blood. Leg muscle samples were taken for glycogen determination as well as samples from breast muscle and liver. Serum from the blood was used to determine the lipoproteins by the method of Hendley et al. (40).

Series 5 - This series consisted of three experiments using only male birds. The treated birds in this series received 6 IU ACTH 3, 6, and 12

hours prior to necropsy. Following sodium pentabarbital anesthesia, 10 cc. of blood was withdrawn into a syringe without the use of an anti-coagulant. Blood was taken up into a heparinized capillary tube from a cut wing vein for hematocrit determination with an International hematocrit centrifuge. Serum from the blood was used for analysis of lipoproteins, fatty acids by the method of Stern and Shapiro (99), and proteins by the method of Gornall, et al. (35) by means of the biuret reaction. The readings were converted to mg. percent by use of a curve prepared by Jack Connally of the O. S. U. Physiology and Pharmacology Department.

Series 6 - Three experiments were carried out in this series, with the treated birds receiving 8 IU ACTH 3, 6 and 12 hours before sampling. The hematocrit, serum lipoprotein, fatty acids and cholesterol were determined as well as the muscle glycogen, all by the same methods as previously mentioned. Because of the possible gradual loss of muscle glycogen when extracting with TCA (100), two samples of muscle of nearly identical weight were taken, one for determination of the TCA fraction and the other for determination of total muscle glycogen after digestion with KOH. Folin-Wu protein-free filtrates were also made from the whole blood as in series 5 for use in determining the blood lactic acid. This was carried out by the method of Barker and Summerson (8).

A Bausch and Lomb Spectronic "20" photometer was used for reading the transmittance of the glucose, lactic acid, cholesterol and ascorbic acid as well as the lipoproteins for series 4. A Beckman DU4 Spectrophotometer was used for the other lipoprotein and fatty acid determinations.

CHAPTER IV

RESULTS AND DISCUSSION

The adrenal gland of mammals secretes increased amounts of certain adrenal cortical hormones following injections of ACTH. Subjecting mammals to stressors has produced similar results. Thus both ACTH injections and various types of stressors will cause enlargement of the adrenal gland, decreased adrenal cholesterol and ascorbic acid and increased amounts of adrenal corticoid secretion.

Although it has been reported that ACTH from mammalian sources stimulated the adrenal of the bird (9) others have questioned the ability of such an "alien" hormone to stimulate the adrenal (45, 96). Stressors such as formaldehyde did not result in increased adrenal activity in birds in one series of experiments (91) but did in another (69). ACE injections have resulted in an increase in the heterophils in birds (92). Similar results have been obtained with cortisone and hydrocortisone as well as with other adrenal hormones such as DOCA and aldosterone (69). Increased acidophil counts have also been observed following ACTH injections and various types of stressors such as restraint, formaldehyde, and cold and wet treated birds (69). These results indicate that the adrenal cortical cells of the bird respond in a manner similar to those of the mammal. Corticosterone has been found to be the most prevalent hormone in the effluent of the adrenal gland of normal fowl. Hydrocortisone, cortisone and aldosterone have also been isolated and these three increased

in amount following ACTH injections while corticosterone decreased (45).

Since it has been shown that a similar mechanism for the control of the adrenal cortical tissue probably occurs in the bird as well as in the mammal, ACTH may be used as a model for determining the effects of various physiological stressors on birds as well as for an indication of the metabolism of birds during conditions of stress.

Carbohydrate Metabolism

Blood Glucose - Adrenocorticotrophin injection was followed by a 25 - 50 percent increase in the level of blood glucose (Table I) which, in series 4, lasted as long as 12 hours. Maximal increases were observed at 6 hours; in series 3 an increase was noted at 6 hours but not at the 12-hour interval.

It will be noted that the control blood glucose levels in series 4 range from 200-210 mg. percent while those in series 3, which were from birds autopsied during the summer months, ranged from 130 - 140 mg. percent. In series 3, zinc hydroxide was used as the protein precipitant while in series 4 Folin-Wu's tungstate filtrate was used. Since Hawk et al. (38) reported lower glucose values with the zinc hydroxide filtrate it is probable that it was the precipitating agent and not the season in which the birds were autopsied that accounted for the lower values in the blood glucose levels.

One source of the increased blood glucose following the injection of ACTH may be the liver. Under conditions of ACTH stimulation there is a high rate of glucose formation from protein sources. This might occur to the extent that the available enzymes for converting glucose to glycogen are loaded to capacity and any glucose formed by phosphorylase activity is unable to re-enter the synthesis pathway and as a result enters

the circulation. In mammals an increased blood glucose, even when due to the actions of adrenal steroids, caused an increased insulin secretion (37) and a consequent inhibition of glucose release from the liver (28). There is the possibility, however, that the control of blood glucose by insulin in the bird is not as effective as in mammals since diabetes does not always occur following pancreatectomy or alloxan administration (91). If this is so the release of liver glucose would not be greatly inhibited.

TABLE I

BLOOD GLUCOSE LEVELS FOLLOWING ACTH INJECTION

Series	Dose, IU	Hours after ACTH Injection 3	6	12
3#	0		(12) 134 ± 27[a]	(12) 139 ± 24
	6		(12) 168 ± 54*	(12) 138 ± 38
	0		(12) 130 ± 33	(12) 128 ± 34
	6		(12) 202 ± 47**	(12) 133 ± 46
4	0	(10) 204 ± 14	(10) 211 ± 13	(8) 204 ± 39
	6	(10) 273 ± 31**	(10) 331 ± 74**	(9) 287 ± 42**

* $P < 0.05$
** $P < 0.01$
\# Birds autopsied during summer months, other were autopsied during winter months
[a] (No. of Birds) mean ± standard deviation in mg. percent

Blood Lactic Acid - No values for blood lactic acid levels in birds have been reported. The average mean values of 28 - 38 mg. percent (range 11-90) found in the control chickens of this experiment (Table II) compare favorably with those found in humans (0 - 40 mg. percent) but are higher than the normal values reported for cattle, horses and dogs where the ranges were from 5 - 30 mg. percent (94). Since considerable variation occurred within each group of each experiment, significant differences between groups were not encountered, but at every time period following the injection of ACTH the 32 - 45 mg. percent mean values for the treated groups were 10 - 20 percent higher than the means for the controls. These

small differences may be of importance since they may reflect an actual low level response to ACTH that has been occurring over a long period of time and as such could represent a considerable difference in glycolysis. There was no indication as to the source of the increased blood lactic acid but since, as will be shown later, the muscle glycogen either did not increase or may in fact have decreased following ACTH injection, the muscles may be implicated. If the various tissues require increased amounts of energy for catabolism and anabolism of proteins following stimulation by glucocorticoids, at least some of this energy could come from the anerobic glycolysis (and concommitant lactic acid formation) of glucose which had its origin either in skeletal muscle glycogen or the increased blood glucose levels. Further, any lactic acid that did escape into the circulatory system from the cells would not re-enter as readily during periods of excess adrenal stimulation as during normal conditions (41).

TABLE II

BLOOD LACTIC ACID LEVELS FOLLOWING ACTH INJECTION

Series	Dose, IU	Hours after ACTH Injection 3	6	12
6	0	(8) 37.9 ± 11.5[a]	(9) 28.0 ± 14.1	(10) 29.3 ± 8.3
	8	(8) 45.3 ± 12.9	(10) 31.9 ± 22.8	(10) 33.8 ± 8.6

[a] (No. of Birds) Mean ± standard deviation in mg. percent.

Liver Glycogen - Liver glycogen increased in every experiment following the injection of ACTH except one (Table III). In this case the 4.4 gm. percent mean for the control was as high as was encountered in any other liver sample whether from injected or control chickens. The extremely high level of glycogen in the livers of this control group may have been

due to a larger amount of feed consumed by the birds of this experiment just prior to removing the feed from them although their muscle glycogen levels were not above those of other groups. The results of this particular experiment suggest that ACTH has no effect on the liver glycogen levels in those instances where the liver has already reached an absolute "limit" of storage, i.e. the storage potential of the liver for glycogen was not altered by ACTH injection. The liver glycogen levels for the other control birds ranged from 0.030 - 1.9 gm. percent and for the injected birds from 0.060 - 4.46 gm. percent.

TABLE III

LIVER GLYCOGEN CONCENTRATIONS FOLLOWING ACTH INJECTION

Series	Dose IU	Hours after ACTH Injection 3	6	12
2	0			(5) 4.425 ± 2.078[a]
	4			(4) 4.123 ± 1.663
	0			(10) 1.171 ± 0.547
	4			(5) 4.245 ± 1.528**
	0			(8) 0.026 ± 0.009
	4			(9) 1.226 ± 0.570**
3	0		(12) 0.332 ± 0.591	(12) 0.031 ± 0.017
	6		(12) 1.864 ± 1.158*	(12) 1.482 ± 1.336*
	0	(4) 0.033 ± 0.018	(12) 0.031 ± 0.012	(11) 0.061 ± 0.110
	6	(5) 0.616 ± 0.682	(12) 1.022 ± 0.733	(12) 1.121 ± 1.414*
4	0	(10) 0.296 ± 0.500	(10) 0.124 ± 0.135	(10) 1.941 ± 0.678
	6	(10) 1.299 ± 0.83**	(10) 2.424 ± 1.483**	(10) 4.459 ± 0.903**

* $P < 0.05$
** $P < 0.01$
[a] (No. of Birds) Mean ± standard deviation in gm. percent

The apparent increase in the liver glycogen concentration in series 3 at the 3-hour interval was insignificant because of the variation within groups. Even though there was more than a 10-fold difference in some of the values (Fig. 1) the lowest concentration of the treated group was higher than the highest concentration in the control group: 0.017 - 0.055 gm. percent in the control group and 0.070 - 1.696 gm. percent in the treated group.

In two of the experiments the liver glycogen of the treated birds when compared to the controls, reached a maximum at 6 hours after the injection of ACTH and then gradually decreased (Fig. 1). In one of the experiments of series 3, the liver glycogen concentration was comparatively much higher at the 12-hour interval than at the 6-hour period. The absolute value for the 12-hour treated group however was not as high as that of the injected group at the 6-hour period.

