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Ultrastructural changes in the uropygial gland of the male Japanese quail, *Coturnix coturnix*, after testosterone treatment

Comparison with the sebaceous gland of the male rat

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Summary. The ultrastructure of the uropygial gland of the male quail was compared to that of the sebaceous gland of the male rat after castration and testosterone treatment of both species. In intact animals, the differentiating cells of these glands displayed almost the same pattern as regards their smooth endoplasmic reticulum, an organelle involved in lipogenesis in both cases. Castration reduced the volume of this organelle, while testosterone administration restored cell morphology to a normal or supranormal level. Finally, this study showed that at ultrastructural level, there is a close functional analogy between the uropygial gland of quail and the sebaceous glands of rats as regards their androgen dependency. Consequently, the uropygial gland might be an attractive model for study of action of androgens on sebaceous-like glands.

Key words: Uropygial gland – Sebaceous gland – Testosterone – Japanese quail – Rat

In birds, the uropygial gland is a holocrine glandular complex which mainly secretes waxes (Jacob and Ziswiler 1982). Despite great differences in size and in location, this gland presents basic similarities with the mammalian sebaceous glands. In fact histological and histochemical studies have shown that the uropygial gland is "a counterpart of mammalian sebaceous glands" (Bradley and Grahame 1960; Das and Ghosh 1959; Kar 1947).

Androgens are known to regulate the activity of the sebaceous glands (Lapiere 1953; Ebling 1957; Strauss and Pochi 1963; Sauter and Loud 1975). In a previous work (Lecaque and Secchi 1982), it was demonstrated in the rat that testosterone acts principally on the vesicular smooth endoplasmic reticulum (sER) by differentiating the sebaceous cells directly involved in sebum synthesis. If the uropygial gland were also a functional equivalent of the sebaceous glands, then it should also be androgen-dependent. In fact, light-microscopy studies have shown that these two cutaneous glands have parallel properties as regards hormonal regulation: Castration reduces the activity of the uropygial gland while testosterone administration to castrates restores it to a normal physiological level (Kar 1947; Maiti and Ghosh 1972; Bhattacharyya et al. 1977).

In preliminary unpublished studies using light microscopy, we showed that the histological features of the uropygial gland of the male quail are similar to those of other species of birds. The ultrastructural investigation described herein was designed to explore further the androgen dependency of the uropygial gland of the Japanese quail. For this purpose we have compared the changes that occur after castration and testosterone administration in the uropygial gland of male quail and in the sebaceous glands of male rats under similar experimental conditions.

Materials and methods

Animals

Groups of 5 male Japanese quail (*Coturnix coturnix*), purchased when 6 weeks old (J. Jouan, Plerin, France), were kept for one week under a short-day photoregime (6L 18D). Bilateral orchidectomy was performed under ketalar anaesthesia. Castrated birds were kept for 5 weeks under 8L 16D prior to testosterone treatment. To induce sexual maturity, intact controls were maintained under a long-day photoregime (18L 6D) for 6 weeks.

Groups of 5 albino male rats of the Sprague Dawley strain (Iffa Credo, France) weighing 200 g were used in this study. Two groups were castrated. Four weeks after castration one of these groups was treated with testosterone and an intact group was used as controls.

Treatment

Testosterone propionate was administered to the quail in subcutaneously implanted capsules of dimethyl polysiloxane (length: 15 mm, internal diameter: 1.5 mm) for 10 days (Abalain et al. 1980). This treatment raised plasma testosterone to 7 ng/ml (twice the physiological level in sexually active quail). This concentration has been shown to be the most effective for stimulating the synthesis of proteins and nucleic acids (Abalain et al. 1980), as well as for activating DNA and RNA polymerases in the cells of the uropygial gland (Abalain et al. 1981, 1983).

