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The influence of various lighting regimes and exogenous adrenal hormones upon blood glucose and electrolyte levels in chicks

Jeffrey C. Herr

To the Graduate Council:

I am submitting herewith a thesis written by Jeffrey C. Herr entitled "The influence of various lighting regimes and exogenous adrenal hormones upon blood glucose and electrolyte levels in chicks." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Animal Science.

H. V. Shirley, Major Professor

We have read this thesis and recommend its acceptance:

R. L. Murphree, R. L. Tugwell

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

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H. V. Shirley, Major Professor

We have read this thesis
and recommend its acceptance:

R L Murphree

R. L. Tugwell

Accepted for the Council:

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THE INFLUENCE OF VARIOUS LIGHTING REGIMES AND EXOGENOUS ADRENAL HORMONES UPON BLOOD GLUCOSE AND ELECTROLYTE LEVELS IN CHICKS

A Thesis
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Jeffrey C. Herr

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ABSTRACT

The objectives of this study were to (1) determine rhythmic changes in blood glucose and electrolytes as influenced by different lighting regimes, (2) determine the influence of exogenous adrenal hormones and ACTH upon blood glucose and electrolyte levels of normally lighted birds, (3) compare blood glucose and electrolyte levels of non-treated birds from different lighting regimes to the blood levels of the treated, normally lighted birds, (4) make some conclusions regarding adrenal gland activity of the various experimental groups, using blood glucose and electrolyte levels as indicators of adrenal function.

Groups of two-day old male and female chicks were subjected to lighting regimes of constant light, constant darkness, and normal light, i.e., 12-hours light:12-hours dark. Likewise, groups of both sex were exposed to normal illumination, but given daily dosages of adrenocortical hormones. The experimental period lasted 21 days. On day 22, blood samples were drawn at 4 hour intervals during a 24 hour period. Serum was extracted and stored via deep freeze until analysis could be performed. Serum samples were analyzed for chloride, bicarbonate, sodium, calcium, potassium, and glucose levels. This was accomplished by means of a sequential multiple analyzer (SMA-660) machine and an atomic absorption-emission spectrophotometer.

An attempt was made to compare the data of the hormone treated groups to that of the various light regime groups. The results indicated that constant light and constant darkness stimulated adrenal function. This was evidenced by the fact that there was an increased retention of sodium and bicarbonate, and an increased excretion of calcium in birds exposed to constant light or darkness. Also, it appeared that normal circadian electrolyte and glucose rhythms were altered by constant light and darkness. It was also apparent that sexes responded differently to the various treatments.

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INTRODUCTION

There has been considerable research conducted regarding lighting regimes and their effects on animals. Most of this type of work done with poultry has dealt with production. Parameters such as onset of maturation, rate of lay, and growth rate have been the primary studies of most light regulating experiments. However, the current study dealt with the influence of various lighting regimes upon adrenal gland function and the resulting blood glucose and electrolyte levels.

A general knowledge of adrenal gland composition and function is necessary to evaluate any physiological changes induced by light or its absence.

The adrenal gland of birds' is composed of many islands of intermixed medullary and cortical tissue associated with the vascular system located in the area of the kidneys (McDonald, 1969).

The medullary portion of the gland is functionally related to the sympathetic nervous system. Stimulation of the sympathetic nervous system via pain, cold, emotional states, or hypoglycemia causes the release of epinephrine or norepinephrine into the blood. Norepinephrine is primarily responsible for circulatory adjustments. Epinephrine is mainly responsible for metabolic changes, especially carbohydrate metabolism and glycogenolysis, the process of breaking down glycogen into constituent glucose units. Epinephrine elevates blood glucose three ways: 1) by breaking down glycogen stores of the liver thereby freeing glucose; 2) by breaking down muscle glycogen into lactic acid which returns to the liver for glucose resynthesis; 3) by stimulating ACTH output from the hypophysis which causes adrenal glucocorticoid release which favors gluconeogenesis and glyconeogenesis. It should also be noted that increased ACTH output causes an increase in the function of epinephrine (Frienden and Lipner, 1971).

In summation, the catecholamines, epinephrine and norepinephrine, are primarily involved with homeostasis, ie., maintenance of blood pressure, regulation of changes in carbohydrate metabolism, and other utilitarian adjustments to meet stressful conditions.

The cortical portion of the adrenal gland is more important than the medullary portion, because it is essential for life, whereas the medullary portion is not (Williams, 1968).

Three main types of steroids are secreted by adrenal cortical tissue. They are the glucocorticoids, mineralocorticoids, and adrenal sex hormones, which to date, have little known importance.

Cortisol and corticosterone are the primary glucocorticoids secreted, and their actions are varied. They affect carbohydrate and protein metabolism to the extent that gluconeogenesis is increased as is protein catabolism. In addition, glucocorticoids exert an anti-inflammatory effect and also inhibit output of ACTH from the pituitary. They also exert lesser effects on water and electrolyte metabolism similar to those of mineralocorticoids. The output of glucocorticoids is at a minimal level in the absence of ACTH, because ACTH influences the formation and release of glucocorticoids. As corticoid levels rise, the output of ACTH is inhibited and vice-versa.

The major mineralocorticoids are deoxycorticosterone, 11-deoxycortisol, and aldosterone. They exhibit their primary effects on the kidney tubules, causing increased resorption and retention of sodium, and increased renal excretion of potassium. They also act to cause increased retention of chloride and water, and increased excretion of phosphorus and calcium. Control of mineralocorticoid secretion is not by the pituitary. It is thought that changes in sodium and potassium concentrations in the blood have a direct effect upon the adrenal cortical tissue. Also, a drop in blood pressure is believed to trigger the renin-angiotensin mechanism which in turn stimulates the glomerulosa cells of the adrenal cortical tissue to secrete mineralocorticoids (Williams, 1968).

Adrenocorticotropic hormone, or ACTH, is produced by the anterior pituitary. It acts by stimulating the adrenal cortex, causing the release of glucocorticoids into the blood stream. However, this stimulation is not vital because adrenal cortical tissue secretes a sufficient amount of hormone to maintain life in the hypophysectomized animal. Stress causes an outpouring of ACTH which in turn causes adrenal cortical tissue to increase its secretion of glucocorticoids.

Some previously observed symptoms of adrenal gland dysfunction have been reported. Hypersecretion of the adrenal cortex is primarily due to the hypersecretion of ACTH, and the symptoms are mainly manifestations of excess glucocorticoids. Protein and fats are converted to glucose. Muscular weakness results due to potassium depletion via diuresis. Also, prolonged high dosages of ACTH or glucocorticoids will cause an increase in the elimination of calcium, phosphorus, and nitrogen which will produce osteoporosis and favor fractures. Prolonged high dosage administration of glucocorticoids inhibits ACTH output to the extent that atrophy of the adrenal gland occurs (Williams, 1968).

High dosages of mineralocorticoids cause large amounts of sodium to be withdrawn from renal filtrate. This retention of sodium carries with it considerable quantities of water also resorbed from renal filtrate, and polydipsia results in an attempt to dilute the excess sodium. As a result, there is an increase in extracellular fluids and edema may follow. An increased resorption of chloride and bicarbonate also occurs since these negative ions tend to follow the positive sodium ions (McDonald, 1969).

Many symptoms of adrenalectomy and mineralocorticoid deficiencies are also known, but since the experiment to be discussed deals with excess hormone levels, a detailed list of such deficiencies is rather unappropriate. However, more often than not, a deficiency will produce the opposite effect of a hypersecretion.

CHAPTER 1

LITERATURE REVIEW

Blood Glucose Characteristics

Normal rhythmic patterns of blood glucose have been observed and reported in normally lighted birds. Smith (1972) reported that blood glucose levels were elevated during periods of illumination, but birds exposed to continuous light showed no rhythmic patterns. Also, birds exposed to continuous darkness had altered glucose rhythms with an elevated period of shorter duration than birds on normal lighting. This work supported previous reports by Smith et al. (1970) which indicated that there was a significant difference between blood glucose levels of chicks during light and dark hours.

Pauly et al. (1967) found that rats kept in constant light or darkness developed free-running blood glucose rhythms. This was thought to occur because of the absence of a light-dark synchronizer.

Batt (1939) found that age exerted a significant effect on normal blood sugar, with young chickens usually having higher levels than the older birds. Breed, sex, season of the year, and egg production produced minimal or nonsignificant differences in blood glucose levels.

Opdyke (1942) obtained an average blood sugar level of

188 mg.% among chicks 30-40 days old. Likewise, Heller and Purcell (1937) obtained values of 230 mg.% in very young chicks.

The influence of intermittent periods of light and dark on rate of growth in chicks has also been studied. Clegg et al. (1951) reported that chicks exposed to alternate 6 hour periods of light and dark were 66 grams heavier at 6 weeks of age than chicks exposed to 12 hours of light and 12 hours of darkness. Likewise, chicks subjected to alternate periods of 2 hours of light and 2 hours of darkness were 102 grams heavier at 6 weeks of age.

Sturkie (1965) states that blood sugar of birds is in the form of D-glucose as in mammals, but the levels are generally twice as high in birds, with the normal levels falling between 175-225 mg.%. Virtually all of the glucose in birds' blood is confined to the plasma, and whole blood analyses do not take into account sex differences. Glucose in the whole blood of males is significantly lower than females, but there is no sex difference in plasma glucose. Therefore, for accurate glucose determinations, only avian plasma should be used.

ACTH and Adrenal Secretion

Nagra et al. (1963) found that administration of proper dosages of mammalian ACTH to birds caused adrenal enlargement and release of the pituitary cortical hormones, corticosterone and aldosterone.

Sources of carbohydrate made available to birds by exogenous adrenal steroids appear to come from non-carbohydrate precursors. In birds, the hyperglycemic and glycogenic effects of corticosterone are due to conversion of protein substrate into carbohydrate (Sturkie, 1965).

Birds have an opague, gelatinous appearing structure which lies within the vertebral column. This glycogen body approximates 5-10% of the total glycogen in the chick. Pharmacological doses of ACTH have a modest glycogenic effect in the "glycogen body," which indicates that avian ACTH may play a role in controlling glycogenesis in the "glycogen body" (Sturkie, 1965).

Most workers agree that plasma corticosteroids in mammals and humans show peak values around or before the time of awakening. Rhythm in plasma corticosteroids completely disappeared in a man who spent three months alone underground, even though he followed a regular cycle of circadian activity, meals, and sleep (Mills, 1966).

