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Morphology of the Skin Glands of the Crab-eating Frog (*Rana cancrivora*)

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ABSTRACT—The skin glands of *Rana cancrivora*, the euryhaline crab-eating frog, were examined using histochemistry and immunohistochemistry. The skin contained three different glands: a mucous gland, a mixed gland and a vacuolated gland. The acinar cells of the mucous gland stained strongly with alcian blue at pH 2.5 (AB) and weakly with periodic acid-Schiff stain (PAS). In the acinus of the mixed glands, several kinds of cells could be identified: (1) cells with abundant cytoplasm which stained with PAS in the transition region between acinus and duct; (2) cuboidal or squamous cells in the apical portion of the acinus; (3) cells filled with carminophilic granules; (4) cells containing many large vacuoles which stained by PAS and AB. In the acinus of the vacuolated glands, the nuclei of the epithelical cells were located peripherally and the large lumen was filled with vacuoles. In all gland types, no specific immunoreactivity was detected by immunohistochemistry for thyrotropin releasing hormone, cholecystokinin 10, neuropeptide Y, neurotensin, somatostatin and 5-hydroxytryptamine. These results indicate that the skin glands of *Rana cancrivora* have a different structure and properties from those of other amphibians studied so far.

INTRODUCTION

Generally, the amphibian skin possesses two types of cutaneous glands: mucous glands and granular glands [4–6, 13, 17–21, 23] and there have been relatively few reports of any other type of amphibian skin gland [3, 5, 6, 13, 18]. The mucous glands produce a mucous secretion which is stained by the periodic acid-Shiff (PAS) method [4, 21]. The granular glands have a lumen filled with numerous granules which contain 5-hydroxytryptamine (5-HT) [22] and amphibian skin peptides [8, 11, 12, 23, 28], which are share common amino acid sequences with mammalian brain-gut peptides, and are divided into several families: angiotensin, bombesin, bradykinins, caeruleins, dermorphins, sauvagine, spasmolysins, tachykinins, thyrotropin releasing hormones (TRH), xenopsins and magainin [2]. The biogenic amines and amphibian skin peptides of the granular glands vary in different species.

The crab-eating frog of South East Asia, *Rana cancrivora*, inhabits both fresh water and brackish water environments [1, 10], and has exceptional tolerance of salinity [4, 15]. Attempts to elucidate the mechanism of this tolerance include some morphological studies of the kidney [25], the internal gills of tadpoles [27] and neuromasts of tadpole skin [26], but little reference has been made to their skin glands. Since it has been proposed that the difference in the morphology and distribution of the skin glands [9] and skin peptides [2]among amphibian species may reflect a physiological adaptation to different environments, the present studies were undertaken to investigate the structure and properties of the skin glands of *R. cancrivora* using histochemical and immunohistochemical techniques designed to detect mammalian brain-gut peptides.

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MATERIALS AND METHODS

Adults of *R. cancrivora*, weighing 19–46 g, were collected around prawn culture-ponds (salinity 33 o/oo) in a mangrove swamp at Ang Sila near Bangkok, Thailand. Samples of the skin, about 10 mm square, were removed from the dorsal and ventral regions of the trunk, fixed in 4% paraformaldehyde (buffered to pH 7.4 with 0.1 M phosphate buffer) overnight at room temperature, dehydrated in a graded series of ethanol, embedded in paraplast, and sectioned at 6 μm thickness. The sections were stained with azan, PAS, alcian blue (AB) at pH 2.5, or the immunohistochemistry using anti-TRH (Polysciences), anti-CCK 10 [16], anti-neuropeptide Y (NPY)(Amershan), neurotensin (NT)(Incstar), anti-somatostatin (Incstar) and anti-5-HT (Incstar) according to a method described previously [23].

RESULTS

Three types of skin glands, mucous glands, mixed glands and vacuolated glands were identified in the stratum spongiosum of the dorsal and ventral skin with azan staining (Figs. 1, 2, 5, 8). In skin from both regions, the mucous glands were more numerous than the mixed glands. When compared with the mucous and the mixed glands, the number of the vacuolated glands was very small. The ducts of three gland types were lined by squamous to cuboidal cells and opened onto the surface of the skin. The acini of these glands appeared to be surrounded by myoepithelial cells.

