A CHARACTERIZATION OF THE CORTICOSTERONE-BINDING-GLOBULIN IN THE PLASMA OF THE DUCK (*ANAS PLATYRHYNCHOS*)

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James Leon Cheshier

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Master of Arts

James L. Cheshire

Author

Approved, August 1973

Eric L. Bradley

Eric L. Bradley

Lawrence L. Wiseman

Lawrence L. Wiseman

Robert E. L. Black

Robert E. L. Black

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ABSTRACT

Following hypophysectomy and subsequent ACTH replacement therapy in the duck (Anas platyrhynchos), the physiological changes observed in body, liver, testes, and adrenal weight, as well as peripheral plasma corticosterone concentration, were essentially the same as those changes reported by Bradley and Holmes (1971). A study of the specific corticosterone binding activity of corticosteroid binding globulin (CBG) was undertaken in order to find out if changes in CBG activity could be the basis for the differences observed by these investigators between the metabolic clearance rates of corticosterone in sham-operated and hypophysectomized ducks.

Electrophoresis was used to characterize the plasma CBG. The greatest amount of tritium labelled corticosterone was associated with the α-globulin fraction of the plasma, thus demonstrating an electrophoretic similarity of duck CBG with several previously characterized mammalian CBG's. Our results did not demonstrate a direct role for ACTH in the control of CBG activity. The observed changes in liver weight after the removal of the adenohypophysis, and the restoration of liver weight after ACTH replacement, seem to be consistent with the notion that rather than ACTH promoted changes in CBG activity, pituitary controlled hepatic metabolism is largely responsible for changes in the metabolic clearance rate of corticosterone.

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INTRODUCTION

Daughaday (1956), Bush (1957), Sandberg, Slaunwhite and Antoniades (1957), and Upton and Bondy, (1958), have demonstrated the presence in human plasma of a specific protein capable of binding corticosteroids with very high affinity and low capacity. This interacting protein, termed "corticosteroid-binding globulin" (CBG) or, alternatively, "transcortin", is clearly distinguished from albumin which has high binding capacity and a low affinity (Seal and Doe, 1963, 1965, 1966; Osorio and Shats, 1968). Slaunwhite and Sandburg (1959) proposed the name "transcortin" for the steroid-binding protein. This name connotes a transport function of the protein which has not been fully established. Therefore, in this paper the more general name corticosteroid-binding globulin (CBG), coined by Daughaday (1956) will be employed. A CBG has been identified in the duck (*Anas platyrhynchos*) (Chader and Westphal, 1968), but it has not been characterized even though the plasma protein components of duck plasma have been known for some time. (Deutsch and Goodloe, 1945).

The exact role of the protein steroid-carrier complex has been debated. Some workers have felt that the complex may function to store steroid in the plasma for protection from adsorption to walls of the vascular system and from chemical breakdown in plasma (Westphal, 1961) and from excessive loss through the kidney (DeMoor, Steeno, & Heyns, 1968). Sandberg, Rosenthal, Schneider and Slaunwhite (1966) have postulated that the protein-bound fraction of the total hormone in plasma is not available for metabolism and that the "biologically effective" fraction is that in the "native" or free state. Thus, protein bound hormones may constitute a sequestered reserve of potential hormone activity.

There are several physiological states in which changes in steroid protein binding occus (Krulik and Svobodova, 1969; DeMoor, Bouillon, and O. Steeno, 1969). It is tempting to speculate that there may be a direct role for the adenohypophysis in the control of CBG activity in light of the observations that pituitary extracts, TSH and thyroid hormone restore to normal the decreased CBG activity observed in hypophysectomized rats (Steinetz and Beach, 1963; Harris, Levine, and Schindler, 1964; D'Angelo and Grodin, 1964). However, there remain differing reports as to whether there is an increase or decrease in plasma CBG activity in rats after hypophysectomy (Westphal, Williams and Ashley, 1962; Gala and Westphal, 1966), and additionally, these observations have been made in a restricted number of species.

Recent studies with hypophysectomized ducks and pigeons have shown there is a highly significant slowing of the metabolic clearance rate (M.C.R.) of injected radioactive corticosterone when compared with sham-operated controls. After ACTH treatment, hypophysectomized birds showed a tendency to return toward a more rapid metabolic clearance rate (Bradley and Holmes, 1971; Chan, Bradley and Holmes, 1972). This decrease in the metabolic clearance of corticosterone from the plasma of the hypophysectomized birds suggests that the catabolism of corticosterone was impaired, but whether this impairment represented a change in hepatic (or other) metabolism, or in the amount of protective protein binding, was not demonstrated. It was therefore decided to characterize the nature of duck plasma corticosteroid binding globulin for comparison with other species and to determine the effects of hypophysectomy and ACTH replacement therapy in hypophysectomized ducks on the specific activity of CBG in order to determine if the changes in metabolic clearance rates observed previously in hypophysectomized and ACTH treated animals could be at least partially explained by ACTH controlled changes in the character or degree of protective protein binding.

MATERIALS AND METHODS

Animal Maintenance

Pekin drakes (*Anas platyrhynchos*), 8-12 weeks of age, were fed a diet of "Chicken grower food" (California Milling Corporation, Los Angeles) with a supply of fresh water *ad libitum*. Food was removed from the cages 12 h before surgery or experimentation. Before surgery, all birds were maintained on a regimen of 12 h light and 12 h darkness at 22°C and 50% relative humidity. After surgery, the ducks were housed in individual cages in a room at 26°C and 45% relative humidity for 5 days and were then returned to the room at 22°C.

