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SOME HISTOCHEMICAL AND BIOCHEMICAL ASPECTS OF SEXUAL DIMORPHISM IN MOUSE SUBMAXILLARY GLANDS

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SOME HISTOCHEMICAL AND BIOCHEMICAL ASPECTS OF SEXUAL DIMORPHISM IN MOUSE SUBMAXILLIARY GLANDS

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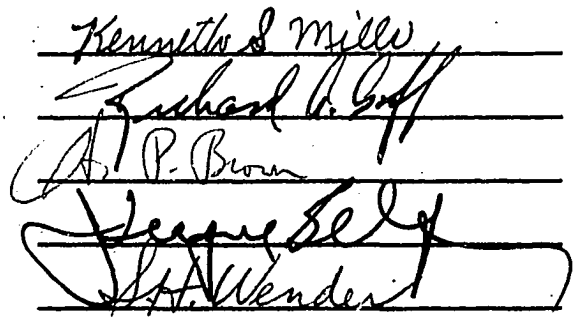

DISSERTATION COMMITTEE

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CHAPTER I

INTRODUCTION

A sex difference in rat submaxillary glands was first reported by F. S. Hammett (1923). Later Lacassagne (1940a) described histological differences in mouse submaxillary glands. Males characteristically have a predominance of terminal tubule cells with basal nuclei and acidophilic granules. Fekete (1941) observed that the rods or basal striations in tubule cells of the female submaxillary glands were lacking in corresponding cells in males. There is a predominance of acinar groups in the submaxillary glands of female mice (Caussé and Lacassagne 1942). Subsequent confirmation of these cellular details was made by Feyel-Cabanes (1947) and Frantz and Kirschbaum (1949).

The production of granules in the terminal tubule cells is influenced by endocrine secretions. Granules in the tubular cells appear first at puberty (Harvey 1952) and develop under the influence of male hormone (Lacassagne 1940c), and to a lesser extent, the thyroid hormone (Arvey and Gabe 1950), estrogen (Lacassagne 1940b, Harvey 1952), and progesterone (Junqueira et al. 1949).

Although the most notable sex-dimorphic difference in submaxillary glands is the greater quantity of granules in the terminal tubule cells of

males, these granules have not been isolated nor has their chemical composition been fully described. Some clues regarding their composition have been furnished by histochemical methods. The granules contain little or no carbohydrates or lipids but do contain protein, tyrosine, (Junqueira et al. 1949, Raynaud 1952) and tryptophan (Glenner and Lillie 1957).

This study is designed to: 1) further characterize the chemical composition of the granules by staining with histochemical reagents specific for protein and amino acids, 2) compare morphological differences, 3) compare histochemically and biochemically, the amounts of protein and tryptophan in the submaxillary gland, and 4) relate these differences to postnatal growth and sexual maturation.

CHAPTER II

MATERIALS AND METHODS

Mice of Jackson laboratory origin from a colony inbred in our laboratories were used in these studies. They were fed a standard Rockland rat diet and given food and water ad libitum. They were killed with chloroform. Right submaxillary glands were used for histochemical study and left ones for biochemical assay. A comparative study of the pancreas (adjacent to the spleen) which does not show sex-dimorphic characteristics (Sreebny et al. 1958) was also made. Seminal vesicle and coagulating gland weights in males and uterine weights in females were employed to determine sexual maturity and effectiveness of gonadectomy or hormone injection.

All organs were weighed on a Roller-Smith torsion balance, accurate to $\pm$ 0.2 mg.

Experimental Animals

A total of 215 mice were used in this study. These animals were divided into 4 groups with 5 to 8 animals in each class, within each group. The animals were distributed as follows:

1) Mice of 10, 20, 30, 35, 45, 60, 65, 70 and 150 days of age were used to study the development of the submaxillary glands from the prepuberal period through sexual maturity. Periods are identified as prepuberal, 10-45 days; puberal, 45-70 days; post-puberal, 70-150 days.

2) Prepuberal castration: Mice were castrated at 20 days of age and autopsied at 65 days to determine the effects of the absence of gonadal hormones prior to the puberal period. They were injected subcutaneously with 0.1 ml sesame oil or oil containing either 0.2 mg testosterone propionate or 0.33 mg estradiol benzoate. Injections were given daily for 10 days prior to autopsy. The groups were: 2.1) males, given testosterone; 2.2) females, given testosterone; 2.3) males, given estradiol; 2.4) females, given estradiol; 2.5) males, given sesame oil; 2.6) females, given sesame oil.

3) Adult castration: Mice were castrated at 120 days of age and autopsied at 150 days of age to determine the effects of the absence of gonadal hormones after development of sex characteristics. Treatment was identical to that described for the prepuberally castrated group.

4) Immature mice were injected with testosterone propionate, to determine the influence of androgens given prior to the time of normal sexual development. The groups were: 4.1) males and 4.2) females injected with 0.1 mg testosterone propionate daily from day 10 through day 19. 4.3) males and 4.4) females injected with 0.05 mg testosterone propionate daily from day 10 through day 29. These animals were compared with normal animals of 20 and 30 days of age.

Histological and Histochemical Methods

Whole submaxillary glands were fixed in buffered formalin for 8 to

10 hours, dehydrated through an alcohol series of 70%, 83%, 95%, 100%; cleared in oil of wintergreen and embedded in paraffin. Sections were cut at 6 to 10 microns, mounted on albumenized slides and dried on a warming plate overnight.

Alcian blue-acid fuchsin stain (Caramia and Angelletti 1962) was used for study of the general structure of the submaxillary glands. Mercuric bromphenol blue stain, Hg-BPB, was used to demonstrate the location of proteins (Bonhag 1953). Napthol Yellow S (Deitch 1955) was used to stain dibasic amino acids. Proteins containing tyrosine were stained and located by Millons' reaction as modified by Rasch and Swift (1960). Protein-bound tryptophan or other 3-indolyl derivatives were visualized with p-dimethylaminobenzaldehyde, p-DMAB, using sodium nitrite as the oxidizing agent (Adams 1957).

Serotonin (5-hydroxytryptamine), if it were bound to an acidic protein or formed the base of a phospholipid, is the only other 3-indolyl derivative likely to be present in fixed tissue (Adams 1957). Unbound serotonin is soluble in aqueous solution and would have been removed as tissues were washed. Submaxillary glands were assayed for serotonin, using the extraction method of Udenfriend et. al. (1955). The density of a sample of the extract was measured with a Klett-Summerson colorimeter, and was compared with that of a standard solution containing 0.8 micromoles of serotonin. The salivary gland extract contained no serotonin.

Histometric Methods

p-DMAB stained sections of submaxillary glands show that tryptophan is concentrated in the granules. An estimate of the relative amount of these granules was necessary to have a basis for comparing the density of granulation as observed in sections stained histochemically with the amount

of tryptophan measured biochemically.

Submaxillary gland sections were stained with p-DMAB. When the section of a tubule appeared as a cross-section and had p-DMAB stained granules, it was counted. The number of tubule sections were counted in a region 1 mm^2 and 6 microns thick. The magnification was 100 X. The results are expressed as the number of tubule cross-sections/mm^3. Sections of submaxillary glands from 4 animals in each class were examined. For each animal, four fields were picked at random from the periphery of a section of the gland. Ten counts made on a single test section averaged 104 sections/mm^3 with a standard deviation of $\pm$ 4. To compare the reliability of this technique, the results were checked with another histometric method. Submaxillary gland sections of both males and females from several experimental groups were sampled. Selected submaxillary gland sections were projected in a microprojector, equipped with a 45^o prism, at a magnification of 250 X. The outlines of terminal tubules with p-DMAB stained granules were drawn on graph paper. The total area of the projected image was calculated. These results were plotted against the cross-section tubule count of the same slide. A straight line was produced which indicates that the two methods are comparable. The method, first described, was considered the most convenient and was used in this study.

Biochemical Assay

Left submaxillary glands of the animals were assayed. At least 50 mg of tissue was required. Single submaxillary glands from immature animals weighed 10 to 20 mg, and those of adults 30 to 50 mg; glands were pooled when necessary. A portion of the pancreas, 50-70 mg, was also assayed. Weighed glands were placed in 50 times their wet weight of cold 0.3% saline, homogenized in glass tissue homogenizers, then centrifuged in the cold at

480 x g for 5 minutes to remove nuclei and broken cell fragments. The supernatant fluid was assayed at room temperature simultaneously with standard solutions and reagent blanks. Averages were determined from duplicate tests.

Tryptophan

Tryptophan was determined by the method described by Graham et. al. (1947). One ml portions of the supernate were pipetted into Klett tubes. Two ml of a solution of 0.5% p-DMAB in 12N HCl were added and tubes allowed to stand for 30 minutes in the dark. Two mls of absolute ethanol and 6 drops of 0.2% $NaNO_2$ in 12 N HCl were then added and the contents of the tubes thoroughly mixed and allowed to stand an additional 30 minutes for color development. The density of the blue color was measured by a Klett-Summerson colorimeter (optimum OD in region of 620 mμ) and compared to the density of standard solutions prepared from a stock of 20 mg DL-tryptophan dissolved in 1% gelatin in NaOH. Protein-bound tryptophan is expressed as micrograms of tryptophan. The greatest variation between duplicate readings is 1.4 μg per 10 mg of submaxillary gland.

Protein

Total protein was measured by the method of Loury et. al. (1951). 0.1 ml portions of the supernate were pipetted into Klett tubes containing 5 ml of alkaline copper tartrate. The density of the blue color was determined after 30 minutes with the Klett-Summerson colorimeter. Egg albumen, 200 μg/0.1 ml, was used as a standard and total protein is expressed as equivalent micrograms. The greatest variation between duplicate readings of unknown samples is 15.5 μg.

CHAPTER III

RESULTS

Sexual Dimorphic Characteristics

Mature Animals

In normal adult mice, 150 days of age, the average weight of the submaxillary glands for males is 110.9 mg (body wt., 23.7 g) and 62.6 mg (body wt., 22.5 g) for females. Mean relative gland weight, mg/10g body weight, is 46.7 mg in males and 27.8 mg in females. Protein averages 157.5 µg/mg in the submaxillary glands of males and 94.0 µg/mg in females. Tryptophan averages 38.9 µg/10 mg in the submaxillary glands of males and 7.9 µg/10 mg in females. In sections of the gland, in males, 165 cross-sections of terminal tubules/mm^3 contain granules with stainable tryptophan and in females 45/mm^3 contain such granules.

In summary, both the absolute and the relative submaxillary gland weights are greater in adult males than in females. The glands of males contain more protein and tryptophan and possess more terminal tubules with granules containing tryptophan than females.

