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Activity of the Adrenal Glands in the Pied Flycatcher and Its Relation to Testicular Regression

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Histological studies on the adrenal glands of the Pied flycatcher from spring migration to the autumn migration period were conducted. Phospholipids and some enzymes (glucose-6-phosphate dehydrogenase, $NADPH_2$-diaphorase, and Ca-activated adenosine triphosphatase) were also studied in the male during the breeding period. The cortical tissue showed low activity as long as the birds were sexually active. The histological, histochemical, and enzymatic results revealed an increase in cortical activity at the time of hatching, a time when the spermatogenesis ceased. The highest values of the histological parameters were, however, found in males caught during the autumn migration. The results also indicated a functional zonation in the cortical tissue. ACTH injections during the beginning of the breeding period did not affect spermatogenesis, testicular size, or seminal vesicles. It did cause hypertrophy in the central zone of the cortical tissue but did not affect the subcapsular zone.

A number of factors has been shown to influence the histology of the avian adrenal glands and a wide variety of functions has been connected with the corticoid hormones, e.g., a relationship between reproduction and cortical activity. For a review of the literature see Ross (1963) and Holmes and Phillips (1976). The circannual adrenal activity of many sedentary species and short-distance migrants has been described, e.g., in the blackbird *Turdus merula* (Fromme-Bouman, 1962) and the cormorant *Phalacrocorax carbo* (Petrescu-Raianu, 1971). The adrenal activity of long-distance migrants has, as far as I know, only been investigated in two species: the white-crowned sparrow *Zonotrichia leucophrys gambelii* (Lorenzen and Farner, 1964) and the white-throated sparrow *Zonotrichia albicollis* (Dusseau and Meier, 1971). The first part of this paper describes seasonal variations in adrenal activity, based upon histological, histochemical, and enzymatic methods, in a long-distance migrant, the Pied flycatcher *Ficedula hypoleuca*. The second part follows on from the possibility of an antagonism between adrenal–gonadal activities (e.g., Greenman and Zarrow 1961; Martin, 1973; Wilson and Follett, 1975) in keeping with my preliminary studies. Therefore the effects of injection of ACTH on the onset of testicular regression in the Pied flycatcher were studied.

MATERIAL AND METHODS

Pied flycatchers were collected at three different places during 1971–1974. One area was situated in north Sweden, at Ammarnäs (65° 58′N) where birds were caught during 1971 and 1972 and two areas in southwest Sweden, at Rävlanda (57° 40′N) and at Åskloster (57° 14′N). In these areas birds were caught during 1972–1974. Spring migrants were collected at Falsterbo bird station in south Sweden during 2 weeks of May 1972. Autumn migrants were collected at Ottenby bird station in southeast Sweden in August 1976. The birds were caught with mistnets or automatic traps in the nesting boxes. Birds collected during the breeding season were captured as soon as possible after their arrival in the area and individually color banded, whereupon they were released. The behavior of the birds was followed by regular, every to every other day, visual observations. Breeding data was recorded by inspection of the nest-boxes at critical periods. Consequently, when the birds were recaptured their breeding status was known. All together 118 males

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and 78 females were examined (Table 1). The observed data were not analyzed on a chronological base, but in relation to the breeding status of the bird, i.e., birds from the nest-building period, the egg-laying period, etc. The area the bird was caught in was not taken into consideration.

The birds were killed by decapitation and the adrenal glands were excised in the field and fixed in Bouin's fixative and after 12 hr removed to 70% ethanol. Histological preparations were made from 7μm paraffin sections and stained with Erlich's hematoxylin–eosin. To assess the activity in the cortical tissue of the adrenal glands, two different methods were used. Measurements of the FCV (fractional cortical volume) were carried out according to the method described by Lorenzen and Farner (1964). As the volume of a cell nucleus is related to the activity of the cell (Oehlert and Schultze, 1960; Citoler *et al.*, 1965) karyometry was also used. Due to the possibility of zonal difference in activity, measurements from the subcapsular zone and central zone were made separately. Microscopic pictures were taken from both zones. The cells were magnified 5500 times and the surface of 50 nuclei from each zone was measured with a planimeter.

