



An appraisal of endocrine activity in strutting sage grouse
by Roger Cecil Thorvilson

A thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree of
MASTER OF SCIENCE in Zoology
Montana State University
© Copyright by Roger Cecil Thorvilson (1969)

Abstract:

An effort was made to appraise differential endocrine activity in reproductively active adult and subadult male sage grouse. Adult sage grouse showed significantly heavier testes than subadults. Pituitaries and thyroids were significantly heavier in subadults. Protein bound iodine (thyroxin) level was significantly higher in the serum of adult birds. There were no significant differences in the weights or histological appearance of adrenal glands in adults and sub adults. Testes weights were shown to be negatively correlated with pituitary, thyroid, and adrenal weights in subadults. Adult grouse showed a positive correlation between testes weights and adrenal weights, but thyroids and pituitaries negatively correlated, with testes and adrenals.

AN APPRAISAL OF ENDOCRINE ACTIVITY IN
STRUTTING SAGE GROUSE

by

ROGER CECIL THORVILSON

A thesis submitted to the Graduate Faculty in partial
fulfillment of the requirements for the degree

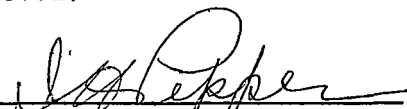
of

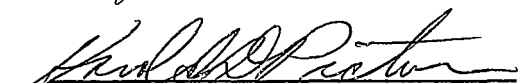
MASTER OF SCIENCE

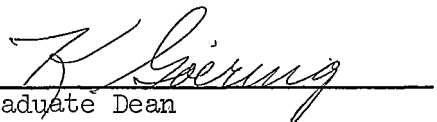
in

Zoology

Approved:


Head, Major Department


Chairman, Examining Committee


Graduate Dean

MONTANA STATE UNIVERSITY
Bozeman, Montana

June, 1969

ACKNOWLEDGMENT

I wish to offer my gratitude to Dr. Harold D. Picton, my advisor and chairman of my committee, for his help throughout this study and his assistance in preparation of the manuscript. I would like to thank Robert E. Urban, who helped me with preparation of adrenal gland sections and with photomicrographs. The Montana Fish and Game Department gave permission to collect the sage grouse. A special thanks is due to Joseph Lucier, who accompanied me on collection trips and provided invaluable assistance in the field.

This study was supported in part by the National Institutes of Health Institutional Funds Program.

TABLE OF CONTENTS

	Page
VITA	ii
ACKNOWLEDGMENT	iii
LIST OF TABLES	v
LIST OF FIGURES	vi
ABSTRACT	vii
INTRODUCTION	1
METHODS	3
Collection and Handling	3
Protein Bound Iodine Determination	4
Adrenal Histology	5
RESULTS	6
DISCUSSION	11
LITERATURE CITED	15

LIST OF TABLES

	Page
Table I. The means, ranges and standard errors of body weights, gland weights, and gland weight/body weight ratios	6
Table II. The means, ranges, and standard errors of gland weights, gland weight/body weight ratios, and PBI transmittance values	7

LIST OF FIGURES

	Page
Fig. 1. Individual gland weight/body weight ratios, body weights, and PBI transmittance values	9
Fig. 2. Adrenal of an adult male collected March 30, 1968	10
Fig. 3. Adrenal of an adult male collected March 30, 1968	10
Fig. 4. Adrenal of a subadult male collected April 21, 1968	10
Fig. 5. Adrenal of a subadult male collected May 12, 1968	10

ABSTRACT

An effort was made to appraise differential endocrine activity in reproductively active adult and subadult male sage grouse. Adult sage grouse showed significantly heavier testes than subadults. Pituitaries and thyroids were significantly heavier in subadults. Protein bound iodine (thyroxin) level was significantly higher in the serum of adult birds. There were no significant differences in the weights or histological appearance of adrenal glands in adults and subadults. Testes weights were shown to be negatively correlated with pituitary, thyroid, and adrenal weights in subadults. Adult grouse showed a positive correlation between testes weights and adrenal weights, but thyroids and pituitaries negatively correlated with testes and adrenals.

INTRODUCTION

In recent years there has been considerable study of the inter-relations between social behavior and endocrine functions in the control of animal reproduction. Christian (1959) and Christian and Davis (1964) have discussed the involvement of the pituitary-adrenocortical axis in an intrinsic control of population size in mammals. They propose that increased social interactions in dense animal populations trigger ACTH release by the pituitary, increased adrenocortical output, and the associated stress responses, which result in reduced reproduction. Highly dominant animals in the social hierarchy are least affected by crowding of populations.

