Sexual Dimorphism of the Miniature Pig
Submandibular Glands

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ABSTRACT

The submandibular salivary glands of 14 Pitman-Moore miniature pigs were examined to determine whether they exhibit sexual dimorphism. Sections of the glands from seven male and seven female animals were stained by several histochemical methods. No morphological sex differences were noted in hematoxylin and eosin-stained sections but differences were found with all of the histochemical methods utilized. Most noticeable was the higher concentration of sialic acid-containing mucosubstances in mucous acini of the female submandibular gland sections when they were stained with either the Alcian blue (pH 2.5), Alcian blue (pH 2.5)-periodic acid-Schiff or periodic acid-phenylhydrazine-Schiff methods. Unlike mucous acini, demilunes of the male submandibular gland possessed a higher concentration of sialic acid-containing mucosubstance than did those of the female. The miniature pig, unlike other animal orders studied, possesses sexual dimorphism at three weeks of age, thus differing markedly from other animals in which sexual dimorphism of the salivary glands does not occur until after the onset of puberty.

Sexual dimorphism of salivary glands was reported first by Lacassagne (’40a) who described a morphological difference between male and female mouse submandibular glands. Lacassagne’s observations were soon confirmed by other investigators (Fekete, ’41; Chaulin-Servinire, ’42). Since 1940, sexual dimorphism of salivary glands has been observed in other mammalian species including rats (Lacassagne, ’40b), gerbils (Abouharb, ’55), Syrian hamsters (Shackleford and Klapper, ’62a), European hedgehogs (Borghi, ’63), desert rats (Shackleford and Schneyer, ’64), crab-eating monkeys (Grod, ’64), rabbits (Spicer and Duvenci, ’64), men (Mandel et al., ’64), agoutis (Hetem, ’67) and Kangaroo rats (Flon et al., ’70). The only report of sexual dimorphism in sublingual glands is in the Syrian hamster (Spicer and Duvenci, ’64). Fava-de-Moraes et al. (’66) have reported a sexual dimorphism in the parotid glands of both the southern fur seal and the southern eared seal (sea lion). In some of these instances sexual dimorphism was indicated solely by morphological features while in other cases it was revealed by histochemical or biochemical characteristics.

The purpose of this study was to determine whether sexual dimorphism exists in the submandibular gland of the miniature pig.

MATERIALS AND METHODS

Seven male and seven female miniature pigs of the Pitman-Moore strain were used. Two-, three-, four-, six-, and nine-month-old animals were purchased from Vita-Vet Laboratories, Marion, Indiana. Three-week-old and one-month-old animals were from litters raised in our animal quarters. All animals were fed a normal diet of commercial pig food with free access to water. They were fasted overnight and killed at approximately the same time in the morning with a captive-bolt pistol to prevent any effects that an anesthetic might have on gland morphology or secretory activity. The submandibular glands were removed and representative samples placed in cold formal-calcium for 18–24 hours. The speci-

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mens were washed briefly in tap water and carried through a routine paraffin embedding procedure. Sections were cut at 8 μ and after mounting on slides were subjected to the following procedures. Hematoxylin and eosin-stained sections were used for morphological study. Mucosubstances with vicinal hydroxyl groups were demonstrated by the periodic acid-Schiff method (Spicer et al., '67). Reaction of pre-existing tissue aldehydes was prevented by employing an amine-aldehyde condensation blocking technique prior to application of the periodic acid-Schiff procedure (Pearse, '68). Glycogen was removed by pretreatment with diastase (malt alpha-amylase) (Lillie, '65). Acid mucosubstances were demonstrated by staining with Alcian blue at pH 2.5 (Mowry, '63). Sialomucins were identified by incubating sections with sialidase for 18 hours at 40°C and then exposing these sections, together with non-sialidase-treated sections, to the Alcian blue staining method (Spicer and Warren, '60). Pretreatment of sections with sialidase prevented the Alcian blue staining of mucosubstance in both acini and demilunes, indicating that it is a sialomucin. Goblet cell mucosubstance was still Alcian blue-positive when stained following sialidase digestion (fig. 6). Periodic acid-Schiff-positive material was highly concentrated in mucous acini of both sexes, while demilunes revealed only slight staining (figs. 7, 8). Diastase pretreatment prevented staining of material localized in the ducts which we assumed to be glycogen. Diastase pre-incubation had no effect on staining of the contents of the acini or demilunes. The periodic acid-phenylhydrazine-Schiff staining revealed the presence of periodate-reactive acid mucosubstance within the mucous acini. The concentration of this material was considerably higher in the mucous acini of the female submandibular gland than in the male gland (figs. 9, 10). It was not clear if demilunes contained a periodate-reactive acid mucosubstance because one could not distinguish the exact boundary between mucous cells and the demilunes when this method was utilized. Mucin of the goblet cells was periodic acid-phenylhydrazine-Schiff positive.