In 1975 histochemical and enzymatic studies of adrenal glands from 36 males were performed. The birds were individually color banded when they arrived in the breeding area and their behavior was observed almost daily during the breeding season. The breeding status of every investigated male was known. The birds were killed by decapitation and the adrenal glands were excised in the field and immediately placed in an ice-cold transport medium, Histocon (Histo-lab, Gothenburg, Sweden), which ensured that the organs did not undergo any autolytic changes or loss of components. Within 24 hr they were prepared for cold microtome sectioning in the laboratory. Freezing was performed in isopentane, chilled to about −140° by liquid nitrogen. The tissues were sectioned at 8 μm. Staining for lipids was done by the OTAN method (Adams, 1959). The enzyme histochemical analyses included the activities of $NADPH_2$-diaphorases, EC 1.6.99.-, according to the method recommended by Chayen *et al.* (1973), of glucose-6-phosphate dehydrogenase (G-6-PDH), EC 1.1.1.49 (Altman, 1968), and of ATPase,EC 3.6.1.3., with the calcium method of Padykula and Herman (1955).

Controls were made after enzyme inhibitory treatments (10% formalin) of tissue sections. Sections were also incubated in media without the respective enzyme substrates. Morphological and histological references from every organ were obtained by staining of neighboring sections with Erlich's hematoxylin–eosin.

In 1976 an additional experiment was performed to see whether there was an interaction of ACTH, or cortical steroids, upon the testicular regression. During the beginning of the egg-laying period 12 males were captured at Rävlanda, divided into two groups, and placed in two large outdoor cages (5 × 3 × 2.5 m). One group ($n=8$) was given daily intramuscular injections of 1.5 IU ACTH, Acton Prolongatum, Ferring, for 14 days (corticosterone was not available). One group ($n=4$) was given the same amount (100 μl) of 0.9% NaCl injections for 14 days. All injections were given early in the morning, 2–3 hr after sunrise. The birds were given free amounts of food (mealworms, ants, and ant pupae) and water. They were killed by decapitation and the organs were removed and transferred to Bouin's fixative. The testes were later weighed on a Mettler precision balance to the nearest 2×10^{-2} mg after which the adrenal glands and seminal vesicles were embedded in paraffin for histological examination. The organs were cut at 7 μm and stained with Ehrlich's hematoxylin–eosin. The FCV and nuclear size of the cortical tissue were measured. The height of 10 epithelial cells in 10 different tubules of the seminal vesicles was measured.

Student's t test was used for the statistical analyses.

RESULTS

Histological Changes

The lowest values of the parameters studied were obtained from males and females caught during spring migration (Table 1 and Fig. 1). Up to the nest-building period, a slight increase of both FCV and nuclear size had occurred in both sexes. This increase was significant for the nuclear size in both zones of the adrenal glands in the female ($P < 0.05$) and for the nuclei in the central zone in the male ($P < 0.05$). No further change was observed until the beginning of the nestling period. At this time there was a significant increase in the male's FCV ($P < 0.05$) and in the size of the nuclei from both the subcapsular zone ($P < 0.05$) and the zentral zone ($P < 0.01$). The high activity in the cortical tissue of the male persisted throughout the nestling period. The maximal values for all parameters were found in males caught during autumn migration. These values were significantly higher than those from any other period ($0.001 < P < 0.05$), except for the nuclear size of the central zone and the

TABLE 1
SEASONAL CHANGES IN NUCLEAR SIZE OF THE INTERRENAL CELLS IN THE PIED FLYCATCHER[a]