Experimental Groups

Hypophysectomized ducks

All surgical procedures, animal treatments and blood collections were carried out at the Central Vivarium, University of California, Santa Barbara. Birds were anaesthetized with intravenously administered sodium pentobarbital and adenohypophysectomized by a transbuccal approach (Bradley and Holmes, 1971). After hypophysectomy the birds were maintained for 14 days before experimentation.

Sham-operated ducks

Sham-operated birds were perpared by the same procedure as the hypophysectomized birds but the contents of the sella turcica were left undisturbed.

ACTH-treated hypophysectomized ducks

Birds which had been hypophysectomized for 14 days were injected intramuscularly, twice daily for 7 days, with 2 i.u. ACTH (H.P.Acthar gel, Armour).

Experimental Procedures

Plasma Collection

Each bird was secured to a restraining apparatus and the brachial artery was cannulated (Intramedic P.E. 160, Clay-Adams). Each bird was injected with 75u sodium heparin in 0.9% NaCl solution and allowed to calm down for 5 minutes prior to the rapid withdrawal of a single 40 ml blood sample. The blood sample was immediately centrifuged at 10,000g for 15 minutes in a refrigerated centrifuge. The plasma was then placed in 20 ml vials and immediately frozen in dry ice and maintained below 60°C during shipment to Williamsburg, Virginia and below - 25°C until analysis.

Total Protein Determination

The total protein concentrations in the terminal plasma samples were determined by the Hycel Biuret procedure (Hycel, Inc. Houston, Texas) using crystallized human albumin (Dade) as a standard. All concentrations were expressed in gm/100 ml plasma.

Determination of plasma corticosterone concentration

Corticosterone was estimated by a modification (Bradley & Holmes 1971) of the method developed and validated by Frankel, Cook, Graber, and Nalbandov (1967). Five ml of plasma was extracted in seven volumes of dichloromethane (Instra-Analyzed, J. T. Baker), washed with 2 volumes each of 0.05 N NaOH and water and immediately chromatographed on Whatman No. 1 paper in cyclohexane: 1, 4 dioxine:methanol:water (100:75:50:25) for 16 h. Fluorescence was measured by a modification of the Zenker and Bernstein (1958) procedure by the addition of 10 ml dichloromethane to the dried eluate before extraction with suphuric acid-ethanol reagent. Crystalline corticosterone (Schwarz/Mann; Division of Becton Dickinson and Co.) was used for standard and the fluorescence of the blank, unknown, and standard solutions were measured in a spectrofluorometer (Aminco-Bowman).

Separation of Plasma Proteins

A modification of the technique described by Westphal and Devenuto (1966) was used to electrophoretically separate the plasma proteins. Two ml of plasma was added to 0.20 μCi of 1,2-H^3-corticosterone (S.A. = 104mCi/mg, Amersham Radiochemical Centre U.K.; distributed by Amersham/Searle) which was in a thin film on the bottom of a 20 ml screw top vial. The mixture was gently shaken at 23°C for an equilibration period of 4 hours.

A buffer for dialysis was prepared by mixing 0.07 μCi 1,2-H^3-corticosterone with 50ml of Tris-sodium barbital buffer (pH 8.8, μ 0.075). The mixture was warmed for 4 h at 45°C wtih frequent mixing and then cooled to room tenperature. The plasma sample which had been previously equilibrated with corticosterone was then placed in dialysis tubing with an 8-12,000 molecular weight retention range (union carbide)and dialized against 50 ml of the tritiated corticosterone containing buffer.

A Gelman electrophoresis apparatus with cellulose acetate strips (Sephraphore III, Gelman) was used to separte the plasma into its component proteins after the removal of non protein bound H^3-corticosterone by dialysis. The cellulose acetate strips were equilibrated with the dialysate of the equilibrated dialysis system described above. Seven and one half μl of the radiocorticoid-equilibrated plasma was applied to each of two duplicate strips to effect a separation of the proteins. A current of 4 mamp per strip was maintained for 3 hours at 23°C. The strips were then dried for 30 minutes at 49°C and in order to locate the protein bands, one strip was stained with amido black 10B (Analytical Chemists, Palo Alto, Calif.). The second strip was cut into sections corresponding to the stained bands on the first strip. Each section was placed in 20 ml scintillation counting vial and eluted with 5 ml of absolute methanol (Instra-Analyzed, J. T. Baker). The eluate was dried and redissolved in Bray's scintillation fluid and counted 20 minutes for tritium in a Nuclear Chicago Series 724 Liquid Scintillation counter.

The percentage distribution of protein in each of the separate bands was determined by measuring the density of each band using a Gelscan Automatic Recording & Integrating Scanner (Models 39372, 39373 Gelman).

The radioactivity of each band was corrected for background by determining the area of each band and subtracting a background value appropriate for that area. Values were expressed as counts per minute per mg protein in each band.

<u>Statistical Methods</u>

All data was analyzed for significant differences using the Student's t-test. An F-test of the ratio of the sums of squares was employed to determine the significance of the variance of means. Significance was considered the 95% level ($P<0.005$) and a $P<0.001$ was considered highly significant. All values are expressed as mean values$\pm$ standard error of the mean (S.E.M.).