The combined Alcian blue-periodic acid-Schiff method conclusively revealed the differences between male and female submandibular glands in the miniature pig. The demilunes were consistently Alcian blue-positive while mucous acini were stained differently in the two sexes (figs. 11, 12). Mucous acini in the male were intensely periodic acid-Schiff-positive as indicated by red coloration (fig. 11). Mucous acini of the female were both Alcian blue- and periodic acid-Schiff-positive, indicating that they were seromucous in both sexes. They were, however, more markedly positive in the glands of the male pigs (figs. 3, 4). Mucous acini of the male glands were unstained by Alcian blue but the mucous acini of the female glands were intensely stained (figs. 3, 4). Occasional serous acini observed in hematoxylin and eosin-stained sections, were shown to be seromucous acini as a result of Alcian blue staining (fig. 3).

Observations

In hematoxylin and eosin-stained sections, the submandibular glands of both male and female miniature pigs gave the appearance of a mixed gland containing mucous acini, serous acini and serous demilunes (figs. 1, 2). The duct system was well developed and consisted of intercalated, striated and excretory ducts. Goblet cells were frequently seen in the excretory ducts. The only difference between male and female glands, when stained with hematoxylin and eosin, was a marked prominence of the demilune cells in the female gland when compared to the male gland (figs. 1, 2).

Staining with Alcian blue (pH 2.5) revealed differences between male and female secretory units. The demilunes were
itive as indicated by the presence of dark blue to purple coloration (fig. 12).

Differences in staining patterns were seen between males and females with all methods used except hematoxylin and eosin and periodic acid-Schiff, at all ages studied. Sections of three-week old male and female glands, stained with either Alcian blue (pH 2.5) (figs. 13, 14), or the Alcian blue-periodic acid-Schiff methods (figs. 15, 16) illustrate these differences.

**DISCUSSION**

The morphological structure of the miniature pig submandibular gland is apparently similar to that seen in the larger domestic pig. Our results support the observation of Shackleford and Klapper ('62b) and Shackleford and Wilborn ('68) that the demilunes of the pig submandibular gland are seromucous. Leppi and Spicer ('67), on the other hand, report that they found no indication of seromucous demilunes in the pig submandibular gland. The differing results may occur because different staining methods or different breeds were used.

Many comments have appeared in the literature pertaining to the age at which sexual dimorphism appears (Flon et al., '70; Smith et al., '71). Age is probably important but ample evidence shows that sexual differences occur in the salivary glands of prepuberal animals (Kronman, '63; Fava-de-Moraes and Nicolau, '66; Smith et al., '71). Some of the differences between male and female submandibular glands were not as apparent in the younger animals in our series but the differences were always present.

The staining of the mucosubstances in the mucous acini, seromucous acini and in demilunes by Alcian blue indicates the presence of an acid mucosubstance. Earlier work suggests that most staining of mucin by Alcian blue in salivary glands can be attributed to the presence of sialic acid (Spicer and Warren, '60; Warren and Spicer, '61) which is indicated by the inability of salivary gland mucin to stain with Alcian blue when the sections have been pretreated with a sialidase solution. The results of our study pertaining to sialic acid content of pig submandibular gland mucosubstance is corroborated by biochemical evidence (Payza et al., '69; De Salegui and Plonska, '69). The high concentration of Alcian blue-positive mucosubstance in mucous acini of glands from female pigs was quite striking when compared to glands of male pigs which were unstained. Ovarian hormones may serve in some way to influence sialic acid incorporation into the mucin molecule as originally suggested by Shackleford and Klapper ('62a). The lower concentration of sialic-acid-containing mucosubstance in the male gland may be due to an inhibitory effect of male sex hormones on sialic acid incorporation into mucin. Devalle et al. ('68) have demonstrated increased sialic acid concentration in the submandibular gland of the male mouse following castration and administration of testosterone propionate to castrated male mice decreased the sialic acid concentration in the submandibular glands.