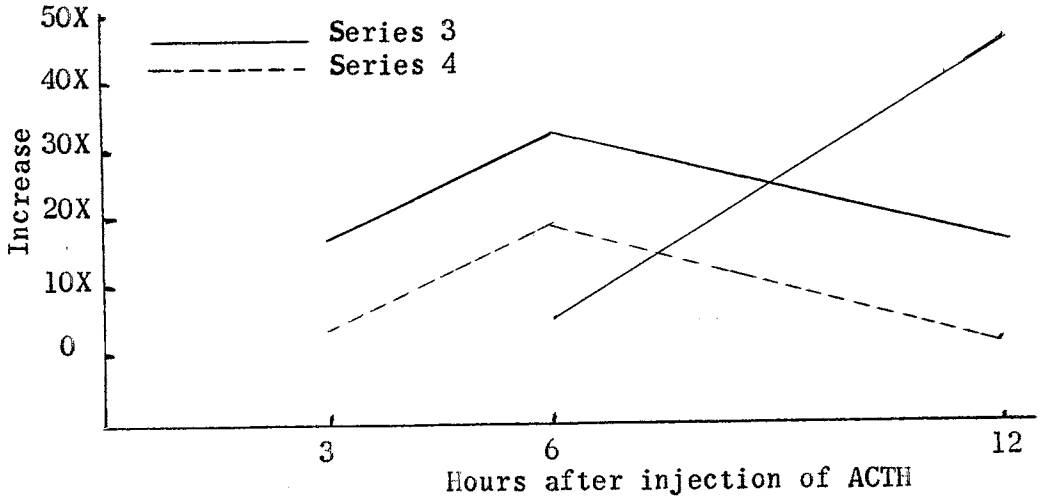

Figure 1. Comparative increase in the liver glycogen concentration of ACTH injected birds over control birds

Muscle Glycogen - Extremely wide variations were encountered between experiments in the white muscle glycogen levels (Table IV and V). The total glycogen concentrations in the control chickens ranged from 21 mg. percent in one group to a high of 568 mg. percent. The values for any one particular series of experiments were more uniform however.

Contrary to the results that have been reported from mammalian experiments, a significantly increased skeletal muscle glycogen following injection of ACTH was never encountered in this work; in fact there was, if not a significant decrease, a general decrease of up to 60 percent in the TCA fraction (Table IV) and a decrease of up to 30 percent in total glycogen (Table V). These decreases were not peculiar to these experiments alone for the unpublished results of experiments by Newcomer (71) with 3-week-old white Leghorn cockerels as well as one preliminary experiment by the writer with the same type of bird have also evidenced this decrease following the injection of ACTH.

It appears that the variations in the total muscle glycogen are largely reflections of the TCA glycogen changes for a comparison of Tables IV and V shows that the direction of change in the TCA glycogen fractions is an indication of, if not wholly responsible for, the change that occurs in the total glycogen. Early work in this field by Bloom et al. (12) indicated that the greatest changes following fasting, feeding or epinephrine injections occurred in the TCA fraction and was termed the labile or "free" fraction; the remaining glycogen that is removed only after KOH digestion was more or less fixed in amount and was termed the "bound" form. Later, by the use of C^{14} labeled glucose, D. Stetten and his co-workers (100-102) demonstrated that the KOH fraction was the more labile, probably being attached to the various cellular enzymes, but relatively constant in amount

TABLE IV

TCA EXTRACTABLE MUSCLE GLYCOGEN CONCENTRATION
FOLLOWING ACTH INJECTION

Series	Dose, IU	Hours after ACTH Injection 3	6	12
2[a]	0			(10) 41.0 ± 20.5[c]
	4			(10) 39.0 ± 29.5
	0			(9) 34.3 ± 24.2
	4			(9) 27.0 ± 16.0
	0			(9) 23.4 ± 10.5
	4			(9) 30.4 ± 23.5
3[b]	0		(12) 11.2 ± 6.1	(12) 15.4 ± 9.7
	6		(12) 9.8 ± 8.9	(11) 15.6 ± 6.4
	0	(4) 20 ± 6	(9) 10.5 ± 8	(7) 7.8 ± 5
	6	(5) 7 ± 2**	(8) 3.5 ± 3*	(11) 7.4 ± 5
4[c]	0	(9) 53 ± 53	(9) 61 ± 97	(8) 68 ± 45
	6	(9) 52 ± 47	(10) 40 ± 56	(10) 53 ± 37
	0[d]	(8) 8 ± 4	(10) 7 ± 3	(8) 23 ± 24
	6	(10) 22 ± 27	(10) 10 ± 11	(9) 16 ± 10
6[c]	0	(10) 89 ± 52	(10) 65 ± 63	(10) 118 ± 48
	8	(10) 55 ± 37	(10) 86 ± 52	(10) 85 ± 46

* $P<0.05$
** $P<0.01$
a Birds autopsied in April
b Birds autopsied (June - September)
c Birds autopsied in winter (November - February)
d Leg (red) muscle, all others breast (white) muscle
e (No. of birds) means ± standard deviation in mg.%

TABLE V

TOTAL MUSCLE GLYCOGEN CONCENTRATION
FOLLOWING ACTH INJECTION

series	Dose, IU	Hours after ACTH Injection 3	6	12
2[a]	0			(10) 189.6 ± 68.9[e]
	4			(10) 184.3 ± 28.8
	0			(10) 164.3 ± 64.0
	4			(9) 165.5 ± 62.5
	0			(9) 158.5 ± 32.7[d]
	4			(9) 169.0 ± 55.9
3[b]	0		(12) 37.3 ± 12.2	(12) 36.6 ± 11.3
	6		(12) 35.5 ± 16.8	(12) 44.4 ± 28.1
	0	(4) 36 ± 14	(9) 25.4 ± 13	(7) 21.0 ± 8.0
	6	(5) 24 ± 7	(8) 14.4 ± 5*	(11) 20.0 ± 9.0
4[c]	0	(9) 288 ± 102	(9) 309 ± 161	(8) 360 ± 21
	6	(9) 314 ± 118	(10) 240 ± 109	(10) 242 ± 91
	0[d]	(8) 187 ± 120	(10) 172 ± 57	(8) 288 ± 43
	6	(10) 196 ± 83	(10) 223 ± 96	(9) 236 ± 83
6[c]	0	(5) 354 ± 65	(10) 568 ± 133	(10) 286 ±127
	8	(6) 274 ± 81	(10) 602 ± 165	(10) 238 ±110

* $P < 0.05$
[a] Birds autopsied in April
[b] Birds autopsied in summer (June - September)
[c] Birds autopsied in winter (November - February)
[d] Leg (red) muscle, all others breast (white) muscle
[e] (No. of birds) Mean ± standard deviation in mg.%

since it picked up new glycogen from the "free" pool as it liberated "bound" glucogen. The results reported here do not disagree with the latter findings but they indicate that a large portion of the glycogen is in a potentially labile state.

In the experiments conducted during the winter months, with the exception of series 6, the white muscle TCA fraction accounted for but 15 - 22 percent of the total muscle glycogen; that is, 78 - 85 percent of the glycogen was in the KOH fraction. In series 6, the percentage of the TCA fraction ranged from 11-41 percent. The birds in this series were fasted for 18 hours prior to autopsy rather than the 6 or 12 hours with all the other birds. Similar results are seen in series 3 where the ranges are from 28 - 55 percent for the TCA fractions.

In nearly every experiment, whether conducted in winter or summer, the ratio (percentage) of the TCA fraction to the total glycogen was reduced, and this reduction was more pronounced in the groups autopsied during the summer. The TCA fraction in the leg muscle made up only 4 - 11 percent of the total muscle glycogen, and the treated group at three hours had nearly a three-fold increase over the corresponding control level.

It is known that the food and/or caloric intake of birds is not as high during the summer months as in the winter. This fact, or the reduction in body energy stores due to prolonged fasting, may indicate that a decreased body energy reserve in some way alters the ratio of free to bound glycogen in the muscles, the alteration being in the direction of an increased TCA fraction. Whatever the mechanism might be for increasing the TCA fraction during the summer or in long fasting periods, ACTH, possibly by the release of adrenal steroids, apparently is able to reverse

it, for it was during the summer that the percentage of the TCA fraction as well as the total glycogen was decreased the greatest amount.

One possible reason for the reduced TCA/total glycogen ratio following ACTH injection might be the fact that the rate of insulin release, or rate of glucose uptake by the tissues, in turn influences the rate of protein synthesis in the cells (33). A decreased energy supply from carbohydrates, or a decreased insulin release because of a decreased blood sugar, would be followed by a decreased protein and glycogenic enzyme activity. This would result in the lower ratio of bound glycogen to the free form as well as a decrease in TCA and total glycogen as seen in series 3. An irritating contradiction to this hypothesis is presented, however, by one experiment by the writer where the control TCA fraction was much higher than any reported in Table IV. Here, however, 3-week old White Leghorns were used. The TCA fraction from the control birds was 43 percent of the total and 34 percent of the total in the injected birds even though the total glycogen decreased by half in the latter group. By way of comparison, Bloor et al. (12) using rat skeletal muscle, extracted about 55 percent of the total glycogen with TCA. The reasons for the higher percentage obtained by the latter workers may be that a higher portion of the rat muscle is in the TCA fraction or due to their method of extraction. They precipitated the glycogen from the TCA homogenate within 20 minutes after the muscle was homogenized. In the present experiments, in an effort to obtain greater extraction, the extraction period was 2 - 4 hours. This longer time may have allowed some destruction of the glycogen (100) although a degradation of this sort should also be manifest in the total glycogen levels.

One other possible reason for the glycogen decrease following injection of ACTH in birds is that increasing amounts of energy were re-

quired to mobilize the muscle proteins as explained earlier. This energy could come from glucose which originates from the stored muscle glycogen or from the increased blood levels. In either case this would result in a reduced net synthesis of glycogen by the muscles.

In the one experimental series where both the breast (white) and leg (red) muscle was analyzed for glycogen, it was found that the average TCA fraction in leg muscle was 1/4 to 1/3 that of breast muscle, while the total glycogen was 1/2 to 2/3 that of breast muscle. This compares favorably with the results of Golden and Long (31) who found leg muscle glycogen to be about 1/3 that of the white muscle. Although statistical differences in series 4 were not observed, the means do not indicate the same responses in red and white muscle glycogen. The averages for both the TCA and total glycogen were higher in the red muscle of injected birds except at the 12-hour interval, and the percentage of TCA to total glycogen is seen increased in the injected birds, especially at six hours, rather than decreased as in the white muscle.

