The Effect of Gonadectomy and Propylthiouracil Treatment on the Submandibular Gland of the Mongolian Gerbil, *Meriones unguiculatus* [1]

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ABSTRACT The histological organization and cytology of the submandibular glands of male and female Mongolian gerbils were described. A sexual dimorphism in the glands, limited specifically to the serous tubules, was noted. In the male, gonadectomy, propylthiouracil administration and a combination of the two treatments resulted in significantly reduced serous tubule diameters. Such reduction was accompanied by a decrease in the number of cells per tubule without any change in the height of individual serous cells. In the female similar treatments were without effect on these parameters of the serous tubules. Experimental treatments did not affect the cytology or the periodic acid-Schiff and Alcian blue reactions of the submandibular glands in either sex.

A sexual dimorphism of the mouse submandibular gland has been recognized (Lacassagne, '40) in which the serous tubule cells of the male are larger and more granular than those of the female. This sexual difference is conditioned, at least in part, by the male hormone as testosterone injections cause a "masculinization" of the serous tubules in the female mouse (Noli, '48). In the male rat, testosterone and thyroxine act jointly to control the morphology of the serous tubules (Grad and Leblond, '49). In this species, castration brings about involution and degranulation of the tubular epithelium (Shafer, '53).

The present study was made to determine (1) whether sexual dimorphism occurs in the serous tubules of the submandibular gland in the Mongolian gerbil, *Meriones unguiculatus*, and (2) whether gonadectomy and/or propylthiouracil administration would modify the morphology of the serous tubules in either sex. A description of the histology and cytology of the gerbil submandibular gland has been included.

MATERIALS AND METHODS

The gerbils, which were three to four months old when purchased from a commercial supplier, were fed on standard rat chow and water. The animals were allowed to acclimate to laboratory conditions for one week prior to use. Twelve males were randomly divided into four equal groups: Group I, intact controls; Group II, animals that had been gonadectomized for one month; Group III, animals fed propylthiouracil (0.1% in drinking water) ad libitum for one month; and Group IV, animals fed propylthiouracil for one month (as in Group III) and gonadectomized two weeks prior to autopsy. Twelve female gerbils were subjected to a similar regimen. Food was removed two days prior to sacrificing the gerbils, although fasting was reported to have little or no effect on the secretory granules of the serous tubules in the rat (Gran and Leblond, '49). Atrophy of the accessory organs of reproduction was noted after gonadectomies. Thyroid glands were weighed to indicate the goitrogenic effect of propylthiouracil. This goitrogen has been shown to exert its peak effect after 30 days in the rat (Sreebny et al., '62).

The animals were killed with chloroform and central portions of their submandibular glands were fixed in Susa for 18–24 hours. All fixed material was then washed in running water for one hour.

[1] Supported in part by National Science Foundation Research grant GB-6435X.

After infiltration, tissues were embedded in either paraffin or glycol methacrylate for sectioning.

Sections were stained with Harris hematoxylin and eosin for routine morphological investigation. The periodic acid-Schiff (PAS) reaction (Humason, '67) was used to demonstrate the presence of carbohydrates, and Alcian blue was employed to detect acid mucopolysaccharides (Humason, '67). Acinar and duct cell types were studied in submandibular glands from all groups.

All measurements were made with an ocular micrometer. In each animal, the diameter and number of cells per tubule were recorded for each of ten serous tubules. At the same time, the height of a single cell from each of the ten serous tubules selected was noted. Only those tubules which were accurately cut in a transverse fashion were selected for measurement. The means of the results from the experimental groups were compared and the significance of differences was determined by Student's t-distribution.

OBSERVATIONS AND RESULTS

In male gerbils, submandibular acini are connected to intralobar ducts by very short intercalated ducts. The intralobar ducts are composed of two portions: the distal serous tubules and the proximal striated ducts, the former being longer than the latter. The striated ducts, in turn, communicate with excretory ducts. This organization is similar to that in the adult rat (Grad and Leblond, '49).

The acini, which comprised about half of each microscopic field, were composed of polygonal cells with basally-positioned, flattened nuclei. These cells were filled with what appeared to be a mucoid substance.