	Male			Female		
	N	CZ	SCZ	N	CZ	SCZ
Spring migration	17	6.00 0.14	5.31 0.13	6	5.61 0.34	4.94 0.19
Nest-building period	21	6.40 0.10	5.43 0.11	8	6.35 0.21	5.53 0.17
Egg-laying period	8	6.34 0.17	5.68 0.25	8	6.16 0.22	5.40 0.17
Incubation, Days 1–7	11	6.08 0.11	5.38 0.15	12	6.41 0.16	5.73 0.12
Incubation, Days 8–14	12	6.06 0.10	5.32 0.11	9	6.38 0.14	5.66 0.09
Nestling, Days 1–7	30	6.81 0.13	5.74 0.13	24	7.06 0.17	5.87 0.14
Nestling, Days 8–14	14	7.25 0.26	6.16 0.15	11	7.54 0.10	6.29 0.18
Autumn migration	5	7.56 0.11	6.57 0.15	—	—	—

[a] Data are given as mean ±SE of planimeter units. CZ, Central zone; SCZ, subcapsular zone.

FCV values from the last part of the nestling period. Unfortunately no adult female was examined from the autumn migration. The female FCV values, from the whole nestling period, differed significantly from the first part of the incubation period, the egg-laying period and the spring migration ($P < 0.05$). During the last part of the nestling period the size of the nuclei, from both zones, differed significantly ($P < 0.01$) from all earlier periods. The first part of the nestling period differed only from the egg-laying period and spring migration ($P < 0.01$).

No significant divergence between the male and the female cycle could be discovered. There were no statistically significant differences between birds caught in different years or in different places provided that birds in the same breeding phase were compared.

Histochemical and Enzymatic Changes

Histochemical and enzymatic studies were performed only in the male. All males ($n=24$) caught before hatching time were in full spermatogenesis, while all males ($n=12$) from the nestling period had regressed testes. No studies were performed on males from autumn migrations.

Birds caught during the prenesting, nest-building, egg-laying, and incubation periods showed a moderate reaction of phospholipids in both zones of the cortical tissue. In four individuals from the incubation period various amounts of unsaturated hydrophobic fatty acids were seen. Males from the nestling period had a high amount of phospholipids in the cortical tissue and no unsaturated hydrophobic fatty acids were seen. The activity of G-6-PDH increased markedly around the time of hatching. During the earlier periods a low to moderate activity was seen in both zones (Fig. 2a). All, individuals from the nestling period showed a high activity in both the subcapsular and the central zone (Fig. 2b). The activity of $NADPH_2$-diaphorase was high during the entire breeding season and

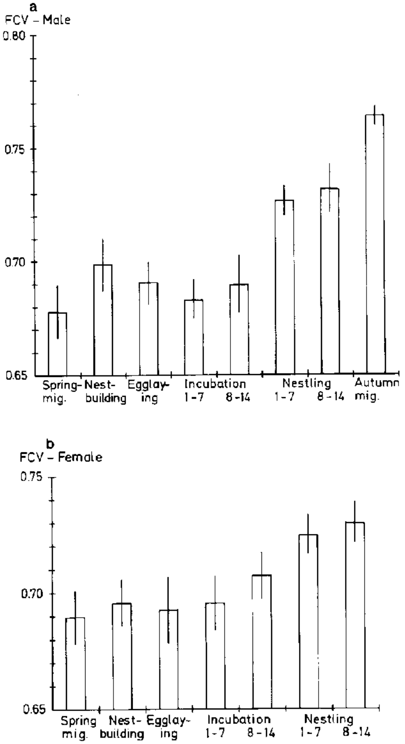

FIG. 1. The fractional cortical values (FCV) in the male (a) and female (b) Pied flycatcher. The number of individuals investigated from each period is shown in Table 1. The bars show the average FCV for the period and the vertical lines indicate standard error.

no differences between the zones could be observed. The ATP:ase activity in individuals from the prenesting, nest-building, and egg-laying periods all showed a weak activity in both the cortical tissue and the vessel walls. All individuals taken during the nestling period showed a strong activity in the subcapsular zone and in most parts of the vessel walls and a moderate activity in the central zone (Fig. 3). A considerable individual variation was seen in adrenals from the incubation period. Some adrenals showed the same pattern as in birds from the nestling period, while others showed a pattern typical for adrenals from, e.g., the egg-laying period.