Position in the social dominance order has been related to the number of successful matings, eggs fertilized, and viable offspring in chickens (Guhl and Warren, 1946). Siegel (1960) found heavier adrenals and pituitaries in cockerel chickens caged at high density levels. Heavier thyroids and adrenals, adrenal lipid depletion, and slow weight gain of testes, with accompanying degenerative changes have been found in socially subordinate male chickens caged with females in groups of six, as opposed to chickens caged as male-female pairs (Flickinger, 1961). Neave and Wright (1968) correlated the adrenal weights of ruffed grouse with population density on drumming sites.

The complex social structure present during the breeding season of the sage grouse has been investigated in considerable detail (Patterson, 1952). However, little work has been done to relate this behavior to

hormonal activity. Patterson (1952) and Eng (1963) have compared testis development of the adult males with that of subadult (yearling) males, but they did not study the other endocrine organs.

Sage grouse behavior during the breeding season has been described by Patterson (1952) and Lumsden (1968). Social dominance is strongly evident throughout the strutting season of the sage grouse. Among the adult males there is a social hierarchy, with a master cock and a few other dominant cocks performing most of the mating. However, all the adults act in repelling subadults from the main strutting ground. Any subadult that ventures onto the ground from the periphery is immediately chased off by an adult. No fighting occurs between adults and subadults, the subadults retreating without observable resistance. Although the subadults are physiologically capable of breeding, the adults effectively keep them from doing so.

It was the purpose of this study to collect both adult and subadult males from wild populations and to look for possible physiological differences between these two groups. No attempt was made to determine dominance patterns within either of the groups. The weights of adrenals, thyroids, pituitaries, and testes were recorded, histological appearances of the adrenals were compared, and an attempt was made to index blood levels of thyroid hormone.

METHODS

Collection and Handling

Thirteen sage grouse were collected from two strutting grounds in Fergus and Petroleum Counties, Montana, from March 30 to May 12, 1968. After a period of observation to distinguish between adult and yearling males, birds were collected with a 22 caliber rifle. Blood was collected from the major vessels and heart. If necessary, clotted blood from the body cavity was also taken. The testes and adrenal glands were excised and placed in separate marked containers. The glands and the blood were then placed on ice. The heads were removed and also put on ice in order to cool the pituitary body. Age determination was confirmed by examination of the outer two primary feathers for characteristic differences as described by Patterson (1952).

The pituitary body and the thyroid glands were excised in the laboratory. The adrenals, thyroids, testes, and pituitary were dissected free of surrounding tissue and weighed to the nearest 0.1 mg on a Mettler precision balance. The adrenal glands were preserved in 10% formalin and the other glands placed in absolute alcohol. Eight to ten hours elapsed from shooting until completion of weighing. The body weight was measured to the nearest 10 gm with a twin pan balance.

The means of gland weights and gland weight/body weight ratios were calculated for the adult and subadult groups, and the differences between means were tested for significance by analysis of variance. Group variances were compared with the F test.

7-12-68
5-1-68
2-1-68

Protein Bound Iodine Determination

Blood which was collected from the birds in the field was allowed to stand approximately twenty four hours. The serum was then drawn off and placed in separate tubes. Initially the serum was stored under refrigeration until used. However, the serum from birds collected April 21 and later was frozen until use. Protein bound iodine determinations were started within two hours after thawing.

The Hycel method (Anon., 1967) of protein bound iodine determination was used to estimate the level of thyroxin in the blood. In this method the protein bound iodine in the serum acts as a catalyst in the decolorization of yellow ceric ammonium sulfate. Protein bound iodine is measured by the rate of change of ceric ion to colorless cerous ion. Transmittance at 435 mu was determined with a Bausch and Lomb Spectronic 20 colorimeter. From each serum sample, three one-ml portions were analyzed for protein bound iodine, and the mean of the transmittance values was recorded.

The preliminary step of removal of inorganic iodine with anionic exchange resin was not employed. Protein bound iodine was determined in several separate aliquots of the serum from a single animal. The exchange resin was used with half of these aliquots; this step was eliminated in determinations with the remaining half. No significant difference in results was found. Since results were comparable and use of the resin introduced a possible source of variability, this step was not used in the following analyses of bird sera.

An analysis of variance was used to compare the transmittance means and variances.