Intercalated duct cells were basically cuboidal in shape with clear cytoplasm and centrally-located nuclei. The serous tubules were quite conspicuous and, in some parts of the sections, they were more prominent than the acini (fig. 1). The cells forming them conformed closely to serozymogenic models. They were columnar (ca. 19.3 $\pm$ 0.7 μ in height, table 2) with basal nuclei and apical granulation (fig. 3). Cells constituting the striated ducts were low columnar types with clear cytoplasm and centrally-situated nuclei. The excretory ducts had both a deep and a superficial layer of epithelial cells. The deep layer included columnar cells with clear cytoplasm and nuclei somewhat below the center of the cell, while the superficial layer was composed of cuboidal cells with clear cytoplasm and centrally-located nuclei.

In PAS preparations, some acinar cells were mildly positive while the majority were negative. In Alcian blue stained material, all acinar cells were negative. The serous tubules yielded a positive reaction with PAS (although much less intense than the positive acinar cells) and a negative reaction with Alcian blue. All other duct cell types were PAS and Alcian blue-negative. PAS-positive secretory material was observed in the lumina of all segments of the duct system except the intercalated ducts. In the female and in all experimental groups, the submandibular histochemistry was the same as that of the normal male gerbil.

Gross observations of the submandibular glands indicated that those from castrated male gerbils were smaller than those from intact controls (actual weights were not recorded in order that immediate fixation might be accomplished). The diameters of the serous tubules were significantly reduced by 19% one month after castration (figs. 3, 5; table 2). Although quantitative studies were not made on the number of tubules per unit area of the sections, the same number of tubules appeared to be present in sections from castrated and control male gerbils. A 26% reduction in the number of cells per serous tubule following castration could possibly account for the reduction in the diameter of these same tubules (table 2). Cytologically, the cells of the serous tubules, as well as other cells comprising the gland, were no different in castrated and control males.

Male gerbils which were fed propylthiouracil for one month showed changes in the submandibular gland that were identical with those following castration. There was a 20% decrease in the mean diameter of the serous tubules accompanied by a 25% decrease in the number of cells per tubule. The mean cell height

TABLE 1

Body, gonad and thyroid weights of the gerbils

Group [1]	Body weight ca. 120 days	Testis weight	Thyroid weight
	gm	*mg*	*mg*
		Males	
I	92.0 ± 1.0	607.3 ± 18.4	6.7 ± 0.3
II	83.3 ± 8.2	—	7.0 ± 0.6
III	86.7 ± 5.9	619.0 ± 5.8	29.3 ± 0.7 [2]
IV	90.7 ± 3.8	—	27.3 ± 0.3 [2]

Group [1]	Body weight ca. 120 days	Ovary weight	Thyroid weight
	gm	*mg*	*mg*
		Females	
I	88.3 ± 5.2	8.3 ± 0.7	7.3 ± 0.7
II	83.0 ± 6.3	—	7.7 ± 0.3
III	85.2 ± 4.0	8.7 ± 0.3	26.0 ± 1.2 [2]
IV	90.3 ± 2.1	—	31.3 ± 0.7 [2]

[1] Group I, normal; II, gonadectomized; III, propylthiouracil-treated; IV, gonadectomized, propylthiouracil-treated.
[2] Mean values (for each sex) significantly different from all groups without propylthiouracil, $P < 0.001$.

in tubules from treated and control gerbils was the same (figs. 3, 6; table 2). There were no structural aberrations in the tubule cells as a result of treatment. Propylthiouracil produced a substantial increase in thyroid weight, but body and testicular weights were unaffected (table 1). Sex accessories from these gerbils appeared no different from those of controls.

The combined treatment of propylthiouracil for one month and castration for the last two weeks resulted in changes in the serous tubules no different from those following either treatment alone. The decrease in the mean diameter of the serous tubules was 25% and the decrease in the cell number per tubule was 28% (figs. 3, 7; table 2).