General Histology

The submaxillary gland is described (Fekete 1941, Junqueira et al. 1949, Harvey 1952) as a compound, branched, tubulo-acinous gland divided by connective tissue septa into several ovoid lobes and lobules.

A large excretory duct leads to the oral cavity. This duct branches into the interlobular ducts which further branch into smaller ducts, which are the intralobular ducts of the individual lobes. The intralobular ducts divide into the terminal tubules which are the sexually dimorphic tubules of the duct system.

Terminal tubules are lined with columnar epithelial cells having central or subcentral nuclei, finely granular cytoplasm and parallel rows of mitochondria between the nucleus and base of the cell.

As the duct system terminates, terminal tubules connect with serous acini composed of small groups of cells, each of which has a deeply staining spherical nucleus at the base. The narrow acinar lumina are seldom seen. These cells possess fine granules in varying amounts and vacuoles are frequently present.

In adult mice, sexual dimorphism is expressed by males having greater quantities of large highly refractive granules within the terminal tubule cells than females. Sections of submaxillary glands from adult males, stained with Alcian blue-acid fuchsin, show the cytoplasm of terminal tubule cells to be densely packed with red staining granules. Basal nuclei appear distorted, cell membranes are indistinct and the basal striations are obscured. In figure 1, a typical section of a gland from an adult male, the red granules photograph as black masses clustered toward the lumen of the tubule. Figure 2 is a section of a gland from an adult female. These sections typically have smaller numbers of terminal tubules containing granules as well as fewer granules per tubule than that observed in adult males.

Histochemistry

Although the nature of fixed tissue is realized to be very different from that of living tissue, histochemical staining methods yield some

information as to the chemical components of the granules in the terminal tubule cells.

Hg-BPB stains granules of the tubular cells a more intense blue than other parts of the cell, indicating a relatively greater amount of protein localized in these granules. Napthol Yellow S, an anionic dye which stains dibasic amino acids yellow, stains the terminal tubule granules more intensely than the cellular cytoplasm. These granules also react with Millons' reagent to form a red pigment indicating the presence of tyrosine. Other cytoplasmic parts of the cells stain a diffuse pink with this stain. Each of these specific histochemical stains colored the granules in tubule cells of the submaxillary glands of males darker than those of females, as a result of the greater number of granules in the male.

Tryptophan is identified as a component of the granule protein in submaxillary glands using p-DMAB-nitrite stain. It produces a dark blue color in the granules, indicating a large amount of tryptophan. Cytoplasm of the terminal tubules and acinar cells is colorless. Submaxillary glands of males have tubular cells crowded with granules containing tryptophan (Fig. 3). Females have fewer tubules and their tubules have fewer granules containing tryptophan than do males (Fig. 4).

Granules in sections of the pancreas from both males and females also produce a dark blue pigment with p-DMAB stain. Males and females appear to have about the same number of granules in these tissue sections.

Performic acid oxidizes tryptophan and thus prevents p-DMAB from reacting with this amino acid and no blue pigment is produced (Toennies 1942). When sections of the submaxillary gland or pancreas are subjected to performic acid oxidation, prior to staining with p-DMAB, no blue color is produced.

Fig. 1. Submaxillary gland, adult male. Alcian blue-acid fuchsin stain.

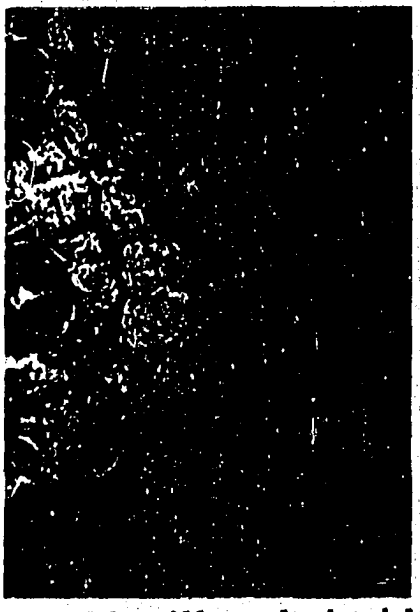


Fig. 2. Submaxillary gland, adult female. Alcian blue-acid fuchsin stain.

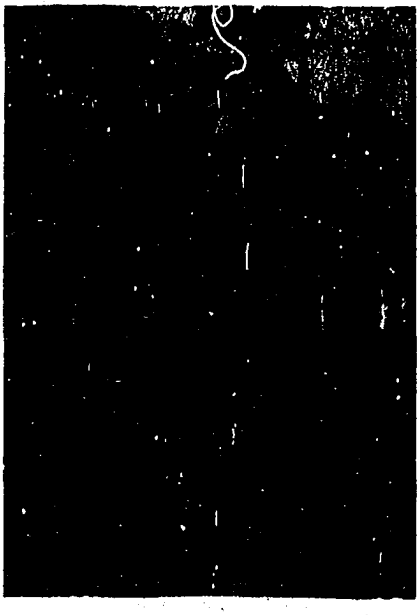


Fig. 3. Submaxillary gland adult male. p-DMAB stain.

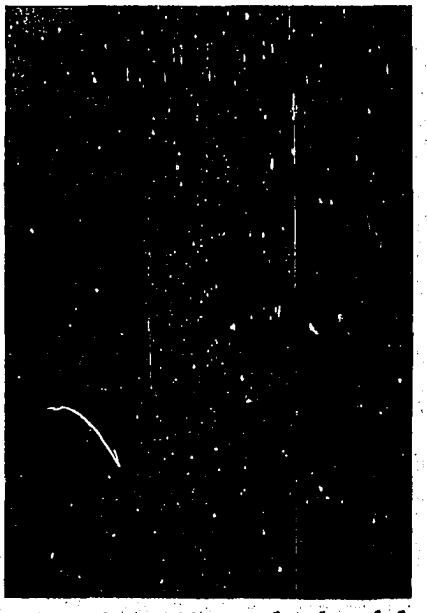
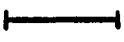


Fig. 4. Submaxillary gland, adult female. p-DMAB stain.



= 50 microns

Postnatal Growth

Females gain weight during the prepuberal and puberal periods at the rate of 0.22 g/day (Fig. 5). Measurements were not made between 70 and 150 days, however the mean body weight of 17.9 g at 70-days (Table 1) is significantly different from the mean weight of 22.5 g at 150-days, indicating that the rate of growth has increased over that of the puberal rate. Males gain weight during the prepuberal period at the rate of 0.22 g/day and 0.35 g/day during puberty (Fig. 5). Males, at 70-days, have a mean body weight of 22.6 g (Table 1) which is not significantly different from that of 23.7 g at 150-days. The growth rate then must be reduced after puberty.

When body weight is compared to age, the slope of the line, 0.22, is the same for females and males during the prepuberal period. During the puberal period, the slope remains the same for females but for males, it increases to 0.35 (Fig. 5). The body weights of males are significantly different from those of females at 65 and 70 days of age. Measurements were not made between 70 and 150 days of age, however a line can be drawn through the respective points which has a slope of 0.06 for females and 0.01 for males. These slope comparisons indicate a decrease in growth rate of males and females and that the male slows down more than the female during the postpuberal period (Fig. 5). So at 150 days there is no significant difference in body weights.

The submaxillary glands of females gain weight at the rate of 0.45 mg/day up to 70 days of age (Fig. 5). Measurements were not made between 70 and 150 days of age, however, the mean submaxillary gland weight of 62.6 mg at 150-days (Table 2) is not significantly different from the mean weight of 56.2 mg at 70-days. Growth rate is slower in the postpuberal period. The submaxillary glands of males grow at the rate of 0.45 mg/day during the

TABLE 1

MEAN BODY WEIGHT FROM DAY 10 THROUGH DAY 150+

Age (days)*	Body weight in grams			
	No. of Animals**	Male	No. of Animals**	Female
10	5	6.4 (5.6- 7.0)	6	5.4 (4.5- 6.5)
20	6	7.8 (6.7- 8.7)	6	7.1 (6.7- 7.3)
30	5	11.6 (11.0-12.0)	5	9.5 (9.2- 9.7)
35	6	11.9 (11.2-12.7)	6	11.2 (10.5-12.0)
45	5	14.6 (14.0-16.4)	5	13.9 (10.4-15.9)
55	6	18.2 (18.0-19.0)	5	14.9 (13.8-17.0)
60	8	19.9 (18.5-22.0)	7	17.7 (16.0-20.5)
65	5	20.4 (17.0-22.0)	5	16.1 (14.5-17.0)
70	6	22.6 (20.5-24.1)	5	17.9 (15.7-19.5)
150	7	23.7 (20.5-28.0)	6	22.5 (18.5-26.0)

* = Prepuberal period, 10-45 days; puberal period, 45-70 days; postpuberal period, 70-150 days.

+ = Mean weights with ranges.

** = Number of individuals in different age groups are the same for the subsequent tables.

prepuberal period and 1.85 mg/day during puberty (Fig. 5). The mean submaxillary gland weight of 110.9 mg at 150-days (Table 2) is not significantly different from the mean weight of 93.9 mg at 70-days, indicating a much slower rate of growth in the postpuberal period.

A plot of submaxillary gland weight as a function of age produces a slope of 0.45 for both females and males during the prepuberal period. In females, this slope remains the same through the puberal period, whereas in males it increases to 1.85 (Fig. 5). Although measurements were not made between 70 and 150 days of age, a line can be drawn through the points which has a slope of 0.08 for females and 0.21 for males (Fig. 5). These slope comparisons indicate a decrease in growth rate of females and males and that the female slows down more than the male. The mean submaxillary gland weight of adult males is 1.8 times heavier than that of adult females.

The mean relative submaxillary gland weight in 10-day females averages 32.1 mg/10 g body weight (Table 2) and decreases 0.06 mg/10g/day during the prepuberal and puberal periods (Fig. 6). Although measurements were not taken between 70 and 150 days of age, the mean relative weight of 27.8 mg at 150-days (Table 2) does not differ significantly from that of 31.4 mg at 70-days. In males, the relative submaxillary weight tends to remain constant during the prepuberal period and increase during puberty, growing at the rate of 0.28 mg/10g body wt./day (Fig. 6). In 150-day males (Table 2) the relative gland is 46.7 mg/10g body wt. and is not significantly different from the mean weight of 41.1 mg/10g body wt. in 70-day males (Fig. 6).