Adrenal Histology

Portions of adrenal glands were sectioned at eight microns and stained with hematoxylin and eosin. Interrenal (cortical) cells were compared as to cell size, separation of cells, degree of vacuolization, and size of nuclei. An interrenal cell/medullary cell ratio was derived from photomicrographs of the adrenal tissue.

RESULTS

The means of body weights, gland weights, gland weight/body weight ratios, and PBI transmittance values are shown in Tables I and II. The adult birds' mean testes weight was significantly greater ($p < 0.01$) than that of subadults. The testes weights relative to body weight were also significantly greater ($p < 0.01$) in the adult group. The adrenal weight/body weight ratios were somewhat greater in the subadult group; however, this difference was not significant. Pituitary weight/body weight ratios were significantly higher ($p < 0.05$) in the subadult grouse. Thyroid weights relative to body weight were also significantly greater ($p < 0.01$) in subadults. The protein bound iodine level in the blood as shown by percent transmittance (percent transmittance is directly proportional to thyroxin concentration) was higher in the adult birds. This difference

Table I. The means, ranges and standard errors of body weights, gland weights, and gland weight/body weight ratios.

	Body Wt. (Kg)	Testes Wt. (Gm) **	Testes(Gm)/ Body(Kg) **	Adrenal Wt. (Mg)	Adrenal(Mg)/ Body(Kg)
<u>Adults</u>					
Mean	2.90	4.272	1.47	163.5	55.4
Range	2.67-3.06	3.17-5.06	1.12-1.80	123.1-234.6	43.6-76.7
S \bar{x}	---	0.24	0.09	---	5.7
N	7	7	7	5	5
<u>Subadults</u>					
Mean	2.40	2.165	0.89	135.7	56.7
Range	2.12-2.70	1.26-3.26	0.56-1.29	127.9-167.6	40.9-62.1
S \bar{x}	---	0.31	0.11	---	3.3
N	6	6	6	6	6

** Difference between means is highly significant ($p < 0.01$).

Table II. The means, ranges, and standard errors of gland weights, gland weight/body weight ratios, and PBI transmittance values.

	Pituitary Wt. (Mg)	Pit. (Mg)/ Body(Kg) *	Thyroid Wt. (Mg)	Thyroid(Mg)/ Body(Kg) **	PBI (% Trans.) *
<u>Adults</u>					
Mean	9.80	3.36	105.0	35.9	14.7(17.3) ^t
Range	7.50-11.7	2.52-4.15	87.1-143.9	29.2-50.8	6.6-18.0
S \bar{x}	---	0.34	---	4.1	2.0
N	5	5	5	5	5
<u>Subadults</u>					
Mean	9.90	4.13	112.2	62.5	8.9(15.3)
Range	7.40-12.1	2.92-4.84	96.9-205.6	38.3-97.0	5.7-17.5
S \bar{x}	---	0.29	---	9.2	2.0
N	6	6	5	5	6

* Difference between means is significant ($p < 0.05$).

** Difference between means is highly significant ($p < 0.01$).

^t Values in parentheses are means of uncorrected transmittance readings.

was significant ($p < 0.05$) when corrected transmittance values were compared.

This correction factor is discussed in the next section.

Variances for the adult and subadult groups did not differ significantly for any of the gland weights or the PBI levels. Thyroid weight relative to body weight varied much more in the subadults than in the adults; the F value of 5.88 approached significance at the 5% level.

An attempt was made to bioassay pituitary activity of both adults and subadults using frogs. Although standard procedures were followed, no activity could be demonstrated. Perhaps this was due to low pituitary activity, a low response of amphibians to bird gonadotropins, or to procedural deficiencies.

Adrenal cortical activity, as indicated by cell size and vacuolization of interrenal cells, did not appear to differ between adult and subadult males. There was considerable variation in the degree of separation of interrenal cells. This separation did not appear to correlate with age, however. As much variation was found within adult and subadult groups as was shown between them. Nor did histological appearance seem to correlate with time or with the adrenal weights. A grid was placed over photographs of adrenal sections, and the proportion of intersects touching interrenal cells was counted. The interrenal-medullary ratios did not differ significantly for adult (mean 2.19; range 1.24-3.33) and subadult (mean 2.31; range 1.85-3.70) birds.