The histological organization and cytology of the female gerbil's submandibular gland was found to be virtually the same as that in the gland of the male. Through low power inspection of microscopic sections, it appeared that the relative number of serous tubules per field was less than that of male sections (figs. 1, 2). The serous tubule diameters of the intact female gerbil glands were approximately 30% smaller than those in the

TABLE 2

Measurements of the serous tubules from submandibular glands of the gerbils

Group [1]	Tubule diameter (micra)	Cells per tubule	Cell height (micra)
		Males	
I	67.4 ± 3.5	17.0 ± 1.4	19.3 ± 0.7
II	54.3 ± 0.7 [2]	12.5 ± 0.3 [3]	19.7 ± 0.2
III	53.8 ± 0.6 [2]	12.8 ± 0.1 [3]	21.0 ± 0.7
IV	50.8 ± 1.8 [2]	12.2 ± 0.5 [3]	19.0 ± 0.5
		Females	
I	47.1 ± 1.5	11.1 ± 0.3	19.9 ± 0.4
II	49.1 ± 2.2	12.2 ± 0.5	19.9 ± 0.4
III	49.9 ± 0.9	12.1 ± 0.4	19.7 ± 0.5
IV	48.1 ± 0.9	11.5 ± 0.4	20.4 ± 0.3

[1] Group I, normal; II, gonadectomized; III, propylthiouracil-treated; IV, gonadectomized, propylthiouracil-treated.
[2] Significant difference from normal, $P < 0.02$.
[3] Significant difference from normal, $P < 0.04$.

normal male gland. The mean number of cells per tubule was 35% less than that in the male. Mean cell heights were the same in both sexes (figs. 3, 4; table 2).

In the female, ovariectomy, propylthiouracil administration or a combination of treatments resulted in no changes in submandibular gland histology or cytology. Likewise, the serous tubules showed no changes in the parameters measured.

DISCUSSION

As in the mouse, the submandibular gland of the Mongolian gerbil was shown to be sexually dimorphic with respect to the serous tubules. In the male, the diameters of these tubules were larger than in the female. It appeared that this difference could be associated with the male hormone since the serous tubules of castrated males closely resembled those of normal females (figs. 4, 5; table 2). It was unlikely that the sexual dimorphism could be attributed to differences in male and female body weights since all animals were approximately the same size (table 1).

Castration and propylthiouracil administration had equivalent effects on the histology of the male submandibular gland, and brought about comparable decreases in serous tubule diameter. Since the decreases observed in Groups II and III were the results of fewer rather than smaller tubule cells, it seemed conceivable that testosterone and, by inference thyroxine, maintained serous tubule size in the normal male with hyperplastic rather than hypertrophic effects.

While hypothyroidism may have explained the effects of propylthiouracil on the serous tubules of Group III males, other possibilities exist. The goitrogen may have acted locally on the submandibular glands. This, however, seems unlikely since no morphological changes were evident in submandibular glands from females that had received the drug. On the other hand, propylthiouracil may have interfered directly or indirectly with the action of testosterone on one of its target organs, viz., the serous tubules. This would account for the lack of an enhanced effect on the submandibular gland in Group IV males when castration and propylthiouracil administration were combined. This mode of action would further explain the goitrogen's lack of effect on the female gland.

It has been shown in rats that castration and thyroidectomy cause a decrease in the granular content of the serous tubule cells along with a displacement of basal nuclei to more central positions (Grad and Leblond, '49). Similar changes occur following thiouracil administration (Nava-Rivera et al., '63). No such cytological aberrations of the serous tubule cells of the male gerbil's submandibular gland were observed in the present study. Possibly these effects are species-specific. However, there is also the possibility that testosterone and thyroxine are positive allosteric effectors for the synthesis of proteolytic enzymes only. If the gerbil's serous tubule cell granules were not protease proenzymes as were the rat's (Sreebny et al., '55; Baker, '58), then, perhaps the lack of the two hormones should not have affected them. Also since the gerbil's serous tubule cells did not experience degranulation following treatment, it is not surprising that their respective nuclei did not undergo the apical migration observed in the rat.

Since the serous tubules of ovariectomized mice increase in size (Chaulin-Servinière, '42), it would follow that the tubules in these rodents are not only stimulated by androgens, but also inhibited by estrogens. Such was not the case in the female gerbil, however, since ovariectomy produced no "masculinization" of the submandibular gland.