When the relative submaxillary gland weight is compared to age, the slope of the line is -0.06 during the prepuberal period for females and 0.0 for males. During puberty, the slope does not change for females but becomes 0.28 for males (Fig. 6). The relative submaxillary gland weights

TABLE 2

MEAN SUBMAXILLARY GLAND WEIGHT AND RELATIVE SUBMAXILLARY GLAND WEIGHT*

Age* (days)	Mg submaxillary gland		Mg submaxillary gland / 10 g body wt.	
	Males	Females	Males	Females
10	20.7 (18.4-23.8)	17.4 (12.0-21.6)	32.3 (29.2-35.1)	32.1 (21.8-38.4)
20	34.8 (30.0-39.0)	33.4 (28.8-38.8)	44.6 (34.9-50.5)	47.2 (41.7-53.4)
30	37.6 (35.4-41.8)	32.7 (26.8-40.0)	32.4 (31.3-38.0)	34.1 (29.1-41.2)
35	47.7 (40.0-56.6)	38.5 (34.0-48.6)	40.0 (35.7-48.3)	34.6 (30.0-44.1)
45	48.3 (46.0-50.0)	42.5 (34.0-48.0)	33.9 (27.2-34.2)	30.7 (24.2-34.6)
55	68.5 (58.0-78.8)	50.0 (48.0-60.0)	37.8 (32.9-47.5)	32.4 (28.9-35.4)
60	77.8 (67.3-90.0)	53.0 (48.0-58.0)	38.0 (35.9-47.0)	28.9 (26.6-31.1)
65	70.2 (60.0-78.0)	53.1 (47.0-58.0)	35.4 (30.2-37.6)	33.4 (30.3-34.5)
70	93.9 (77.0-108.0)	56.2 (51.0-61.0)	41.1 (35.0-45.9)	31.4 (29.4-32.2)
150	110.9 (89.2-140.0)	62.6 (54.0-73.0)	46.7 (43.1-52.6)	27.8 (21.2-30.4)

+ = Mean weights with ranges.

* = Prepuberal period, 10-45 days; puberal period 45-70 days; postpuberal period, 70-150 days.

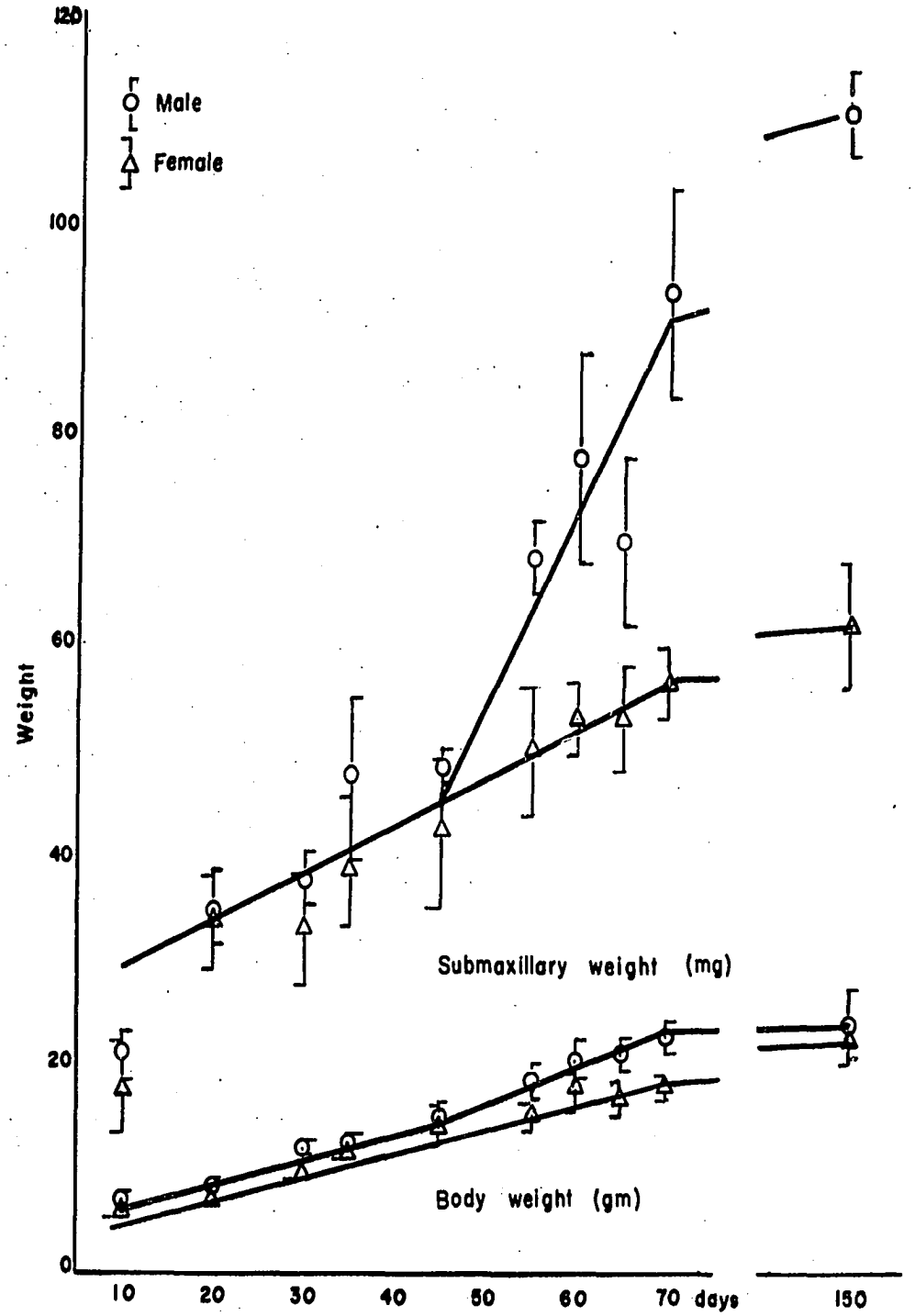


Fig. 5. Body weight and submaxillary gland weight as a function of age. Values are expressed as means with standard deviation.

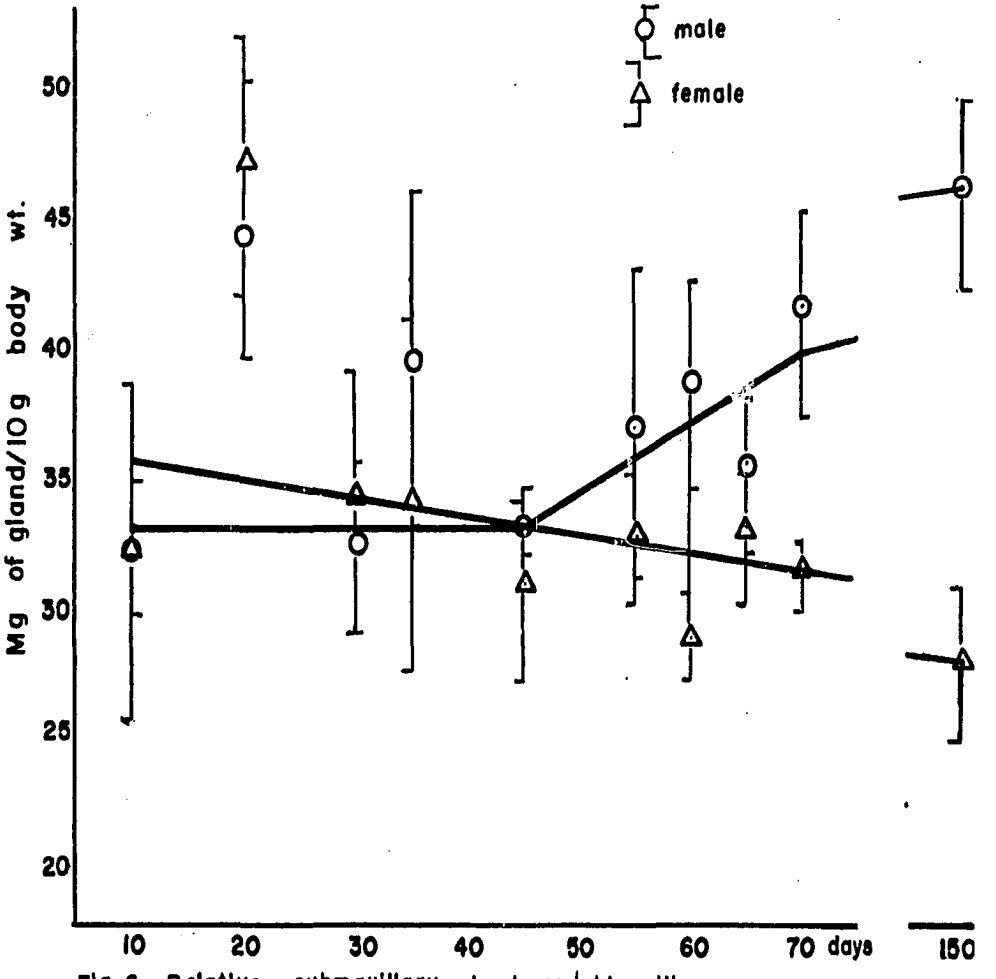


Fig. 6. Relative submaxillary gland weight with age.
Values are expressed as means with standard deviation.

of males and females are not significantly different at 45-days, but are different at 70-days and at 150 days of age. The relative submaxillary gland weight of adult males is 1.7 times greater than that of adult females.

Weights of accessory sex organs were not recorded until 30 days of age. After this, these organs gain weight at the rate of 0.30 mg/day in females and 0.37 mg/day in males through the remainder of the prepuberal period (Table 3). Uterine weight fluctuates during puberty. No attempt was made to associate these changes with the estrus cycle. Seminal vesicle-coagulating gland weights in males increase 2.5 mg/day during puberty. These organs average 85 mg in 150-day males, and 72.0 mg in 70-day males. These weights (Table 3) are not significantly different, indicating decreased growth in the postpuberal period.