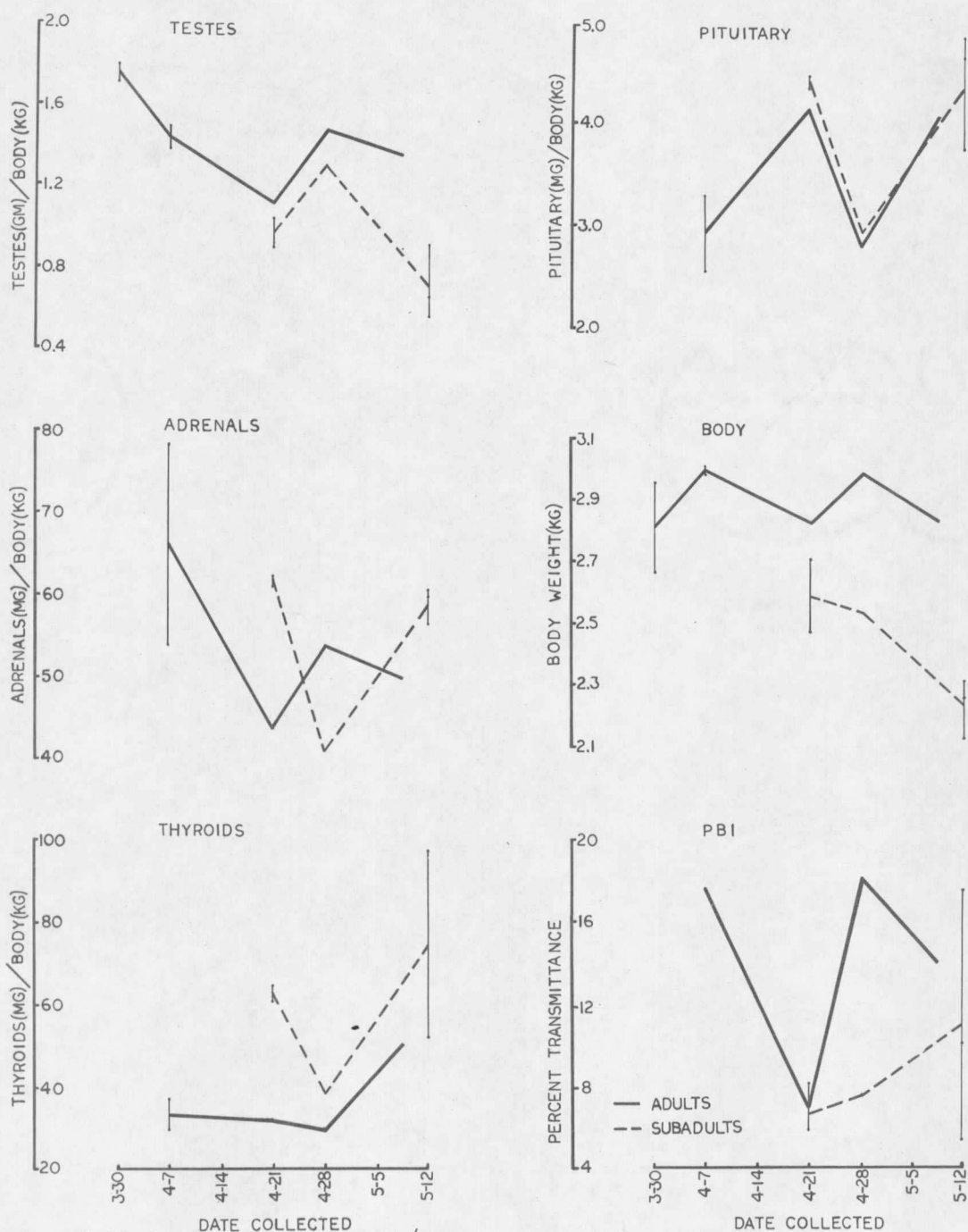
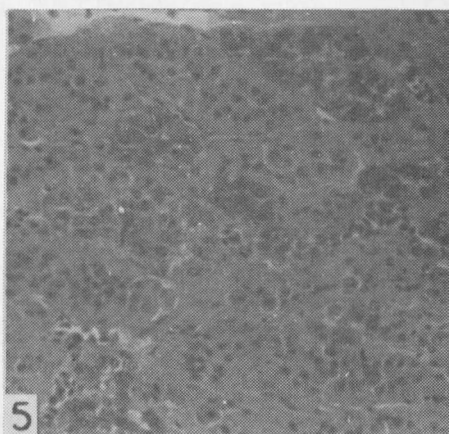
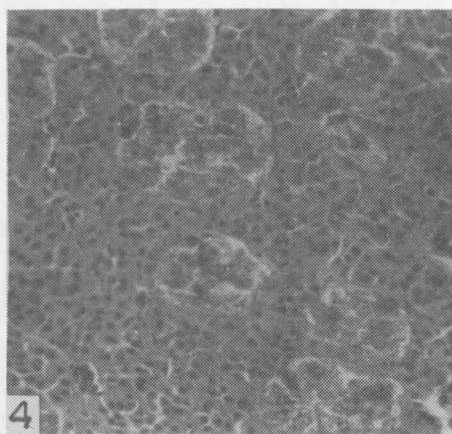
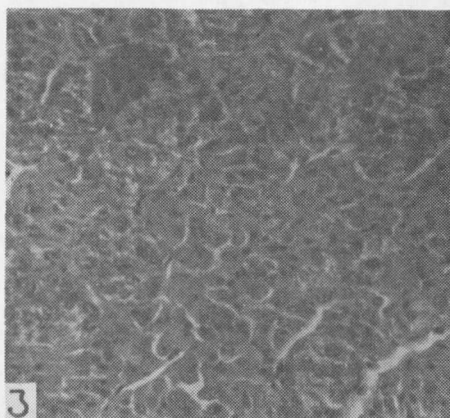
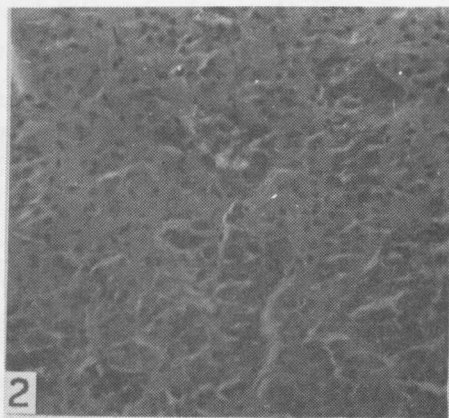