The failure of sialidase pretreatment to prevent Alcian blue staining of goblet cell mucin may be explained by the fact that there are multiple forms of sialic acid (Scheinthal and Bettelheim, '68), some of which are resistant to some sialidases (Spicer and Warren, '60). The mucin of the goblet cell may not be a sialomucin.

The periodic acid-phenylhydrazine-Schiff method revealed the presence of a periodate reactive acid mucosubstance in the submandibular gland mucous acini of both males and females with a higher concentration occurring in the female. This method has been shown by Spicer ('61) and Spicer et al. ('67) to be relatively selective for the demonstration of sialo- and sulfomucins. Because we have been unable to demonstrate sulfomucins in these glands we must assume that the periodic acid-phenylhydrazine-Schiff positive material is a sialomucin.

We feel that there is a distinct possibility that these differences result from the actions of sex hormones on the salivary glands as indicated by many earlier studies (Chretien, '66; Disher and Elias, '67; Calissano and Angeletti, '68; Devalle et al., '68).

Even though the exact mechanism of interaction between the endocrine glands and the salivary glands is not known, the salivary gland-endocrine interrelationship
is well documented as well as other salivary glands, may well be under the control of a combined hormonal action of a very complicated nature (Raynaud, '64; Ohlin, '66; Devalle et al., '68; Calissano and Angeletti, '68).

LITERATURE CITED


Mowry, R. W. 1963 The special value of methods that color both acidic and vicinal hydroxyl groups in the histochemical study of mucins. With revised directions for the colloidal iron stain, the use of alcin blue G8X and their combinations with the periodic acid-Schiff reaction. Ann. N. Y. Acad. Sci., 106: 402-423.


PLATE 1
EXPLANATION OF FIGURES

1 Submandibular gland, nine-month-old male. Demilunes are indicated by arrows. Hematoxylin and eosin. × 180.

2 Submandibular gland, nine-month-old female. Demilunes are indicated by arrows. Hematoxylin and eosin. × 180.

3 Submandibular gland, nine-month-old male. A seromucous acinus is indicated by an arrow. Alcian blue (pH 2.5). × 180.

4 Submandibular gland, nine-month-old female. Alcian blue (pH 2.5). × 180.

5 Submandibular gland, nine-month-old female. A goblet cell is indicated by an arrow. Alcian blue (pH 2.5). × 180.

6 Submandibular gland, nine-month-old female. A goblet cell is indicated by an arrow. Alcian blue (pH 2.5)-sialidase-incubated section. × 180.


8 Submandibular gland, nine-month-old female. Periodic acid-Schiff. × 180.
PLATE 2
EXPLANATION OF FIGURES

9 Submandibular gland, nine-month-old male. Periodic acid-phenyl-
hydrazine-Schiff. × 180.

10 Submandibular gland, nine-month-old female. Periodic acid-phenyl-
hydrazine-Schiff. × 180.

11 Submandibular gland, nine-month-old male. Demilunes are indicated
by arrows. Alcian blue (pH 2.5)-periodic acid-Schiff. × 180.

12 Submandibular gland, nine-month-old female. Demilunes are indi-
cated by arrows. Alcian blue (pH 2.5)-periodic acid-Schiff. × 180.

13 Submandibular gland, three-week-old male. Alcian blue (pH 2.5).
× 180.

14 Submandibular gland, three-week-old female. Alcian blue (pH 2.5).
× 180.

15 Submandibular gland, three-week-old male. Alcian blue (pH 2.5)-
periodic acid-Schiff. × 180.

16 Submandibular gland, three-week-old female. Alcian blue (pH 2.5)-
periodic acid-Schiff. × 180.