TABLE 3

MEAN ACCESSORY SEX ORGAN WEIGHTS IN MILLIGRAMS

	Days of age						
	30	45	55	60	65	70	150
Males*	2.9	8.5	42.0	49.0	47.0	72.0	85.0
Females*	8.1	12.6	14.5	42.0	125.0	18.5	126.0

* Males = seminal vesicle and coagulating gland

* Females = uterus

The amount of protein incorporated in the submaxillary glands of 10-day females averages 103.4 µg/mg and decreases 0.19 µg/mg/day during the prepuberal and puberal periods (Fig. 7). Protein in 150-day females averages 94.0 µg/mg (Table 4) and is significantly different from 83.4 µg/mg at 70-days. Protein incorporation increases in the postpuberal period in

TABLE 4

MEAN MICROGRAMS OF PROTEIN PER MILLIGRAM OF GLAND+

Age* (days)	Submaxillary gland		Pancreas	
	Male	Female	Male	Female
10	98.2 (91.8-105.0)	103.4 (99.0-109.0)c	140.2 (138.3-141.5)	142.0 (135.4-149.3)
20	97.3 (96.4- 99.6)	100.5 (98.0-105.3)	128.1 (123.7-131.5)	116.3 **
30	110.0 (107.4-116.3)	104.6 (92.6-106.8)	136.5 (123.6-146.5)	138.5 (128.0-152.3)
35	105.3 (104.8-110.7)	93.6 (86.9-101.3)	124.3 (115.3-131.6)	141.3 (134.8-153.0)
45	99.9 (86.9-108.2)	96.9 (86.1-101.3)	133.7 (120.5-138.3)	144.9 (129.5-167.3)
55	119.4 (109.8-124.7)	97.0 (96.3-108.1)	.....	.....
60	128.0 (98.2-147.1)	94.8 (91.4- 97.9)	152.8 (137.3-165.8)	141.2 (138.6-150.4)
65	123.0 (122.0-124.6)	99.7 (90.4-104.8)	139.6 (107.9-154.7)	115.3 (87.4-118.8)
70	136.8 (128.8-146.8)	83.4 (82.4- 86.3)	154.0 (150.0-158.3)	148.0 (132.0-164.0)
150	157.5 (149.9-191.6)	94.0 (81.0-108.4)	145.0 (140.3-148.6)	143.0 (132.2-177.8)

\+ = means with ranges
* = prepuberal period, 10-45 days; puberal period, 45-70 days; postpuberal period, 70-150 days.
** = pooled glands
... = not recorded

females. In males, at 10-days, protein incorporation averages 98.2 µg/mg and decreases 0.19 µg/mg/day during the prepuberal period. During puberty, incorporation of protein in the submaxillary glands increases to 1.44 µg/mg/day. Measurements were not made between 70 and 150 days, however the average of 157.5 µg/mg at 150-days is significantly different from the average of 136.8 µg/mg at 70-days. The rate of protein incorporation by the submaxillary gland of males during the postpuberal period is 0.26 µg/mg/day (Table 4).

When protein/mg of submaxillary gland is compared to age, the slope of the line is -0.19 during the prepuberal period for both males and females. During puberty, the slope continues for females and changes to 1.44 for males. Measurements were not made between 70 and 150 days, although lines drawn through their respective points have slopes of 0.26 for males and 0.13 for females (Fig. 7). The submaxillary glands of males average 1.7 times as much protein as those of adult females (Table 4).

The amount of protein incorporated by the pancreas in males and females of each age group is variable. The mean rates of incorporation do not indicate any effect of age or sex on the protein content of the pancreas (Table 4).

The amount of tryptophan in the submaxillary glands of 10-day females averages 6.2 µg/10 mg and decreases 0.06 µg/10 mg/day during the prepuberal period. During puberty, tryptophan incorporation increases to 0.13 µg/10 mg/day (Fig. 7). Measurements were not made between 70 and 150 days, however the quantity of 7.9 µg/10 mg at 150-days (Table 5) is not significantly different from 7.5 µg/10 mg in 70-day females. Tryptophan incorporation rate decreases during the postpuberal period in females. Males incorporate tryptophan at the rate of 0.09 µg/10 mg/day during the pre-

puberal period and 1.03 μg/10 mg/day during puberty (Fig. 7). The rate of tryptophan incorporation in the submaxillary glands of males increases 11-fold during puberty. Tryptophan averages 38.9 μg/10 mg of submaxillary gland in 150-day males (Table 5) and is not significantly different from the average of 34.0 μg/10 mg in 70-day males. Tryptophan incorporation decreases during the postpuberal period.

In a plot of the amount of tryptophan as a function of age, the slope of the line during the prepuberal period is -0.06 for females and 0.09 for males. During puberty, the slope is 0.13 for females and 1.03 for males (Fig. 7). Although measurements were not made between 70 and 150 days, lines drawn through their respective points have slopes of 0.0 for females and 0.06 for males (Fig. 7). Submaxillary glands of adult males have 5 times as much tryptophan as those of adult females.

Incorporation of tryptophan in the pancreas is not significantly different in males and females. The rates of incorporation vary within the prepuberal, puberal and postpuberal periods (Table 5) and do not appear to be influenced by the age of the animal.

Granules containing detectable amounts of tryptophan first appear in terminal tubule cells of the submaxillary glands of females at 45 days of age. During the puberal period in females, they appear at the rate of 1.05 tubules/mm^3/day. These tubules average 45/mm^3 at 150-days and this is not significantly different from the mean of 33/mm^3 for 70-day females. In males, these granules first appear at 35 days of age. The tubules containing these granules appear at the rate of 1.3 tubules/mm^3/day during the remainder of the prepuberal period and increase to a rate of 3.64/mm^3/day during puberty (Fig. 8). Measurements at 70 and 150 days show the mean number of tubules at 150-days is 165/mm^3 (Table 6) and is significantly

TABLE 5

MEAN MICROGRAMS OF TRYPTOPHAN PER TEN MILLIGRAMS OF GLAND+

Age* (days)	Submaxillary gland		Pancreas	
	Male	Female	Male	Female
10	5.6 (5.4- 5.9)	6.2 (6.0-6.9)	20.4 (19.6-20.7)	22.5 (21.2-23.2)
20	6.0 (5.4- 6.8)	6.0 **	25.0 (24.0-25.8)	24.0 **
30	6.8 (6.0- 7.0)	5.7 (4.5-6.7)	29.7 (26.7-31.6)	32.0 (25.2-33.8)
35	7.6 (7.4- 7.8)	4.7 (4.6-5.1)	25.9 (23.4-28.4)	27.0 (26.2-28.4)
45	8.0 (5.9-11.2)	3.9 (3.6-4.7)	27.5 (24.6-30.0)	23.9 (20.0-25.6)
55	12.4 (10.5-16.2)	6.2 (6.0-7.5)	....	....
60	25.6 (20.6-31.9)	8.0 (7.0-8.8)	32.8 (21.8-37.5)	26.7 (23.7-28.3)
65	27.0 (26.4-29.9)	6.4 (5.6-7.1)	24.4 (20.1-30.8)	19.5 (13.8-30.8)
70	34.0 (23.0-36.7)	7.5 (6.8-8.1)	29.7 (25.9-32.6)	32.8 (29.4-34.6)
150	38.9 (22.9-44.8)	7.9 (7.0-9.2)	26.7 (15.6-33.6)	27.0 (19.6-39.2)

\+ = means with ranges
* = Ages: prepuberal period, 10-45; puberal period, 45-70; postpuberal period, 70-150.
** = pooled glands
... = not recorded

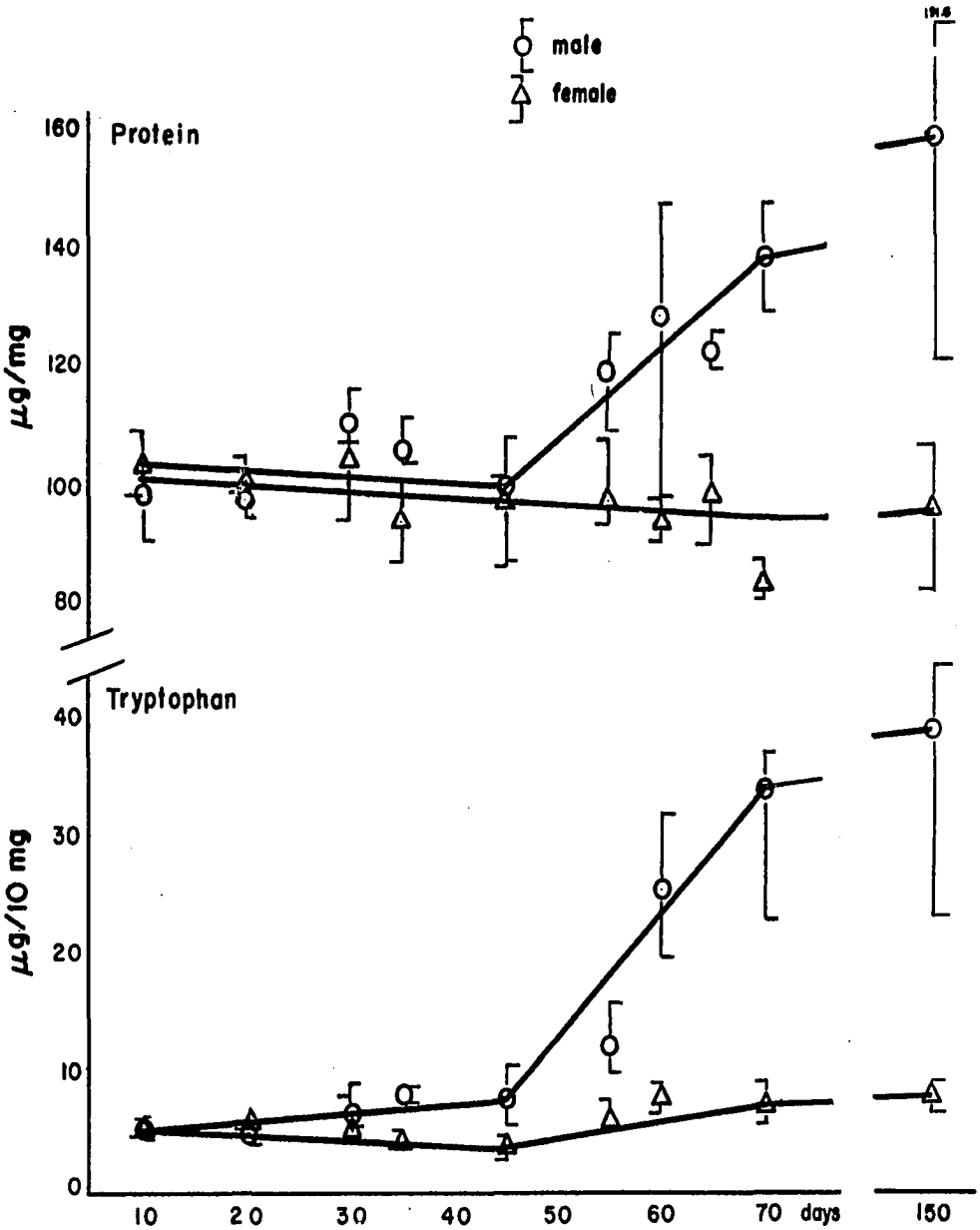


Fig. 7. Submaxillary gland protein and tryptophan with age. Values are expressed as means with ranges.

different from the mean of 128/mm^3 at 70 days.

A plot of the number of terminal tubules as a function of age produces a line with a slope of 0.0 in early puberty and 1.3 for males during the late prepuberal period. They are not observed in females during either part of this period. During puberty, the slope of the line is 1.05 for females and 3.64 for males (Fig. 8). Although measurements were not made between 70 and 150 days of age, lines drawn through these respective points have slopes of 0.15 for females and 0.46 for males (Fig. 8). Adult males have 3.6 times as many terminal tubules with granules containing tryptophan as adult females.