Perkoff et al. (1959) concluded that the adrenal rhythm reflects a rhythm in ACTH secretion. This has been demonstrated by measurement of plasma ACTH concentration, the variations of which are of adequate size to cause the variations found in plasma corticosteroid concentration.

It has been concluded that the avian pituitary plays a major role in controlling the output of corticosterone by the adrenal, although the adrenals of chickens are capable of functioning independently of pituitary regulation to a limited extent (Nagra et al. 1963). Two weeks after hypophysectomy, the "resting" level of corticosterone in the effluent blood from the adrenals of hypophysectomized chickens was 40% lower than in intact fowl. The output of corticosterone by hypophysectomized birds or intact chickens increased during the time a series of hourly blood samples were collected from the adrenal vein. However, the magnitude of the response in the hypophysectomized birds was considerably less than that of the intact birds. Intravenous administration of ACTH to hypophysectomized chickens or to intact birds elevated the amount of corticosterone in adrenal venous blood.

Garren et al. (1961) studied the adrenal response of young chickens to ACTH as influenced by dosage and frequency of injection. The effect of level, injection interval, and suspending vehicle on the response of young chicks to ACTH was investigated. ACTH in saline caused the greatest responses, e.g., increased adrenal weight, decreased bursa weight, and decreased body weight gains. Generally speaking, a greater response was obtained when a given daily dosage was divided in doses at 2 hour intervals than when given at intervals of 4, 6, and 8 hours. In some instances, a daily dosage of ACTH that failed to give a discernible response in a short period of time did so after a longer period of time. ACTH suspended in gel was shown to be slightly less effective.

Brown et al. (1958) investigated the effects of surgical trauma and exogenous ACTH and adrenal cortical hormones on water balance, electrolytes, and gluconeogenesis in chickens. They found that DCA (desoxycorticosteroid acetate) treatment resulted in polyuria, polydipsia, and sodium and potassium retention. Cortisone acetate caused loss in weight, polyuria, and increased excretion of sodium, potassium, total nitrogen, and uric acid. ACTH prevented normal weight gains, and caused an increase in uric acid and total nitrogen excretion along with an increase in liver glycogen. Although there was no change in electrolyte excretion, the sodium and potassium presumed to be released by tissue catabolism made it appear probable that there was retention of electrolytes by the kidneys. ACTH also caused a small amount of adrenal hypertrophy. These data indicate that the hormones secreted by avian adrenals produce effects similar to those shown in mammals.

Circadian Rhythms and Light

As was previously mentioned, mammals and birds apparently do exhibit 24 hour circadian rhythms in adrenal cortical secretion. Critchlow (1963) reported no prominent peaks of corticosterone levels in the plasma of rats kept in constant light. However, the highest levels observed were comparable to those observed under normal illumination. Constant light may have some leveling effect as evidenced by the elevation of trough levels. There was, however, a suggestion of a 24 hour periodicity with some phase shift. Thus, it appears that constant light affects the secretory activity of the adrenal cortex and tends to exert some leveling effect on the 24 hour variations in corticosterone.

Other work reported states that a temporal shift in environmental lighting is associated with a corresponding shift in the rhythm of adrenal cortical function in mice (Halberg et al. 1959). This suggests that the nervous system is involved in mechanisms underlying this rhythmicity.

Light has been shown to be a stimulant of the pituitary in the work of Benoit and co-workers as reviewed by Sturkie (1954). The data collected in such studies indicated that limited light may be more stimulating to the anterior pituitary function of chicks than an abundant light environment. An increased pituitary function was evidenced by an increased thyroid size, increased adrenal size, and increased gonad size.

CHAPTER 2

MATERIALS AND METHODS

Cross-bred female and male broiler-type chicks obtained from Central Soya Hatchery, Chattanooga, were used in this experiment. The experimental design is presented in Table 1.

At 2 days of age, chicks of each sex were divided into 18 groups of 9 birds each. Fourteen groups of each sex were then distributed randomly among 3 electrically heated brooders in a room that received alternating periods of 12 hours of light and 12 hours of darkness (12L:12D). Two groups of each sex were placed in brooders in a room that received constant light (24L:0D). Likewise, two groups of each sex were put in brooders located in a constant darkness room (0L:24D). All the chicks were vaccinated via their drinking water against Newcastle disease and infectious bronchitis. They were allowed feed and water ad libitum during the entire experiment. The feed was a commercial chick starter mash (Table 2).

The birds were grown for 2 weeks, at which time various hormone treatments were begun for those chicks exposed to 12L:12D. The 24L:0D and 0L:24D groups received no hormone treatments.

Two groups of males and two groups of females received

TABLE 1

EXPERIMENTAL DESIGN

Treatment	Sex	No. Groups	Birds/Group
12L:12D	M	2	9
	F	2	9
24L:0D	M	2	9
	F	2	9
0L:24D	M	2	9
	F	2	9
ACTH**	M	4*	9
	F	4*	9
DEX.**	M	4*	9
	F	4*	9
DES.**	M	4*	9
	F	4*	9

* Two groups received a low dose and two groups received a high dose.

** Birds were exposed to normal lighting (12L:12D).

ACTH = adrenocorticotropic hormone
DEX. = dexamethazone sodium phosphate
DES. = desoxycorticosterone acetate

TABLE 2

EXPERIMENTAL DIET

Feedstuff	Amount %
Yellow corn	63.60
Alfalfa meal, 17%	2.50
Fish meal	2.50
Soybean oil meal, 50%	25.50
Ground limestone	0.60
Defluorinated rock phosphate	1.50
Salt	0.48
Manganese sulfate	0.02
Vitamin mix	0.02
Coccidiostat premix	2.50
Calculated Analysis:	
Crude protein, %	21.54
Productive energy, C/lb.	943.0
Methionine, %	0.408
Cystine, %	0.313
Calcium, %	0.960
Phosphorus, %	0.692
Available phosphorus, %	0.449
Manganese, mg./lb.	31.20
Vitamin A, I.U./lb.	5349
Vitamin D, I.C.U./lb.	340.0
Riboflavin, mg./lb.	3.01
Niacin, mg./lb.	27.78
Pantothenic acid, mg./lb.	6.67
Choline, mg./lb.	718.0

.02cc's (low dose, 1.6 U.S.P. units) of ACTH, and two groups of each sex received .04cc's (high dose, 3.2 U.S.P. units) of ACTH.

Two groups of each sex received .02cc's (low dose, .08mg.) of a synthetic glucocorticoid (dexamethazone sodium phosphate), and two groups of each sex received .04cc's (high dose, .16mg.) of the glucocorticoid.

Also, two groups of each sex received .04cc's (low dose, 0.2mg.) of a synthetic mineralocorticoid (desoxycorticosterone acetate), and two groups of each sex received .08cc's (high dose, 0.4mg.) of the drug.

Two groups of females and two groups of males served as controls. They were treated with physiological saline.

All the birds treated were injected daily, either subcutaneously or intramuscularly. Injections were administered in the morning, but the time varied from day to day. Injections were repeated for 21 days and then discontinued.

On day 22 of the experiment, blood samples were taken at 4 hour intervals during a 24 hour period, the first samples being taken at 7:00 am. During each sampling period, two birds from each group were bled. This included 12 groups of hormone treated chicks, 2 control groups, 2 groups from the constant light room, and 2 groups from the constant darkness room. In other words, 36 birds were used per sampling period, half of them being females and half males.

Special care was taken to handle the chicks as quietly and as gently as possible in an attempt to keep extreme nervousness and excitement among the birds at a minimum. Approximately 4cc's of blood was drawn from each chick by means of cardiac puncture. The blood was then allowed to stand at room temperature for approximately 30 minutes to permit clotting. The samples were then centrifuged at 1500 R.P.M. for at least 20 minutes, and approximately 2cc's of serum was extracted and placed in labeled vials. The vials were then placed in a freezer (-20 degrees C.) for storage until the samples could be analyzed.

Several weeks later, the serum samples were thawed and levels of sodium, potassium, calcium, chloride, bicarbonate, and glucose were measured. This was accomplished by means of a sequential multiple analyzer machine (SMA-660) and an atomic absorption-emission spectrophotometer at The University of Tennessee Memorial Research Center.

Analyses of the data was accomplished by means of The University of Tennessee Computer Center's Statistical Analysis System (SAS).

CHAPTER 3

RESULTS AND DISCUSSION

Preliminary analyses of the data indicated that numerous and complicated statistical interactions were involved. Sexes responded differently in respect to hormones and to light regimes. There were also some dose versus treatment interactions among the injected birds, and time versus light interactions among the light regime groups.

There were no significant treatment-dose interactions among males for any of the parameters studied, and there were no significant differences between high or low dose birds.

There were, however, significant treatment-dose interactions for sodium and glucose levels among females. The ACTH and dexamethazone treated birds had a higher mean level of sodium for the high dose injection (vs. low dose), while desoxycorticosterone produced a lower mean level for the high dose injection. Similarly, the ACTH and desoxycorticosterone treated birds had a higher mean level of glucose for the high dose injection (vs. low dose), while dexamethazone produced a lower mean level for its high dose injection. However, for both sexes, there were no significant overall dose mean differences in electrolyte and glucose levels.

Since the light regime groups did not involve parameters such as different dose levels of injected hormones, a direct comparison between injected birds and light regime birds would have been statistically inaccurate. Therefore, it was decided that the most proper method of analysis would be to compare the sexes and types of treatments independently, and then try to make some basic assumptions and comparisons based on characteristics evidenced in the various treatments.

Hence, the ACTH, dexamethazone, and desoxycorticosterone treated birds were compared with each other, and the 24L:0D, 0L:24D, and 12L:12D groups were compared with one another.

For the hormone injected groups, the overall electrolyte and glucose levels for the 24 hour sampling period were used for analysis. Since exogenous hormone injections would alter or interfere with any normal circadian electrolyte or glucose levels, individual readings at the four hour sampling intervals would have been of little value in so far as comparison of rhythms between the two types of treatments, i.e., injected versus light regime groups.

Among the light regime groups, however, overall electrolyte and glucose levels were compared as well as levels recorded at each sampling interval (time-treatment differences). The latter was done in an attempt to determine circadian rhythms in normal birds, and to see how constant light or constant darkness might affect such rhythms.

