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Response of the Chicken Pineal Gland, Blood and Reproductive Organs to Darkness[1]

L. Z. MCFARLAND, W. O. WILSON AND C. M. WINGET
Departments of Anatomy and Poultry Husbandry, University of California, Davis, California 95616 and NASA, Ames Research Center, Moffett Field, California 94035

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ANATOMICAL and embryological relationships between the pineal and diencephalon and between the pituitary and the diencephalon have been evaluated in detail for many different animals (Gladstone and Wakeley, 1940; Ariens Kappers and Schade, 1965). Recently Owman (1964) described certain physiological functions in the rat pineal, and Quay (1965) summarized the structural and cytological differences between the avian and mammalian pineal organ. Pineal extracts have successfully decreased castrate-pituitary-hypertrophy in the male chicken (Shellabarger, 1952). Rowan's (1926) classical work with the slate-colored junco focused attention on the influence of light on neuroendocrine mechanisms. Farner (1964) has reviewed the photoperiodic control of the bird's reproductive cycle, and the relationship between light, hypothalamus, and adenohypophysis has been reviewed by Bullough (1959), Donovan and Harris (1955), and Hague (1964). Histochemical studies of the pineal gland indicate acid and alkaline phosphatase as well as other enzyme systems to be present in the pineals of the pig, horse, goat and sheep (Mikami, 1951). Morphological changes within the rat pineal parenchyma in response to light have been reported by Roth *et al.* (1962) and Quay (1956) has

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reported changes in mice maintained in constant darkness.

We were interested in determining what effect prolonged exposure to darkness would have on the pineal and other processes after preconditioning the chicken to a 14 hour light: 10 hour dark regimen.

MATERIALS AND METHODS

A group of Leghorn×New Hampshire pullets were kept in individual cages and preconditioned for 112 days with incandescent light of 14 hours illumination and 10 hours darkness (14L:10D). They were then divided into two groups: one group was placed in total darkness for 56 days; the other group was maintained on the 14L:10D schedule for a similar period. Both groups were fed the same laying ration and were given water *ad libitum*.

Nine pullets were killed from the dark treated group and 11 from the light treated group after obtaining a terminal body weight. A 5 ml. blood sample was drawn into a heparinized syringe for clinical hematologic studies, and each chicken was then killed with an overdose of sodium pentobarbital and decapitated. The ovary and oviduct were dissected from the carcass, blotted and weighed. The pineal gland was dissected, weighed and then placed in 10% formalin for subsequent microscopic examination. Paraffin sections were stained with hematoxylin and eosin or the periodic acid-Schiff reaction and frozen sections were stained with sudan black B reaction for lipid. The pinealocyte cell volume was estimated by counting the number of nuclei per standard field using an ocular grid at a magnification of 1080 diameters. Four such fields were counted from each of six pineals chosen at random from the individuals in each of the two groups. Variance and covariance methods were used [illegible] the data.

RESULTS

Those chickens deprived of light had significantly smaller bodies, pineal glands, ovaries and oviducts than those chickens receiving 14L:10D illumination (Table 1). The weight of the ovaries and oviducts were most severely affected, in fact, those chickens kept in darkness had not laid any eggs at the time of death whereas the chickens illuminated laid an average of eleven eggs per chicken during the 56 day experimental period. The light deprived chickens lacked any mature or maturing ovarian follicles. Several atretic follicles were observed, indicating resorbance of the follicular yolk due to the absence of gonadotropic action. The oviduct had regressed in size and appearance to that typically observed in the non-laying hen with a non-active ovary.

Microscopic examination of the pineal gland of chickens kept in darkness indicated a substantial loss of parenchymal cell volume, as reflected by the significantly greater number of nuclei per area examined (Table 2). Furthermore, the pineal parenchymal cells of light deprived birds had fewer cytoplasmic lipid granules and a complete loss of the interlobular sudanophilic material, which was quite prominent in the illuminated chickens (Table 2). The luminal apex of the columnar parenchymal cells of both the illuminated and light deprived chickens had a "brush border" that stained intensely with the periodic acid-Schiff re-

TABLE 1.—*The effect of darkness on the mean weight of the body, pineal gland, ovary and oviduct*

Organ	Lighting schedule		P
	14 hr. light N=11 Organ wt. (g.)	0 hr. light N=9 Organ wt. (g.)	
Body	1,837±100*	1,447±94*	<0.025
Ovary	50.77±5.36	0.76±0.07	<0.001
Oviduct	43.00±2.61	1.48±0.80	<0.001
Pineal gland	3.7 ±0.19†	2.9 ±0.24†	<0.05

* Standard error.

action indicating the presence of a glycoprotein. However there was no significant difference noted in the amount of PAS material present between the two groups (Table 2).