RESULTS

Body and organ weights (Table 1)

Both the hypophysectomized and the ACTH treated hypophysectomized birds showed a significantly greater body weight loss following surgery than the sham-operated control birds. The weights of the testes, the adrenal glands, and the liver from the hypophysectomized birds were signigicantly lower than the corresponding values in the sham-operated birds. After replacement therapy with ACTH, the weight of the testes from the hypophysectomized birds continued to decline until at autopsy the mean testicular weight was 24% of the corresponding value for the sham-operated birds. The adrenal gland weights from hypophysectomized birds treated with ACTH remained significantly low with respect to the sham-operated controls. The livers of the ACTH-treated hypophysectomized birds increased in weight and were not significantly different from the sham-operated controls.

Peripheral plasma corticosterone (Table 2)

There was a highly significant decrease in peripheral plasma corticosterone concentration in the hypophysectomized birds compared with the sham-operated birds. After hypophysectomized birds were given replacement therapy with ACTH the plasma corticosterone concentration had increased and was not significantly different than the sham-operated values.

TABLE 1

Organ weights (means ± s.e.m.) recorded at autopsy from sham-operated and hypophysectomized birds 14 days after surgery, and from hypophysectomized birds kept for 14 days, then treated for 7 days with ACTH.

	Body weight	Organ weights at autopsy		
Treatment	Terminal (g)	Testes (mg)	Adrenal (mg)	Liver (g)
Sham-operated (9)	2903 ±84.49	509 ±22.65	290 ±19.67	57 ±7.29
Hypophysectomized (10)	2066**** ±94.61	135**** ±17.77	155**** ±12.65	33** ±2.37
Hypophysectomized + ACTH (8)	2013**** ±117.13	122**** ±10.39	175**** ±19.05	$46^{n.s.}$ ±6.79

**$P < 0.02$, ****$P < 0.001$ and $^{n.s.}$=Not significant, with respect to the corresponding value for sham-operated controls. (These data courtesy of J. Devlin, University of California)

TABLE 2

Peripheral plasma corticosterone concentration and total protein concentration (means ± s.e.m.) in the plasma of sham-operated and hypophysectomized birds 14 days after surgery, and in hypophysectomized birds kept 14 days and then treated for 7 days with ACTH.

Treatment	Peripheral Plasma Corticosterone µg/100ml	Total Plasma Protein gm/100ml
Sham-operated (9)	5.57 ±1.16	3.42 ±0.09
Hypophysectomized (10)	0.73**** ±0.12	2.13**** ±0.12
Hypophysectomized + ACTH (8)	2.68$^{n.s.}$ ±0.94	3.48$^{n.s.}$ ±0.27

****$P < 0.001$ and $^{n.s.}$ = not significant, with respect to the corresponding value for sham-operated controls.

Total plasma protein concentration (Table 2)

No significant differences were observed between the total protein concentrations in the plasma of the hypophysectomized birds treated with ACTH and the corresponding value in the sham-operated birds. However, after hypophysectomy, a highly significant decrease in the total plasma protein concentration was observed.

Fraction of the total protein in each plasma protein band (Table 3; Fig. 1,2,3,; Appendix 1)

There were no significant differences observed between hypophysectomized and ACTH-treated animals with respect to the location of any of the plasma protein components after electrophoretic separation (Apendix 1).

Also there were no observed differences in any of the identified plasma components of hypophysectomized birds with the corresponding components in the sham-operated birds and there were no significant differences between the gamma, beta, and albumin plasma components of the hypophysectomized birds treated with ACTH with respect to those same components in the sham-operated birds. However, in the ACTH treated hypophysectomized birds the alpha 2 component was significantly decreased and the alpha 1 component was significantly increased with respect to the corresponding sham-operated control value.

Corticosterone-Binding specific activity of the plasma protein components Table 4, Fig. 1,2,3)

There were no significant differences among the very small amounts of tritiated corticosterone associated with each of the gamma, beta, alpha 2, and albumin plasma components of the sham-operated birds, the hypophysectomized birds, and the hypophysectomized birds that received

TABLE 3

The fraction of total protein in each electrophoretogram component determined directly by integration of the area under the curve (means ± s.e.m.).

	Plasma Protein component				
Treatment	Gamma	Beta	Alpha 2	Alpha 1	Albumin
Sham-operated (9)	0.1455 ±0.03	0.2844 ±0.03	0.1543 ±0.01	0.0348 ±0.001	0.3754 ±0.02
Hypophysectomized (10)	$0.1934^{n.s.}$ ±0.04	$0.2373^{n.s.}$ ±0.01	$0.1164^{n.s.}$ ±0.02	$0.0589^{n.s.}$ ±0.03	$0.3973^{n.s.}$ ±0.05
Hypophysectomized + ACTH (8)	$0.1666^{n.s.}$ ±0.04	$0.2576^{n.s.}$ ±0.02	0.1219*** ±0.01	0.0560* ±0.01	$0.3977^{n.s.}$ ±0.01

*$P < 0.05$, ***$P < 0.01$ and $^{n.s.}$ =not significant, with respect to the corresponding value for controls.

TABLE 4

Specific binding activity of each plasma protein component expressed as counts per minute per mg protein (means ± s.e.m.) for each plasma component.