Chloride

The Effects of Light Regimes

No significant differences were found between the 24L:0D, 0L:24D, or 12L:12D groups of either sex in regard to overall serum chloride during the 24 hour sampling period (Table 3). The means were 113.8, 112.3, and 112.3 MEq/L respectively for males. For females, the respective means were 113.5, 111.8, and 112.7 MEq/L. Also, there were no significant time-treatment differences present in either sex (Table 3).

The Effects of Hormone Injections

There were no significant differences in the overall chloride levels between the three groups of hormone injected males (Table 4).

Among females, the ACTH (114.3 MEq/L) and desoxycorticosterone (115.3 MEq/L) groups were significantly higher than the dexamethazone (112.0 MEq/L) group (Table 4).

Discussion

The results indicated that chloride blood levels were not affected by light regimes. Both sexes had similar mean levels.

For reasons unknown, the female injected groups displayed significant differences in mean chloride levels. The male injected groups had levels almost identical to

TABLE 3

EFFECT OF LIGHT REGIMES AND SEX ON CIRCADIAN SERUM LEVELS OF CHLORIDE

Time	Sex	24L:0D	0L:24D	12L:12D
		MEq/L	MEq/L	MEq/L
0300	M	111.5[a]	112.0[a]	110.0[a]
0700	M	115.0[a]	114.5[a]	112.0[a]
1100	M	117.5[a]	110.5[a]	115.0[a]
1500	M	113.5[a]	113.0[a]	112.5[a]
1900	M	113.5[a]	110.0[a]	112.5[a]
2300	M	111.5[a]	113.5[a]	112.0[a]
Mean*	M	113.8[a]	112.3[a]	112.3[a]
0300	F	113.0[a]	112.0[a]	113.5[a]
0700	F	114.5[a]	114.0[a]	113.0[a]
1100	F	109.5[a]	116.0[a]	113.0[a]
1500	F	111.0[a]	111.5[a]	116.0[a]
1900	F	113.0[a]	111.5[a]	111.5[a]
2300	F	111.5[a]	111.5[a]	110.5[a]
Mean*	F	113.5[a]	111.8[a]	112.7[a]

Means within each column with different superscripts are significantly different at the .05% level of probability.

*Means within row with different superscripts are significantly different at the .05 % level of probability.

TABLE 4

MEAN ELECTROLYTE VALUES FOR INJECTED BIRDS

Treatment	Sex	Cl^-	HCO_3	Na^{++}	Ca^{++}	K^+	Glucose
		MEq/L	MEq/L	MEq/L	Mg.%	MEq/L	Mg.%
ACTH	M	113.3^a	18.3^b	155.6^a	7.1^b	5.9^a	269.2^a
DEX.	M	112.5^a	18.2^b	155.7^a	8.9^a	6.2^a	263.8^a
DES.	M	111.8^a	21.4^a	156.7^a	7.3^b	6.2^a	253.9^b
ACTH	F	114.3^a	18.3^a	156.9^a	7.2^b	6.1^a	261.9^a
DEX.	F	112.0^b	18.6^a	156.2^a	8.5^a	6.1^a	265.0^a
DES.	F	115.3^a	19.6^a	159.2^a	6.9^b	6.0^a	248.3^b

Means within each column with different superscripts are significantly different at the .05 % level of probability.

ACTH = adrenocorticotropic hormone
DEX. = dexamethazone sodium phosphate
DES. = desoxycorticosterone acetate

those of the light regime groups, while the female injected groups had levels slightly higher than the light groups. Among females, the fact that the mineralocorticoid treated group had the highest chloride level was expected since chloride retention is an action of mineralocorticoids.

Apparently, chloride regulation in females is more easily influenced by mineralocorticoid secretion than in males.

However, it appears that neither constant illumination nor constant darkness produces enough change in mineralocorticoid secretion to alter chloride levels in either sex.

The fact that there were no significant time differences in chloride levels among the light regime groups of either sex suggests that there is not a discernible chloride rhythm present in young birds.

Bicarbonate

The Effects of Light Regimes

Among males, the overall serum bicarbonate means for the 24L:0D (20.4 MEq/L) and 0L:24D (20.4 MEq/L) groups were significantly higher than the 12L:12D (19.1 MEq/L) group (Table 5). There were also significant time differences present in each group (Table 5).

Among females, the overall bicarbonate mean for the 0L:24D (22.6 MEq/L) group was significantly higher than the

TABLE 5

EFFECT OF LIGHT REGIMES AND SEX ON CIRCADIAN SERUM LEVELS OF BICARBONATE

Time	Sex	24L:0D	0L:24D	12L:12D
		MEq/L	MEq/L	MEq/L
0300	M	21.0[ab]	20.0[b]	21.5[a]
0700	M	19.5[cd]	19.5[b]	18.0[b]
1100	M	20.5[bc]	23.5[a]	15.5[c]
1500	M	19.0[d]	19.5[b]	21.5[a]
1900	M	20.5[bc]	19.5[b]	19.0[b]
2300	M	22.0[a]	20.5[b]	19.0[b]
Mean*	M	20.4[a]	20.4[a]	19.1[b]
0300	F	19.5[b]	21.5[cd]	17.5[d]
0700	F	16.5[d]	20.5[d]	18.0[cd]
1100	F	18.5[bc]	25.0[a]	18.5[bc]
1500	F	19.5[b]	24.0[ab]	19.5[ab]
1900	F	17.5[cd]	23.0[b]	20.5[a]
2300	F	23.0[a]	21.5[cd]	19.5[ab]
Mean*	F	19.1[b]	22.6[a]	18.9[b]

Means within each column with different superscripts are significantly different at the .05% level of probability.

*Means within row with different superscripts are significantly different at the .05% level of probability.

24L:0D (19.1 MEq/L) and 12L:12D (18.9 MEq/L) groups (Table 5). There were also significant time differences within each group (Table 5).

The Effects of Hormone Injections

Among males, the overall bicarbonate level for the desoxycorticosterone group (21.4 MEq/L) was significantly higher than the ACTH (18.3 MEq/L) and dexamethazone (18.2 MEq/L) groups (Table 4).

There were no significant differences among females (Table 4).

Discussion

The fact that male mineralocorticoid treated birds had a significantly higher bicarbonate level than the ACTH and glucocorticoid treated groups was expected since mineralocorticoids are known to increase the resorption of bicarbonate. Hence, the significantly higher levels found in males subjected to constant illumination and darkness suggest that a hypersecretion of mineralocorticoid was occurring as a result of constant light or darkness.

Females appeared to be responding to a mineralocorticoid hypersecretion only during constant darkness. If hypersecretion did occur during constant light, it apparently did not increase mineralocorticoid output enough to alter bicarbonate levels in the blood.

Circadian rhythms were apparent in the bicarbonate levels of both sexes. Among males, the normally lighted birds showed peak levels around 3 am. and 3 pm. The constant light birds reached their peak values during the late night and early morning hours. The constant darkness birds reached a single peak value around 11 am. (Figure 1). It appears that constant light and constant darkness both have a leveling effect upon bicarbonate levels, except for one rather short span of elevation during a 24 hour period. The peak value for constant light males occurred during the same time period that one of the normally lighted peaks was evident. However, the one peak value for the constant darkness males was completely out of phase with those of the normal birds'.

Among females, the normally illuminated birds showed only one peak value which occurred around 7 pm. The constant light females also showed only one peak value, but it was slightly out of phase, occurring about 11 pm. The constant darkness birds exhibited elevated levels from about 11 am. until 6 pm. (Figure 2). Again, it appears that the constant light bicarbonate rhythm was more similar to the normal female rhythm, even though it was slightly out of phase. As was the case with males, the constant darkness rhythm is completely out of phase.