The rats were injected subcutaneously with testosterone propionate in doses of 250 µg/rat/day for 7 days, a treat-

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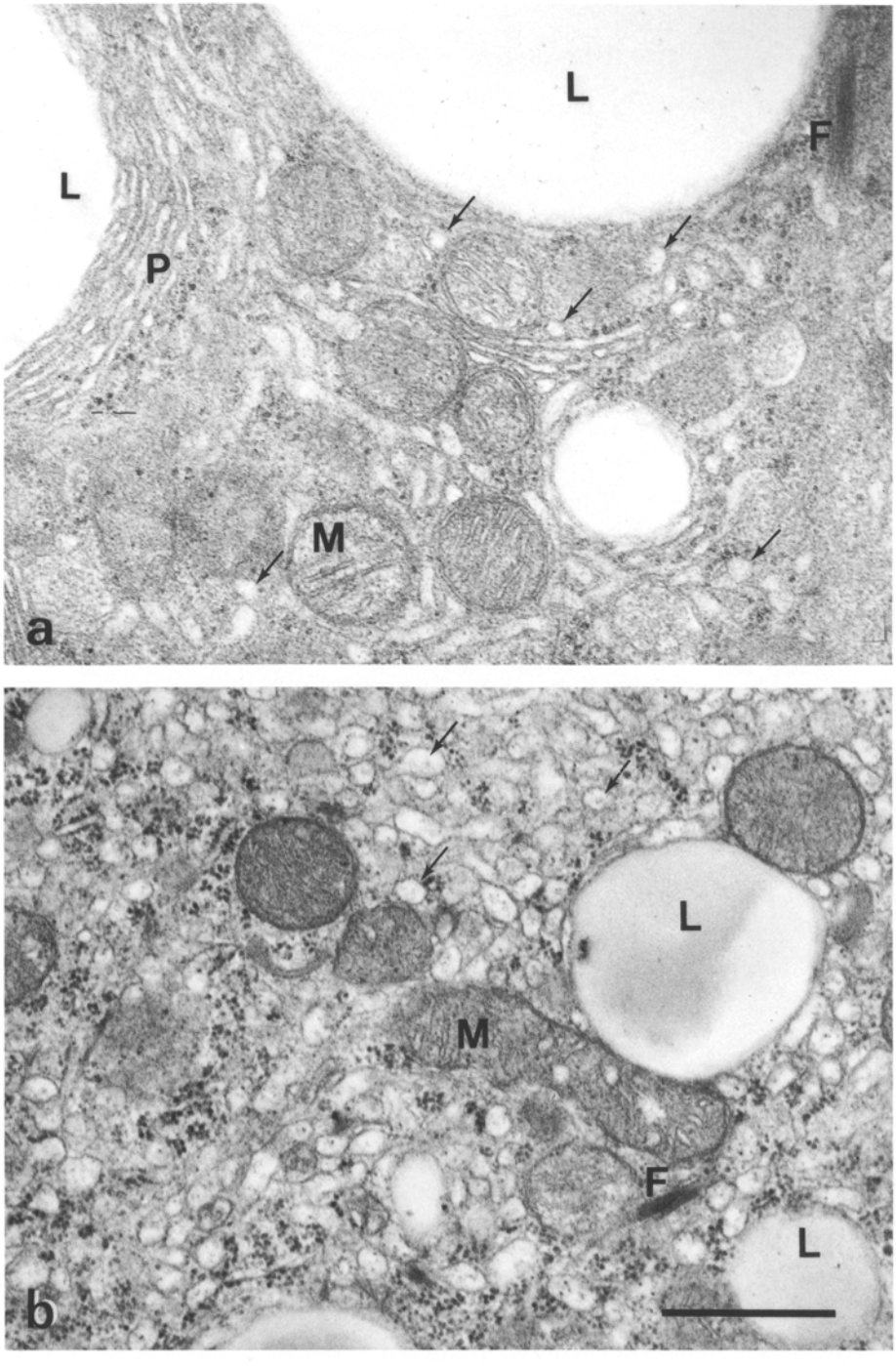

Fig. 1 a, b. Part of the cytoplasm of intermediate cells in control uropygial and sebaceous glands:
a Uropygial gland of male quail. The cytoplasm contains large lipid droplets (*L*) without a limiting membrane. Whorled profiles (*P*) of smooth endoplasmic reticulum (sER) and small infrequent vesicles (↗) can be observed.
b Sebaceous gland of male rat. Lipid droplets are smaller than those observed in the uropygial gland. Numerous sER vesicles (↗) are clearly visible. *F* filamentous bundle, *M* mitochondrion. Scale bar: 0.5 μm

ment previously shown to activate the sebaceous glands (Lecaque and Secchi 1982).