TABLE 6

NUMBERS OF TERMINAL TUBULES WITH GRANULES CONTAINING TRYPTOPHAN*

	Days of age							
	30	35	45	55	60	65	70	150
Males	0	32 (30-37)	45 (35-50)	91 (80-97)	113 (92-124)	104 (96-111)	128 (93-149)	165 (155-171)
Females	0	0	12 (10-21)	----	27 (16- 31)	33 (26- 38)	33 (21- 50)	45 (40- 51)

* = expressed as means, with ranges, of tubules per mm^3 of submaxillary gland

-- = not recorded

Summary. The overall patterns of body growth and submaxillary growth differ in females and males. During the prepuberal period, the respective growth rates are the same for both sexes. During puberty, growth continues at the same rate in females but increases in males. In the postpuberal period, growth decreases in both sexes.

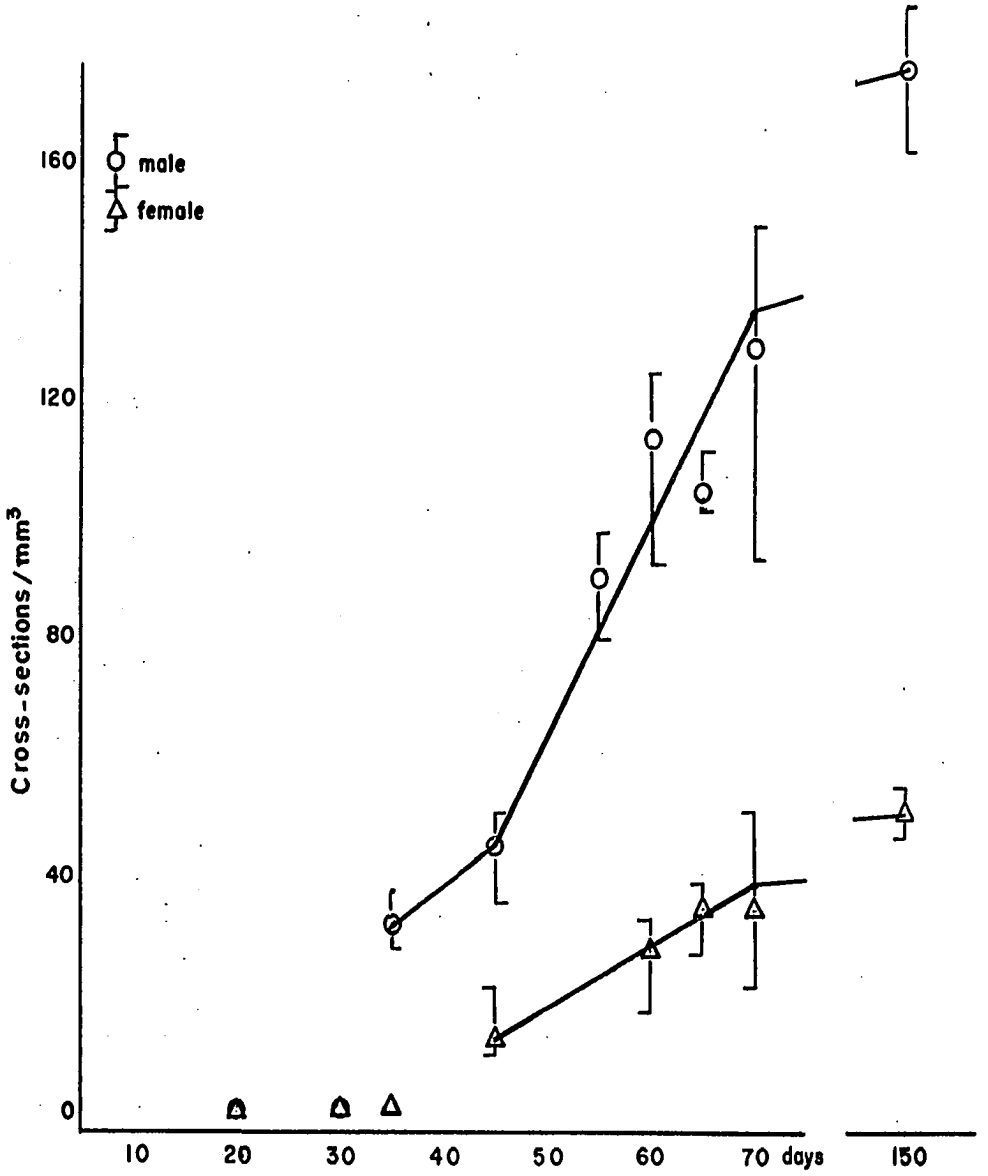


Fig. 8. Terminal tubules with granules containing tryptophan as a function of age. Values are expressed as means with ranges.

Relative submaxillary gland weight decreases during the prepuberal, puberal and postpuberal periods in females. In males, there is no significant change in relative gland weight during the prepuberal period, although an increase is observed during puberty. Relative submaxillary gland weight is greater in adult males than in adult females.

Accessory sex organ growth increases during puberty in both males and females.

The highest rate of protein and tryptophan incorporation is observed during puberty in males. During the puberal period in males, the rates of incorporation of both protein and tryptophan increase above that of the prepuberal period. In females, during puberty, incorporation of protein decreases whereas that of tryptophan increases. The amount of protein and tryptophan incorporated per day decreases in the postpuberal period of males. A slight increase in protein incorporation concurrent with a decrease in tryptophan incorporation is observed in females during the postpuberal period.

Incorporation of protein and tryptophan in the pancreas does not appear to be influenced by age or sex of the animal.

Granules containing tryptophan appear earlier in the terminal tubules of submaxillary glands of males than in those of females. The number of cross-sections of these tubules increase in both males and females as the animals get older, and the rate at which they appear is greater in males than in females.

Effects of Castration

Prepuberally castrated males. When males are castrated at 20 days and examined at 65 days, the submaxillary gland does not grow as fast and the relative gland weight decreases 21 percent of normal. Similarly protein incorporation per mg in these glands decreases 24 percent. The

incorporation of tryptophan per 10 mg is reduced 74 percent. A 77 percent reduction in number of terminal tubules with granules containing tryptophan parallels the decrease in tryptophan incorporation (Table 7, 15).

The amount of protein and of tryptophan in the pancreas of prepuberally castrated males (Table 7) is normal.

The relative seminal vesicle-coagulating gland weight decreases to 91 percent below normal (Table 7, 15).

Adult castrated males. Relative submaxillary gland weight in adult castrated males decreases to 24 percent below normal. There is a concurrent 33 percent decrease in the incorporation of protein per mg by the submaxillary gland. During this same time, tryptophan per 10 mg decreases 57 percent. The number of terminal tubules with granules containing tryptophan decreases 38 percent (Table 7, 15).

Adult castrated males have normal amounts of protein and tryptophan in the pancreas (Table 7, 15). The gross weight of the pancreas was not measured.

Relative weights of the accessory sex organs are reduced to 85 percent below normal (Table 7, 15).

Prepuberally castrated females. Females castrated at 20 days and examined at 65 days have a 15 percent reduction in relative submaxillary gland weight. Incorporation of protein is normal, 107.6 µg/mg of gland. Tryptophan incorporation per 10 mg decreases 27 percent whereas the number of terminal tubules with granules containing tryptophan decreases 72 percent (Table 8, 15).

The pancreas of these females has normal amounts of protein and tryptophan (Table 8).

Relative uterine weight decreases to 77 percent below normal (Table 8, 15).

TABLE 7

CHANGES OBSERVED IN CASTRATION OF PREPUBERAL MALES AND ADULT MALES+

	Autopsied at 65 days		Autopsied at 150 days	
	Normal	Prepuberal castrate	Normal	Adult castrate
Relative accessory sex organ wt. mg/10g body wt.*	23.0 (13.7- 31.4)	2.06 (1.03- 3.6)	27.4 (15.4- 32.6)	4.2 (3.07- 7.2)
Submaxillary gland				
Absolute wt. of gland	70.0 (60.0- 78.0)	54.0 (50.0 - 57.0)	110.9 (105.6-130.0)	92.4 (81.6 - 96.0)
Relative $\frac{\text{wt. mg of gland}}{\text{10 g body wt.}}$	35.0 (28.9- 36.4)	27.8 (25.2 - 32.0)	46.7 (42.1- 52.6)	35.5 (29.9 - 47.2)
Terminal tubules # X-sections with tryptophan/mm^3	104.0 (96 - 111)	24.0 (18 - 32)	165.0 (155 -171)	103.0 (96 -123)
µg protein/mg gland	123.0 (120.0-130.0)	93.0 (89.8 - 95.2)	157.5 (149.9-191.6)	104.8 (91.3 -114.8)
µg tryptophan/10mg gland	27.6 (26.4- 29.9)	7.2 (6.2 - 8.4)	38.9 (22.9- 44.8)	16.9 (14.0 - 18.7)
Pancreas				
µg protein/mg gland	145.0 (140.0-148.0)	127.2 (111.1 -136.0)	139.6 (107.9-154.7)	153.9 (106.1 -180.3)
µg tryptophan/10 mg gland	24.4 (20.1- 30.8)	21.9 (13.4 - 30.6)	26.6 (15.6- 33.6)	24.3 (15.6 - 33.6)

+ = mean values with ranges
* = seminal vesicle-coagulating gland weight

Adult castrated females. Relative submaxillary gland weight in adult castrated females decreases to 22 percent below normal. There are normal amounts of protein and tryptophan in the submaxillary glands. Similarly, there are normal numbers of terminal tubules with granules containing tryptophan (Table 8, 15).

The pancreas of these females has normal amounts of protein and tryptophan (Table 8).

Relative uterine weight decreases 48 percent (Table 8).

Summary. Relative gland weight decreases in both prepuberally and adult castrated males. This is accompanied by reduced protein and tryptophan incorporation. Examination of p-DMAB stained sections show fewer terminal tubules with granules containing tryptophan than normal. Incorporation of protein and tryptophan in the pancreas is normal. Relative accessory sex organ weights are significantly reduced.

In prepuberally castrated and adult castrated females, relative submaxillary gland weights decrease. The mean amount of protein in the submaxillary gland of these females is normal. In prepuberally castrated females, the reduced incorporation of tryptophan per 10 mg parallels the decrease in the number of terminal tubules with granules containing tryptophan. In adult castrated females, both the incorporation of tryptophan and terminal tubule number are normal. In the pancreas, of both prepuberally castrated and adult castrated females, the incorporation of protein and tryptophan is normal. Castration reduces the relative accessory sex organ weights in both prepuberally castrated and adult castrated females.