ACTH Experiment

ACTH injections did not change the testicular activity. All testes in the ACTH group, as well as in the control group, were actively producing sperm at the end of the experiment. There was no significant difference in the weight of the testes between the two groups. Nor was there any difference in the height of the epithelium in the seminal vesicles between the two groups (Table 2).

A significant increase ($P < 0.01$) in the size of the nuclei in the central zone of the cortical tissue occurred after the ACTH injections. The subcapsular zone seemed unaffected (Table 2).

Birds from the ACTH group had a significantly ($P < 0.05$) lower body weight than those from the control group (Table 2).

DISCUSSION

The view that secretorial alterations in the cortical cells are reflected in structural alterations seems to be generally accepted. Fractional cortical volume (FCV) and nuclear size have been commonly used as histological indicators of secretorial activity in avian adrenal glands (e.g., Fromme-Bouman, 1962; Gorman and Milne, 1970). Using these parameters as indicators of the cortical steroid production in the Pied flycatcher, this study revealed a significant increase in cortical activity during the nestling period in both sexes.

G-6-PDH is involved in the pentose phosphate shunt and several authors have suggested that it plays an important role in the mechanism stimulating cortical steroid synthesis as it catalyzes the oxidation of glucose 6-phosphate and thereby providing $NADPH_2$ (Haynes and Berthet, 1957; Grant, 1960; Arvy, 1971). The G-6-PDH ac-

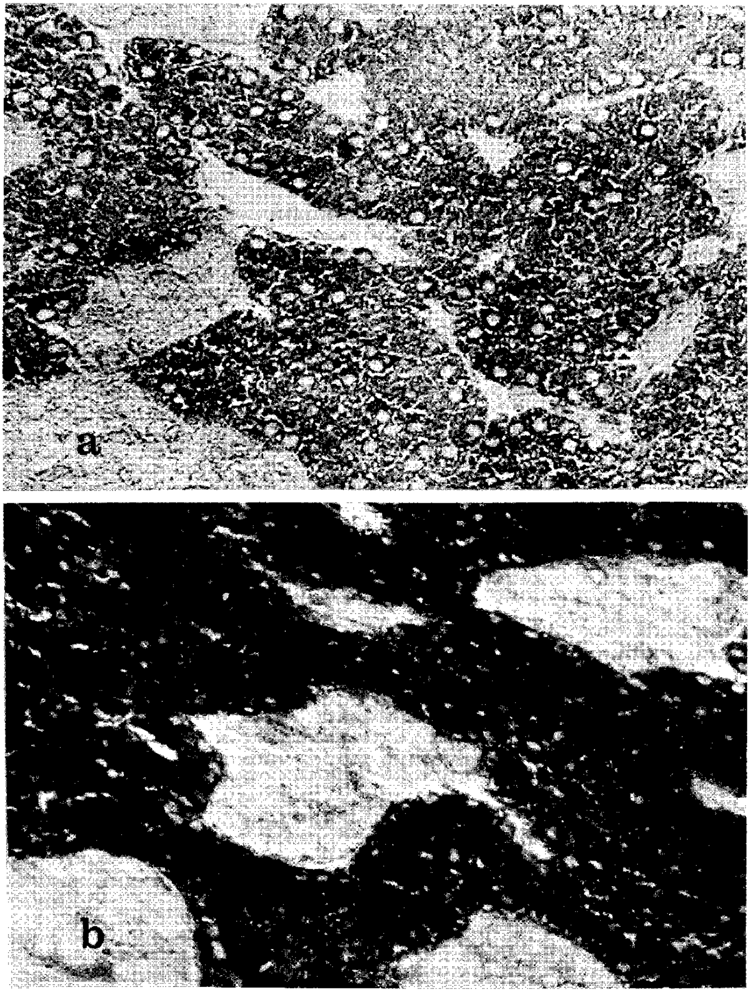

FIG. 2. The activity of glucose-6-phosphate dehydrogenase in the adrenal glands of the Pied flycatcher during the incubation period (a, × 650) and during the nestling period (b, × 600).