Histological procedures

At the end of treatment quail were killed by cervical dislocation. The uropygial glands were quickly removed, opened to discard lipid secretions and cut into small pieces. From the rats, biopsies of dorsal skin were taken from the interscapular region. In both cases, samples were immediately fixed by immersion in 4% glutaraldehyde buffered to pH 7.4 in 0.1 M phosphate buffer at 4° C. Specimens were then rinsed in the buffer, cut into small fragments and post-fixed for 1 h in phosphate-buffered 1% osmium tetroxide at 4° C, dehydrated with a series of graded ethanols and embedded in Epon 812. Blocks were cut with glass or diamond knives. Sections 1 μm thick were mounted on glass slides and stained with 0.5% toluidine blue in sodium borate for light-microscopic examination. Ultrathin sections were mounted on Formvar-coated grids, stained with uranyl acetate and lead citrate in an Ultrostainer LKB for examination with an Emiskop 101 Siemens electron microscope at 80 kV.

Only the basal end of the uropygial gland tubule (Zone I according to Lucas and Stettenheim 1972) and the basal portion of the sebaceous gland were taken into account, because these were the sections previously shown to be the most active in both birds (Lucas and Stettenheim 1972) and rats (Lecaque and Secchi 1982).

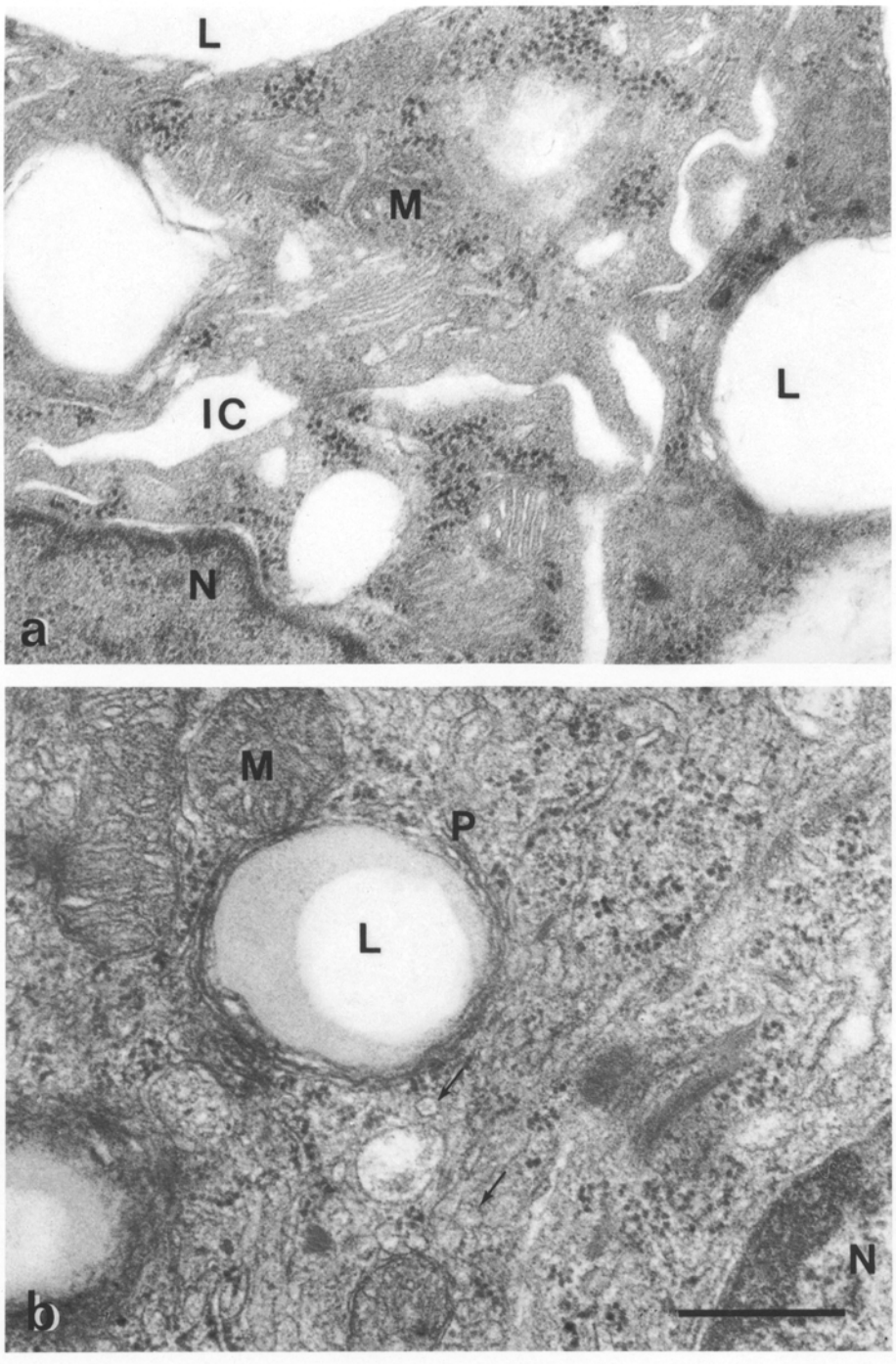

Fig. 2a, b. Effect of castration on the intermediate cells of uropygial and sebaceous glands:
a Uropygial gland of male quail. Large lipid droplets (*L*) are still present. Smooth endoplasmic reticulum vesicles (sER) are no longer visible and small mitochondria (*M*) display a dense matrix. The intercellular spaces (*IC*) are enlarged.