Effects of Androgen

Immature males. Intact males that received testosterone daily for 10 days when compared to normal show a 23 percent reduction in relative

TABLE 8

CHANGES OBSERVED IN CASTRATION OF PREPUBERAL FEMALES AND ADULT FEMALES+

	Autopsied at 65 days		Autopsied at 150 days	
	Normal	Prepuberal castrate	Normal	Adult castrate
Relative accessory sex organ wt./10g body wt.*	78.5 (56.0- 96.9)	17.7 (7.6- 31.6)	55.6 (48.9- 68.4)	29.1 (24.8- 38.7)
Submaxillary gland				
Absolute wt. mg of gland	53.1 (47.0- 58.0)	43.5 (34.0- 43.5)	62.6 (59.0- 73.0)	49.0 (45.0- 56.0)
Relative $\frac{\text{wt. mg of gland}}{\text{10 g body wt.}}$	33.0 (30.3- 34.5)	28.1 (23.4- 31.5)	27.8 (21.2- 30.4)	21.8 (20.7- 33.0)
Terminal tubule # X-sections with tryptophan/mm^3	32 (26 - 38)	9 (6 - 12)	45 (40 - 51)	44 (34 - 48)
µg protein/mg gland	99.7 (90.4-104.8)	107.6 (91.1-119.1)	94.0 (81.0-108.4)	98.0 (80.6-116.0)
µg tryptophan/10mg gland	6.4 (5.6- 7.1)	4.7 (4.2- 5.2)	7.9 (7.0- 9.2)	7.5 (7.6- 10.2)
Pancreas				
µg protein/mg gland	115.3 (87.4-118.1)	148.2 (143.5-152.0)	143.0 (122.2-177.8)	137.6 (130.7-160.3)
µg tryptophan/10 mg gland	19.5 (13.8- 30.8)	24.6 (13.1- 33.6)	27.0 (19.6- 39.2)	26.0 (16.4- 34.0)

+ = mean values with ranges
* = uterine weight

submaxillary gland weight. The amount of protein incorporated per mg of gland is normal. On the other hand, the amount of tryptophan per 10 mg of gland increases significantly, 82 percent. Terminal tubules with granules containing tryptophan are not observed in normal 20-day males but average 71 tubules/mm^3 in testosterone injected males (Table 9, 15).

When testosterone is given to intact immature males for a longer period of time, day 10 to day 29, relative submaxillary gland weight increases 34 percent. The amount of protein/mg is not significantly increased but tryptophan per 10 mg increases 316 percent. Terminal tubules with granules containing tryptophan average 104/mm^3 in testosterone injected males, although they are not observed in normal males at 30 days of age (Table 9, 15).

The incorporation of protein and tryptophan in the pancreas is not significantly different from normal in either the 20-day old or the 30-day old males which were given testosterone (Table 9).

Relative accessory sex organ weight in 30-day old injected males increases 2300 percent (Table 9, 15).

Immature females. Intact females that received testosterone daily for 10 days show a 27 percent reduction in relative submaxillary gland weight. Protein incorporation per mg decreases 15 percent and tryptophan incorporation per 10 mg of gland increases 50 percent. Terminal tubules with granules containing tryptophan are not observed in normal 20-day females but average 37 tubules/mm^3 in testosterone injected females (Table 10, 15).

When testosterone injections are extended over a 20 day period, the relative submaxillary gland weight of these females is normal. There is a significant 11 percent increase in protein incorporation per mg by the submaxillary gland and tryptophan per 10 mg increases 583 percent. Terminal

TABLE 9

INFLUENCE OF TESTOSTERONE ON INTACT IMMATURE MALES+

	Autopsied at 20 days		Autopsied at 30 days	
	Normal	Injected[1]	Normal	Injected[2]
Relative accessory sex organ wt. mg/10 g body wt.*	.....	29.4 (17.6-52.9)	2.5 (2.2- 3.3)	60.0 (58.6- 74.6)
Submaxillary gland				
Absolute wt. mg of gland	34.8 (30.0- 39.0)	24.1 (20.8-28.2)	37.6 (35.4- 41.8)	65.6 (53.8- 77.2)
Relative wt. mg of gland / 10 g body wt.	46.0 (34.7- 50.3)	35.4 (32.1-38.6)	32.5 (31.3- 38.0)	43.7 (37.1- 54.7)
Terminal tubule # X-sections with tryptophan/mm^3	0	71 (63 -76)	0	104 (83 -112)
µg protein/mg of gland	97.3 (96.4- 99.6)	100.8 (**)	110.0 (107.4-116.3)	105.0 (88.8-118.0)
µg tryptophan/10mg gland	6.0 (5.4- 6.8)	10.9 (**)	6.8 (6.0- 7.0)	28.3 (21.8- 31.6)
Pancreas				
µg protein/mg gland	129.0 (128.3-130.4)	125.0 (**)	136.5 (123.6-146.5)	138.3 (128.8-148.8)
µg tryptophan/10 mg gland	25.0 (24.0- 25.8)	24.0 (**)	29.7 (26.7- 31.6)	32.4 (28.4- 33.4)

\+ = mean values with ranges
1 = 0.1 mg, daily for 10 days prior to autopsy
2 = 0.05 mg, daily for 20 days prior to autopsy
* = seminal vesicle-coagulating gland weight
** = pooled glands

tubules with granules containing tryptophan average 94/mm^3 in testosterone treated females, although they are not observed in normal females at 30 days of age (Table 10, 15).

The incorporation of protein and tryptophan in the pancreas is not significantly different from normal in either the 20-day old or the 30-day old females which were given testosterone (Table 10).

Relative uterine weight increases 280 percent in 30 day old females which are given testosterone propionate from day 10 through day 29 (Table 10, 15).

Prepuberally castrated injected males. Administration of testosterone to prepuberally castrated males results in normal values for relative submaxillary gland weight, incorporation of protein and tryptophan, and terminal tubules with granules containing tryptophan (Table 11, 15).

The amount of protein and the quantity of tryptophan in the pancreas of these males is normal (Table 11).

Relative accessory sex organ weights are normal in prepuberally castrated males which have been treated with testosterone (Table 11, 15).

Adult castrated injected males. In adult castrated males, treated with testosterone, the relative submaxillary gland weight, amount of protein and quantity of tryptophan, and terminal tubules with granules containing tryptophan are equal to those of normal adult males (Table 11, 15).

The pancreas of these males has normal amounts of protein and tryptophan (Table 11).

In adult castrated males, given testosterone, the relative weight of the accessory sex organs increases 104 percent (Table 11, 15).

Prepuberally castrated injected females. When androgen is administered to prepuberally castrated females, the relative submaxillary gland

TABLE 10

INFLUENCE OF TESTOSTERONE ON INTACT IMMATURE FEMALES+

	Autopsied at 20 days		Autopsied at 30 days	
	Normal	Injected[1]	Normal	Injected[2]
Relative accessory sex organ wt. mg/10g body wt.*	.....	23.0 (21.6- 29.5)	8.5 (7.4- 10.5)	32.3 (28.5- 36.1)
Submaxillary gland				
Absolute wt. mg of gland	33.4 (28.2- 38.8)	20.9 (15.4- 28.6)	32.7 (26.8- 40.0)	48.3 (34.8- 76.4)
Relative wt. mg of gland	47.0 (41.7- 53.4)	34.3 (26.0- 42.6)	34.4 (29.1- 41.2)	36.3 (24.7- 40.9)
Terminal tubules # X-sections with tryptophan/mm^3	0	37 (28 - 41)	0	94 (76 -101)
µg protein/mg gland	100.5 (98.0-105.3)	85.0 (**)	104.6 (92.6-106.8)	116.0 (112.0-128.8)
µg tryptophan/10mg gland	6.0 (**)	9.0 (8.6- 10.9)	5.7 (4.5- 6.7)	38.9 (24.2- 41.2)
Pancreas				
µg protein/mg gland	116.3 (**)	125.0 (114.0-132.0)	138.5 (128.0-152.3)	104.0 (100.0-112.0)
µg tryptophan/10 mg gland	24.0 (**)	26.2 (23.5- 36.2)	32.0 (25.2- 33.8)	25.2 (23.1- 26.2)

\+ = mean values with ranges
1 = 0.1 mg, daily for 10 days prior to autopsy
2 = 0.05 mg, daily for 20 days prior to autopsy
* = uterine weight
** = pooled glands

TABLE 11

EFFECTS OF TESTOSTERONE ON PREPUBERALLY CASTRATED AND ADULT CASTRATED MALES+

	Autopsied at 65 days		Autopsied at 150 days	
	Normal	Prepuberal castrate	Normal	Adult castrate
Relative accessory sex organ wt. mg/10g body wt.*	23.0 (13.7- 31.4)	25.7 (19.4- 27.7)	27.4 (15.4- 32.6)	55.9 (39.7- 74.0)
Submaxillary gland				
Absolute wt. mg of gland	70.0 (60.0- 78.0)	78.8 (75.0- 84.0)	110.9 (105.6-130.0)	106.7 (96.0-117.0)
Relative wt. mg of gland / 10 g body wt.	35.0 (28.9- 36.4)	38.3 (35.4- 40.9)	46.7 (42.1- 52.6)	42.0 (36.5- 47.8)
Terminal tubule # X-sections with tryptophan/mm^3	104 (96 -111)	98 (74 -116)	165 (155 -171)	153 (146 -164)
µg protein/mg gland	123.0 (122.0-124.0)	117.6 (113.2-126.0)	157.5 (149.9-191.6)	173.1 (152.8-196.8)
µg tryptophan/10mg gland	26.6 (26.4- 29.9)	25.7 (23.6- 28.0)	38.9 (22.9- 44.8)	45.5 (33.4- 61.8)
Pancreas				
µg protein/mg gland	145.0 (140.0-148.0)	140.4 (134.2-144.0)	139.6 (107.9-154.7)	145.1 (129.8-174.1)
µg tryptophan/10 mg gland	24.0 (20.1- 30.8)	27.0 (25.2- 28.0)	26.6 (15.6- 33.6)	28.8 (18.3- 33.3)

+ = mean values with ranges
* = seminal vesicle-coagulating gland weight

weight is similar to that of the normal female. Protein incorporation per mg is significantly increased 17 percent and tryptophan incorporation per 10 mg increases 155 percent. These increases are accompanied by a 225 percent increase in numbers of terminal tubules with granules containing tryptophan (Table 12, 15).

The incorporation of protein and tryptophan in the pancreas is normal (Table 12).

When prepuberally castrated females are injected with testosterone, relative uterine weights decrease 55 percent (Table 12, 15).