	Plasma protein component				
Treatments	Gamma	Beta	Alpha 2	Alpha 1	Albumin
Sham-operated (9)	1549 ±766.56	47 ±47.11	837 ±562.38	51,387 ±6142.78	741 ±298.84
Hypophysectomized (10)	1164 n.s. ±430.16	162 n.s. ±110.04	683 n.s. ±415.39	43,636 n.s. ±3901.04	764 n.s. ±255.75
Hypophysectomized + ACTH (8)	1214 n.s. ±651.42	0	475 n.s. ±314.71	29,822*** ±4076.74	414 n.s. ±217.51

***$P < 0.01$ and n.s. = not significant, with respect to the corresponding value for controls.

FIGURE 1

A composite electrophoretic separation pattern of the relative protein densities of plasma from all sham-operated birds. The crossed bars on the peaks indicate the magnitude of the S.E.M. for the mean of the values used to determine the peak height and location. The dotted vertical lines indicate the relative areas taken to represent the various protein components. The height of the bar graph under each component peak indicates the mean value for the amount of H^3-corticosterone associated with protein (cpm/mg) within the component area.

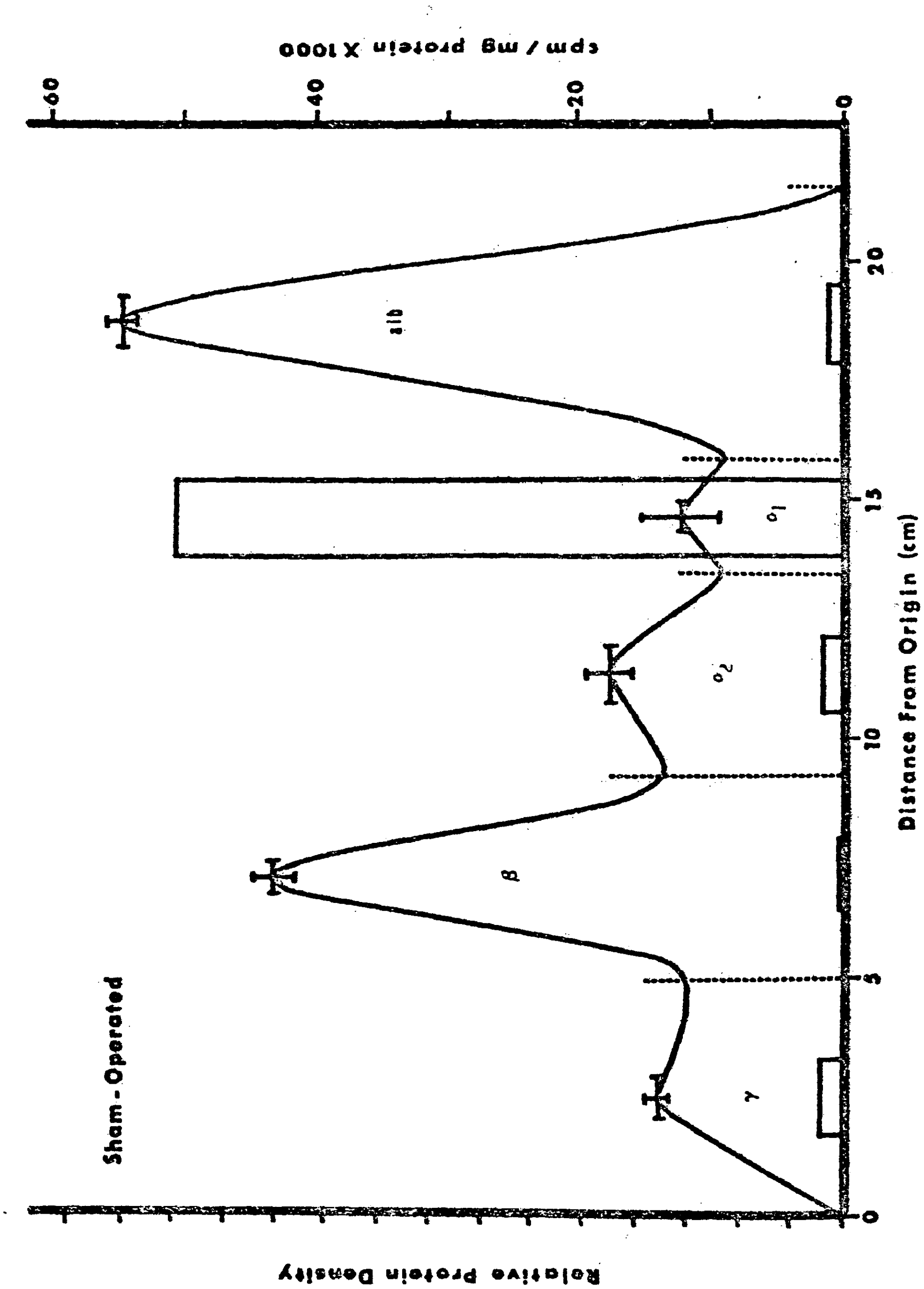

Sham - Operated
Relative Protein Density
cpm / mg protein X 1000
Distance From Origin (cm)
0
5
10
15
20
0
20
40
60
γ
β
α2
α1
alb

FIGURE 2

A composite electrophoretic separation pattern of the relative protein densities of plasma from all hypophysectomized birds. The crossed bars on the peaks indicate the magnitude of the S.E.M. for the mean of the values used to determine the peak height and location. The dotted vertical lines indicate the relative areas taken to represent the various protein components. The height of the bar graph under each component peak indicates the mean value for the amount of H^3-corticosterone associated with protein (cpm/mg) within the component area.