b Sebaceous gland of male rat. Lipid droplets (*L*) are still observed and very few sER profiles (*P*) are visible around them. Vesicles (↗) have decreased in number and size.
M mitochondrion, *N* nucleus. Scale bar: 0.5 μm

Results

In the uropygial gland, a section passing through zone I, showed the presence of three different cell layers: basal or undifferentiated, intermediate or differentiating, and mature or totally differentiated. The same layers were observed in the sebaceous gland in the same sequence. Our ultrastructural study focused on the first cell layer and on the intermediate cells from which the lipid droplets originate.

1) Intact animals (controls)

In both the uropygial and sebaceous glands, the basal cells were seen to form a single layer on the basement membrane surrounding the alveolus. They were elongated in shape with a small rim of cytoplasm around an ovoid nucleus containing a large nucleolus. In the cytoplasm, the rough endoplasmic reticulum (rER) and free ribosomes predominated. Profiles of sER were infrequent and mitochondria randomly distributed. Numerous desmosomes connected the basal cells to one another and to the intermediate cells.

In the quail, the intermediate cells of the uropygial gland contained numerous lipid droplets in their cytoplasm and these droplets seemed about four times larger than those in the corresponding cells of the sebaceous glands in the rats (Fig. 1a). In neither cases were limiting membranes observed around the lipid droplets. Only numerous profiles of sER cisternae were present in whorls around them. A very few areas displayed small sER vesicles; these areas