Adult castrated injected females. When adult castrated females are injected with testosterone, the relative submaxillary gland weight increases significantly 41 percent. This is accompanied by a 59 percent increase in protein incorporation per mg and a 378 percent increase in tryptophan incorporation per 10 mg of gland. During this time, the number of tubules with granules containing tryptophan increases 238 percent (Table 12, 15).

The amount of protein and tryptophan in the pancreas of testosterone injected females is normal (Table 12).

Relative uterine weight decreases 42 percent in adult castrated females when they are injected with testosterone (Table 12, 15).

Summary. Relative submaxillary gland weight decreases in immature males and females, given testosterone from 10 to 20 days of age. Protein incorporation per mg is normal in the submaxillary glands of these males, however it is reduced in females. The incorporation of tryptophan per 10 mg and the numbers of terminal tubules with granules containing tryptophan exceed normal values in both sexes. When testosterone injection is extended to 30 days of age, relative submaxillary gland weights increase

TABLE 12

EFFECTS OF TESTOSTERONE ON PREPUBERALLY CASTRATED AND ADULT CASTRATED FEMALES+

	Autopsied at 65 days		Autopsied at 150 days	
	Normal	Prepuberal castrate	Normal	Adult castrate
Relative accessory sex organ wt. mg/10g body wt.*	78.5 (56.0- 96.9)	35.2 (30.8- 47.8)	55.6 (48.9- 68.4)	32.5 (25.6- 44.6)
Submaxillary gland				
Absolute wt. mg of gland	53.1 (47.0- 58.0)	64.0 (58.0- 70.0)	62.6 (59.0- 73.0)	84.8 (68.0- 97.0)
Relative $\frac{\text{wt. mg of gland}}{\text{10 g body wt.}}$	33.0 (30.0- 34.5)	35.1 (32.5- 38.8)	27.8 (21.2- 30.4)	39.3 (35.4- 42.1)
Terminal tubule X-sections with tryptophan/mm^3	32 (26 - 38)	104 (90 -111)	45 (40 - 51)	152 (138 -167)
µg protein/mg gland	99.7 (90.4-104.8)	117.1 (112.9-123.6)	94.0 (81.0-108.4)	149.8 (133.6-160.8)
µg tryptophan/10mg gland	6.4 (5.6- 7.1)	16.3 (12.9- 17.6)	7.9 (7.0- 9.2)	37.8 (26.7- 42.8)
Pancreas				
µg protein/mg gland	115.3 (87.4-118.1)	157.6 (140.8-167.8)	143.0 (122.2-177.8)	141.1 (120.4-159.1)
µg tryptophan/10 mg gland	19.5 (13.8- 30.8)	17.0 (14.0- 19.0)	27.0 (19.6- 39.2)	28.3 (17.6- 34.7)

+ = mean values with ranges
* = uterine weight

significantly in both males and females. Protein incorporation per mg increases significantly in females but not in males. Tryptophan incorporation per 10 mg and the numbers of terminal tubules with granules containing tryptophan are markedly increased. The incorporation of protein and tryptophan in the pancreas is normal in immature mice treated with testosterone. Relative accessory sex organ weights surpass normal weights in these injected males and females.

Administration of testosterone to prepuberally castrated and adult castrated males results in relative submaxillary gland weight, protein and tryptophan incorporation, and numbers of terminal tubules with granules containing tryptophan which approximate normal values. The amount of protein and tryptophan in the pancreas is normal. Relative accessory sex organs weights are equal to or surpass normal weights.

When testosterone is given to castrated females, the relative submaxillary gland weight is significantly increased in adult castrated females but is normal in prepuberally castrated females. The incorporation of protein and tryptophan in the submaxillary gland and the numbers of terminal tubules with granules containing tryptophan surpass normal values. Incorporation of protein and tryptophan in the pancreas of these females is normal. Relative uterine weights are reduced in both groups of females.

Effects of Estrogen

Prepuberally castrated injected males. In males castrated prepuberally and treated with estrogen, the relative submaxillary gland weight decreases 17 percent below normal. Similarly, protein incorporation per mg of gland decreases 19 percent and tryptophan incorporation per 10 mg decreases 79 percent. The number of terminal tubules with granules containing tryptophan decreases 48 percent (Table 13, 15).

TABLE 13

EFFECTS OF ESTRADIOL ON PREPUBERALLY CASTRATED AND ADULT CASTRATED MALES+

	Autopsied at 65 days		Autopsied at 150 days	
	Normal	Prepuberal castrate	Normal	Adult castrate
Relative accessory sex organ wt. mg/10g body wt.*	23.0 (13.7- 31.4)	4.9 (2.9- 6.6)	27.4 (15.4- 32.6)	7.8 (4.3- 13.0)
Submaxillary gland				
Absolute wt. mg of gland	70.0 (60.0- 78.0)	53.2 (48.0- 58.0)	110.9 (105.6-130.0)	91.2 (82.0-106.0)
Relative $\frac{\text{wt. mg of gland}}{\text{10 g body wt.}}$	35.0 (28.9- 36.4)	29.2 (28.0- 30.5)	46.7 (42.1- 52.6)	39.4 (32.9- 47.7)
Terminal tubule X-sections with tryptophan/mm^3	104 (96 -111)	54 (34 - 74)	165 (155 -171)	105 (77 -123)
µg protein/mg gland	123.0 (122.0-124.0)	100.2 (95.6-108.8)	157.5 (149.9-191.6)	120.2 (112.0-130.6)
µg tryptophan/10mg gland	27.6 (26.4- 29.9)	5.7 (4.2- 6.4)	38.9 (22.9- 44.8)	27.0 (18.2- 29.6)
Pancreas				
µg protein/mg gland	145.0 (140.0-148.0)	127.6 (118.2-134.1)	139.6 (107.9-154.7)	135.6 (105.0-187.1)
µg tryptophan/10 mg gland	24.4 (20.1- 30.8)	18.2 (17.6- 28.0)	26.6 (15.6- 33.6)	25.0 (13.4- 30.4)

+ = mean values with ranges
* = seminal vesicle-coagulating gland weight

Protein and tryptophan incorporation in the pancreas is normal (Table 13).

Relative weights of the accessory sex organs are reduced 79 percent (Table 13, 15).

Adult castrated injected males. In adult castrated males, administration of estrogen, when compared with normal males, results in a 16 percent decrease in relative submaxillary gland weight. Protein incorporation per mg in the submaxillary gland decreases 24 percent and tryptophan incorporation per 10 mg decreases 31 percent. Similarly the number of terminal tubules with granules containing tryptophan decreases 36 percent (Table 13, 15).

The pancreas of adult castrated males, treated with estrogen, has normal amounts of protein and tryptophan (Table 13).

Relative accessory sex organ weight is reduced 72 percent below normal (Table 13, 15).

Prepuberally castrated injected females. Relative submaxillary gland weight is normal in females castrated prior to puberty and then injected with estrogen. Protein incorporation per mg is normal, however tryptophan incorporation per 10 mg increases 39 percent. There are normal numbers of terminal tubules with granules containing tryptophan in the submaxillary gland (Table 14, 15).

The amount of protein and tryptophan in the pancreas of these females is normal.(Table 14).

Injection of estrogen results in normal relative accessory sex organ weight (Table 14, 15).

Adult castrated injected females. Administration of estrogen to adult castrated females results in normal relative submaxillary gland

TABLE 14

EFFECTS OF ESTRADIOL ON PREPUBERALLY CASTRATED AND ADULT CASTRATED FEMALES+

	Autopsied at 65 days		Autopsied at 150 days	
	Normal	Prepuberal castrate	Normal	Adult castrate
Relative accessory sex organ wt./10g body wt.*	78.5 (56.0- 96.9)	69.0 (36.1- 76.8)	55.6 (48.9- 68.4)	134.8 (92.2-150.0)
Submaxillary gland				
Absolute wt. mg of gland	53.1 (47.0- 58.0)	51.2 (50.0- 56.0)	62.6 (59.0- 73.0)	60.7 (49.0- 78.0)
Relative $\frac{\text{wt. mg of gland}}{10\text{-g body wt.}}$	33.0 (30.3- 34.5)	31.3 (30.3- 35.0)	27.8 (21.2- 30.4)	28.3 (26.6- 29.8)
Terminal tubule # X-sections with tryptophan/mm^3	32 (26 - 38)	35 (26 - 43)	45 (40 - 51)	65 (49 - 72)
µg protein/mg gland	99.7 (90.4-104.8)	105.4 (97.3-112.6)	94.0 (81.0-108.4)	115.9 (99.8-133.3)
µg tryptophan/10 mg gland	6.4 (5.6- 7.1)	8.9 (6.7- 11.7)	7.9 (7.0- 9.2)	18.4 (13.4- 22.4)
Pancreas				
µg protein/mg gland	115.3 (87.4-118.1)	133.5 (143.5-152.0)	143.0 (122.2-177.8)	143.4 (113.1-174.7)
µg tryptophan/10 mg gland	19.5 (13.8- 30.8)	13.1 (12.6- 14.0)	27.0 (19.6- 39.2)	26.7 (13.2- 33.4)

+ = mean values with ranges
* = uterine weight

weights. Although the incorporation of protein per mg is normal, tryptophan per mg of gland increases 133 percent. This is accompanied by a 44 percent increase in numbers of terminal tubules with granules containing tryptophan (Table 14, 15).

Protein and tryptophan incorporation are normal in the pancreas of adult castrated females, treated with estrogen (Table 14).

Relative accessory sex organs weight in these females increases 142 percent over normal (Table 14, 15).

Summary. Administration of estrogen to the castrated male fails to bring its relative submaxillary gland weights, incorporation of protein per mg and tryptophan per 10 mg and numbers of terminal tubules with granules containing tryptophan to normal values. The incorporation of protein and tryptophan by the pancreas is normal. Relative weights of the accessory sex organs are below normal, in both prepuberally castrated and adult castrated males.

After the period of estrogen administration, the relative submaxillary gland weight and the incorporation of protein per mg of gland is normal in castrated females. This is accompanied by significant increases in tryptophan incorporation per 10 mg of gland and in numbers of terminal tubules with granules containing tryptophan. Protein and tryptophan incorporation in the pancreas of castrated females, treated with estrogen are normal. Relative uterine weight is normal in prepuberally castrated females and surpasses normal in adult castrated females treated with estrogen.