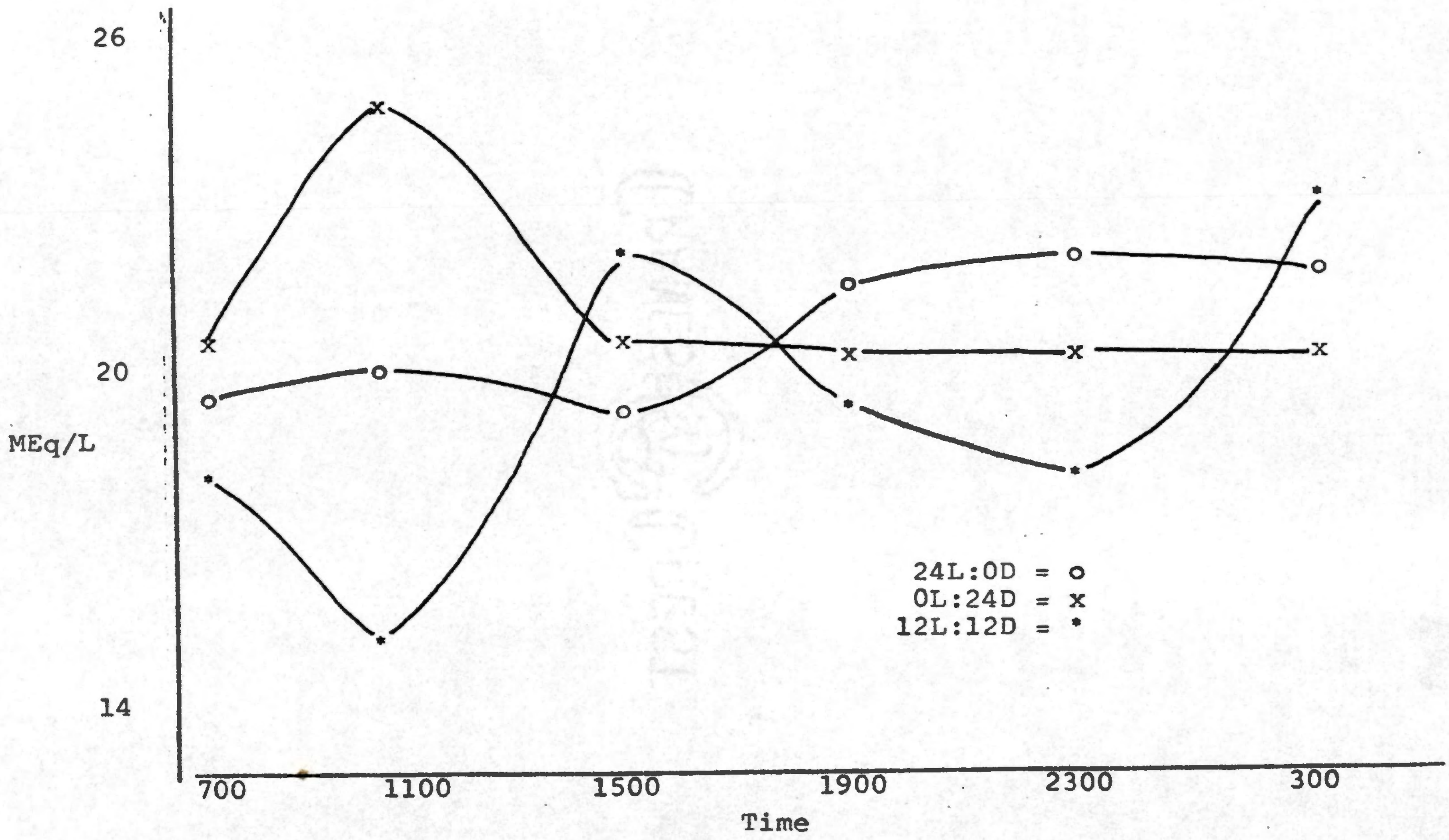

FIGURE 1

CIRCADIAN SERUM BICARBONATE LEVELS OF MALES

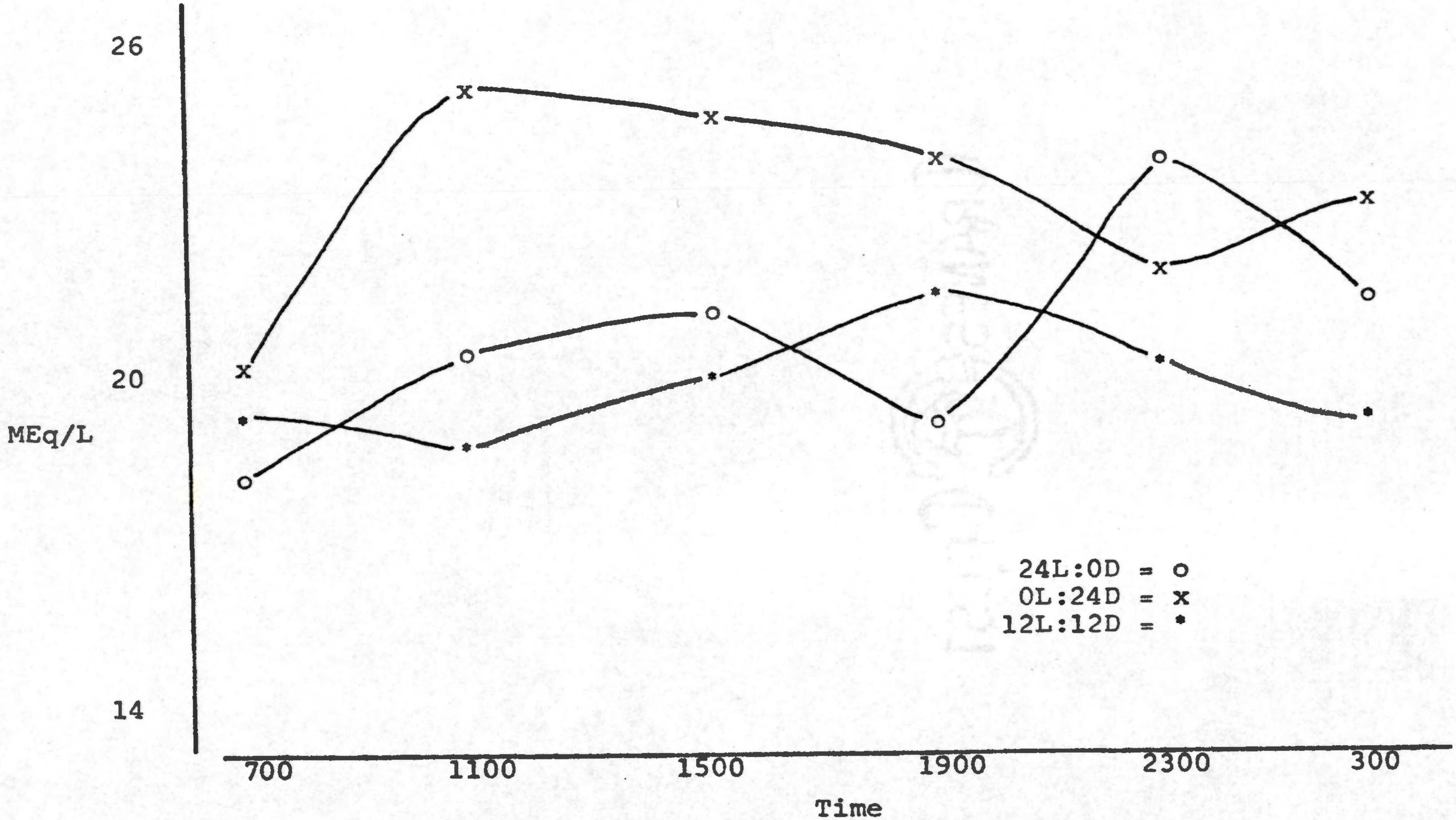

FIGURE 2

CIRCADIAN SERUM BICARBONATE LEVELS OF FEMALES

Sodium

The Effects of Light Regimes

Among males, the overall serum sodium mean for the 24L:0D group (155.3 MEq/L) was significantly higher than the 0L:24D (151.0 MEq/L) and 12L:12D (151.4 MEq/L) groups (Table 6). There were also significant time differences present in each light group (Table 6).

Among females, the overall means for the 24L:0D (154.2 MEq/L) and 12L:12D (154.7 MEq/L) groups were significantly higher than the 0L:24D (150.4 MEq/L) group (Table 6). There were also significant time differences in each group (Table 6).

The Effects of Hormone Injections

There were no significant sodium level differences between any of the injected groups of either sex (Table 4, p. 21).

Discussion

The mean sodium levels of both sexes of the injected groups did appear to be significantly higher than those of the normally lighted birds'. Hence, it was assumed that sodium retention had occurred in each of the hormone treated groups since that is a primary action of mineralocorticoids, and to a lesser extent, ACTH and glucocorticoids.

The fact that constant light males exhibited highly

TABLE 6

EFFECT OF LIGHT REGIMES AND SEX ON CIRCADIAN SERUM LEVELS OF SODIUM

Time	Sex	24L:OD	OL:24D	12L:12D
		MEq/L	MEq/L	MEq/L
0300	M	154.0[cd]	150.0[b]	150.5[b]
0700	M	159.0[ab]	156.0[a]	150.5[b]
1100	M	162.5[a]	155.0[a]	155.5[a]
1500	M	155.5[bc]	150.5[b]	151.0[b]
1900	M	151.5[de]	143.5[c]	151.0[b]
2300	M	149.0[e]	151.0[b]	150.0[b]
Mean*	M	155.3[a]	151.0[b]	151.4[b]
0300	F	153.0[bc]	151.0[a]	156.5[a]
0700	F	158.5[a]	153.5[a]	158.0[a]
1100	F	156.0[ab]	154.0[a]	158.5[a]
1500	F	151.0[c]	149.5[ab]	154.0[ab]
1900	F	153.5[bc]	146.0[b]	150.5[b]
2300	F	153.0[bc]	148.5[ab]	150.5[b]
Mean*	F	154.2[a]	150.4[b]	154.7[a]

Means within each column with different superscripts are significantly different at the .05% level of probability.

*Means within row with different superscripts are significantly different at the .05% level of probability.

significant sodium levels suggests sodium retention and is probably due to hypersecretion of a mineralocorticoid. Females kept in constant darkness had significantly lower sodium levels than the normally lighted birds'. This seems to indicate a hypofunction of mineralocorticoids and a decreased retention of sodium. However, Brown (1958) found that Cortisone Acetate, a glucocorticoid, caused an increased excretion of sodium. Thus, it may be that constant darkness caused a hypersecretion of glucocorticoids and the resultant low sodium level.

Rhythmic sodium patterns were evident in both sexes. Among males, the constant light and constant darkness rhythms were very similar to the normal rhythm with peak values occurring around 11 am. Apparently, constant light or darkness had no effect upon circadian sodium rhythms in males (Figure 3).

Likewise, among females, the constant light and darkness rhythms mimicked the normal rhythm for females (Figure 4).

Calcium

The Effects of Light Regimes

Among males, the overall mean serum calcium level for the 24L:0D group (8.1 mg.%) was significantly higher than the 0L:24D (6.9 mg.%) and 12L:12D (7.1 mg.%) groups (Table 7). There were also significant time differences in each