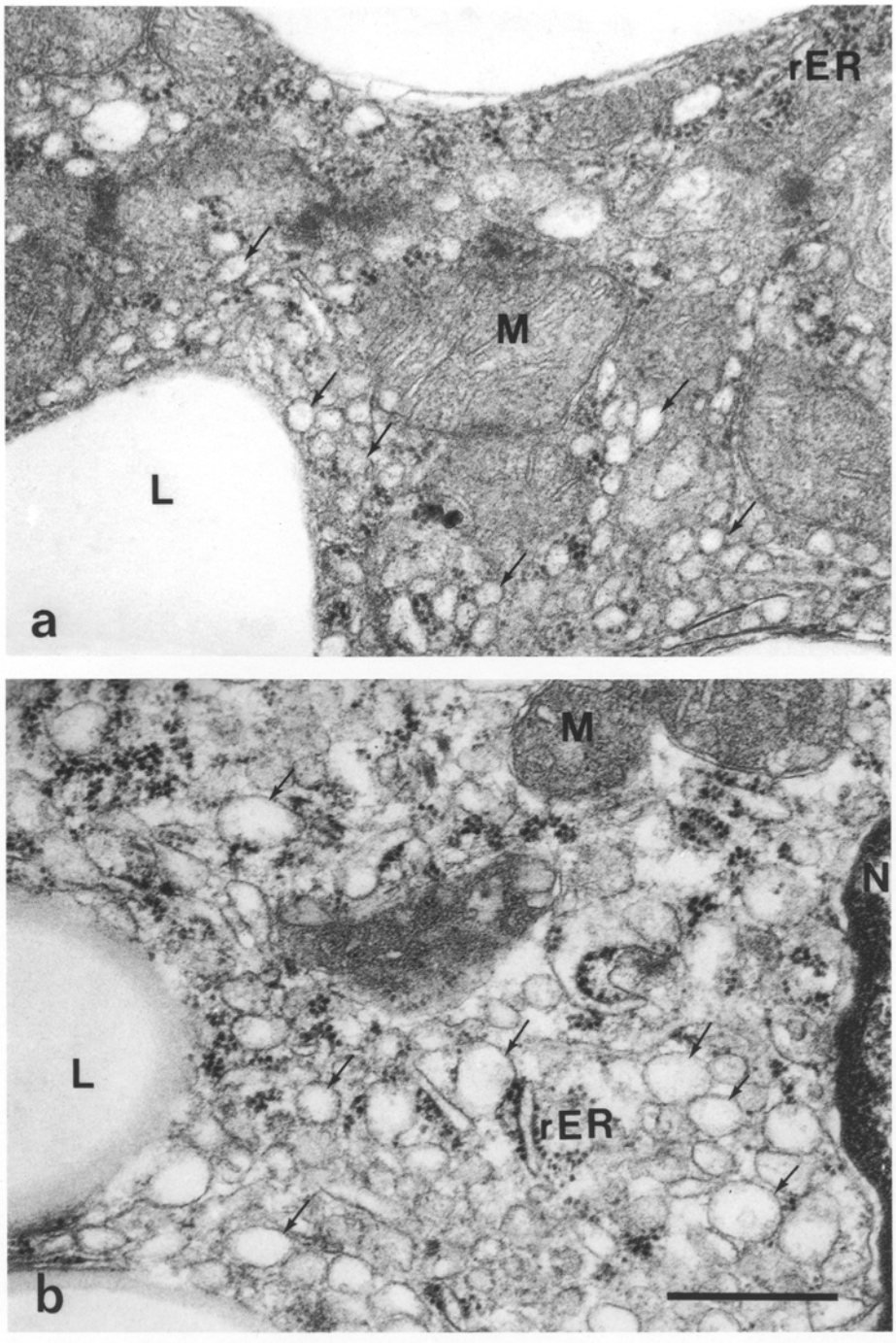

Fig. 3a, b. Effect of testosterone on the intermediate cells of uropygial and sebaceous glands in castrated animals.
a Uropygial gland of male quail. Numerous smooth endoplasmic reticulum vesicles (sER) (↗) fill up the cytoplasm. The mitochondria (*M*) have recovered their normal size.
b Sebaceous gland of male rat. The cytoplasm contains numerous large sER vesicles (↗). *L* lipid droplet, *M* mitochondrion, *N* nucleus, rER: rough endoplasmic reticulum. Scale bar: 0.5 μm

were even fewer in the uropygial glands than in the sebaceous glands (Fig. 1b). As observed in rat sebaceous glands, rER profiles in quail uropygial gland were only seen in rare instances and the Golgi apparatus was small and located near the nucleus. In quail only, large bundles of filaments were seen along the periphery of intermediate uropygial gland cells.

2) After castration

The dark cytoplasm of the flattened basal cells of the uropygial gland contained only free ribosomes and small mitochondria with dense matrices (Fig. 2a). The nuclei were slightly indented, with condensed chromatin. A similar picture was observed in the basal cells of the sebaceous glands (Fig. 2b).