General Considerations - ACTH injection seems to be followed by an overall increase in the transfer of energy and protein materials from the peripheral tissues to the liver and possibly to some other organs. This transfer is evidenced by the reported catabolism of proteins in skeletal muscle and their subsequent accumulation and conversion to glycogen in the liver and visceral organs. The experiments reported here also indicate an increase in the liver glycogen stores which often is accompanied by a decrease of that compound in the white skeletal muscle. In additon to the carbohydrate and protein mobilization there was an increased lipid concentration in the serum. Repeated ACTH injections have resulted in an accumulation of lipids in the liver (3) as well as their possible

increased utilization in egg formation (59). To accomplish these transfers, if increased blood lactic acid levels are an indication of increased metabolism, certain organs must be required to increase their rate of energy consumption. This transfer however need not mean that some organs are being deprived of energy and material resources for there is also a simultaneous increase of these same substances in the circulatory system from which they may draw.

If the function of ACTH by way of the adrenal cortical hormones is to help the organism withstand the effects of stress then some reason must be found to explain why the massive shifting of materials from one organ to another occurs and why the formation of greater carbohydrate stores takes place at the expense of the skeletal muscle proteins. Teleologically one possibility that may be suggested is that the concentration of these materials in the liver, and possibly in some of the other visceral organs, provides a convenient location from which to redistribute these substances as needed later. This would be advantageous in that the liver may have a uniform response to various stimuli whether they be nervous or humoral. Also, the liver, by virtue of its high concentration of many metabolic enzymes and its ability to grow and regenerate, may be an organ that can rapidly mobilize materials if sudden demands for them should be made.

Shifting materials out of the peripheral tissues would not necessarily deprive these tissues of necessary nutrients since the increased blood sugar levels that occur during these stressful conditions would also increase insulin output (37). Glucose could be taken into the cells very readily as required and the insulin in turn, along with the increased blood sugar, could increase the rate of protein synthesis in

these same cells. This does not necessarily mean that an increased protein catabolism (glucocorticoid action) is being counteracted by an increased anabolism (insulin action). It may indicate that there is a shift in the basic activities of the cells with those proteins of least survival value being eliminated and others increased. The decreased muscle glycogen in the ACTH injected birds (but not in mammals) may represent an unloading by these cells of material that is not required under those conditions in which they would be stimulated in this way. They are "clearing the decks for action." It is not generally agreed that the "well-fed" individual is the healthiest.

The more pronounced reduction in muscle glycogen in the birds raised in the summer indicates that the energy stores of the bird may contribute to the way that the bird responds to ACTH injections. Further work may profitably be carried out in this area by altering the energy content of the feed or by investigating the effects of fasting for different time periods on the response to ACTH injections.

Lipid Metabolism

Lipoproteins - The protein bound lipids of the experimental groups of series 4 showed a highly significant increase when compared to the control groups 6 and 12 hours but not 3 hours after the injection of 6 units of ACTH (Table VI). This marked increase was not observed in series 5 and 6 where 6 and 8 IU ACTH respectively were injected into the birds. It should be pointed out that the ACTH used in series 5 was outdated and although perhaps most of its activity may still have been present there was not enough response to show significance. In series 6 the fact that the birds were fasted 18 hours prior to autopsy rather than only 12 hours

as was done with the other groups is another variable that must be considered as a possible reason for the reduced differences following the injection of ACTH. In both series 5 and 6, no response was seen at 6 hours while at 3 hours the differences were comparatively large.

TABLE VI

SERUM LIPOPROTEINS AND TOTAL FATTY ACID LEVELS FOLLOWING ACTH INJECTION

Series	ACTH IU	Hours after ACTH Injection 3	6	12
4	0	(9) 367 ± 50[a]	(9) 293 ± 69	(8) 414 ± 67
	6	(7) 403 ± 86	(10) 537 ± 93*	(6) 599 ± 53*
5	0	(10) 389 ± 114	(9) 421 ± 59	(10) 465 ± 81
	6	(10) 443 ± 130	(10) 406 ± 78	(9) 500 ± 65
6	0	(10) 332 ± 38	(10) 373 ± 64	(10) 416 ± 66
	0	(10) 357 ± 42	(10) 372 ± 41	(10) 428 ± 72
		Serum Total Fatty Acids, meq./100 cc.		
6	0	(10) 1.132 ± 0.13	(8) 1.000 ± 0.12	(10) 1.254 ± 0.2
	8	(10) 1.372 ± 0.18*	(8) 1.58 ± 0.11	(10) 1.405 ± 0.2

* $P < 0.01$

[a] (No. of birds) means ± standard deviation in mg. percent

An interesting observation is that in those birds autopsied 3 hours after the injection of ACTH in series 6 (Table VII), half of each group were of each sex; an analysis of variance of the lipoproteins indicated that the males had a higher serum lipoprotein level (359 mg. percent) than did the females (304 mg. percent), ($P < 0.005$). The differences at 3 hours due to ACTH injection were placed at $P = 0.08$ which, though not classed as significant, indicated a fairly high level of probability that ACTH did

increase this level. Lorenz et al. (59) reported no sex differences in the serum lipids of 15-week-old birds but reported nearly a 10-fold increase in the lipid fraction during laying.

TABLE VII

LEVELS OF SERUM LIPOPROTEINS AND SERUM TOTAL FATTY ACIDS FOLLOWING ACTH INJECTION IN MALE AND FEMALE BIRDS[a]

Dose IU	Serum Lipoproteins, mg.%		Total Fatty Acids, meq/100 c.c.	
	Male	Female	Male	Female
0	359 ± 26[b]	304 ± 26	1.215	1.049
8	381 ± 37	334 ± 35	1.424	1.320*

* $P < 0.02$ when compared to female control group

a Five birds in each group. These are the same birds as listed in Table VI, Series 6, at three hours

b Mean ± standard deviation

Total Fatty Acids - The total fatty acids responded to ACTH injection in a manner quite similar to the lipoproteins (Table VII). The greatest increase (0.240 meq/100 cc. serum) was noted 3 hours following the injection of ACTH ($P = 0.005$). Here, as in the lipoproteins there was a higher total fatty acid content in males than in females (1.215 meq/100 cc. in males compared to 1.049 meq/100 cc. in females). An analysis of variance of the means of the fatty acid concentrations also indicated a treatment effect due to ACTH; most of this difference was due to the fatty acid increase in the females.

From the results obtained in the lipoprotein and fatty acid analyses one may conclude that the serum lipid levels are higher in males than in females at six weeks of age but that capacity of the males for lipid mobilization is not as great. If this is true of older birds it may indicate

a means whereby the females can readily mobilize energy during the laying season; indeed one of the first reports of adrenal gland activity implicated it with ovulation (80).

The non-esterified fatty acids represent the fraction of the lipids that is most readily available to meet cellular energy requirements. This fraction probably does not account for any of the lipid increase however since previous work has indicated that the NEFA decreases whenever blood glucose levels increase (24). Cholesterol, as will be seen later, also contributes to the serum lipid increase. Since the total fatty acids increase to a greater extent than do the lipoproteins following ACTH injections it follows that some of the other lipid fractions must decrease. That the increased fatty acids could arise from the lipid depots is suggested by the work of Achotz and co-workers (90). They observed an increased NEFA release from the epididymal fat bodies of rats injected with ACTH. Although they restricted their study to the NEFA it may be that other lipid fractions were released or made available for mobilization.

Blood Cholesterol - An inspection of Tables VIII and IX reveals that there was considerable variation in the cholesterol values in response to injection of ACTH. The cholesterol values in Table VIII represent plasma levels while in Table IX serum levels are indicated. The high values for the controls seem above normal when compared with some reported values of 50 - 150 mg% (94) but are comparable with the results obtained by Slover (93). There was some increase ($P = 0.08$) in the serum cholesterol at 3 hours but no differences at 6 and 12 hours following the injection of ACTH. If the adrenal gland requires greater amounts of cholesterol for hormone synthesis it is not reflected in the plasma cholesterol. This may be due to the fact that the released adrenal

hormones in turn may stimulate an increased plasma cholesterol (27) and this action masks any increased cholesterol uptake that may occur in the gland. It might be significant to note that at six hours when serum cholesterol means of the control and treated groups were identical the lipoprotein means were also identical. This evidence, however meager, indicates a possible prepotent effect on the lipoproteins.

TABLE VIII

PLASMA AND ADRENAL CHOLESTEROL LEVELS SIX HOURS FOLLOWING ACTH INJECTION

Dose, IU	Plasma	Adrenal
0	(10) 65 ± 10[a]	(10) 2.88 ± 0.56
4	(10) 58 ± 16	(10) 2.38 ± 0.55
0	(4) 70 ± 4	(5) 3.15 ± 0.48
4	(9) 70 ± 15	(10) 2.68 ± 0.36*

* $P < 0.06$

[a] (No. of Birds) Mean ± standard deviation in mg. percent

TABLE IX

SERUM CHOLESTEROL LEVELS FOLLOWING ACTH INJECTION

Dose, IU	Hours after Injection 3	6	12
0	(10) 168 ± 26[a]	(10) 103 ± 19	(10) 144 ± 35
8	(10) 191 ± 25	(8) 102 ± 4	(10) 166 ± 44

[a] (No. of birds) Mean ± standard deviation in mg. percent

Adrenal Cholesterol - The adrenal cholesterol was lower in the injected birds 6 hours after ACTH (Table VIII). This is in agreement with the concept that the adrenal gland increases the utilization of this compound for hormone synthesis following stress or ACTH stimulation (93).

Adrenal Ascorbic Acid

All of the values for the adrenal ascorbic acid levels (6.5 - 52 mg. percent) are much lower than are reported in the literature (Table X). probably the main reason for this is that other tissues were first taken from all of the birds in the experiment before attention was given to the adrenals, consequently much of the ascorbic acid may have been lost. The results then at most are comparative.

TABLE X

ADRENAL ASCORBIC ACID LEVELS FOLLOWING ACTH INJECTION

Series	ACTH IU	Hours after ACTH Injection 3	6	12
1	0			(7) 6.5 ± 2.9[a]
	4			(10) 9.2 ± 4.8
8	0	(5) 56.1 ± 15.3	(8) 31.6 ± 32.9	
	8	(5) 58.0 ± 20.7	(8) 51.9 ± 16.8	

[a] (No. of birds) Mean ± standard deviation in mg.%

No significant differences were noted at any of the time intervals but there was a 60 percent difference in the means at 6 hours. This difference is in the direction of an increase in the injected bird rather than a decrease as would be expected if any change occurred. Perek and

Eckstein (74) found an adrenal ascorbic acid depletion in laying hens 1½ hours following the injection of ACTH. Slover (93) detected a decrease 12 hours after injection but not at 3 or 6 hours, and an adrenal ascorbic acid decrease was noted in mammals for from 1 - 12 hours (57).