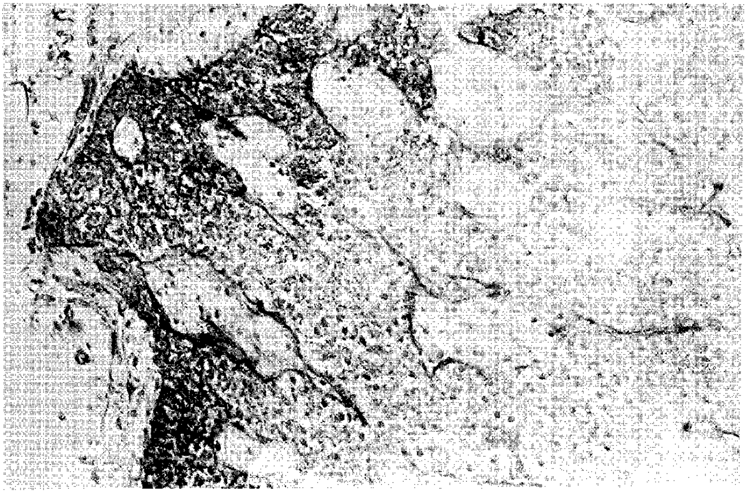

FIG. 3. The activity of Ca-activated ATPase in the adrenal glands of the Pied flycatcher during the nestling period, × 400.

tivity also seems to be under the control of the adenohypophysis. ACTH increases the activity of this enzyme in the adrenals (Koritz and Peron, 1958; Greenberg and Glick, 1960; Studzinski *et al.*, 1961). The change in the G-6-PDH activity during the breeding season in the Pied flycatcher thus indicated an increased secretion of adrenal steroids at the time of hatching. The very high activity of $NADPH_2$ in the cortical tissue of the Pied flycatcher is not surprising as it is required for the synthesis of steroid hormones (Chayen *et al.*, 1973). An increase, as seen in the G-6-PDH activity, would also have been expected in $NADPH_2$ but did not occur. This was probably due to a high overall activity of $NADPH_2$-diaphorase, whereby small fluctuations in the activity was masked. The diaphorase registrations were consequently of little

TABLE 2
EFFECTS OF ACTH ON SEXUALLY ACTIVE MALE PIED FLYCATCHERS[a]

	N	Mean Testicular Weight (mg)	Height of Seminal Vesicle Epithelium (μm)	Adrenals CZ	Adrenals SCZ	Body Weight (g)
Control group	4	31.39	13.59	5.80	5.15	12.55
		0.31	0.26	0.80	0.35	0.20
ACTH group	8	24.87	13.34	7.28*	5.73	11.80**
		5.02	0.83	0.11	0.41	0.19

[a] Data are given as mean ± SE. Data from the adrenal glands are given as planimeter units.
* $P < 0.01$.
** $P < 0.05$.

value when studying variations in the synthesis of adrenal steroids. The adrenal phospholipid content was found to increase at the same time as the G-6-PDH activity. A lot of studies on lipids in the adrenals of birds have been done. Conclusions about phospholipids cannot be drawn from the demonstration of lipids as such. However, it is likely that the amount of phospholipids reflects the corticotrophic secretion as it has been shown that the amount of phospholipids in the adrenals of domestic pigeon was dependent of ACTH (Sinha and Gosh, 1961). The high activity of ATPase in the vessel walls during the nestling period also indicates a high secretorial activity of the adrenal cells (Arvy, 1971). The histochemical and enzymatic studies thus confirmed the histological results.

The increase in cortical activity occurred simultaneously with the onset of testicular regression, at the time of hatching. It is suggested by Silverin (unpublished) that in the Pied flycatcher there is a feedback of testosterone on the LH release mechanism, while the FSH secretion is under the control of a different mechanism. The cause of testicular regression is still obscure. As it is known that cortical hormones affect gonadal activity (e.g., Wilson and Follett, 1975) in some bird species and due to the above mentioned reverse relationship, the possibility that the increase in cortical activity was responsible for the onset of testicular regression was not excluded. Nagra *et al.* (1960) and Péczely (1972) have shown that ACTH injections strongly increase the amount of corticosteroids in the blood. The ACTH injections in the Pied flycatcher proved to be effective as the central zone of the cortical tissue underwent hypertrophy and the body weight decreased significantly. It can therefore be concluded that there was an increased secretion of cortical steroids in the Pied flycatcher, but, under the prevailing conditions, it seemed unlikely that the cortical hormones or their intermediates had any visible effect on the testes or seminal vesicles.