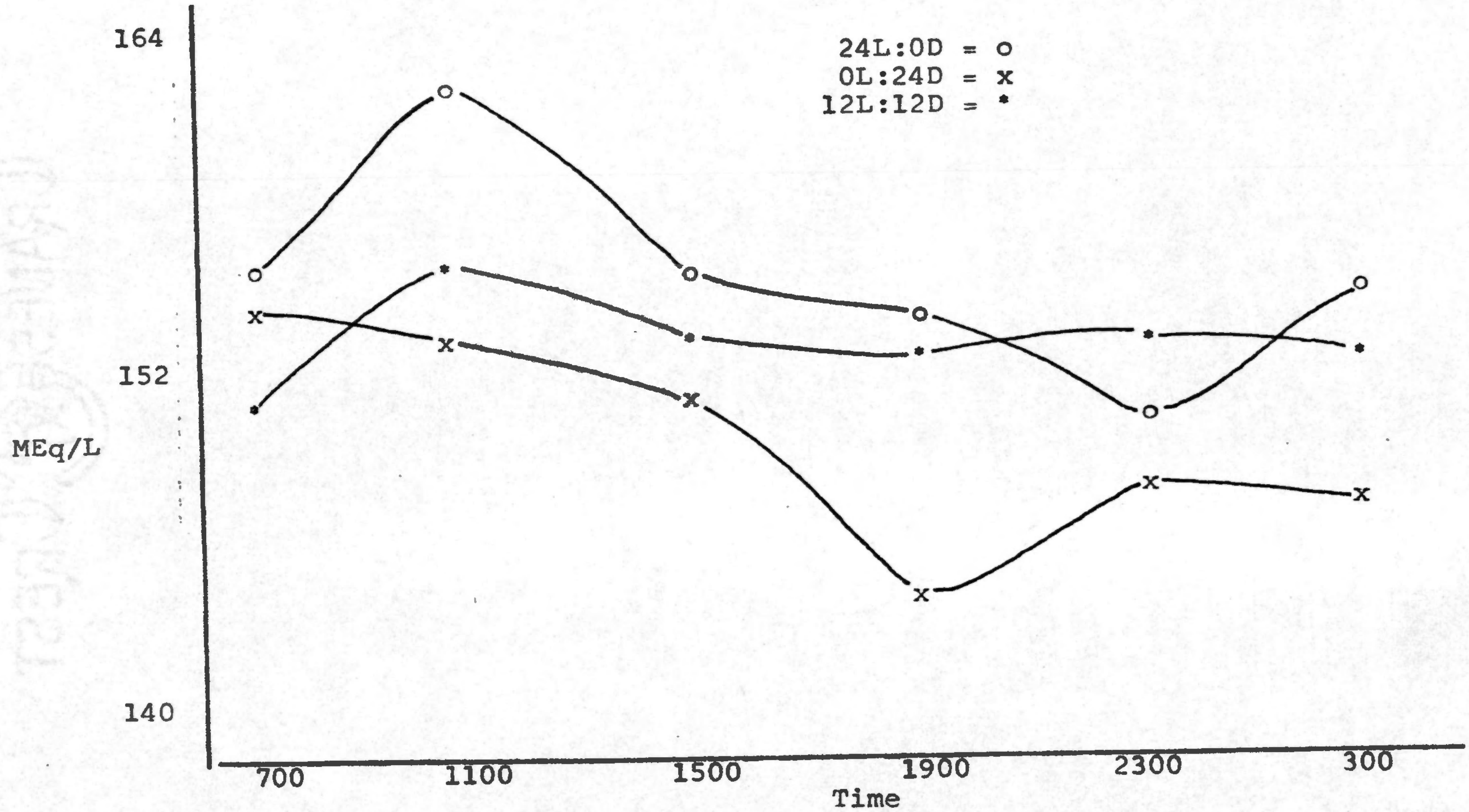

FIGURE 3

CIRCADIAN SERUM SODIUM LEVELS OF MALES

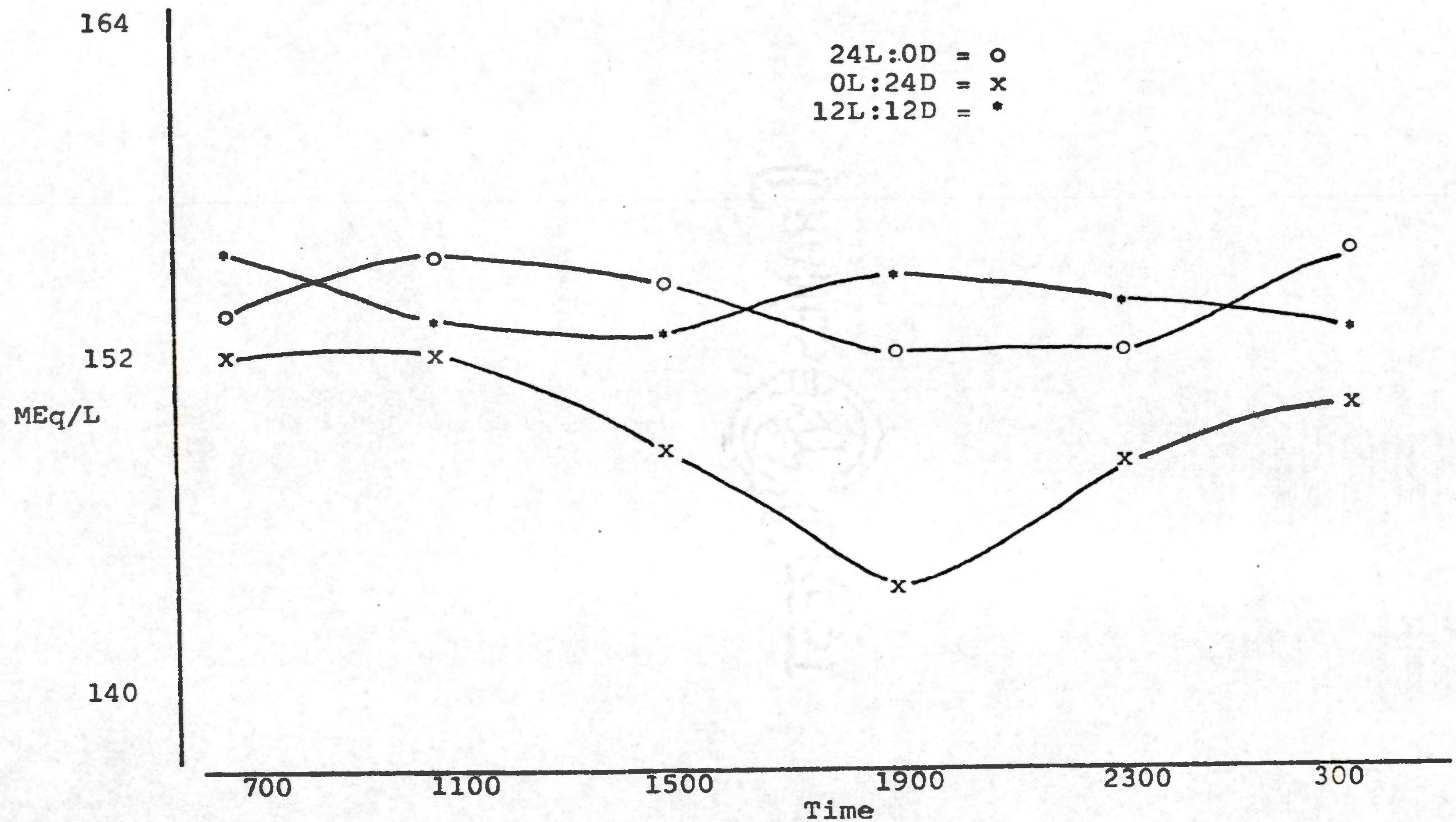

FIGURE 4

CIRCADIAN SERUM SODIUM LEVELS OF FEMALES

TABLE 7

EFFECT OF LIGHT REGIMES AND SEX ON CIRCADIAN SERUM LEVELS OF CALCIUM

Time	Sex	24L:0D	0L:24D	12L:12D
		Mg.%	Mg.%	Mg.%
0300	M	7.7[c]	6.1[b]	5.9[bc]
0700	M	7.6[c]	10.4[a]	9.9[a]
1100	M	11.4[a]	6.5[b]	8.9[a]
1500	M	7.3[c]	6.6[b]	7.2[b]
1900	M	8.6[b]	4.9[c]	5.2[c]
2300	M	6.1[d]	6.8[b]	5.3[c]
Mean*	M	8.1[a]	6.9[b]	7.1[b]
0300	F	6.9[b]	5.3[d]	7.4[b]
0700	F	8.4[a]	6.7[c]	8.6[a]
1100	F	7.3[b]	10.4[a]	9.4[a]
1500	F	6.6[b]	8.2[b]	6.5[bc]
1900	F	6.5[b]	4.6[d]	5.7[c]
2300	F	5.8[b]	4.9[d]	5.2[c]
Mean*	F	6.9[a]	6.6[a]	7.1[a]

Means within each column with different superscripts are significantly different at the .05 % level of probability.

*Means within row with different superscripts are significantly different at the .05 % level of probability.

group (Table 7).

The Effects of Hormone Injections

Among males, the overall calcium level for the dexamethazone group (8.9 mg.%) was significantly higher than the ACTH (7.1 mg.%) and desoxycorticosterone (7.3 mg.%) groups (Table 4, p. 21).

Likewise, for females, the dexamethazone group (8.5 mg.%) was significantly higher than the ACTH (7.2 mg.%) and desoxycorticosterone (6.9 mg.%) groups (Table 4, p.21).

Discussion

The significantly elevated serum calcium levels of the dexamethazone treated birds were expected, since prolonged administration of glucocorticoids is known to cause an increased elimination of calcium from bone, and hence, an increased level of blood calcium. Turner and Bagnara (1971) state that the antianabolic action of glucocorticoids shows up in bone as a profound decrease in collagen synthesis and accelerated bone resorption or dissolution. Apparently, normal absorption of calcium by bone and other calcium utilizing tissues does not occur.

The fact that male birds exposed to constant light had significantly elevated calcium levels would seem to indicate calcium excretion. This would lead one to believe that a hypersecretion of ACTH or glucocorticoids had occurred, and it is the author's belief that such was the case.

There were no apparent effects upon the calcium levels of females exposed to the different lighting regimes.

Circadian calcium rhythms were evident in both sexes. The normally lighted males had single peak values around 7 am., followed by steady declines in blood calcium throughout the rest of the day. The constant light males also had their peak values around 7 am., and their levels flucuated slightly thereafter. Constant darkness males exhibited these same patterns, but their rhythms were slightly out of phase (Figure 5).

Normally illuminated and constant darkness females had very similar rhythms with peak values occurring around mid-morning. Constant light females had slightly elevated levels during the early morning, but levels appeared to be fairly constant throughout the 24 hour period (Figure 6).

Potassium

The Effects of Light Regimes

Among males, the overall means for the 0L:24D (6.7 MEq/L) and 12L:12D (6.8 MEq/L) groups were significantly higher than the 24L:0D (6.2 MEq/L) group (Table 8). However, there were no significant time differences in any of the groups (Table 8).