Regarding the histochemistry of the submandibular gland, it appeared on the basis of the PAS reaction that acinar cells were not monotypic. The acinar cells responding positively to PAS apparently contained quantities of carbohydrates as one would expect in mucoid cells. Those cells which reacted negatively either had expelled their contents or simply had never elaborated a demonstrable carbohydrate product. If these cells had expelled their contents, then one would have expected them to experience some degree of exhaustion atrophy. This did not occur. Moreover, if the cells were PAS-negative because they had released their products, then such material would probably have been visible in the ductal elements. PAS-positive material was never observed in the ducts proximal to the acini (the intercalated ducts), but this was not surprising when one considered the length of these ducts. While PAS-positive material was found in the lumina of the serous tubules, it could not be concluded that the material was of acinar origin since these tubules themselves were probably capable of secretion.

The granules of the serous tubules, presumably zymogenic, seemed to be carbohydrate-protein complexes since they reacted positively to PAS. The remaining duct cell types which did not contain these granules were PAS-negative. While this is similar to observations in other rodents (Leblond, '50), it is not necessarily typical of all mammals. For example, in the principal submaxillary gland of the vampire bat, *Desmodus rotundus*, the striated ducts are PAS-positive (DiSanto, '60). Also, in the principal submaxillary gland of the frugivorous bat, *Artibeus jamaicensis*, both the striated and intercalated duct cells are PAS-positive, the reaction not being localized in a granular portion (Wimsatt, '56).

ACKNOWLEDGMENTS

The author wishes to express his appreciation to Professors Samuel L. Leonard and William A. Wimsatt for their suggestion of the problem and advice during the course of study. Their assistance in the preparation of the manuscript is gratefully acknowledged. The technical assistance of Mr. Anthony L. Guerriere also is most appreciated.

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PLATE 1

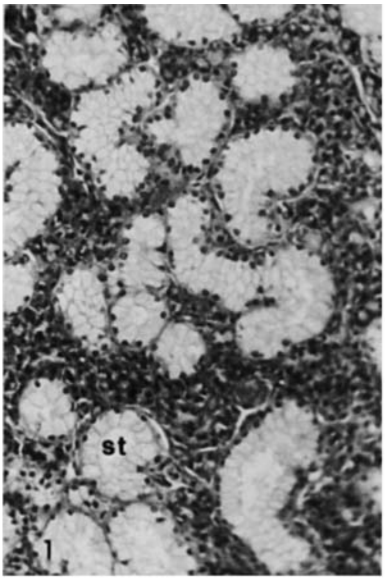
EXPLANATION OF FIGURES

1 Section through the submandibular gland of a normal male gerbil. st, serous tubule; hematoxylin and eosin; × 160.

2 Section through the submandibular gland of a normal female gerbil. st, serous tubule; hematoxylin and eosin; × 160.

3 Serous tubule from a normal male submandibular gland. Note size and number of cells per tubule. st, serous tubule; a, acinar cell; hematoxylin and eosin; × 640.