The intermediate cells in the uropygial gland were retracted, with an irregular cytoplasmic border. The intercellular spaces were enlarged and contained small interdigitating cellular processes. The dark cytoplasm showed free ribosomes, numerous lipid droplets, small mitochondria, but only a few whorled profiles and sER vesicles. In the sebaceous glands, there was a large decrease in contents of the smooth vesicles of castrated quail compared with the intact animals. In both cases, the nuclei were indented and exhibited condensed chromatin.

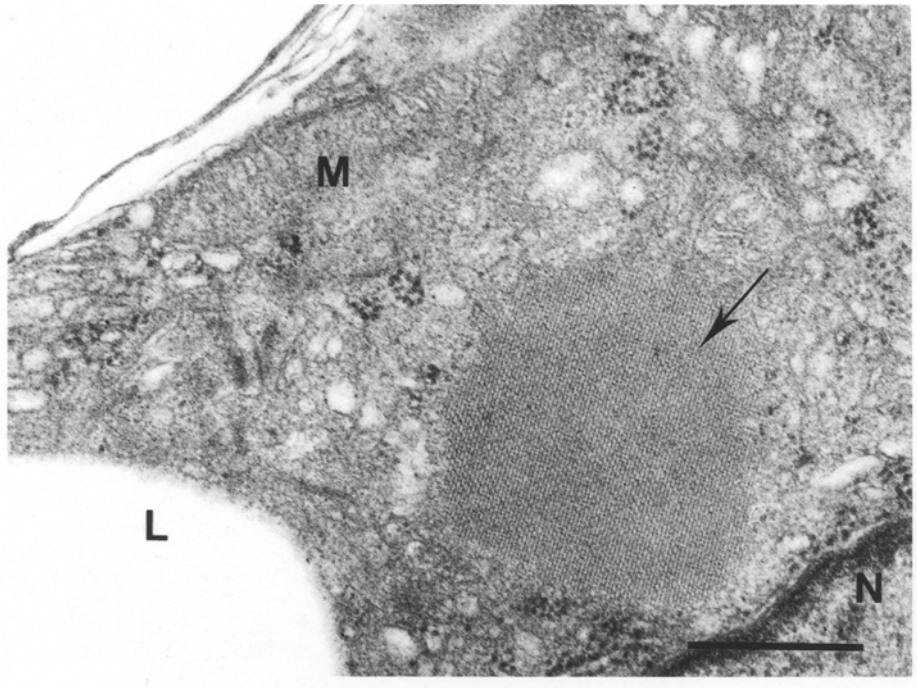

Fig. 4. Effect of testosterone on the intermediate cells of the uropygial gland in castrated male quail. Pseudocrystalline bodies (↗) are observed in the cytoplasm. *L* lipid droplet, *M* mitochondrion, *N* nucleus. Scale bar: 0.5 μm

3) After testosterone propionate

After castration followed by testosterone propionate administration, the ultrastructure of the basal cells in both species was found to resemble that of the controls. The most striking feature of the uropygial gland was the appearance of a large quantity of small sER vesicles scattered throughout the cytoplasm of the intermediate cells (Fig. 3a). In some of these cells, the number of vesicles seemed to be as large as that observed in the sebaceous gland cells of testosterone-treated rats (Fig. 3b). The lipid droplets in the quail gland were not surrounded by the whorled sER profiles observed in the rat after this treatment. Mitochondria with clear matrices were abundant. The Golgi apparatus was not well developed. In the uropygial gland, unlike the sebaceous gland, pseudocrystalline inclusions with a surface area of 0.5 to 1 mm^2 were observed in the cytoplasm of some cells (Fig. 4). The nature of these inclusions has not yet been clarified.