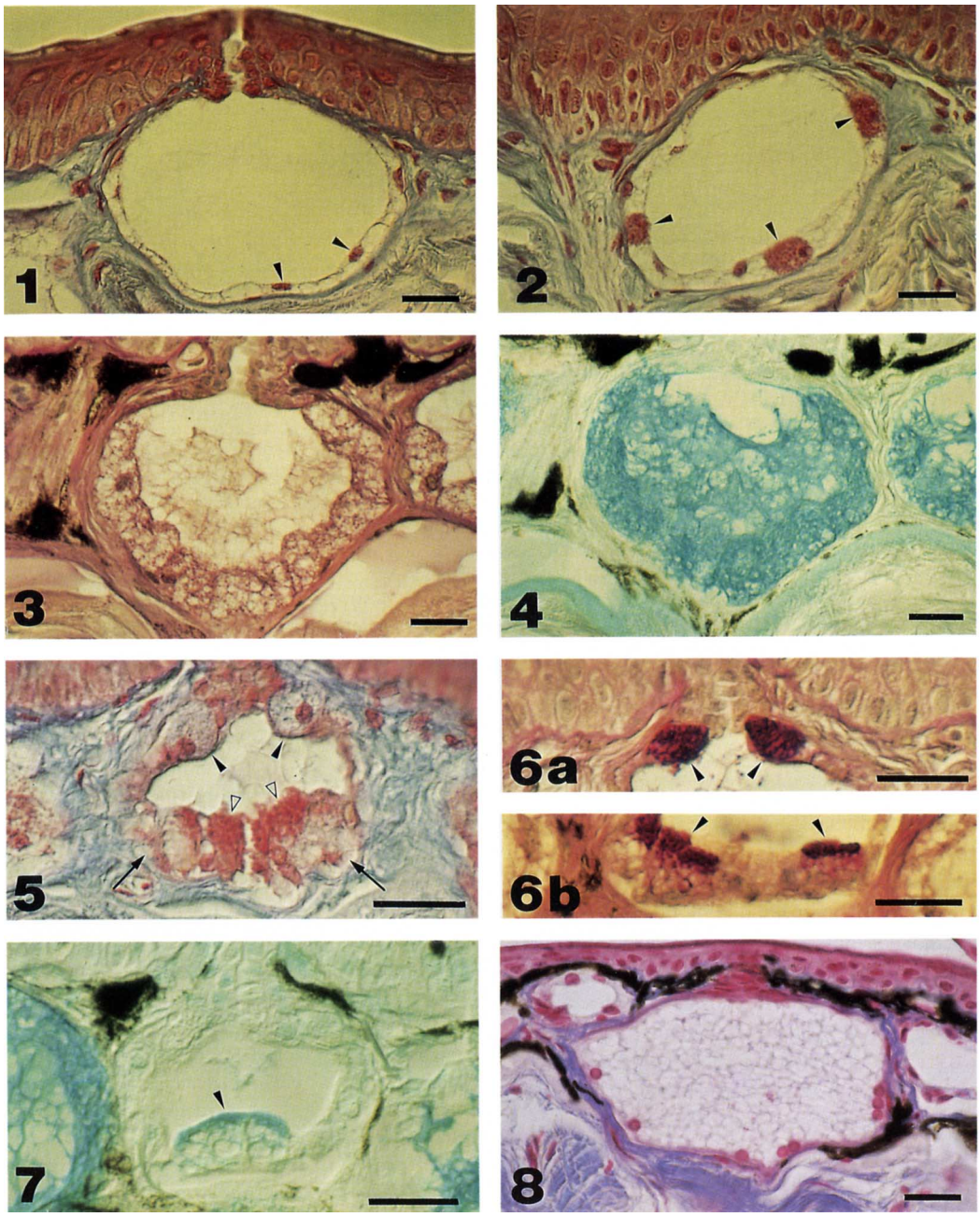

FIG. 1–9. Histochemistry of the skin glands in the ventral skin (Figs. 1,2,5,6) and dorsal skin (Fig. 3,4,7,8) of *Rana cancrivora*. The sections were viewed with differential interference microscopy except for Fig 8. Bars=20 μm.

FIG. 1. Section through a mucous gland stained with azan, in which acinar cells with pale and foamy cytoplasm are seen. Note that the nuclei are located apically (arrowheads).

FIG. 2. Section through a mucous gland stained with azan, showing the presence of three cells with carminophilic granules (arrowheads) among the pale acinar cells.

FIG. 3. Section through a mucous gland stained with PAS. The intensity of the PSA reaction is weak in the lumen.

FIG. 4. Section through a mucous gland stained with AB. In the mucous gland, the lumen and acinar cells are stained aquamarine.

FIG. 5. Section through a mixed gland stained with azan, showing three different types of cellular components: cells stained with aniline blue (arrowheads), cells with carminophilic granules (open arrowheads) and cells with clear vacuoles (arrows).

FIG. 6. Section through a mixed gland stained with PAS. (a) In the transition region between duct and acinus, strongly PAS-positive cells are found (arrowheads). (b) In the basal portion of the acinus, vacuolated cells are stained with PAS, particularly in their apical region (arrowheads).

FIG. 7. Section through a mixed gland stained with AB. The AB reaction is seen in the apical portion of the vacuolated cells (arrowhead).

FIG. 8. Section through a vacuolated gland stained with azan. The nuclei are located at the periphery and the lumen is filled with vacuoles.