No reason can be given for the seeming anomaly in the results reported here for adrenal ascorbic acid. It is thought by some that ascorbic acid may combine with adrenal steroids in peripheral tissues and that this combination inhibits the excretion of the steroids. Conversely, if the results reported here are real, there is the possibility that a similar combination may inhibit the destruction of any ascorbic acid in the adrenal gland and for this reason a larger concentration of measurable ascorbic acid remains in those birds that have been stimulated to release large amounts of steroids. Although no specific account was made of the delay in time between killing the bird and removal of the adrenal glands, the maximal time lapse for the three hour birds was 3½ hours, about 5 hours for those of the 6 hour interval and possibly as much as 8 hours for those at the 12 hour interval. For the concept that the adrenal steroids protect the ascorbic acid from being destroyed to be accepted however, a complex of the adrenal hormones with the acid would have to be demonstrated in the adrenal gland or in its effluent. Lowenstein and Zwemmer (77) suggested such a combination but Long (75) later discounted it and the idea has not been revived since. In rats the adrenal ascorbic acid depletion is followed by an increase that may reach as much as 50 percent of normal 12 hours following the injection of ACTH (57). There are no prior observations reported that might account for the increases reported here to be compared with those increases seen in the rat after ACTH injections.

Serum Proteins

There was a significant increase from 909 - 1025 mg. percent in the total serum protein concentration at three hours following the injection of ACTH (Table XI), but the apparent increases 6 and 12 hours following the injections were not significant. An increase in the albumin fraction accounted for the greater portion of the increase at 3 hours but not at the other two time intervals. The serum globulin means were increased slightly in the injected birds. ACTH has been shown to increase the number of lymphocytes in birds for as long as 12 hours (68). This increase may also be accompanied by an increased lymphocyte destruction as in mammals (26) and a concomittant increase in the serum gamma globulin fractions. Another and probably more important source for the increased protein is the liver. It is known that liver protein synthesis increases following ACTH or adrenal steroid injections (33). This may enable the liver to synthesize and release more albumins and alpha and beta globulins.

The serum protein concentrations reported here range from one-half to one-fifth the values that have been reported elsewhere for birds (94) and as such represent comparative rather than absolute values.

A comparison of the plasma electrophoretic patterns does not alter the protein picture to any great extent. The results which are expressed as the percentage of the total plasma proteins seem to indicate a definite trend only in the fibrinogen-gamma globulin fraction (Table XII). In each instance the percentage of this fraction is greater in the injected groups. To what extent the fibrinogen itself contributes to this increase cannot be determined from these curves but an explanation of the increase in the total globulin fraction seen in the results of the

TABLE XI

SERUM ALBUMIN, GLOBULIN AND TOTAL PROTEIN CONCENTRATIONS FOLLOWING ACTH INJECTION[a]

ACTH IU	Hours after ACTH Injection 3	6	12
		Albumin, mg.%	
0	504 ± 112	512 ± 80	601 ± 96
6	568 ± 51	501 ± 86	603 ± 74
0	405 ± 81	498 ± 145	388 ± 56
6	458 ± 94	530 ± 112	439 ± 84
0	909 ± 112	1010 ± 133	989 ± 98
6	1025 ± 84*	1031 ± 93	1053 ± 53

* $P<0.05$

[a] Values determined by means of biuret reaction. Eight to ten birds in each group

TABLE XII

THE PERCENTAGE COMPOSITION OF THE PLASMA PROTEINS SIX AND TWELVE HOURS FOLLOWING ACTH INJECTION

Dose, IU	Sex	Time	Albumin	$\alpha_1+\alpha_2$	β	$\phi+\gamma$	Unaccounted
0	M	6	25.1	32.2	12.9	18.1	11.7
4			18.0	28.5	18.0	22.6	12.9
0	F	6	17.5	34.5	29.9	12.4	5.7
4			24.9	27.7	26.0	15.6	5.8
0	M	12	23.6	45.5	15.5	9.1	6.3
4			22.8	42.1	11.2	14.2	9.7
0	F	12	13.7	46.2	17.1	12.0	5.8
4			13.9	51.3	12.2	13.9	11.0

biuret method would demand that the majority of the increase be due to the globulin component. The lack of agreement for the albumin fractions when the two methods were utilized may be due to the fact that some of the albumins in the electropheretic curves had lagged behind with the fast globulin fractions or were not fully developed on the electrophoretic strip. The reported range for albumin in birds is between 38 and 46 percent which is roughly 2 times the 14 - 25 percent range obtained on these curves.

Blood Cellular Responses

Hematocrit - The only significant alteration obtained in the hematocrit as a result of ACTH injections was a decrease from 32.0 to 29.6 percent packed red cells that occurred six hours following the injections (Table XIII, Series 6). Several other decreases in the means were observed in the injected birds when compared with the controls but there were also some increases noted. Significant decreases were noted, however, in series 1 between the 3-hour and the 12-hour counts in both the control and injected groups. During this 9 hour period the mean control hematocrit dropped from 34.7 to 27 (22 percent) and the injected hematocrit from 32.1 to 28.4 (12 percent) in one experiment while in the other experiment in series 1 the control hematocrit dropped from 33.1 to 27.9 (16 percent) and in the injected group from 32.1 to 29.6 (9 percent). In both of these experiments it is noteworthy that the mean hematocrit for the injected group was lower than the controls at 3 hours following ACTH injections but that the situation was reversed at the 12 hour interval. Sturkie and Newman (105) interpreted a similar decrease in the hematocrit to be due to an increased plasma volume which followed an

increase in the serum sodium. The results reported here indicate that the stress due to successive cardiac puncture reduced the hematocrit to a greater extent than did ACTH but that ACTH inhibited the large decrease that is seen in the control group.

TABLE XIII

HEMATOCRITS FOLLOWING ACTH INJECTION AND DURING CARDIAC PUNCTURE

Series	ACTH IU	Hours after ACTH Injection 3	6	12
1	0	(10) 43.7 ± 2.5[b]	(10) 26.0 ± 6.5	(10) 27.0 ± 3.6
	4	(10) 32.1 ± 2.0	(10) 30.4 ± 4.3	(10) 28.4 ± 2.6
	0	(10) 33.1 ± 2.4	(10) 30.5 ± 2.9	(10) 27.9 ± 5.1
	4	(10) 32.1 ± 1.7	(.0) 29.3 ± 1.6	(9) 29.6 ± 1.9
5[a]	0	(7) 26.1 ± 1.4	(7) 28.5 ± 2.1	(7) 27.5 ± 1.7
	6	(10) 27.1 ± 2.8	(9) 26.0 ±10.0	(10) 26.4 ± 1.4
6[a]	0		(8) 32.0 ± 2.6	(10) 32.3 ± 3.3
	8		(10) 29.6 ± 1.5*	(9) 31.7 ± 2.4

* $P < 0.05$

[a] Different groups of birds were utilized for each time period in this series

[b] (No. of birds) Mean ± standard deviation in mg. percent

Acidophils - In the first experiment (female birds) to determine the effect of ACTH injections on the acidophil count, no significant difference between the control and the injected groups were found until 12 hours following the injections (Table XIV). During this period of time the acidophil count in the injected group increased from 20.2 to 50.5 cells/ 0.009 mm^3. An increase in the acidophils of the control group also occurred but here the increase was from 18.4 to 35.7 cells. In the injected

male birds the acidophil count was always significantly higher (9 - 31 cells/0.009 mm^3) than in the corresponding control group, not withstanding the fact that the acidophil count of the control group increased from 17.9 to 41.8 cells/0.009 mm^3.

TABLE XIV

ACIDOPHIL COUNTS FOLLOWING ACTH INJECTIONS AND DURING REPEATED CARDIAC PUNCTURE

Series	Sex	Dose, IU			
1	F	0	(10) 18.4 ± 3.9[a]	(10) 38.6 ± 23.7	(10) 35.7 ± 10.6
		4	(7) 20.2 ± 2.0	(7) 51.2 ± 22.4	(7) 59.5 ± 30.0*
	M	0	(10) 17.9 ± 18.2	(10) 28.9 ± 12.6	(10) 41.8 ± 14.9
		4	(10) 27.1 ± 8.3*	(10) 45.9 ± 15.3*	(10) 73.6 ± 31.6**

* $P < 0.05$
** $P < 0.01$
a (No. of birds) Mean No. of Cells/0.009 mm^3 ± standard deviation. The same birds were used throughout one experiment.

The increased acidophil count observed in series I following ACTH injections and cardiac puncture have also been reported by others to occur following ACTH injections (68, 92) and restraint (68). An analysis of co-variance of the regression lines (Fig. 2) formed by the increased means obtained in the acidophil counts, indicates that ACTH increased the acidophil counts in both males ($P < 0.005$) and in females ($P < 0.03$). An analysis of the regression lines indicated that successive cardiac puncturing also increased the acidophil count ($P = 0.001$). Since hemodilution occurred simultaneously with the acidophilia the absolute count increased to a greater extent than suggested by the results shown in

Table XIV. The acidophilia probably is due mainly to an increase in the heterophils (68). This could mean that ACTH stimulation caused a discharge of heterophils either from the bone marrow or from peripheral "storehouses." If the former is true this would presume that the bone marrow maintains a great capacity for a sudden alteration of production on one particular cell type. Whether or not the bone possesses this potentiality is not known.