Discussion

From a histological point of view, the uropygial gland of the Japanese quail resembles that of other birds (Jacob and Ziswiler 1982). Moreover, the general organization of acini in this gland exhibits striking similarities with that of the sebaceous gland. At the subcellular level, the present results demonstrated the similarity between these glands. In particular, the intermediate cells in which lipid synthesis occurs exhibited numerous lipid droplets and a well developed sER in both cases. However in the uropygial gland, these droplets were larger and seemed to compress the cytoplasm. Furthermore, in the uropygial gland, unlike in the sebaceous glands, a whorled endoplasmic reticulum was seen and the sER vesicles were smaller and fewer than in the sebaceous gland. This ultrastructural organization might be closely related to the function of the uropygial gland: Thus several authors have suggested that the vesicles and whorled profiles visualized in the sebaceous glands play some part in lipogenesis (Hibbs 1962; Bell 1974), and according to Morohashi (1968), lipid material is probably synthesized by the agranular reticulum and conveyed to the Golgi apparatus. In this respect the slight differences observed in the present study between the two glands examined do not seem to be of crucial physiological importance.

As yet, morphological changes in the uropygial gland of castrated and testosterone-treated male birds have only been studied by light microscopy. Maiti and Ghosh (1972) reported that castration of male birds retarded this gland's activity and also caused hypoplasia, reduced lipid transformation and accelerated cell loss. Subsequent administration of testosterone to the castrated birds restored uropygial gland activity to a normal level. The present study using electron microscopy clearly demonstrates the changes occurring in the cytoplasmic structures of the intermediate cells of this gland in Japanese quail after castration and testosterone therapy, and supplies ultrastructural evidence for its androgen dependency. Our work also provides conclusive evidence for the existence of correlations between these two kinds of holocrine-type cutaneous glands, since both exhibit the same subcellular pattern and react similarly to castration and testosterone treatment.

The physiological function of the uropygial gland is still largely a matter of speculation. Since Rutschke (1960) presented unequivocal evidence that duck feathers remain waterproof even six weeks after uropygial gland removal, the general opinion has been that the main function of the uropygial gland is not water repellence, contrarily to what was previously believed (Kossmann 1871; Weitzel 1951). Instead, it is thought that the secretion of the uropygial gland might help to make the keratin flexible, although its functions probably cover a broader spectrum of plumage hygiene (Jacob and Ziswiler 1982). Such possibilities are consistent with the fact that natural selection has allowed this gland to be maintained even in terrestrial birds like quail.

It is well known that in mammals, specialized sebaceous glands act as organs of communication (Mykytowycz et al. 1974). This might constitute another functional similarity between the two glands studied here, since the androgen dependency of the uropygial gland suggests that its function is to some extent linked to sexual activity and perhaps to mating behaviour. We previously showed that in quail, the quality of this gland's secretions varies with its androgenic status. More precisely, testosterone stimulates the uropygial gland's production of dodecane-2,3-diol (Abalain et al. 1984). In contrast with earlier claims, it is now well established that many avian species have a very well developed olfactory sense (Wenzel 1971), and it is therefore tempting to revert to the old idea that the uropygial gland is a scent gland (Paris 1913). If this were true, its secretions might be connected with pheromonal communication during the breeding season, and preliminary studies have indeed suggested that this connection exists in geese (Würdinger 1978) and ducks (Jacob et al. 1979). At the present time, it has not been proven that dodecane-2,3-diol is connected with pheromone communication in quail; further studies are needed to settle this issue.

From another point of view, the uropygial gland is a very attractive model, because the use of biochemical methods to study hormonal action in sebaceous glands may involve difficulties in mammalian models, on account of the wide-spread distribution of these glands in the skin. The uropygial gland, on the contrary, offers a very reliable model for these studies, as there is only one in each bird and in the quail it provides 200 mg of tissue. The cavity in the center of this gland allows large quantities of secreted material to be obtained more easily from quail than sebaceous gland secretions from mammalian models. As already mentioned, we recently showed dodecane-2,3-diol to be a specific marker of androgen action in the uropygial gland (Abalain et al. 1984). We also recently developed an accurate method for measuring occupied and unoccupied androgen receptors (Amet et al. 1986) which are also present in the uropygial gland (Amet et al. 1982). Consequently, the uropygial gland could be used as a suitable tool for quantitative evaluation of androgen action in sebaceous-like organs. In this respect it could be of great value in the development of screening tests for antiandrogenic drugs, that act on the sebaceous gland but are devoid of general side effects.

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