The mucous glands were lined mainly by simple squamous or cuboidal cells with cytoplasm which is pale and foamy after azan staining (Fig. 1). The nuclei were flat and lay mainly in the apical portion of the cells. In smaller mucous glands, the acinar epithelial cells were tall pyramidal to columnar in shape and their nuclei at times were located basally. Occasionally, cells with carminophilic granules were distributed among the pale acinar cells (Fig. 2). The acinar cells stained light pink with PAS (Fig. 3) and aquamarine with AB (Fig. 4), and the secretion within the lumen also stained weakly with PAS and strongly with AB. In the acinar cells, both methods chiefly stained fine granules, but not the contents of vacuoles.

The acinus of a mixed gland was composed of the following kinds of cells (Fig. 5). Firstly, in the transition region between acinus and duct, there were cells with abundant cytoplasm that stained blue with azan (Fig. 5) and dark purplish-red with PAS (Fig. 6a). Secondly, cuboidal to squamous cells lightly stained with carmine were present in the apical portion of the acinus. Thirdly, cells containing carminophilic granules were located basally in the acinus. Finally, cells with many large vacuoles were located in the basal and lateral portions of the acinus, and stained dark purplish-red with PAS (Fig. 6b) and aquamarine with AB (Fig. 7), particularly in their apical region. Occasionally, cells stained with aniline blue were present basally and laterally.

The vacuolated cells in the mixed glands were stained by anti-somatostatin. However, when the antibody was preincubated with synthetic somatostatin 28, the immunostaining was still present. Since this antibody is generated in a rabbit against somatostatin conjugated to keyhole limpet hemocyanin, the antibody was also peincubated with hemocyanin. This treatment abolished the immunostaining, indicating that the immunoreaction by anti-somatostatin is due to non-specific reaction. No other immunoreactivity (TRH, CCK 8, NPY, NT and 5-HT) was detected in all gland types.

In the acinus of the vacuolated glands, the rounded nuclei of the epithelial cells were located peripherally, and the boundaries between these cells were not clear (Fig. 8), suggesting they form a syncytium. The wide lumen was pale after azan staining and was filled with vacuoles. In the relatively small glans, however, a few fine carminophilic granules were seen in the lumen.

DISCUSSION

In *R. cancrivora*, we found three types of glands: mucous glands, mixed glands and vacuolated glands. The present histochemical study has shown that the skin glands of *R. cancrivora* are different in some respects from those of the other amphibians so far studied.

The mixed glands contained several kinds of cells, in which at least two types of cell could be classified as mucous cells which stained with PAS and were located at the ductal pole, and serous cells filled with carminophilic granules; vacuolated cells are the exception to this classification. There have been relatively few reports concerning the presence of skin glands containing both mucous cells and serous cells [5, 6, 18]. Some of the morphological features of the seromucous glands described by Mills and Prum [18] in *R. pipiens, R. temporaria* and *R. catesbeiana* resembled those of the mixed glands seen in the present study. These include the presence in the seromucous glands of a distinct type of cell at the transition region between acinus and dust, and some large clear vacuoles in the basal cells of the acinus. However, it is not clear whether the seromucous glands are identical to the mixed glands, since the reports by Mills and Prum [18] include no histochemical data.

The glands with pale and foamy cells seen after azan staining were identified as mucous glands on the basis of their staining properties with PAS and AB, which suggested the presence of acid and neutral glycoconjugates [21]. However, these glands differ somewhat in morphology from those well-known mucous glands in which the nuclei are located basally. In the present study, the nuclei of the mucous glands were distributed apically and, additionally, some of the acinar cells contained carminophilic granules. Although the precise function of these mucous glands remains unknown, it is generally accepted that mucous glands keep the skin moist and their secretion serves as a lubricant in the water [9, 20]. Further, it has been proposed that they are involved in ion transport across the frog skin [24], which has been found to be reduced in *R. cancrivora* compared with *R. temporaria* [7].

Unlike most amphibians, typical granular glands were not detected in the skin of *R. cancrivora*. There has been one previous report of the absence of granular glands from *R. angolensis* [17]. However, the possibilities remain that a small number of granular glands are present, perhaps confined to a small area of the skin, that the staining substances of the granules dissolve away during staining procedures, or that the granules are released under a certain physiological condition. In this respect, it is note that the vacuolated glands resemble in part granular glands of some species, such as *Xenopus*, in which the epithelium form a syncytium [8]. It has also been reported that the granular glands of *Xenopus* have many vacuoles in the lumen at vacuolated stage [12]. To verify whether a granular gland occur in this species, further immunohistochemical study for detecting amphibian peptides should be needed, and in future, the name "vacuolated gland" may be need to be changed to a more appropriate name.

In conclusion, these unique characteristics of the skin glands of this species allow us to assume that they are associated with the frog's exceptional adaptation to salinity.

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