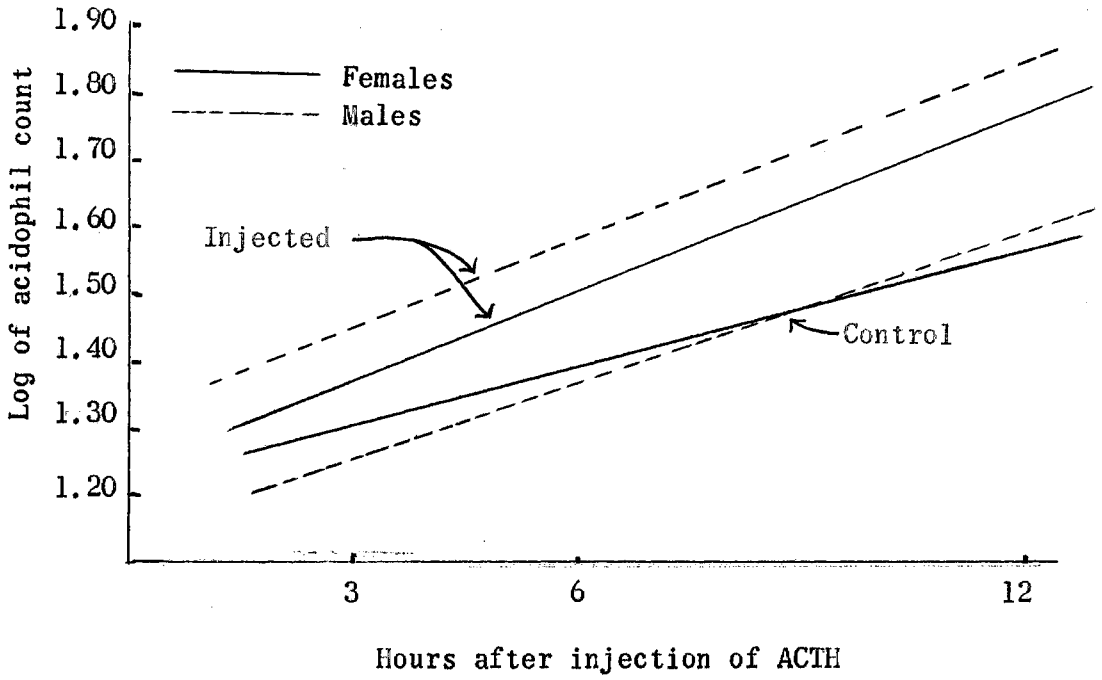

Figure 2. Log of acidophil counts in control and ACTH injected birds during 3 successive cardiac punctures

CHAPTER V

SUMMARY AND CONCLUSIONS

Six-week-old, Silver Oklabar chickens were injected with either 4, 6, or 8 IU ACTH in a commercial gelatin solution; control birds were injected with an equal volume of the gelatin solution. Blood and/or tissue samples were taken from the birds at 3, 6 or 12 hours following the injection and analyzed for various metabolic and blood cell parameters.

ACTH injection was followed by a 25 - 50 percent increase in the blood sugar level for as long as 12 hours after the injection. Simultaneously there was a non-significant increase in blood lactic acid levels and a 20 - 30 fold increase in the liver glycogen levels while the total skeletal muscle glycogen either did not differ or decreased as much as 30 percent below that of the control level. Most of the decrease was due to the TCA extractable fraction, which in series 3 decreased as much as 60 percent. The TCA fraction was reduced to a greater extent following injection of ACTH in birds autopsied during the summer months than in the winter months. Seasonal differences in the ratio (percentage) of the TCA fraction to the total glycogen were also found. The ratio of the TCA fraction to the total glycogen in those chickens autopsied during the summer ranged from 28 - 50 percent; a decrease of 16 - 22 percent was observed in those autopsied during the winter except in those birds that were fasted for longer than 12 hours wherein the range was from 11 - 41 percent. ACTH injection did not elicit a significant change in the leg muscle glycogen.

Along with the increased carbohydrate mobilization there was also an increase in the concentrations of the blood lipids and proteins. Injection of ACTH resulted in an increase of up to 15 percent in the serum lipoprotein and serum total fatty acid levels. In one experiment where both male and female birds were used, the males had more than a 10 percent higher serum lipid level (359 vs. 304 mg. percent) but the females responded to the ACTH injections with a greater relative increase than did the males.

Increased adrenal cortical activity in the treated birds was evidenced by a decrease of 16 percent (3.15 - 2.68 mg. percent) in the adrenal cholesterol, a precursor for corticoid synthesis, 6 hours after the injection of ACTH; blood cholesterol levels were not significantly altered by the ACTH injection.

An increase from 909 to 1025 mg. percent in the total serum protein occurred 3 hours after the injection of ACTH. Non-significant increases in the total serum protein levels were noted in the injected birds 6 and 12 hours following the injection of ACTH. Except for the 3-hour period where albumin was responsible for the major portion of the total protein increase, the globulin fractions accounted for all of the increases noted. Analysis of the electrophoretic patterns showed that the fibrinogen plus gamma globulin fraction of the plasma proteins was increased at 6 and 12 hours following the injection of ACTH. The $alpha_1$ plus $alpha_2$ globulin fraction decreased about 4 percent in the treated birds during this same time.

Non-significant increases of 2 - 20 mg. percent were found in the adrenal ascorbic acid concentrations following the injection of ACTH. Because of the time interval that elapsed between the death of the birds

and the removal of their adrenal glands it was suggested that the increased level might be due to the prevention of the destruction of ascorbic acid by the higher concentrations of adrenal cortical hormones in the ACTH injected birds.

ACTH injection was followed by a 2 - 7 percent decrease in the hematocrit. Three, repeated cardiac punctures reduced the hematocrit 16 - 22 percent in the control birds but only 9 - 12 percent in the treated birds.

The acidophil counts were increased by both ACTH injection and by repeated cardiac puncture. The counts in the control birds increased from 18 cells/0.009 mm^3 at 3 hours to more than 35 cells/0.009 mm^3 at 12 hours. The acidophil counts in the ACTH injected birds were 2 - 30 cells/ 0.009 mm^3 higher than those of the control birds at the corresponding time interval.

The results reported here show that the injection of ACTH, by way of increased adrenal cortical stimulation, was followed by increases in the concentrations of glucose, lipids and, to a lesser degree, of proteins in the blood. Simultaneously the body's energy stores were mobilized to the liver at the expense of those stores in the skeletal muscle.

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A P P E N D I X

Series 1

HEMATOCRIT AND ACIDOPHIL COUNTS OF BLOOD OBTAINED BY CARDIAC PUNCTURE FROM CONTROL AND ACTH# INJECTED 6-WEEK-OLD PULLETS

Bird	Hematocrit			Acidophils"		
Control	3 Hr.[a]	6 Hr.	12 Hr.	3 Hr.	6 Hr.	12 Hr.
19301	36.2	24.1	21.7	12.0	34.0	50.0
19305	25.0	31.6	27.2	24.5	47.0	49.0
19306	34.0	20.9	21.0	16.0	8.0	28.0
19308	35.8	19.0	30.4	11.0	16.0	26.0
19314	39.0	13.5	33.0	24.0	72.0	40.0
19315	35.1	34.9	28.0	21.0	43.5	48.0
19318	36.6	31.0	28.0	22.5	28.0	28.0
19594	31.2	28.0	26.0	16.5	32.5	35.0
19595	33.5	30.4	28.7	22.0	83.0	33.0
19598	30.5	27.0	25.8	14.5	22.0	20.0
Average	34.7	26.0	27.0	18.4	28.6	35.7
S.D.	2.53	6.54	3.63	3.87	23.7	10.6
Experimental						
19303	31.3	30.0	33.0	19.0	89.5	55.5
19310	31.3	28.0	27.0	20.0	45.0	47.0
19311	32.0	30.6	30.9	34.0	21.0	50.5
19312	33.5	32.6	28.1	18.0	50.0	35.0
19316	33.2	24.9	26.6	23.5	71.0	124.0
19317	28.1	30.1	26.8	19.0	40.0	67.0
19319	31.5	28.0	27.0	9.0	42.0	38.0
19591	32.0	26.0	28.0			
19592	31.9	39.8	25.0			
19593	36.4	33.8	32.0			
Average	32.1	30.4	28.4	20.2	51.2	59.5*
S.D.	1.95	4.29	2.62	7.38	22.4	30.1

* $P < 0.05$, compared to control at same time interval
\# 4 IU ACTH
" Acidophils reported as cells/0.009 mm^3
[a] Hours after injection of ACTH

Series 1

HEMATOCRIT AND ACIDOPHIL COUNTS OF BLOOD OBTAINED BY CARDIAC PUNCTURE FROM CONTROL AND ACTH# INJECTED 6-WEEK-OLD COCKERELS

Bird	Hematocrit			Acidophil"		
Control	3 Hr.[a]	6 Hr.	12 Hr.	3 Hr.	6 Hr.	12 Hr.
19535	37.0	36.6	32.4	23.0	26.0	53.0
19536	33.3	31.0	28.9	30.0	25.0	40.0
19540	30.0	25.3	24.5	14.0	30.0	39.0
19541	33.3	32.5	33.0	9.5	23.0	36.0
19542	30.0	30.5	25.0	20.0	59.0	71.5
19547	33.5	29.8	31.0	17.0	17.5	52.0
19548	35.2	32.0	33.0	19.0	34.0	37.5
19549	32.0	29.5	30.2	12.5	14.5	15.0
19554	30.0	28.4	18.2	17.5	37.0	32.0
19558	35.7	29.1	22.5	16.0	23.0	42.0
Average	33.1	30.5	27.9	17.9	28.9	41.8
S.D.	2.40	2.95	5.07	18.2	12.6	14.9
Experimental						
19531	30.5	26.0	28.4	21.0	35.0	71.0
19533	29.6	29.8	27.6	24.0	36.5	55.0
19544	30.0	31.5	29.0	23.5	56.0	47.0
19545	32.0	29.6	30.4	35.0	47.0	94.0
19546	29.7	27.2	27.5	19.0	39.0	50.0
19550	32.0	29.2		24.0	40.0	153.0
19551	33.0	29.9	29.2	47.0	61.0	80.0
19557	33.7	29.5	30.0	25.5	28.0	70.0
19559	34.0	30.0	32.6	29.0	79.0	65.0
19560	33.7	30.4	32.3	23.0	37.0	50.0
Average	32.1	29.3	29.6	27.1*	45.9*	73.6**
S.D.	1.66	1.58	1.85	8.3	15.3	31.6

* $P < 0.05$, compared to control at same time interval
** $P < 0.01$, compared to control at same time interval
\# 4 IU ACTH
" Acidophils reported as cell/0.009 mm^3
a Hours after injection of ACTH

Series 1

BLOOD AND ADRENAL CHOLESTEROL LEVELS OF CONTROL AND ACTH# INJECTED 6-WEEK-OLD PULLETS 6 HOURS AFTER INJECTION

Control			Experimental		
Bird No.	Blood mg.%	Adrenal gm.%	Bird No.	Blood mg.%	Adrenal gm.%
19304	72	3.96	19561	70	2.75
19307	66	3.06	19562	68	2.24
19309	60	2.51	19564	56	3.20
19320	63	3.10	19573	68	1.87
19567	66	1.90	19577	70	1.95
19569	70	2.34	19578	24	2.52
19572	70	2.87	19582	60	3.14
19587	83	3.13	19585	63	2.54
19589	56	3.18	19586	34	2.00
19600	44	2.76	19588	63	1.58
Average	65	2.88	Average	57.6	2.38
S.D.	10.4	.56	S.D.	15.9	.55