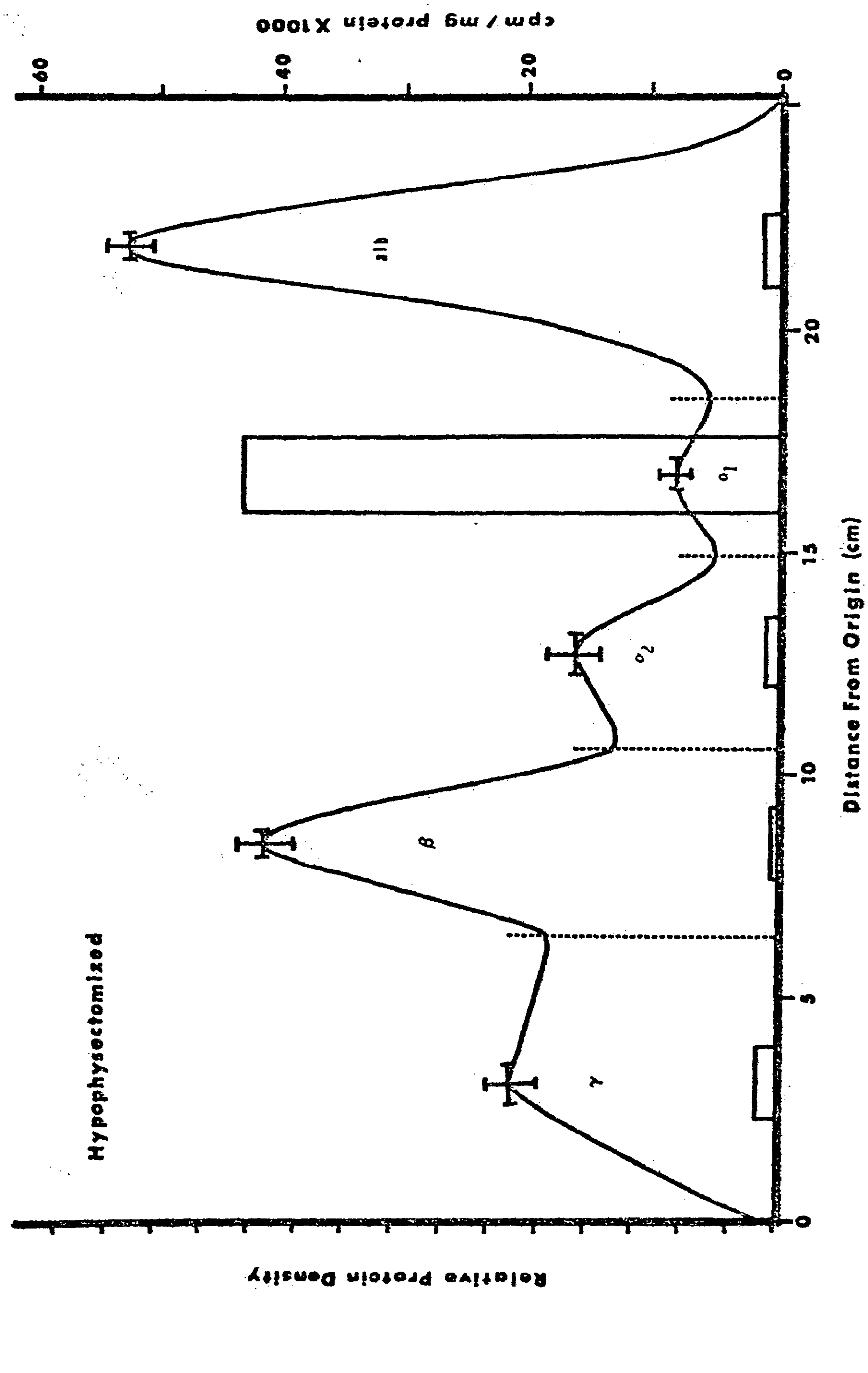
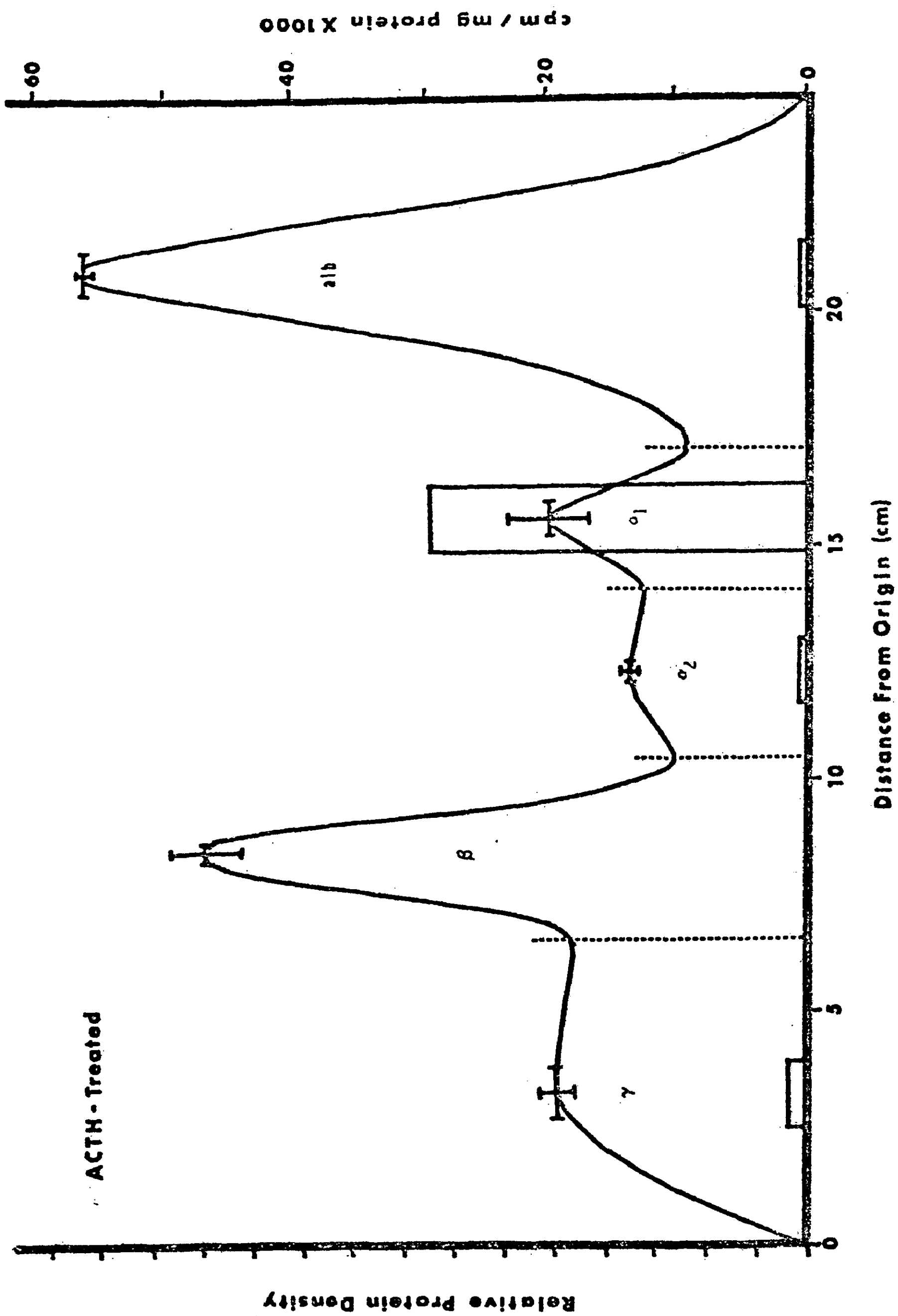