The fact that the increase in cortical activity occurred at the same breeding phase in both sexes makes it likely that the functional significance of this increase is the same in both sexes. Charnov and Krebs (1974) speculate that, in order to maximize the individual's fitness, it is of adaptive value for the parents, especially in short-lived birds, to "work hard" in raising the brood, even at the expense of increasing their own mortality rate. It is reasonable to suggest that if the parents "work hard" in raising the brood they will have to use the body's own energy reserves during this period. One effect of this would be a decrease in body weight. The body weight of both sexes in the Pied flycatcher does decrease during the nestling period (Silverin, unpublished data). It is known that an increased cortical activity causes a decrease in the body weight of birds (e.g., Baum and Meyer, 1960; Bradley and Holmes, 1971). The high cortical activity in the Pied flycatcher during the nestling period might therefore be an adaptation to an increased need to utilize the body's own energy reserves during this period.

The results obtained in the Pied flycatcher coincide with the findings in the white-crowned sparrow in showing low cortical activity when the birds show maximal sexual activities and a high cortical activity when the birds are in the refractory period (Lorenzen and Farner, 1964). Measurements on seasonal variations of plasma corticosteroids in another strong migrant, the white-throated sparrow, also agree with these findings (Dusseau and Meier, 1971). Contrary to the results from strong migrants, maximal gonad activity coincides with maximal cortical activity in several sedentary species and weak migrants, e.g., the starling *Sturnus vulgaris* (Burger, 1938), blackbird (Fromme-Bouman, 1962), common gull *Larus canus* (Thybusch, 1965/66), mallard and the domestic duck *Anas platyrhynchos* (Höhn *et al.*, 1965), Gambel's quail *Lophortyx gambelii* (Raitt, 1968), Eastern rosella *Platycercus eximius*

(Hall, 1968), house sparrow *Passer domesticus* (Moens and Coessens, 1970), cormorant (Petrescu-Raianu, 1971), common eider *Somateria mollissima* (Gorman and Milne, 1971). The differences between strong migrants and sedentary species or weak migrants are difficult to evaluate, but it is known that cortical hormones are involved in the development of seasonal migratory behavior in the white-crowned sparrow (Meier *et al.*, 1965). This is also indicated in the rose-colored starling (Naik and Georg, 1963) and in the mallard (Höhn *et al.*, 1965). This may also be the case in the Pied flycatcher as the highest values of FCV and nuclear size were found in males from the autumn migration period.

Light microscopic studies, ultrastructural, and histochemical investigations have shown that there is a morphological and functional difference between a subcapsular and a central zone in the cortical tissue in many bird species (e.g., Nagra *et al.*, 1960; Sinha and Ghosh, 1961; Ghosh, 1962; Kondics and Kjaerheim, 1966; Bhattacharyya *et al.*, 1972; Cronshaw *et al.*, 1974; Bhattacharyya, 1975; Bhattacharyya *et al.*, 1975) while in other species no difference between the two zones could be observed (Nagra *et al.*, 1960; Bhattacharyya *et al.*, 1972; Bhattacharyya, 1975; Bhattacharyya *et al.*, 1975). In the Pied flycatcher only cells in the central zone were hypertrophic after the ACTH injections, a result in agreement with the findings on pigeon (Kondics, 1965; Péczely, 1972). A distinct cytochemical zonation as to the distribution of Ca-activated ATPase was seen in male Pied flycatchers during the nestling period. These results together with the histological results, suggest that there is a functional zonation in the adrenal glands of the Pied flycatcher.

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