Among females, there were no significant differences in the overall levels of potassium in the three groups (Table 8). There were significant time differences in

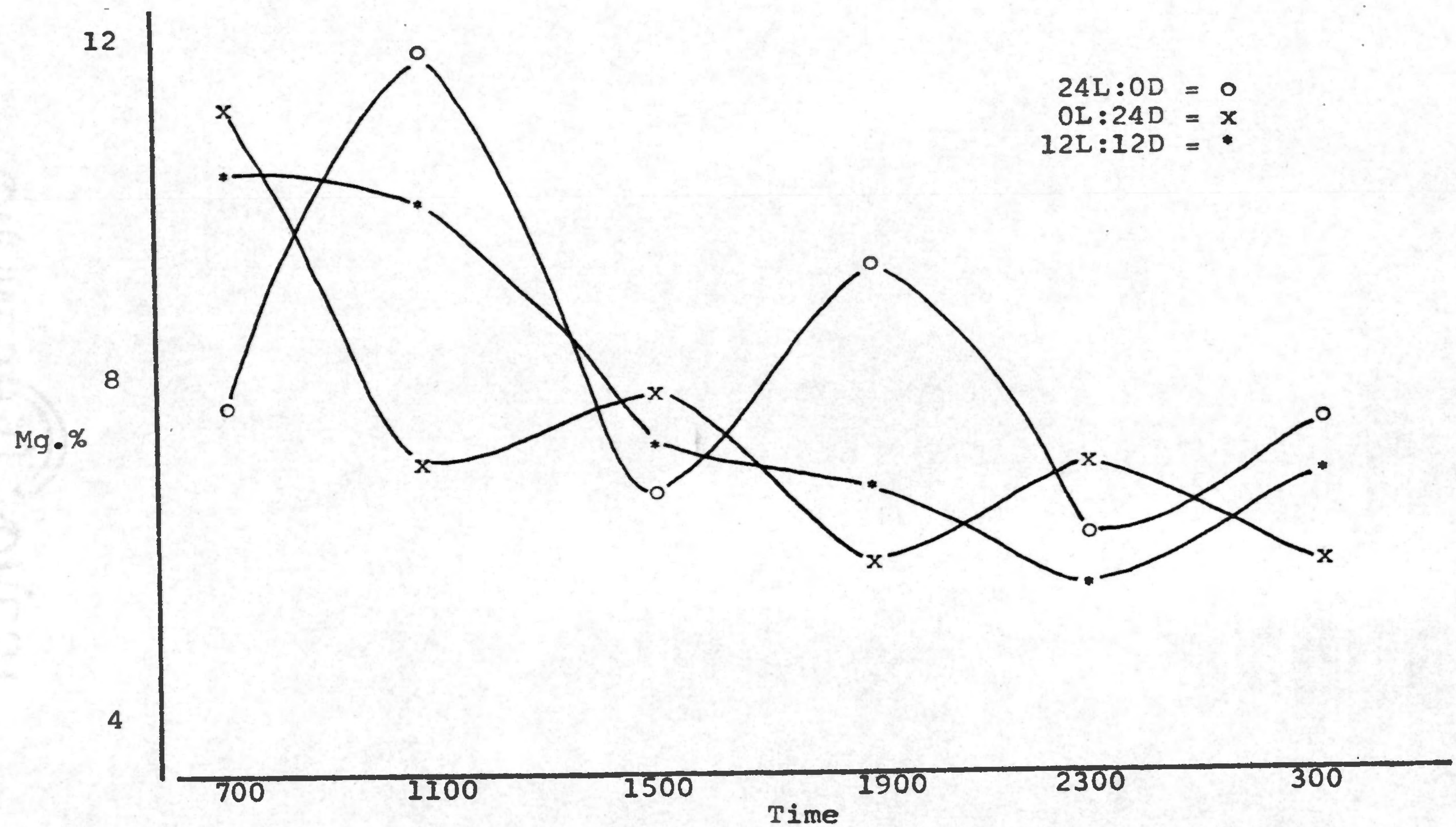

FIGURE 5

CIRCADIAN SERUM CALCIUM LEVELS OF MALES

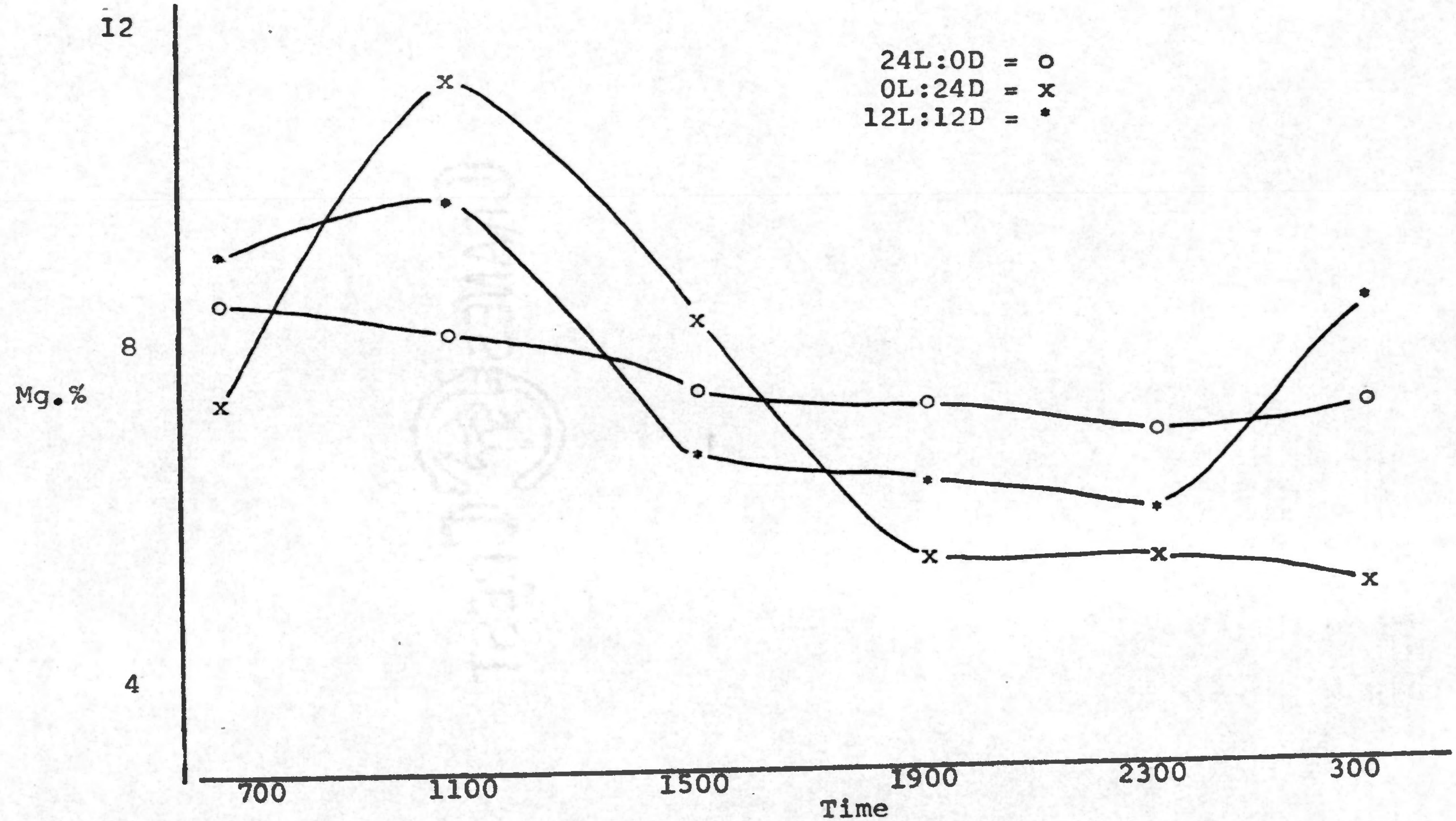

FIGURE 6

CIRCADIAN SERUM CALCIUM LEVELS OF FEMALES

TABLE 8

EFFECT OF LIGHT REGIMES AND SEX ON CIRCADIAN SERUM LEVELS OF POTASSIUM

Time	Sex	24L:0D	0L:24D	12L:12D
		MEq/L	MEq/L	MEq/L
0300	M	5.7^{a}	6.9^{a}	6.6^{a}
0700	M	5.9^{a}	6.7^{a}	6.9^{a}
1100	M	6.8^{a}	6.2^{a}	6.8^{a}
1500	M	6.5^{a}	6.8^{a}	6.8^{a}
1900	M	5.8^{a}	6.9^{a}	6.9^{a}
2300	M	6.3^{a}	6.5^{a}	6.9^{a}
Mean*	M	6.2^{b}	6.7^{a}	6.8^{a}
0300	F	6.3^{bc}	5.4^{b}	5.6^{a}
0700	F	6.3^{bc}	8.2^{a}	6.4^{a}
1100	F	5.8^{c}	5.6^{b}	6.1^{a}
1500	F	6.6^{b}	5.8^{b}	6.1^{a}
1900	F	6.4^{b}	5.8^{b}	6.1^{a}
2300	F	7.3^{a}	6.2^{b}	6.3^{a}
Mean*	F	6.4^{a}	6.2^{a}	6.1^{a}

Means within each column with different superscripts are significantly different at the .05 % level of probability.

*Means within row with different superscripts are significantly different at the .05 % level of probability.

the 24L:0D and 0L:24D groups, but not in the 12L:12D group (Table 8).

The Effects of Hormone Injections

There were no significant differences in potassium levels between any of the injected groups of either sex (Table 4, p. 21).

Discussion

Apparently, neither the amount of exogenous hormones administered, nor the effect of constant light or darkness was sufficient enough to alter potassium mobilization.

There were no circadian rhythms evident among males. Females exposed to normal light also failed to exhibit circadian rhythms. However, constant light females had flucuating rhythms with peak values occurring around 11 pm. Constant darkness females had constant values almost identical to normal birds', except for a major peak which occurred around 10 am. (Figure 7).

Glucose

The Effects of Light Regimes

Among males, the overall serum glucose means for the 0L:24D (256.7 mg.%) and 12L:12D (255.0 mg.%) groups were significantly higher than the 24L:0D (246.3 mg.%) group (Table 9). There were also significant time differences present in each group (Table 9).