Hypophysectomized
cpm / mg protein X1000
0
20
40
60
alb
β
γ
α1
α2
Relative Protein Density
0
5
10
15
20
Distance From Origin (cm)

FIGURE 3

A composite electrophoretic separation pattern of the relative protein densities of plasma from all hypophysectomized plus ACTH birds. The crossed bars on the peaks indicate the magnitude of the S.E.M. for the mean of the values used to determine the peak height and location. The dotted vertical lines indicate the relative areas taken to represent the various protein components. The height of the bar graph under each component peak indicates the mean value for the amount of H^3-corticosterone associated with protein (cpm/mg) within the component area.


ACTH - Treated
cpm / mg protein X 1000
60
40
20
0
Relative Protein Density
Distance From Origin (cm)
0
5
10
15
20
alb
α1
α2
β
γ

ACTH replacement. The amount of the total radioactivity associated with the alpha 1 peak was in excess of 93% in all treatments. No significant differences were observed between the specific binding activity expressed as cpm of H^3-corticosterone per mg of protein in the alpha 1 component of the hypophysectomized birds plasma and the corresponding value in the sham-operated birds. There was, however, a significant decrease observed in the cpm of H^3-corticosterone per mg of protein in the alpha 1 component of the hypophysectomized birds treated with ACTH when compared with the corresponding control value.

DISCUSSION

The effects of hypophysectomy and ACTH replacement therapy demonstrated in this study are essentially the same as those obtained in the recent studies on hypophysectomized ducks by Bradley and Holmes (1971). The terminal body weights of the sham-operated animals were not significantly different between these studies nor were there differences in the declines observed in the body weights after hypophysectomy. However, the testes and adrenal weights of sham-operated ducks in this study were significantly higher ($P<0.05$ and $P<0.001$, respectively) than those reported by Bradley and Holmes (1971). These differences may be due to a seasonal variation in the weights of these organs but the differences are probably not important because all of the changes observed after hypophysectomy and ACTH replacement therapy were not significantly different from those obtained earlier (Bradley and Holmes, 1971).

The significant declines in the testes, adrenal and liver weights in the hypophysectomized animals clearly indicate the effects of the loss of the adenohypophysial trophic hormones known to influence these organs. In the hypophysectomized ducks treated with ACTH, those functions under direct ACTH influence tended to return in the direction of the sham-operated values, whereas the testes weight continued to decline in the absence of pituitary gonadotrophin.

The peripheral plasma corticosterone concentrations observed for sham-operated ducks were not significantly different from those values reported by Bradley and Holmes (1971) for sham-operated ducks sampled 2-8 minutes after restraint. They were, however, significantly higher ($P<0.05$) than the values they reported for animals allowed to rest 65 minutes following restraint before sampling, thus indicating the important role of the intact pituitary in mediating a plasma corticosterone "stress" response.