Fig. 1. Individual gland weight/body weight ratios, body weights, and PBI transmittance values. Vertical lines indicate range.



- Fig. 2. Adrenal of an adult male collected March 30, 1968; Adrenal wt. ratio 53.8. Medullary cells stain dark. Interrenal cells are quite compact and sinusoids are small. H&E. X230.
- Fig. 3. Adrenal of an adult male collected March 30, 1968; Adrenal wt. ratio 76.7. Interrenal cells more separated and somewhat larger than in Fig. 1. Hematoxylin and eosin. X230.
- Fig. 4. Adrenal of a subadult male collected April 21, 1968; Adrenal wt. ratio 61.1. Interrenal cells compact and small. H&E. X230.
- Fig. 5. Adrenal of a subadult male collected May 12, 1968; Adrenal wt. ratio 56.2. Interrenal cells small and very compact. H&E. X230.

DISCUSSION

Significantly heavier pituitaries and thyroids in subadults may be due more to age difference per se than to behavior-related responses. This supposition would be supported by the lack of significant differences in the weights and histological appearance of adrenal glands. The adrenal cortex has consistently been the primary endocrine organ showing response to psychological stress. Siegel (1959) found hypertrophy of adrenal glands in crowded female chickens, but no significant differences in thyroid or pituitary weights. In a later study (1960), at higher density levels, he found significantly heavier pituitaries, as well as adrenals, in crowded cockerels. Christian and Davis (1964) state that in comparing adrenal weight changes with changes in populations, adrenal changes due to reproductive condition must be considered, and only adrenals from animals of similar reproductive status can properly be compared. In this study it would seem extremely difficult to separate hormonal effects due to age difference from those due to social stress. Testes development occurs earlier in the breeding season in adults than in subadults (Eng, 1963). Therefore, gonadotrophin release by the pituitary should reach high levels in adults earlier than in subadults. Significantly heavier pituitaries in subadults perhaps are due to reduced gonadotrophin release in adults at the time of my collections, while subadult pituitaries were still at a high level of activity.

The gland weight/body weight ratio may introduce bias where animals of different ages are compared. Siegel (1960) reported that adrenal and

pituitary gland weight/body weight ratios declined significantly with age, in chickens studied from five weeks to seventeen weeks of age. It is possible that sage grouse could show a similar decline in these ratios as body weight continued to increase after the first year of age.