4 IU ACTH

Series 1

BLOOD AND ADRENAL CHOLESTEROL LEVELS OF CONTROL AND ACTH# INJECTED 6-WEEK-OLD COCKERELS 6 HOURS AFTER INJECTION

Control			Experimental		
Bird No.	Blood mg.%	Adrenal gm.%	Bird No.	Blood mg.%	Adrenal gm.%
19503	74	3.89	19502	72	2.86
19509	68	2.56	19504	70	2.60
19510		3.13	19507	79	3.35
19514	70	2.98	19512	104	2.76
19525	66	3.18	19515	66	2.57
			19519	64	2.21
			19521	58	2.43
			19522	52	2.98
			19526		2.15
			19530	68	2.86
Average	69.5	3.15	Average	70.3	2.68*
S.D.	3.5	.48	S.D.	14.8	.36

* P = 0.06
# 4 IU ACTH

Series 1

ADRENAL ASCORBIC ACID CONCENTRATIONS OF CONTROL AND ACTH# INJECTED WEEK OLD COCKERELS 12 HOURS AFTER INJECTION

Control		Experimental	
Bird	Ascorbic Acid, mg.%	Bird	Ascorbic Acid, mg.%
19590	9.309	19313	8.605
19596	6.867	19565	5.5749
19563	3.704	19566	9.524
19576	1.961	19568	12.463
19579	9.816	19571	18.657
19580	5.614	19574	2.830
19583	8.226	19575	3.754
		19584	13.609
		19597	9.051
		19599	7.233
Average	6.498		9.170
S.D.	2.900		4.800

4 IU ACTH

Series 2

LIVER GLYCOGEN AND BREAST MUSCLE GLYCOGEN OF CONTROL AND ACTH# INJECTED 6-WEEK-OLD COCKERELS 12 HOURS AFTER INJECTION

Bird No.	Liver Glycogen gm.%	Muscle Glycogen TCA mg.%	KOH mg.%	Total mg.%
Control				
84	3.9741"	42.8	189.8	232.6
168	4.7456	21.4	77.0	98.3
105	1.3066	28.7	103.8	132.6
165		87.4	233.1	320.5
97		34.6	179.1	263.8
149		28.7	128.0	156.7
155		32.2	115.8	148.0
4630		26.7	152.2	178.9
139	6.7500	41.7	141.4	183.1
79	5.3469	66.1	115.4	181.4
Average	4.425	41.0	143.6	189.6
S.D.	2.078	20.5	46.3	68.9
Experimental				
125	4.5930	48.5	117.2	219.6
4632		95.9	242.5	338.4
77		19.6	102.4	121.9
136		10.2	92.2	102.4
4633	2.2824	41.0	164.0	205.0
101		5.4	53.8	59.2
174		7.5	66.3	74.0
122		54.6	157.9	212.5
123	3.0015	69.7	194.6	264.3
162	6.6142	44.4	171.8	245.7
Average	4.123	39.7	141.7	184.3
S.D.	1.663	29.5	60.4	28.8

4 IU ACTH, groups autopsied in April

Series 2

LIVER GLYCOGEN AND BREASE MUSCLE GLYCOGEN OF CONTROL AND ACTH# INJECTED 6-WEEK-OLD PULLETS 12 HOURS AFTER INJECTION

Bird No.	Liver Glycogen gm.%	Muscle Glycogen TCA mg.%	KOH mg.%	Total mg.%
Control				
126	0.950	29.9	83.9	113.8
166	1.474	43.1	219.5	262.6
143	0.712	26.2	104.1	130.3
4628	1.007	17.0	124.3	141.3
130	1.386	43.6	174.8	218.4
110	0.166	18.9	63.8	82.7
86	1.758	56.2	159.9	216.1
113	1.029	3.8	88.7	92.5
106	0.992	17.4	130.5	147.9
146	2.234	87.2	150.1	237.3
Average	1.171	34.3	130.0	164.3
S.D.	0.547	24.2	47.4	64.0
Experimental				
127		55.7	208.8	264.5
175				
173	4.608	27.9	131.7	159.6
150		16.9	143.8	160.7
164	4.272	10.7	86.4	97.1
109	1.757	28.7	131.0	159.7
147		15.0	101.6	116.6
4626	5.933	27.0	144.8	171.8
4627		12.3	84.2	96.5
159	4.656	49.0	213.9	262.9
Average	4.245**	27.0	138.5	165.5
S.D.	1.528	16.0	47.7	62.5

** $P < 0.01$

4 IU ACTH, groups autopsied in April

Series 2

LIVER GLYCOGEN AND BREAST MUSCLE GLYCOGEN OF CONTROL AND ACTH[#] INJECTED 6-WEEK-OLD CHICKENS 12 HOURS AFTER INJECTION

Bird No.	Liver Glycogen gm.%	Muscle Glycogen TCA mg.%	KOH mg.%	Total mg.%
Control				
132	.028	5.3	80.2	85.7
172	.033	33.3	147.8	181.1
92		21.2	111.8	113.0
4631	.027	22.8	147.5	170.3
104	.39	22.9	168.8	191.7
83	.016	40.6	155.2	195.8
124	.032	26.6	130.6	157.2
167				
118	.021	26.0	145.2	171.2
4629	.015	11.8	128.8	140.6
Average	.026	23.4	135.1	158.5
S.D.	.009	10.5	26.4	32.7
Experimental				
157	2.036	36.1	145.8	181.9
134	.853	34.3	154.3	188.6
133	.698	19.8	134.2	154.0
78	1.103	12.7	84.4	97.1
135	1.212	24.7	100.3	125.0
115	2.151	23.6	108.6	132.2
161	1.727	89.2	204.9	294.1
152	.548	19.7	157.6	177.3
154	.706	13.8	157.1	170.9
121			79.8	
Average	1.226**	30.4	132.7	169.0
S.D.	.570	23.5	39.2	55.9

** $P < 0.01$

[#] 4 IU ACTH, groups autopsied in April

Series 3

BLOOD GLUCOSE, LIVER GLYCOGEN AND BREAST MUSCLE GLYCOGEN OF CONTROL AND ACTH# INJECTED 6-WEEK-OLD COCKERELS 6 HOURS AFTER INJECTION

Bird No.	Blood Glucose mg.%	Liver Glycogen gm.%	Muscle Glycogen TCA mg.%	KOH mg.%	Total mg.%
Control					
Z96-22	68	0.555	27.7	27.7	55.4
Z96-14	140	0.039	7.5	16.8	24.3
No Band	146	1.791	13.3	30.0	43.3
Z96-15	118	0.043	10.4	13.3	23.6
Z96-18	152	0.063	6.5	13.7	20.2
Z96-16	164	0.036	9.8	23.6	33.4
Z96-19	158	0.013	5.1	25.0	30.1
Z96- 7	146	0.041	11.5	17.9	29.4
Z96-15	146	1.278	7.7	36.4	44.1
Z96- 1	102	0.034	12.0	38.6	50.6
Z96- 7	124	0.039	6.7	47.4	54.1
Z96- 3	140	0.052	15.7	22.7	38.4
Average	133.5	0.332	11.2	26.1	37.3
S.D.	27	0.591	6.1	10.5	12.2
Experimental					
Z96-24	152	3.670	8.7	45.3	54.0
Z96-13	108	3.033	9.2	17.3	26.5
Z96- 5	172	1.200	9.7	31.9	41.6
Z96-17	184	1.885	5.8	15.1	20.9
Z96-20	244	1.371	9.5	14.5	23.9
Z96-23	164	1.857	7.1	14.2	21.2
Z96-19	114	0.051	6.6	15.7	22.3
Z96-21	120	0.029	36.7	36.7	73.4
Z97- 8	118	3.391	8.3	17.2	25.5
Z97-12	152	1.479	11.0	43.3	54.4
Z97-11	282	2.384	00.0	31.4	31.4
Z97- 6	202	3.022	5.0	25.5	30.6
Average	167.8	1.864**	9.8	25.7	32.5
S.D.	54	1.158	8.9	11.7	16.8

** $P < 0.01$

6 IU ACTH, groups autopsied in June

Series 3

BLOOD GLUCOSE, LIVER GLYCOGEN AND BREAST MUSCLE GLYCOGEN OF CONTROL AND ACTH# INJECTED 6-WEEK-OLD COCKERELS 12 HOURS AFTER INJECTION

Bird No.	Blood Glucose mg.%	Liver Glycogen gm.%	Muscle Glycogen TCA mg.%	Muscle Glycogen KOH mg.%	Muscle Glycogen Total mg.%
Control					
Z96- 3	196	.071	9.9	13.1	23.0
Z96- 2	158	.031	26.0	20.0	46.0
Z96- 1	120	.007	7.7	16.2	23.9
Z98-19	158		22.8	14.3	37.1
Z98-16	140	.018	9.9	20.5	30.4
Z98-10	140	.022	6.7	24.7	31.4
Z98- 9	152	.048	26.6	26.6	53.1
Z98- 2	152	.030	10.8	32.5	43.3
Z98-13	152	.029	10.6	20.6	31.1
Z98- 5	146	.029		21.0	21.0
Z98-21	158	.036	28.0	21.0	48.9
Z98- 6	98	.020	26.0	23.4	49.4
Average	139.3	.031	15.4	21.2	36.4
S.D.	23.5	.017	9.7	5.3	11.3
Experimental					
Z96-12	102	.235	2.2	20.2	22.4
Z96- 6	155	1.274	8.3	21.8	30.0
Z98-23	158	.003			25.7
No Band	164	4.255	22.1	24.6	36.7
Z98-25	190	.159	16.4	20.7	37.1
Z96-10	202	3.078	18.9	112.9	131.7
Z96- 9	68	1.376	22.5	17.9	40.4
Z96-11	50	.255	14.3	15.1	29.4
Z960 8	164	2.481	12.2	24.4	36.6
Z96- 5	124	.970	19.1	38.3	57.4
Z96- 4	124	2.488	12.9	14.8	27.7
Z96- 7	178	1.210	22.2	25.1	47.3
Average	138.3	1.482**	15.6	30.5	44.4
S.D.	36.6	1.336	6.4	28.1	28.1

** $P < 0.01$
# 6 IU ACTH, groups autopsied in June

Series 3

LIVER GLYCOGEN AND BREAST MUSCLE GLYCOGEN OF CONTROL AND ACTH# INJECTED 6-WEEK-OLD CHICKENS 3 HOURS AFTER INJECTION

Bird No.	Liver Glycogen gm.%	Muscle Glycogen TCA mg.%	KOH mg.%	Total mg.%
Control				
Z 98-17	.0173			
Z 98-18	.0546	18.8	10.0	28.8
Z 98- 4	.0184	26.4	26.4	52.7
Z100-17	.0427	21.6	18.4	40.0
Z100-19		12.9	8.9	21.7
Average	.033	20.0	16.	36.
S.D.	.018	6.0	10.	14.
Experimental				
Z 98-22	1.6960	3.5	16.8	20.3
Z 98-14	.1224	8.0	8.0	15.9
Z 98-20	.3181	4.7	16.9	21.5
Z100- 4	.0700	11.0	17.1	28.1
z100-11	.8741	5.6	26.9	32.4
Average	.616	7.0**	17.0	24.0
S.D.	.682	2.0	7.	7.