The peripheral plasma corticosterone concentrations in hypophysectomized ducks were significantly lower ($P<0.001$) than their corresponding sham-operated controls, but they were also significantly higher than those values reported by Bradley and Holmes (1971). Most probably this difference is accounted for in differences in technique and methodology between laboratories rather than an indication of incomplete hypophysectomy. Bradley and Holmes (1971) used 10ml of plasma for their corticosterone determinations, but only 5 ml of plasma was used in this study. Thus, in our samples there was a possibility of a greater amount of non-specific fluorescence relative to the very low plasma corticosterone concentration, thereby giving an inordinately higher value for corticosterone.

Treatment with ACTH caused the expected increase in plasma corticosterone concentration in hypophysectomized animals. This response was not of the magnitude observed by Bradley and Holmes (1971) and has caused us to suspect that the treatment regimen was not the same or that the ACTH potency was unaccountably different.

Following adenohypophysectomy, the total plasma protein concentrations were significantly lower than the corresponding values for sham-operated birds (Table 2). This change was apparently not due to a large decrease in any single plasma protein component because no significant differences were observed between the amount of any component of hypophysectomized bird plasma compared with that same component of sham-operated bird plasma (Table 3). Although this might imply a significant increase in the plasma volume to dilute the existing protein, other studies in the duck have shown no significant changes in the extracellular fluid volume, blood volume or hematocrit following hypophysectomy (Bradley and Holmes, 1971). Thus, it would seem that there is a quite general decrease in the mass of all plasma protein components following the removal of the pituitary. It may be important to note that only the alpha 1 protein component values from hypophysectomized animals, though significantly different from the sham-operated values, showed significant variation ($F = 3.50$; $P < 0.05$) from the variance of the sham-operated values, perhaps indicating a differential response to the loss of the pituitary with respect to this component.

When hypophysectomized animals were treated with ACTH, two significant changes in the alpha globulin fractions were observed, wherein the alpha 2 component decreased and the alpha 1 component increased with respect to sham-operated values. Therefore, there appear to be significant changes that can be made on the amount of the protein fraction containing CBG relative to the other plasma proteins by this ACTH preparation acting in the absence of all other adenohypophysial hormones.

Corticosterone is the principal corticosteroid in the duck (de Roose, 1961; Donaldson, Holmes & Stockenko, 1965; Sandor and Lanthier, 1963). Therefore, tritium labeled corticosterone was chosen as the radioactive tracer for these studies. The quantitative determination of CBG activity in plasma is difficult due to the very low concentration of this protein in the blood, the spontaneous dissociation of the complex of CBG with the radiolabeled corticosteroid, the possible interference from other binding proteins, and the competitive or non-competitive binding inhibition by other steroid hormones which may or may not be known as to structure or quantity (Westphal, 1971).

Electrophoretic procedures are well suited for a qualitative demonstration of steroid binding to proteins. The principal merit of electrophoresis lies in the ability to characterize the plasma protein component to which the steroid is bound. However, the rapid dissociation of steroid protein complex is very troublesome using paper strip electrophoresis. When this technique was first used for the study of serum protein interaction with estrogens, progesterone, and cortisol (Szego and Roberts, 1955; Westphal, 1955, 1956) considerable trailing of steroid was observed, even for complexes of relative high affinity. Similar difficulties were reported by other authors (Upton and Bondy, 1958; Rosembaum, Christy, and Kelly, 1966) using paper-electrophoretic techniques.

Daughaday (1956) overcame the steroid-protein complex dissociation difficulties during electrophoretic migration by using equilibrium paper electrophoresis. The principle of the procedure is to perform the electrophoresis in an environment which contains the dissociable steroid at the concentration of the unbound portion. This is done by dialyzing the protein-steroid solution (plasma with labeled steroid) to be electrophoresed against the electrophoretic buffer until equilibrium is reached. Actually, there is continuous dissociation and association of the steroid-protein complexes, but, the net result is the apparent absence of dissociation (Westphal, 1971). When we used this technique with duck plasma, it was found that the labeled corticosterone migrated primarily with the alpha 1 globulin in the sham-operated controls, the hypophysectomized birds, and the hypophysectomized birds given ACTH (Table 4, Fig. 1,2,3.) The small amounts of the labeled steroid found in the other plasma components and at the origin have been considered by others to be due to trailing and sample application and not indicative of other binding protein components (Westphal and Devenuto, 1966).

Westphal, Williams, and Ashley (1962) reported an increased CBG activity in rats after hypophysectomy and adrenalectomy. But in another study no increase in CBG activity was observed following adrenalectomy and hypophysectomy in rats, and an increase in endogenous ACTH did not alter CBG activity. In fact, CBG was shown to respond inversely to the corticosteroid level; increased perepheral corticosteroid concentration resulted in decreased CBG activity, and vice versa (Gala & Westphal, 1966). DeMoor, Heyns, Baelen and Steeno (1968) also found a low CBG activity in patients with adrenocortical hyperplasia which can be indicative of high ACTH levels.