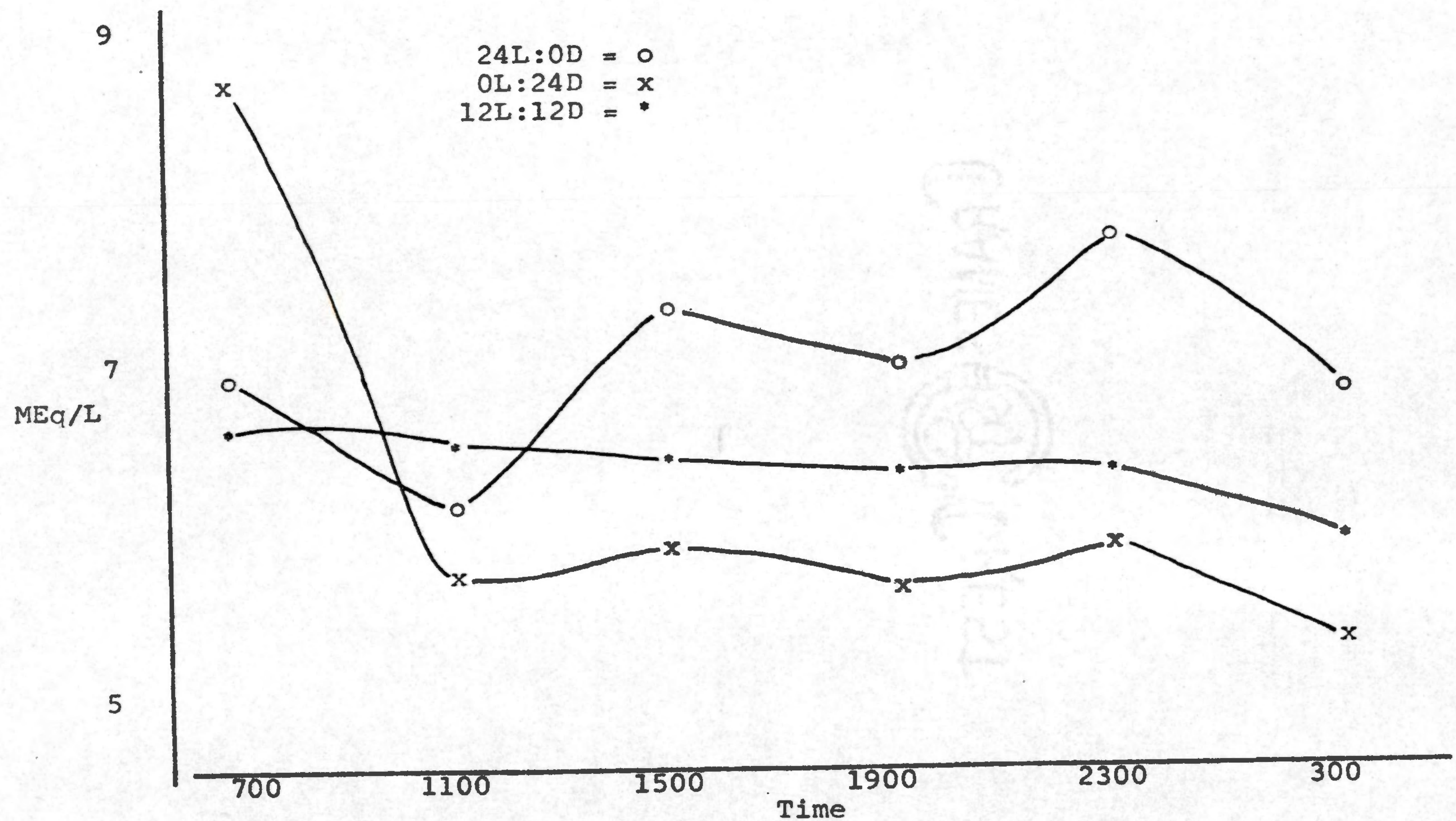

FIGURE 7

CIRCADIAN SERUM POTASSIUM LEVELS OF FEMALES

TABLE 9

EFFECT OF LIGHT REGIMES AND SEX ON CIRCADIAN SERUM LEVELS OF GLUCOSE

Time	Sex	24L:0D	0L:24D	12L:12D
		Mg.%	Mg.%	Mg.%
0300	M	245.0^{b}	272.5^{a}	247.5^{c}
0700	M	250.0^{b}	260.0^{b}	267.5^{ab}
1100	M	262.5^{a}	267.5^{a}	262.5^{b}
1500	M	237.5^{c}	247.5^{c}	240.0^{c}
1900	M	245.0^{b}	247.5^{c}	240.0^{c}
2300	M	237.5^{c}	245.0^{c}	272.5^{a}
Mean*	M	246.3^{b}	256.7^{a}	255.0^{a}
0300	F	242.5^{b}	247.5^{c}	277.5^{a}
0700	F	255.0^{a}	247.5^{c}	247.5^{d}
1100	F	247.5^{b}	257.5^{b}	265.0^{b}
1500	F	245.0^{b}	265.0^{a}	257.5^{c}
1900	F	242.5^{b}	232.5^{d}	245.0^{d}
2300	F	247.5^{b}	247.5^{c}	252.5^{c}
Mean*	F	246.7^{b}	249.6^{b}	257.5^{a}

Means within columns with different superscripts are significantly different at the .05% level of probability.

*Means within row with different superscripts are significantly different at the .05% level of probability.

Among females, the overall glucose mean for the 12L:12D group (257.5 mg.%) was significantly higher than the 24L:0D (246.7 mg.%) and 0L:24D (249.6 mg.%) groups (Table 9). Again, there were significant time differences within each group (Table 9).

<u>The Effects of Hormone Injections</u>

Among males, the overall glucose levels for the ACTH (269.2 mg.%) and dexamethazone (263.8 mg.%) groups were significantly higher than the desoxycorticosterone (253.9 mg.%) group (Table 4, p. 21).

Likewise, for females, the ACTH (261.9 mg.%) and dexamethazone (265.0 mg.%) groups were significantly higher than the desoxycorticosterone (248.3 mg.%) group (Table 4, p. 21).

<u>Discussion</u>

Both sexes of the ACTH and dexamethazone injected birds had what appeared to be significantly higher glucose levels than the normally lighted birds'. This effect was anticipated since blood glucose elevation is an action of ACTH and glucocorticoids.

The fact that constant light males and constant light and constant darkness females exhibited significantly lower glucose values than the controls indicates that a hyposecretion of ACTH and glucocorticoids had occurred. However, it is the author's belief that such may not be the case.

Sturkie (1954) states that light has been shown to be a stimulant of the pituitary. Also, studies indicated that limited light may be more stimulating to the anterior pituitary function of chicks than an abundant or continuous light environment. This increased function was evidenced by increased thyroid, gonad, and adrenal size. It is also believed that prolonged hypersecretion of hormones by a gland may lead to exhaustion and temporary cessation of the glands activity (Williams, 1968).

It may have been then in this experiment that constant light and constant darkness caused a prolonged stimulation of ACTH and resultant glucocorticoid secretion which initially would have produced high blood glucose levels. However, pituitary and adrenocortical unresponsiveness may have eventually occurred during the experimental period. This would have reduced protein catabolism and gluconeogenesis, and blood sugar levels would have dropped to levels near or slightly below normal.

Perhaps a blood sample taken early in the experimental period, or several samples taken during the course of the experiment would have been helpful in determining the actual effects of constant light and darkness upon serum glucose. Unfortunately, samples were only taken at the end of the experiment.

Circadian glucose rhythms were apparent in both sexes.

Among males, the normally lighted birds showed prominent peak values around 7 am. and 11 pm. The constant light birds had a single peak value around 11 am., but then glucose levels dropped and remained constant throughout the rest of the day. The constant darkness males displayed a rhythm almost identical to that of normal birds', but it was slightly out of phase (Figure 8).

The normally illuminated females displayed a glucose rhythm very much like the males, with peak values occurring at 11 am. and 3 am. This agrees with work done by Batt (1939), who states that sex produced minimal or non-significant differences in blood glucose rhythms. The constant light females appeared to have lost their glucose rhythm. The constant darkness females displayed prominent peak values around 3 pm., followed by slight flucuations throughout the rest of the day (Figure 9). These findings support work by Smith (1972), who stated that birds exposed to continuous light showed no rhythmic patterns, and birds exposed to constant darkness had altered glucose rhythms.

Conclusions

The results of this experiment indicate that constant light and constant darkness stimulate adrenal gland function via pituitary release of ACTH and subsequent release of glucocorticoids. There is also an apparent hypersecretion of mineralocorticoids. These assumptions are based on