** $P < 0.01$

6 IU ACTH, groups autopsied in June

Series 3

BLOOD GLUCOSE, LIVER GLYCOGEN, AND BREAST MUSCLE GLYCOGEN OF CONTROL AND ACTH# INJECTED 6-WEEK-OLD COCKERELS 6 HOURS AFTER INJECTION

Bird No.	Blood Glucose mg.%	Liver Glycogen gm.%	Muscle Glycogen TCA mg.%	KOH mg.%	Total mg.%
Control					
Z100- 9	158	.027			
Z100-18	120	.027	20.5	17.6	38.1
Z100-21	82	.025	23.6	23.6	47.3
Z100- 5	172	.045	3.1	7.8	10.9
Z100- 6	143	.029			
Z100- 8	98	.040			
No Band 4	114	.009	19.4	19.7	39.5
Z100-15	164	.031	4.6	10.0	14.6
Z100-12	172	.018	4.9	10.9	15.8
Z100-20	78	.058	4.8	18.6	23.4
Z100- 7	134	.027	8.3	14.7	22.9
Z100-1	124	.033	4.7	11.7	16.4
Average	129.9	.031	10.5	15.0	25.4
S.D.	33	.012	8.	5.	13.
Experimental					
Z 97- 4	108	.450			
Z 99- 1	158	1.328			
Z 97- 2	258	.249	4.0	7.4	11.4
Z 99- 3	228	1.840			
Z 99- 5	216	1.388	1.9	9.5	11.4
Z 99- 8	236	1.310			
Z 99-10	164	1.749	10.9	6.1	17.0
Z 97- 1	244	2.310	1.8	18.3	20.1
Z100-13	172	.022	.0	8.6	8.6
Z100- 3	158	.305	3.6	17.2	20.8
Z100-16	222	.535	3.7	12.4	16.2
Z 99- 4	250	.769	2.1	7.7	9.9
Average	202.2**	1.022**	3.5*	10.9	14.4*
S.D.	47	.733	3.	5.	5.

* $P < 0.05$
** $P < 0.01$
\# 6 IU ACTH, groups autopsied in June

Series 3

BLOOD GLUCOSE, LIVER GLYCOGEN AND BREAST MUSCLE GLYCOGEN OF CONTROL AND ACTH# INJECTED 6-WEEK-OLD PULLETS 12 HOURS AFTER INJECTION

Bird No.	Blood Glucose mg.%	Liver Glycogen gm.%	Muscle Glycogen TCA mg.%	KOH mg.%	Total mg.%
Control					
Z99-24	134	.017	2.4	22.9	25.3
Z99-17	172	.024			
Z99-14	128	.047	6.9	5.2	11.0
Z99-16	134		10.0	17.7	27.7
Z99-18	134	.024			
Z99- 6	41	.016	16.2	16.2	32.4
Z99-11	152	.025	9.0	12.2	21.2
Z99- 9	128	.046			
Z99- 2	94	.025			
Z99- 3	128	.392	4.0	13.2	17.2
Z99- 7	125	.017			
Z99-12	164	.037	5.7	7.8	13.5
Average	127.8	.061	7.75	13.6	21.3
S.D.	34	.110	5.	6.	8.
Experimental					
Z100-24	161	3.640	7.7	10.3	18.0
Z100-23	184	3.516	4.8	4.8	9.6
Z 99-22	140	.015	0.0	12.4	12.4
Z 99-19	146	.023	4.3	12.3	16.6
Z 99-20	114	.046	4.6	11.5	16.1
Z 99-23	60	.025	10.3	14.9	25.2
Z100-22	102	.027	12.8	20.9	33.6
Z100-25	41	1.188	14.8	19.3	34.0
No Band 3	202	2.557			
Z 99-15	128	.034	5.1	14.1	19.9
Z 99-21	172	.634	12.5	15.0	27.5
Z 99-13	140	1.752	4.7	6.3	11.0
Average	132.5	1.121*	7.4	12.95	20.36
S.D.	46	1.41	5.	5.	9.

* $P < 0.05$
\# 6 IU ACTH, groups autopsied in June

Series 4

BLOOD GLUCOSE, SERUM LIPOPROTEINS, LIVER GLYCOGEN AND MUSCLE GLYCOGEN OF CONTROL AND ACTH# INJECTED 6-WEEK-OLD COCKERELS 3 HOURS AFTER INJECTION

Bird No.	Blood Glucose	Lipoproteins	Liver Glycogen	Breast Muscle Glycogen TCA	Breast Muscle Glycogen KOH	Breast Muscle Glycogen Total	Leg Muscle Glycogen TCA	Leg Muscle Glycogen KOH	Leg Muscle Glycogen Total
Control									
9179	222"	445	0.061	9	266	275	17	406	423
9195	202	445	0.413	14	351	365	5	114	119
9183	196	347	0.070	69	239	308	7	258	265
9188	222	391	0.203	34	81	115	7	144	151
9192	216	347	0.254	182	96	278	6	218	224
9197	190	293	0.052	36	309	345			
9193	196	347	0.037				6	33	38
9166	182		0.063	74	398	472	10	72	82
9163	212	347	1.688	30	186	216	6	184	190
9146	202	347	0.117	28	192	220			
Average	204	367	0.296	53	235	288	8	179	187
S.D.	13.6	50.2	0.5	54	108	102	4	118	120
Experimental									
9187	314	347	1.842	148	92	240	8	58	66
9186	250	347	0.561	39	326	365	8	79	87
9181	237	293	0.539	17	118	135	11	173	184
9198	280	553	0.998		235		9	266	277
9196	280	445	2.209	63	331	394	97	72	169
9001	296	445	1.190	23	224	247	5	266	271
9170	250		0.553	111	290	401	15	170	185
9175	224	391	1.022	10	507	517	30	306	336
9173	304		3.068	21	285	306	15	176	191
9165	296		1.011	36	167	203	23	172	195
Average	273**	403	1.299**	53	258	314	22	179	196
S.D.	30.8	86	0.83	47	120	118	27	86	83

** $P < 0.01$

6 IU ACTH, groups autopsied in November

" Liver glycogen in gm. percent, all other values in mg. percent

Series 4

BLOOD GLUCOSE, SERUM LIPOPROTEINS, LIVER GLYCOGEN AND MUSCLE GLYCOGEN OF CONTROL AND ACTH# INJECTED 6-WEEK-OLD COCKERELS 6 HOURS AFTER INJECTION

Bird No.	Blood Glucose	Lipoproteins	Liver Glycogen	Breast Muscle Glycogen TCA	Breast Muscle Glycogen KOH	Breast Muscle Glycogen Total	Leg Muscle Glycogen TCA	Leg Muscle Glycogen KOH	Leg Muscle Glycogen Total
Control									
9105	202"	203	.078	16	350	366	7	154	161
9156	230	281	.256	22	363	385	5	187	192
9104	202	281	.060				8	143	151
9123	230	203	.467	81	527	608	13	146	159
9121	196	281	.137	18	151	169	7	260	267
9144	216	422	.047	22	122	144	9	163	172
9127	208	281	.051	51	207	258	3	234	237
9137	222	344	.034	10	382	392	5	197	202
9149	196	344	.047	12	76	88	8	110	118
9132	207		.057	312	55	367	8	58	66
Average	211	293	.0124	61	248	309	7	165	172
S.D.	13	69	.135	97	163	161	26	58	57
Experimental									
9172	414	563	2.381	18	412	430	8	227	235
9155	360	563	1.755	15	162	177	3	275	278
9119	402	500	5.900	185	106	291	8	222	230
9158	242	718	2.492	8	297	305	6	56	62
9128	310	563	0.940	12	86	98	19	277	296
9145	280	344	0.307	6	82	88	5	179	184
9176	414	500	3.051	8	287	295	6	190	196
9185	360	563	2.313	78	227	305	2	79	81
9200	196	500	2.315	18	125	143	8	330	338
9177	332	563	2.782	54	212	266	38	296	334
Average	331**	537**	2.424**	40	200	240	10	213	223
S.D.	74	93	1.483	56	108	109	11	90	96

** $P < 0.01$
# 6 IU ACTH, groups autopsied in November
" Liver glycogen in gm. percent, all other values in mg. percent

Series 4

BLOOD GLUCOSE, SERUM LIPOPROTEINS, LIVER GLYCOGEN AND MUSCLE GLYCOGEN OF CONTROL AND ACTH# INJECTED 6-WEEK-OLD COCKERELS 12 HOURS AFTER INJECTION

Bird No.	Blood Glucose	Lipo-Proteins	Liver Glycogen	Breast Muscle Glycogen TCA	Breast Muscle Glycogen KOH	Breast Muscle Glycogen Total	Leg Muscle Glycogen TCA	Leg Muscle Glycogen KOH	Leg Muscle Glycogen Total
Control									
9107	228"	391	.989	26	501	527	22	239	261
9106	153	347	1.075	158	52	210	8	278	286
9117	250	445	2.694	32	157	189	17	225	352
9115		391	.925					266	
9103	211	391	2.744	81	408	489	7	235	242
9120		445	2.093						
9113	178	347	2.321	84	660	744	12	230	242
9152	242		1.904	71	247	318	80	270	350
9102	216		1.721	18	57	75	15	280	295
9164	150	553	2.948	75	249	324	22	250	272
Average	203.5	414	1.941	68	292	360	22	253	288
S.D.	39	67	.678	45	216	216	24	43	43
Experimental									
9125	254		4.920	26	161	182	10	167	178
9124	269	662	4.640	25	167	192	24	242	266
9168			4.500	20	303	323	24	230	254
9111	346	609	4.118	63	224	287	8	124	132
9122	300	608	5.123	83	347	430	11	287	298
9112	262	608	5.649	60	203	263	9	376	385
9101	276	608	2.562	46	171	217	19	168	187
9108	365		4.382	141	83	224	2	138	140
9109	266	500	3.374	32	63	95	34	251	285
9157	248		4.299	33	172	205		389	
Average	287.3**	599	4.457**	53	189	242	16	237	236
S.D.	41.6	53	0.903	37	88	91	10	93	83