In the duck we observed no significant difference in the specific protein binding activity found in the CBG containing alpha 1 component of the plasma of the hypophysectomized bird when compared with the sham-operated value. However, when ACTH replacement treatment was given to hypophysectomized birds there was a decrease in the specific activity observed in the alpha 1 component of the plasma (Table 4, Fig. 1 & 3). This compares favorably with the results obtained by Gala and Westphal (1966) for the rat. It is possible that in the hypophysectomized duck ACTH stimulation of the adrenal may result in an increased corticosteroid release, thereby promoting a decrease in CBG activity. On the other hand although there was no significant difference between the mean value of what we have characterized as the specific binding activity for hypophysectomized versus sham-operated controls, there was a distinct trend showing that the individual values for hypophysectomized birds were below the individual values for sham-operated birds. Thus, it may be that in the ACTH-treated hypophysectomized duck the lowered CBG activity we observed was not a direct result of ACTH treatment, but also a result of the continued absence of thyroid stimulating hormone for an additional 7 days. It has been established that thyroid hormone is involved in the control of the synthesis and the activity of the CBG protein (Labrie, Pelletur, and Fortier, 1967), and we have shown in a preliminary study using an intact duck that triiodothyronine promotes an increase in CBG activity after 14 days of oral administration (50 mg/day).

Because the results of our study do not demonstrate a direct role for ACTH in the control of CBG activity, the answer to what physiological adjustments may take place to cause the reported changes in the metabolic clearance rate of corticosterone now seem likely to be found in an understanding of what changes in hepatic metabolism occur as a consequence of hypophysectomy. The blood concentration of the adrenal cortical hormones, like corticosterone, is stablilized by a negative-feedback control of adrenal cortical secretion rate, so that changes in the corticosteroid concentration in blood. The concentration of these steroids in blood depends upon both the rate of secretion of the hormones into the blood, and their rate of removal (Ingle, Higgins and Kendall, 1938; Sayers and Sayers, 1948). The removal of adrenal steroids is accomplished almost entirely by the Δ^4-steroid hydrogenases, which inactivate the steroids via A-ring reduction in the liver (Mills, 1962). Urquhart, Yates, and Herbst (1959) have concluded that there is a parallel relationship between the _in vivo_ capacity of liver to inactivate corticosteroids and the adrenal cortical secretion rate. Urquhart, Yates and Herbst (1959) found that neither ACTH nor adrenal cortical hormones increased the capacity of the liver to inactivate corticosteroids. They concluded that their observations of parallel changes in adrenal size and hepatic capacity to inactivate corticosteroids demonstrate the existence of an hepatic control of adrenal cortical secretion rate.

Extreme prolongation of corticosteroid half-lives in plasma following hepatectomy has been observed (Yates, 1965). This hepatic regulation of adrenal cortical function is apparently mediated through the anterior pituitary (ACTH) negative-feedback control which stabilized plasma corticosteroid concentration: if plasma hormone levels rise, the rate of removal drops (Yates, 1965). After hypophysectomy, the controlling influence of the liver on adrenal cortical function appears to be lost (Urquhart, Yates, and Herbst, 1959).

The observations we have made of changes in liver weight after the removal of the pituitary seem to be entirely consistent with the notion that hepatic metabolism is largely responsible for changes in the metabolic clearance rate of corticosterone. Both these studies and those of others on ducks and pigeons (Bradley and Holmes, 1971; Chan, Bradley and Holmes, 1972), have indicated that there is a significant loss of liver weight following hypophysectomy and there is a liver weight restoration after ACTH replacement therapy to values not significantly different from controls. Although the results of these studies do not eliminate ACTH from a schema for the control of CBG activity, they do indicate that any action of ACTH on CBG is indirect, and that probably the loss of thyroid stimulating hormone is most directly responsible for the diminution of CBG activity after hypophysectomy.

The adenohypophysis is obviously central in the control of corticosterone concentration in the duck. The direct effects of the pituitary and ACTH on corticosterone produced from the adrenal have been demonstrated (Bradley and Holmes, 1971), and we have shown that with the loss of pituitary function, CBG activity, and presumably hepatic metabolic activity, are also diminished. Our suspicions are now quite strong that in the duck, as in mammals, CBG activity is directly dependent upon hepatic metabolism.

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APPENDIX 1

Values for all electrophoretic separation scans comparing component peak location (mm) from point of application and distance from each successive peak, thereafter.

	Plasma protein component				
Treatment	Gamma	Beta	Alpha 2	Alpha 1	Albumin
Sham-operated (9)	26 ±2.30	47 ±3.73	43 ±6.16	34 ±3.27	41 ±5.76
Hypophysectomized (10)	33 n.s. ±2.60	54 n.s. ±3.18	38 n.s. ±4.97	34 n.s. ±4.87	53 n.s. ±2.60
Hypophysectomized + ACTH (8)	33 n.s. ±2.77	55 n.s. ±2.01	44 n.s. ±2.53	40 n.s. ±4.21	52 n.s. ±4.92

n.s. = not significant, with respect to sham-operated controls.

VITA

James Leon Cheshier

Born in Star City, Arkansas, August 2, 1949. Graduated from Fayetteville High School, Fayetteville, Arkansas, June 1967. Received B. A. degree in zoology from the University of Arkansas, Fayetteville, Arkansas, June 1971. Entered graduate program at the College of William and Mary, Williamsburg, Virginia, September 1971. During tenure as graduate student, served as teaching assistant at College of William and Mary, 1971-1973. Candidate for Master of Arts degree in Biology 1972-1973.