The most dramatic hematologic change noted in chickens kept in darkness was a reduction in the estimated total plasma protein and the plasma phospholipoprotein complex. There was a slight increase in the number of RBC, hematocrit and hemoglobin, however, the mean corpuscular hemoglobin concentration did not differ from illuminated chickens. (Table 3).

TABLE 2.—*Effect of darkness on pineal histology*

Observation	Lighting schedule	
	14L:10D	OL:24D
Sudanophilic particles in pinealocytes	moderate	occasional
Sudanophilic particles in interlobular septae	intense	occasional
Periodic acid-Schiff positive particles in pinealocytes	intense	moderate
Number of pinealocyte nuclei per microscopic field	117±4	151±4 ($P<.001$)

TABLE 3.—*Effect of darkness on hematologic values*

	Treatment	
	14L:10D	OL:24D
RBC, No./mm.^{3}	1.5±.1	2.2±.2
Hematocrit, %	22.6±.1	26.7±.6
Hemoglobin, g./100 ml.	7.6±.2	8.7±.2
MCV, μ^3	15.1±.1	12.1±.2
MCH, $\mu\mu$g.	50.6±.2	39.5±.4
MCHC	33.6±.4	32.6±.6
Total plasma protein, g./100 ml.	9.2±.9	4.2±.2
Phospholipoprotein, g./100 ml.	5.7±1.7	0.4±.1

DISCUSSION

Subjecting chickens to relatively long scotoperiods effectively interferes with various biorhythmic processes. In this experiment, pullets conditioned to 14 hours light: 10 hours dark and then subjected to constant darkness for an eight week period ceased to lay eggs completely. In a similar experiment (Wilson and Woodard, 1958), laying hens deprived of light for only four weeks had a 43% decrease in egg production. Total darkness does not prevent pullets from coming into egg production (Rider, 1938), but there appears to be a delay in time of maturity and the chickens are smaller (King, 1962).

The body weight of chickens deprived of light for varying periods of time was consistently less than that of chickens receiving light. Whether this is the result of a reduced appetite, a reflection of the inability to consistently locate food in total darkness, or other factors is not known.

The pineal gland of the chicken is profoundly effected by light deprivation. Not only is there a significant decrease in weight of the gland and an associated loss of parenchymal cell volume, but also the light dependent melatonin synthesizing cytoplasmic enzyme, hydroxy-indole-O-methyl transferase (HIOMT), is greatly reduced (Axelrod *et al.*, 1964). In contrast, rats deprived of light for seven weeks had greatly increased pineal gland weight (Wurtman *et al.*, 1963) as well as an elevated HIOMT activity. Constant illumination of the rat decreases pineal cytoplasmic basophilia and the size of the pinealocyte nuclei (Roth *et al.*, 1962). We (Winget *et al.*, 1967) have shown also that chickens deprived of light have a significant decrease in plasma and pituitary alkaline phosphatase; plasma, diencephalic and pituitary acid phosphatase; and plasma and diencephalic cholinesterase activities. Only diencephalic alkaline phosphatase activity was significantly elevated in the light deprived state.

The absence of lipid in the pinealocyte cytoplasm was striking in light deprived chickens. Severe lipid depletion of the pineal has also been observed in rats with hormonal imbalances such as hy-

pophysectomy, combined castration and adrenalectomy and administration of angiotensin II or propylthiouracil (Hungerford *et al.* 1962) and with sodium deficiency (Panagiotis and Hungerford, 1961).

An ultrastructural study of the chicken pineal (Fujie, 1968) demonstrated numerous cytoplasmic processes (microvilli) extending into the luminal portion of the lobule. These undoubtedly account for the PAS-positive "brush border" observed in this study. Furthermore, it was observed that short daily photoperiods (4L:20D) reduced the amount of cytoplasm, granules, lipid droplets and lysosomes in the pinealocyte. Based on the present study, one should predict a more marked ultrastructural change in the pineal of chickens deprived of light for prolonged periods.

The foregoing discussion emphasizes the need to further elucidate the interrelationships between photoperiodicity, pineal structure and function, and other physiological processes in the avian species.

SUMMARY

Twenty chickens were preconditioned to a 14L:10D lighting regimen for 112 days, then half were placed in total darkness for 56 days and the other half continued on the 14L:10D schedule. Exposure to this duration of darkness significantly decreased the volume of pinealocytes and the concentration of the sudanophilic lipid material in the interlobular septa and pinealocytes. Darkness also reduced the body weight of chickens as well as casuing a complete cessation of egg laying. The number of red blood cells increased, whereas the refractive index and the grams percent of phospholipoprotein decreased.

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