Effects of Androgens upon DNA Synthesis in the Uropygial Glands of Quails —Immunohistochemical Studies with Bromodeoxyuridine—

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Avian uropygial glands are holocrine glands organically homologous to mammalian sebaceous glands⁵, moreover, like sebaceous gland, uropygial glands are said to be androgen-dependent^{1,7-10,12,14,16}. One possible method for investigating the secretory activity of uropygial glands consists in determining the frequency of mitosis in the basal cells of the secretory tubules. Conventional techniques for this purpose include the observation of mitotic activity using colchicine^{7,13,15,18} and the determination of proportions of marked cells resulting from the nuclear uptake of [³H]-thymidine during the period of DNA synthesis^{11,12}. On the other hand, monoclonal antibodies against 5-bromo-2'-deoxyuridine (BrdU), a thymidine analogue developed by GRATZNER⁴, permit determination of the proportion of marked cells by immunohistochemical methods^{3,17} in a

short period of time.

In the present study, DNA synthesis in the basal cells of the secretory tubules of the uropygial glands of castrated and androgen-treated quails was investigated by immunohistochemical techniques using BrdU. Since the shallow and deep portions of the uropygial glands are histologically different⁶, the differences between the two portions with respect to androgen dependence were also investigated.

Materials and Methods

The experimental birds comprised 20 Japanese male quails (*Coturnix coturnix japonica*) four weeks of age. Ten of these quails were castrated. The experimental groups consisted of a untreated group (N group), an androgen treated group (T group), a castrated group (C

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group), and a group treated with androgen after castration (C + T group), with 5 quails in each group. The androgen preparation used was an olive oil solution of testosterone propionate (Sigma Chemical Co.), injected daily (0.2 mg/0.1 ml/day) into the superficial pectoral muscle until the birds were 8 weeks of age. Upon completion of the period of treatment, a solution of BrdU in physical saline (40 mg/kg body weight) was injected into the peritoneal cavity of each bird, and the uropygial gland was excised 1 hour later. The glands were fixed in 70% ethanol for 2 hours, then embedded in paraffin by the ordinary method, and 3 μ m sections were prepared.

The sections were processed by the immunohistochemical procedure of DOLBEARE *et al.*³⁾ and MORSTYN *et al.*¹⁷⁾. The sections were deparaffinized and deproteinized by digestion with 0.4 mg/ml of pepsin in 0.1 N hydrochloric acid for 3 minutes at 37°C to expose the DNA. The DNA was then converted to short-chain form by treatment with 2 N hydrochloric acid for 60 minutes at 37°C to expose the BrdU. After neutralization with 0.1 M sodium borate, the specimens were treated with 10% normal horse serum for 20 minutes at room temperature to prevent nonspecific reactions. These specimens were incubated for 2 hours at room temperature with 100 fold diluted anti-BrdU antibody (Becton Dickinson Co.), and then for 30 minutes at room temperature with 200 fold diluted biotinized anti-mouse antibody (Vector Co.). They were followed by treatment with avidin-biotin-peroxidase complex (Vector Co.) for 60 minutes at room temperature. Coloration was carried out by using 3-3'-diaminobenzidine tetrahydrochloride, and finally the specimens were counter-stained with hematoxylin.

In the prepared sections, 5,000 basal cells in the shallow and deep portions of the secretory tubules were counted separately, and the respective proportions of cells displaying a positive reaction were determined. Student's

t-test was employed for statistical processing of the data obtained.

Results and Discussion

The nuclei of the BrdU-positive basal cells in the secretory tubules were distinguished by brown staining. The observed proportions of these positively marked cells relative to the total number of basal cells counted are shown in Table 1. That is, the proportions of BrdU-positive basal cells in both the shallow and deep portions of the secretory tubules were increased by administration of androgen and decreased by castration. On the other hand, there was no significant difference in this respect between the control group and the group treated with androgen after castration. Moreover, the proportion of marked cells was higher in the deep portion as compared with the shallow portion for all the experimental groups.

As regards the difference between the proportions of marked cells observed by the [³H]-thymidine method and by the BrdU method, no reports concerning this point have appeared in connection with either avian uropygial glands or mammalian sebaceous glands, but the results obtained by the two methods are said to agree well each other in the case of mouse intestinal epithelium^{2,19)}. The results of KUSUHARA *et al.*¹¹⁾, who investigated the proportion of marked basal cells in the secretory tubules of the uropygial glands of quails 1

Table 1. Proportions of marked basal cells in secretory tubules of uropygial gland

Group	Shallow portion	Deep portion
N	1.29 $\pm$ 0.07 ^{a,b}	5.88 $\pm$ 0.26 ^{a,b}
T	2.37 $\pm$ 0.11 ^{a,c,e}	8.34 $\pm$ 0.28 ^{a,c,e}
C	0.78 $\pm$ 0.06 ^{b,c,d}	3.88 $\pm$ 0.18 ^{b,c,d}
C+T	1.20 $\pm$ 0.13 ^{d,e}	5.98 $\pm$ 0.08 ^{d,e}

Mean $\pm$ standard deviation (%).

A letter a,b,c,d, or e attached to any two groups indicates a statistical difference at the significance level of 1%.

hour after administration of [^3H]-thymidine, almost identical with the corresponding results obtained in the present study using BrdU.

The activity of the uropygial gland is said to be controlled by sex hormones, particularly by androgens^{1,7-10,12,14,16}. According to the results of studies employing colchicine to investigate the frequency of mitotic figures during a specified period (5-6 hours) following administration^{7,13,15,18}, the number of mitotic figures was increased by testosterone administration but decreased by castration in all cases; furthermore, administration of testosterone after castration resulted in greater mitotic activity as compared with the controls. KUSUHARA and ISHIDA¹² administered sex hormones to the domestic fowls and counted the proportion of marked cells in the deep portion of the uropygial gland 1 hour later, using [^3H]-thymidine; that was decreased by castration, but increased by administration of androgen after castration. Identically similar results were obtained from uropygial glands of quails in the present study, again indicating that DNA synthesis in the basal cells of the secretory tubules of avian uropygial glands is indeed controlled by androgens. Furthermore, KUSUHARA and ISHIDA¹² also reported that the rate of secretion by the uropygial gland was accelerated by administration of androgens but retarded by castration in the examination of the temporal course of the changes of DNA synthesis. In the present study, only the proportion of marked basal cells was investigated, but nevertheless the data obtained tended to corroborate the results of KUSUHARA and ISHIDA¹² concerning the effects of androgens in stimulating quail uropygial gland secretion.

Furthermore, in the present study, the deep portion of the uropygial gland displayed a higher proportion of marked cells than the shallow portion in all the experimental groups, suggesting that secretory function is intrinsically more active in the deep portion.

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