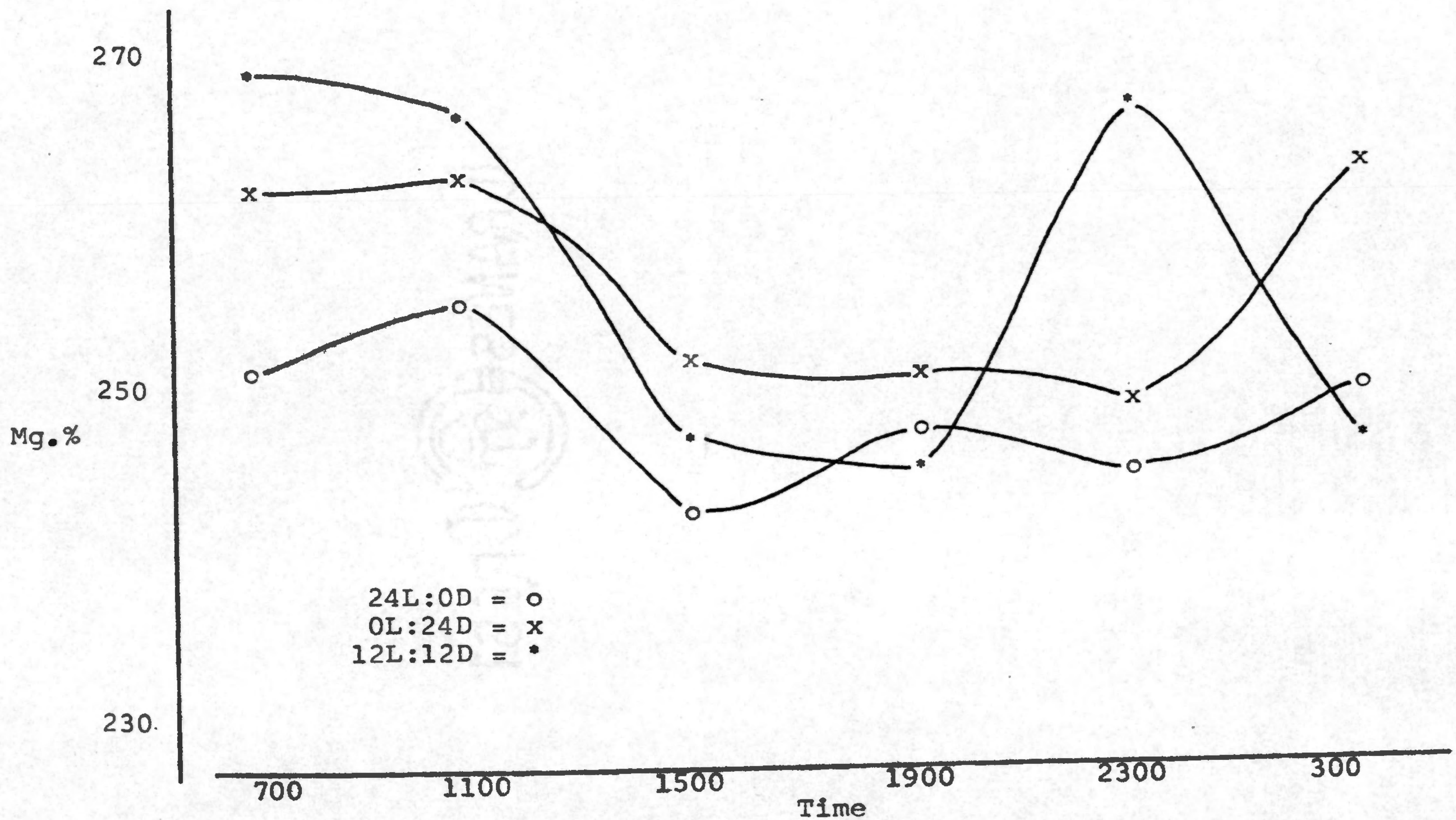

FIGURE 8

CIRCADIAN SERUM GLUCOSE LEVELS OF MALES

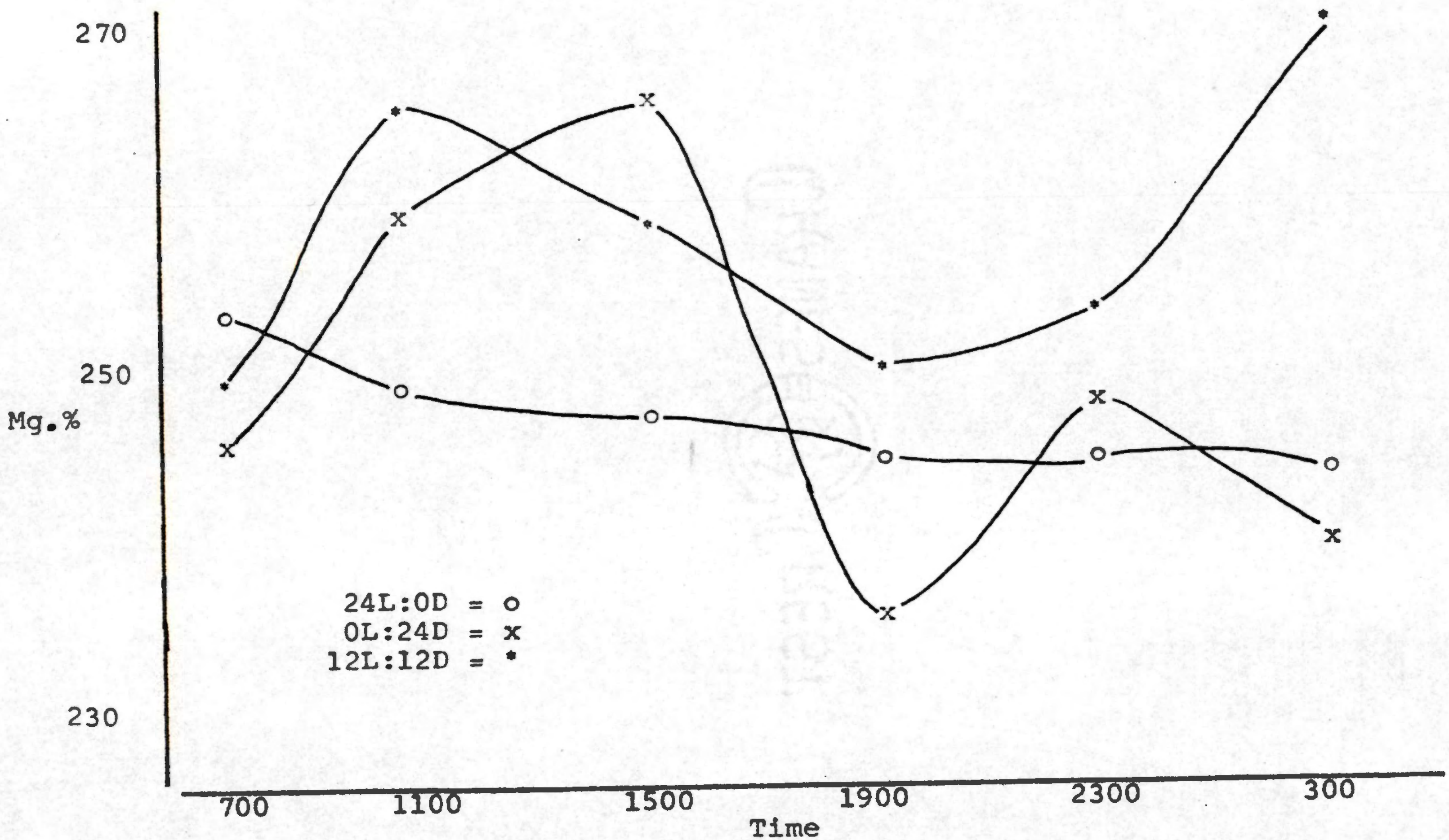

FIGURE 9

CIRCADIAN SERUM GLUCOSE LEVELS OF FEMALES findings which indicate increased retention of bicarbonate and sodium, and increased excretion of calcium.

Also, it is apparent that these different lighting regimes affect or cause electrolyte and glucose circadian rhythms to vary from the normal rhythms. Figures 1-9 are the basis for this assumption.

It can also be concluded that sexes differ in their responses and sensitivity to constant light or darkness and also to exogenous adrenal hormone injections. However, it is not known why many of these differences occur.

CHAPTER 4

SUMMARY

1. Twelve groups of two-day old male and female broiler-type chicks were subjected to lighting regimes of constant light, constant darkness, and normal light, i.e., 12L:12D. Likewise, 24 groups of both sexes were exposed to normal illumination, but given daily dosages of adrenocortical hormones. The experiment lasted 21 days.

2. Serum samples were analyzed for chloride, bicarbonate, sodium, calcium, potassium, and glucose levels.

3. The data of the hormone treated groups was compared to that of the light regime groups.

4. The results indicated that constant light and constant darkness stimulated adrenal function. This was evidenced by the fact that there was an increased retention of sodium and bicarbonate, and an increased excretion of calcium in birds exposed to constant light or darkness.

5. Normal circadian electrolyte and glucose rhythms were altered by constant light and constant darkness.

6. Different sexes were found to respond differently to the various treatments.

LIST OF REFERENCES

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Batt, H.R., 1939. The blood sugar levels of the fasting domestic fowl. Am. J. Physiol. 126:429.

Brown, K.I., D.J. Brown, and R.K. Meyer, 1958. Effect of surgical trauma, ACTH and adrenal cortical hormones on electrolytes, water balance, and gluconeogenesis in male chickens. Am. J. Physiol. 192:43.

Clegg, R.E. and P.E. Sanford, 1951. The influence of intermittent periods of light and dark on growth rate of chicks. Poultry Sci. 30:760.

Critchlow, V., 1963. The role of light in the neuroendocrine system. Advances in Neuroendocrinology, ed. A.V. Nalbandov, University of Illinois Press, Urbana, pp. 378-402.

Frienden, E., and H. Lipner, 1971. Biochemical Endocrinology of the Vertebrates, Prentice-Hall Inc. Englewood Cliffs, N.J.

Garren, H.W., C.H. Hill, and M.W. Carter, 1961. Adrenal response of young chickens to ACTH as influenced by dosage and frequency of injection. Poultry Sci. 40:446.

Halberg, F., C.P. Barnum, R.H. Silber, and J.J. Bittner, 1958. 24-hour rhythms at several levels of intergration in mice on different lighting regimes. Proc. Soc. Exptl. Bio. Med. 97:897.

Heller, V.G., and L. Purcell, 1937. Chemical composition of the blood of the hen during its life cycle. J. Bio. Chem. 118:549-553.

Mason, J.W., 1959. Physiological influences on the pituitary-adrenal cortical system. Rec. Prog. Hormone Research 15:345.

McDonald, L.E., 1969. Veterinary Endocrinology and Reproduction, Lea and Febiger Co., Philadelphia, Pa.

Mills, J.N., 1966. Human circadian rhythms. Physiol. Rev. 46:128.

Nagra, C.L., J.B. Birnie, J. Baum, and R.K. Meyer, 1963. The role of the pituitary in regulating steroid secretion by the avian adrenal. Gen. and Comp. Endo. 3:274.

Newcomer, W.S., 1959. Effects of hypophysectomy on some functional aspects of the adrenal gland of the chicken. Endo. 65:133.

Opdyke, P.F., 1942. Response of fasted and non-fasted chicks to insulin. Endo. 31:363-370.

Pauly, J.E., 1967. Circadian rhythms in blood glucose of rats. Am. J. Anat. 120:627-636.

Perkoff, G.T., K. Eik-Nes, C.A. Nugent, H.L. Fred, R.A. Nimer, L. Rush, L.T. Samuels, and F.H. Tyler, 1959. Studies of the diurnal variation of plasma 17-hydroxycorticosteroids in man. J. Clin. Endo. Metab. 19: 432-443.

Smith, C.J.V., 1972. Blood glucose levels in young chickens: The influence of light regimes. J. Poultry Sci. 51:268-273.

Stamler, J., R. Pick, and N.L. Katz, 1951. Effects of corticoids and ACTH on fluid exchange, blood pressure, and glycemia. Fed. Proc. 10:131.

Sturkie, P.D., 1965. Avian Physiology, 2nd ed. Comstock Publishing Associates, Division of Cornell University Press, New York.

Turner, C.D., J.T. Bagnara, 1971. General Endocrinology, 5th ed. W.B. Saunders Co., Philadelphia, Pa., pp. 247-254.

Williams, R.H., 1968. Textbook of Endocrinology, W.B. Saunders Co., Philadelphia, Pa.

VITA

Jeffrey Curtis Herr, son of Paul Richard and Thelma (Alexander) Herr was born in Lancaster, Pennsylvania on February 28th, 1952. He attended elementary school in East Petersburg, Pennsylvania, and junior high school in Landisville, Pennsylvania. He was graduated from Hempfield High School, Landisville, Pennsylvania in 1970.

He attended North Carolina State University from 1970 to 1974, receiving the degree of Bachelor of Science in Poultry Science in May, 1974.

He entered graduate school at The University of Tennessee in September, 1974, and received the Master of Science degree with a major in Animal Science in August, 1976.

He was married May 25th, 1974, to Christine Rae Shepardson, daughter of Harry and Roberta (Reese) Shepardson III.