4 Serous tubule from a normal female submandibular gland. Note size and number of cells per tubule. st, serous tubule; a, acinar cell; PAS-hematoxylin; × 640.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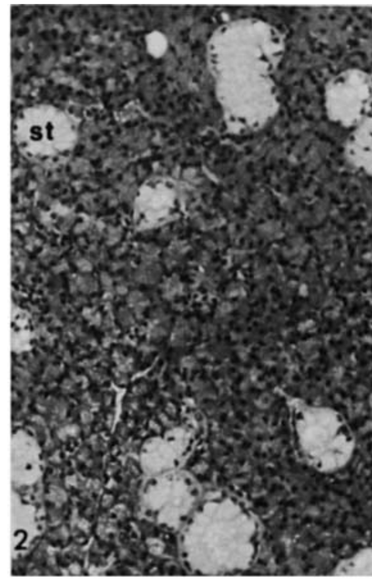
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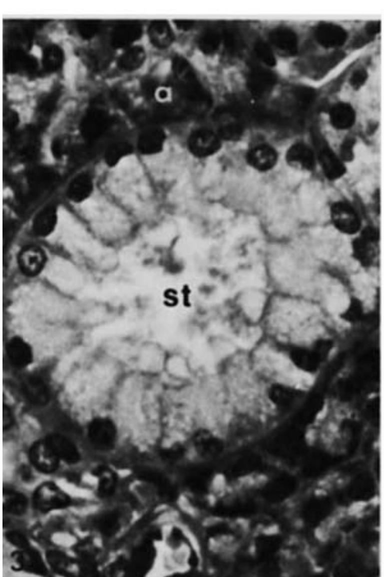
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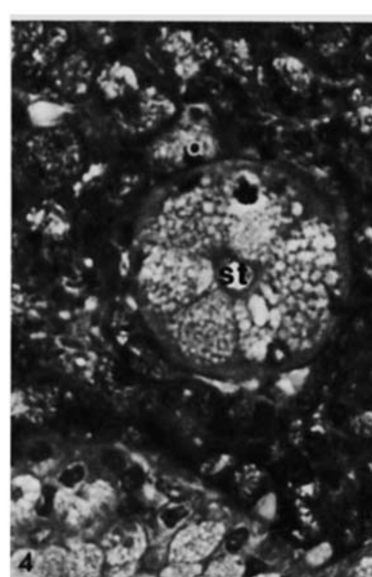
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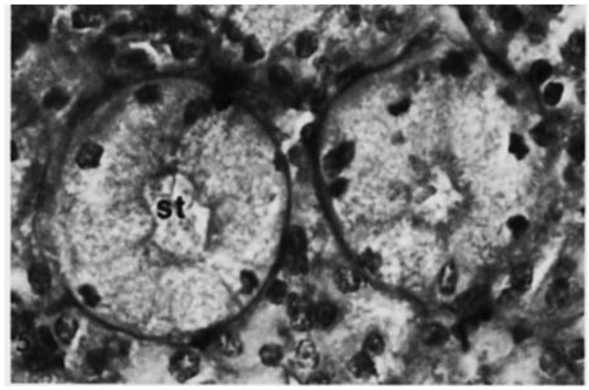
PLATE 2

EXPLANATION OF FIGURES

5 Serous tubules (st) from a Group II (castrated) male gerbil. Note the decrease in tubule diameter and cells per tubule when compared with the normal condition. PAS-hematoxylin; × 640.

6 Serous tubules (st) from a Group III (propylthiouracil-treated) male gerbil. Note the similarity between these serous tubules and those from castrated male gerbils, viz., tubule diameter and cells per tubule. PAS-hematoxylin; × 640.

7 Serous tubules (st) from a Group V (castrated, propylthiouracil-treated) male gerbil. Note the similarities among serous tubules of male gerbils from Groups II, III and IV. PAS-hematoxylin; × 640.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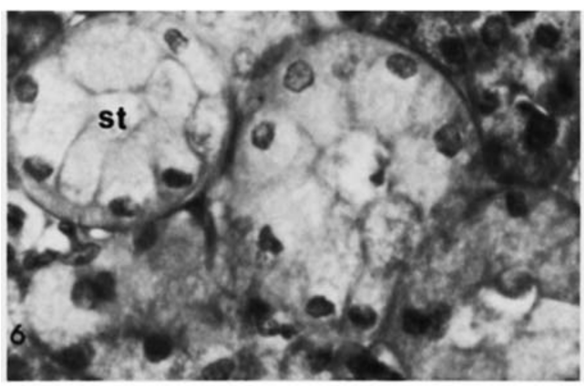
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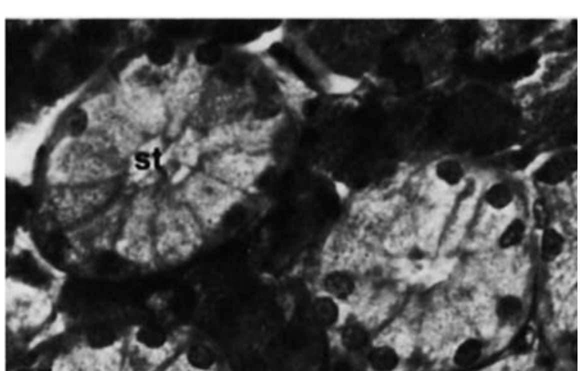
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