** $P < 0.01$

6 IU ACTH, groups autopsied in November

" Liver glycogen in gm. percent, all other values in mg. percent

Series 5

HEMATOCRIT AND SERUM PROTEIN LEVELS OF CONTROL AND ACTH# INJECTED 6-WEEK-OLD COCKERELS

Control Groups					Treatment Groups (6 IU ACTH)				
Birds	Hem.[a]	Alb.[b]"	Glob.[c]	Total[d]	Bird	Hem.	Alb.	Glob.	Total
Three Hours									
21		596	554	1150	31	30			
22	24	470	344	814	32	32	596	504	1100
23		512	396	908	33	27	554	646	1200
24	27	554	446	1000	34	25	470	488	958
25	27	470	488	958	35	28	554	404	958
26		344	428	772	36	26	596	362	958
27	26	512	302	814	37	25	596	362	958
28	25	428	428	856	38	26	613	412	1050
29	27	596	362	958	39	25	596	404	1000
30	27	554	302	856	40		512	538	1050
Ave.	26	504	405	908		27	568	458	1026*
Six Hours									
41	29	428	530	958	51	31	554	496	1050
42	30	470	580	1050	52	28	596	628	1224
43	30	596	454	1050	53	24	512	446	958
44	28	554	260	814	54	28			
45	27	470	780	1250	55	30	512	488	1000
46	27	596	404	1000	56	29	470	488	598
47	30	596	554	1150	57	30			
48	32				58		428	530	958
49	27	512	396	908	59	30	344	756	1100
50	25	386	522	908	60	30	596	404	1000
Ave.	29	512	498	1010		29	502	530	1031
Twelve Hours									
1	28	596	504	1100	11	29	620	456	1076
2	25	638	218	856	12	28	722	278	1100
3	27	638	488	1126	13	25	512	538	1050
4	30	554	496	1050	14	26	638	362	1000
5	27	512	396	908	15	26			1150
6	27	512	328	840	16	26	470	580	1050
7	29	814	186	1000	17	25	596	404	1000
8		554	496	1050	18	28	638	368	1000
9		512	446	958	19	25	638	362	1000
10		680	320	1000	20	26	596	504	1100
Ave.	27	601	388	989		26	603	439	1053

* P = 0.05 compared to control mean at 3 hours
\# 6 IU ACTH, (Hormone was 6 months outdated)
" All protein values are in gm. percent; values to nearest whole number
[a] Hematocrit
[b] Albumin
[c] Globulin
[d] Total Protein

Series 6

BLOOD LACTIC ACID, SERUM TOTAL FATTY ACID, LIPOPROTEINS AND CHOLESTEROL ADRENAL ASCORBIC ACID AND BREAST MUSCLE GLYCOGEN OF CONTROL AND ACTH# INJECTED 6-WEEK-OLD CHICKENS 3 HOURS AFTER INJECTION

Bird	Sex	Lactic Acid	Serum TFA[a]	Serum Lip-p[b]	Serum Chol.[c]	Serum A.A.[d]	Muscle Glycogen TCA	Muscle Glycogen Total
Control								
51	M		1.242"	370	180	46.8	79.0	
51	F	19.7	1.187	345	185		140.1	466.1
53	F	39.4	0.976	290	152		44.0	
54	F	33.0	1.098	310	156		131.2	380.0
55	F	59.8	1.054	300	147		168.1	
56	M	33.0	1.131	345	156	63.0	31.3	227.7
57	M	45.4	1.131	345	156	59.5	110.0	408.5
58	F	36.9	0.932	275	143		2.0	
59	M	35.9	1.375	400	231	75.4	97.3	286.9
60	M		1.198	335	180	35.5	89.5	
Average		37.9	1.132	331.5	168.6	56.1	89.3	353.8
S.D.		11.5	0.129	38.2	26.4	15.3	51.9	64.5
Experimental								
41	M		1.475	355	196	80.0	67.8	163.7
42	M	61.8	1.164	345	166	47.7	71.3	349.7
43	M	66.1	1.275	370	180	77.0	35.0	380.0
44	F	35.9	1.342	290	176		46.5	211.3
45	F	40.0	1.309	345	191		70.9	
46	F	36.9	1.187	345	171		49.3	273.5
47	M	51.5	1.586	435	219	54.6	64.8	265.6
48	F		1.597	380	213		5.0	
49	F	31.3	1.164	310	166		4.5	
50	M	38.8	1.619	400	238	30.5	133.2	
Average		45.3	1.37**	357.5	191.5	58.0	54.8	274.0
S.D.		12.9	0.183	41.8	24.7	20.7	37.1	81.4

** P = 0.01 compared to control mean
# 8 IU ACTH, groups autopsied in February
" TFA in meq/100 cc., all other values in mg. percent
[a] Total fatty acids
[b] Lipoproteins
[c] Cholesterol
[d] Adrenal ascorbic acid

Series 6

BLOOD LACTIC ACID AND HEMATOCRIT, SERUM TOTAL FATTY ACIDS, LIPOPROTEIN AND CHOLESTEROL, ADRENAL ASCORBIC ACID AND BREAST MUSCLE GLYCOGEN OF CONTROL AND ACTH# INJECTED 6-WEEK-OLD COCKERELS 6 HOURS AFTER INJECTION

Bird	Lactic Acid	Hemat-ocrit	TFA[a]	Serum Lip-p[b]	Chol.[c]	Adr. A.A.[d]	Muscle Glycogen TCA	Total
Control								
11	20.9"	34	1.009	403	118	5.4	82.7	624.0
12	30.7	31	0.866	334	88	109.4	171.5	593.8
13	15.5		0.999	357	98	23.8	98.2	644.0
14	40.6	28	0.932	345	104		163.9	282.7
15	24.4	34	1.009	391	104	23.4	16.3	627.8
16	39.7	31	0.999	345	95	17.8	61.9	461.2
17	23.2		0.886	345	85	38.9	13.7	720.8
18	34.6	35	1.299	541	148	13.3	9.6	441.5
19	28.4	29	0.966	311	88	21.1	17.3	685.3
20	34.6	34	1.052	357	107		13.1	600.0
Average	29.3	32	1.002	373	103	31.6	64.8	568.1
S.D.	8.3	2.6	0.120	64	18.6	32.9	62.9	133.4
Experimental								
1	29.9	32	0.986	403	107	43.3	99.6	570.6
2	25.5	30	1.132	403	98	43.3	127.9	848.9
3	37.7	27		426		30.0	12.7	548.7
4	36.8		1.109	403	104	44.6	15.9	303.9
5	36.3			288		77.6	17.8	691.9
6	45.9	31	1.332	368	98	61.9	133.8	768.3
7	37.0	30	1.166	380	107	45.9	121.5	712.5
8	23.2	28	1.052	368	104	39.2	96.3	651.9
9	21.1	29	1.185	334	101	72.6	87.2	460.5
10	44.9	30	1.299	345	98		147.1	460.5
Average	33.8	29.6	1.158	372	102	51.9	85.9	601.8
S.D.	8.6	1.5	0.110	41	4	15.8	51.8	164.7

* $P < 0.05$
\# 8 IU ACTH, groups autopsied in January
" Hematocrit in percent, TFA in meq/100 cc, all other values in mg. percent
[a] Total fatty acids
[b] Lipoproteins
[c] Cholesterol
[d] Adrenal ascorbic acid

Series 6

BLOOD LACTIC ACID AND HEMATOCRIT, SERUM TOTAL FATTY ACIDS, LIPOPROTEIN AND CHOLESTEROL AND BREAST MUSCLE GLYCOGEN OF CONTROL AND ACTH# INJECTED 6-WEEK OLD COCKERELS 12 HOURS AFTER INJECTION

Birds	Lactic Acid	Hemat-ocrit	Serum TFA[a]	Serum Lip-p[b]	Serum Chol.[c]	Muscle Glycogen TCA	Muscle Glycogen Total
Control							
21	11.5"	32.5	1.065	380	176	106.2	375.8
22	30.3	35	1.042	345	114	179.5	418.5
23		32	1.364	415	152	140.4	329.9
24	12.9	32	1.142	400	139	148.0	221.8
25	21.9	28	1.109	400	147	108.3	315.4
26	28.5	34	1.209	470	147	166.5	412.3
27	41.4		1.320	483	134	149.8	366.3
28	42.6	36	1.164	300	111	93.9	256.5
29	20.3	35	1.741	520	219	73.4	136.2
30	52.9	26	1.386	450	103	19.3	26.8
Average	28.0	32.3	1.254	416.3	144	118.5	286.0
S.D.	14.1	3.34	0.207	66.0	35	48.4	127.0
Experimental							
31	16.2	29	1.164	400	130	110.6	367.7
32	43.7	30.5	1.309	470	257	46.2	242.5
33	11.5		1.475	380	130	108.3	184.3
34	29.4	28	1.075	345	99	114.6	250.3
35	29.4	34	1.797	610	151	148.2	423.6
36	35.0	33	1.419	415	185	18.8	186.3
37	25.1	30	1.530	415	166	101.5	279.7
38	11.0	33	1.298	400	156	99.1	246.2
39	27.6	34	1.375	435	185	93.0	172.6
40	89.9	34	1.609	415	201	6.6	21.9
Average	31.9	31.7	1.405	428.5	166	84.7	237.5
S.D.	22.8	2.36	0.212	72.0	44	45.5	110.3

8 IU ACTH, groups autopsied in February

" Hematocrit in percent, TFA in meq/100 cc., all other values mg. percent

[a] Total fatty acids

[b] Lipoproteins

[c] Cholesterol

VITA

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Doctor of Philosophy

Thesis: SOME EFFECTS OF ACTH ON METABOLISM AND BLOOD CELLS IN THE CHICKEN

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Biographical:

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