In Fig. 1, individual gland weights and body weights are compared with respect to time. Adrenal, thyroid, and pituitary development appears to be negatively correlated with testes weights in the subadults. This relationship agrees with the results of Siegel (1959, 1960) and Flickinger (1961), who found heavier adrenals, thyroids, and pituitaries and smaller testes in heavily stressed chickens, as compared to animals in non-stressed groups, or highly dominant animals in the stressed groups. In adult sage grouse, however, adrenal weights showed a close positive correlation with testes weights. Thyroid and pituitary glands in adults were negatively correlated with testes, as in subadults. Conclusions drawn from relationships shown in Fig. 1 are not highly reliable because of the small sample size involved. An extreme gland weight of one animal could considerably alter the apparent correlations.

In the protein bound iodine analysis, a replacement bottle of ceric reagent was used when the initial bottle was depleted. Results of subsequent analyses suggested that this ceric reagent differed from the first in its light transmittance. The mean of transmittance values obtained for four sage grouse sera tested with the new reagent was 12.9% greater than that of the seven grouse previously tested with the old

reagent. Controls run with this new reagent had a mean 12.8% greater than the mean of controls run with the old. For statistical analysis, therefore, 12.8 was subtracted from the transmittance values obtained in PBI analyses with the new reagent bottle.

Lipid depletion as indicated by small cortical cells with reduced vacuolation, has been interpreted as evidence of adrenal stimulation in humans (Symington et al., 1957). Cell size in the sage grouse adrenals did not vary significantly. If differences in interrenal cell separation and extent of sinusoids indicate adrenal activity differences, this variation may be due to individual positions in the dominance order.

No general patterns of endocrine activity were conclusively determined. However, the following picture is suggested: The pituitary-adrenal axis is activated earlier in the year in the adults than in subadults. The testes and thyroids also are activated earlier. Subadults are faced with an established adult breeding hierarchy when they become physiologically ready for reproduction. According to this view the activation of the pituitary axis is the primary response and the thyroid activity is basically a supportative reaction. Sex steroids from the testes carry reproductive activity past the pituitary peak. This staggered reproductive readiness in age groups could prevent failure of reproduction even if the adult breeding period were completely disrupted.

Future study in this area might be aimed at intensive analysis of the adult sage grouse endocrinology, including evaluation of blood levels

of sex hormones. Relationships will become clearer as methods are devised for more direct analysis of hormone activities.

LITERATURE CITED

- Anon. 1967. Hycel cuvette P.B.I. determinations. Hycel Inc., Houston, Texas. 12 pp.
- Christian, J. J. 1959. The roles of endocrines and behavioral factors in the growth of mammalian populations. In "Comparative Endocrinology" (A. Gorbman, ed.), pp. 71-97. Wiley, New York.
- Christian, J. J. and D. E. Davis. 1964. Endocrines, behavior, and population. *Science* 146(3651): 1550-1560.
- Eng, R. L. 1963. Observations on the breeding biology of male sage grouse. *J. Wildl. Mgmt.* 27(4): 841-846.
- Flickinger, G. C. 1961. Effect of grouping on adrenals and gonads of chickens. *Gen. and Comp. Endo.* 1: 332-340.
- Guhl, A. M. and D. C. Warren. 1946. Number of offspring sired by cockerels related to social dominance in chickens. *Poultry Sci.* 25: 460-472.
- Lumsden, H. G. 1968. The displays of the sage grouse. Research Report (Wildlife) No. 83. Dept. of Lands and Forests, Ontario, Canada.
- Neave, D. J. and B. S. Wright. 1968. Ruffed grouse adrenal weights related to population density. *J. Wildl. Mgmt.* 32(3): 633-634.
- Patterson, R. L. 1952. The sage grouse in Wyoming. Wyoming Game and Fish Commission and Sage Books Inc., Denver. 341 pp.
- Siegel, H. S. 1959. The relation between crowding and weight of adrenal glands in chickens. *Ecology* 40: 495-498.
- Siegel, H. S. 1960. Effect of population density on the pituitary-adrenal cortical axis of cockerels. *Poultry Sci.* 39: 500-510.
- Symington, T., A. R. Currie, V. S. O'Donnell, J. K. Grant, E. G. Oostler, and W. G. Whyte. 1957. Hyperplasia and tumors of the human adrenal cortex: Histology, enzymes change, and corticoid production. In "Ciba Foundation Colloquia on Endocrinology" Vol. 12, pp. 102-115. Little, Brown and Co., Boston, Massachusetts.

MONTANA STATE UNIVERSITY LIBRARIES



3 1762 10005223 0



N 378

T 399

COP 2