TABLE 15

A SUMMARY OF THE EFFECTS OF CASTRATION, TESTOSTERONE INJECTION, AND ESTROGEN INJECTION ON MALE AND FEMALE MICE*

	Relative Submaxillary weight		Submaxillary Protein µg/mg		Submaxillary Tryptophan µg/10 mg		Terminal** tubules/ mm^3		Relative wt. accessory sex organ	
	♂ Male	♀ Female	♂ Male	♀ Female	♂ Male	♀ Female	♂ Male	♀ Female	♂ Male	♀ Female
CASTRATION										
Prepuberal	↓ 21	↓ 15	↓24	0	↓ 74	↓ 27	↓ 77	↓ 72	↓ 91	↓ 77
Adult	↓ 24	↓ 22	↓33	0	↓57	0	↓ 38	0	↓ 85	↓ 48
TESTOSTERONE INJECTED										
Intact										
Immature 20-day	↓ 23	↓ 27	0	↓ 15	↑ 82	↑ 50	--	--		
Immature 30-day	↑34	0	0	↑ 11	↑316	↑583	--	--	↑ 2300	↑ 280
Castrated										
Prepuberal	0	0	0	↑ 17	0	↑ 155	0	↑ 225	0	↓ 55
Adult	0	↑ 41	0	↑ 59	0	↑378	0	↑ 238	↑ 104	↓ 42
ESTROGEN INJECTED										
Castrated										
Prepuberal	↓ 17	0	↓ 19	0	↓ 79	↑ 39	↓ 48	0	↓ 79	0
Adult	↓ 16	0	↓24	0	↓31	↑ 133	↓ 36	↑ 44	↓ 72	↑ 142

* = Values are given as deviation of percent from normal. ↑ = increase ↓ = decrease 0 = no significant change.

** = Terminal tubules with granules containing tryptophan

-- = Terminal tubules with tryptophan are not present in normal; but in injected animals,
20-day males = 71/mm^3 20-day females = 37/mm^3
30-day males = 104/mm^3 30-day females = 94/mm^3

CHAPTER IV

DISCUSSION AND CONCLUSIONS

In general, the major sex-dimorphic characters in submaxillary glands of adult mice include: 1) gross weight, 2) terminal tubule diameter, 3) height of tubular cells, and 4) degree of granulation in terminal tubule cells (Lacassagne 1940a, Junqueira et al. 1949, Harvey 1952). As a result of the studies presented in this paper, the list of differences may now be extended to include 5) number of cross-sections of terminal tubules/mm^3 with granules containing tryptophan, 6) quantity of protein and 7) amount of tryptophan.

Staining of the granules in terminal tubule cells with Hg-BPB indicates their protein nature. The blue color is more intense in the granules than in the acinar or tubular cell cytoplasm, indicating a higher concentration of protein in these granules. Further, the granules in the submaxillary glands of males appear to stain more intensely and to appear more plentifully than in females. Thus, the submaxillary glands of males are histochemically richer in protein than those of females. Biochemically, submaxillary glands of adult males have more protein than those of adult females.

The protein of the granules in the terminal tubules contain dibasic amino acids in greater concentration than adjacent tissue since Napthol Yellow stains the granules a darker yellow than the acinar or terminal

tubule cytoplasm. Tyrosine is also present in greater concentration in the granules than in the cytoplasm, as shown by their reaction with Millons' reagent (Junqueira et al. 1949). Although this use of Millons' reagent has been criticized (Raynaud 1952) in that other phenolic groups might be stained my study verifies the presence of tryosine in the granules with a modified Millons' reagent (Rasch and Swift 1960). Furthermore, the granules of the tubules of males stain a darker red than those of females.

The granules of the terminal tubules also contain tryptophan, as indicated by their reaction with p-DMAB stain. The color appears more intense and there appears to be more granules in the tubules of adult males than in adult females. Tryptophanwas identified in the submaxillary glands of male mice by Glenner and Lillie (1957). They also identified tryptophan as a component of bovine pancreas. Marshall (1954) reported that the zymogen granules in bovine pancreas contain 5.4 percent tryptophan. The granules in the pancreas of mice also stain with p-DMAB, and the density of granules appear to be the same in males and females.

Body weight and submaxillary gland weight in females increases gradually during the prepuberal and puberal periods. However, submaxillary gland growth is slower than body growth resulting in decreased relative submaxillary gland weight during these periods.

In females although the submaxillary gland weight increases with age, the incorporation of protein per mg decreases in the prepuberal and puberal periods. The gland is getting bigger but the relative amount of protein in the gland is smaller. This also implies that the relative amounts of non-protein such as water, carbohydrates or lipids, increases during puberty in females.

The measurement of total protein includes the portion of protein

which has tryptophan as a part of the molecule. The decrease in total protein in the submaxillary gland of females in the prepuberal period is accompanied by a decrease, to a lesser extent, of tryptophan. When the amounts of protein or tryptophan are plotted against age and the slopes of the lines compared, the slopes show that during puberty tryptophan incorporation increases but the rate of protein incorporation does not change. This indicates an accelerated accumulation of tryptophan in the gland during this period.

The time of the increase in tryptophan incorporation coincides with the appearance of granules containing detectable tryptophan in the terminal tubules of the submaxillary glands of females. In p-DMAB stained sections of the glands, tryptophan is concentrated in the large granules of the terminal tubules. The increase in tryptophan incorporation and the subsequent increase in granules containing tryptophan are concurrent with the increase in uterine weight. This suggests some influence of female gonadal hormones on tryptophan incorporation and granule production. This does not exclude the influence of general organ development nor the influence other endocrine glands may have upon the submaxillary gland.

In males, body weight increases during the prepuberal period with an increase in the rate of growth during puberty. The submaxillary glands have a similar pattern of growth. In graphs of body weight and submaxillary gland weight compared to age, the slopes of the lines during puberty show that the submaxillary gland grows faster than the body. Relative submaxillary gland weight, as a consequence of disproportionate growth of the submaxillary gland, increases during this period.

During the prepuberal period in males, although the gland is gaining weight, the incorporation of protein per mg decreases. Increases in

weight during this period results from the increase in non-protein components of the gland. During puberty, both the rates of submaxillary growth and incorporation of protein increase. The increase in rate of protein incorporation over the prepuberal rate is greater than the concurrent increase in submaxillary gland growth rate. The submaxillary glands of males become increasingly richer in protein during puberty.

In males, the decrease in protein incorporation during the prepuberal period is accompanied by an increase in tryptophan incorporation. During the puberal period, both the rate of protein incorporation and the rate of tryptophan incorporation increase. When protein and tryptophan incorporation are plotted against age and the slopes are compared during puberty, protein incorporation has a slope of 1.44 and tryptophan incorporation has a slope of 1.03. Since the measurement of protein-bound tryptophan is included in the measurement of total protein, the difference between the two slopes represents a slope for protein which does not contain tryptophan or a slope of 0.41. Protein-bound tryptophan then, is incorporated at a faster rate than other protein in the submaxillary glands of males during puberty.

Examination of p-DMAB stained sections indicate that tryptophan is being concentrated in the large granules of the terminal tubules. Granules containing tryptophan first appear in the terminal tubules of normal males at 35 days of age. The number of these tubules increases gradually during the remainder of the prepuberal period. The rate at which they appear increases during puberty. When tryptophan incorporation per 10 mg of gland and number of tubules are plotted against age and the slopes compared, the rate of tryptophan incorporation increases 11-fold and the rate of appearance of the terminal tubules increases 3.4 fold during puberty. This suggests

that the volume of granules per tubule is increasing during this period.

In males, the accessory sex organs gradually increase in weight in the late prepuberal period. The rate of growth increases during puberty and decreases in the postpuberal period. This pattern of growth is typical for those organs, as secondary sex organs, which depend on the male sex hormone for their development (Sreebny et al. 1955). The increase in tryptophan incorporation and the increase in granules containing tryptophan are concurrent with the increase in accessory sex organ weight. This suggests that male gonadal hormones influence the incorporation of protein-bound tryptophan and the synthesis of granules containing tryptophan in the terminal tubules of the submaxillary glands of males. The influence of general organ development or the influence of other endocrine glands can not be excluded.

When the patterns of growth for males and females are compared, the relative submaxillary gland weight in females decreases during the prepuberal period whereas in males it remains constant. The rate of relative gland growth increases in males during puberty but does not change in females. The relative submaxillary gland weight in males is significantly heavier than in females during the puberal and postpuberal periods. Males incorporate a disproportionately greater amount of tissue as submaxillary gland than females.

Incorporation of protein per mg in the submaxillary glands of females and males decreases during the prepuberal period. The rate of incorporation remains the same in females and increases in males during puberty. After 45-days of age, males have 1.7 times more protein per mg of submaxillary gland than females of the same age. The absolute weight of the gland is heavier in males than in females of these ages.

When the water, lipid and non-lipid fractions were measured in the submaxillary glands of adult mice (Atkinson et. al. 1959), the relative amount of these fractions were found to be the same, although the wet weight of the submaxillary glands of males were significantly heavier than those of females. Considering the greater abundance of terminal tubules in the submaxillary glands of males, Atkinson and his group concluded that the principal factor effecting sex-dimorphism in the gland is the absolute difference in tissue mass due primarily to the differential growth of tubular elements.

The incorporation of tryptophan per 10 mg decreases in the submaxillary gland of females and increases in males during the prepuberal period. During puberty, the rate of tryptophan incorporation increases in both sexes, however the increase is greater in males than in females. The rate of tryptophan incorporation in males during the prepuberal period is the same as that in females during puberty. The greater increase in tryptophan incorporation by the submaxillary glands of males over that of females could result from the increase in gonadal androgens in males.

Granules containing tryptophan appear in the terminal tubules of males at 35 days of age and in those of females at 45 days of age. Harvey (1952) reported that acidophilic granules appear in the tubular cells when the animals are between 20 and 35 days of age. These granules are not detected by p-DMAB staining until later. This could mean that although the granule is formed, tryptophan is not incorporated until later or that the amount of tryptophan is below the sensitivity of the p-DMAB stain. Acidophilic granules in the terminal tubules of males becomes more abundant than those of females at about 30 to 35 days of age (Harvey 1952). The biochemically detectable amount of tryptophan in the submaxillary gland of

males and females becomes significantly different at 35 days of age, although in females, granules containing tryptophan do not appear until later.

When numbers of terminal tubules with granules containing tryptophan are plotted against age and the slopes are compared, the rate of appearance of these tubules for males in the prepuberal period is the same for that of females during puberty. Furthermore, the rate of appearance during puberty is greater in males than in females. The rate of appearance parallels the rate of tryptophan incorporation in the gland as measured by biochemical analysis. Not only are the terminal tubules with granules containing tryptophan more numerous in males than in females, but the density of the granules per cross-section appear to be greater (Fig. 4,5). These observations indicate that greater amount of tryptophan in submaxillary glands of males, measured histochemically, histometrically, and biochemically, as compared to females, is influenced by the additional androgens produced by males during and following puberty.

The amount of protein per mg from a portion of the pancreas does not change significantly with age in males or females. Further, when the amount of tryptophan per 10 mg incorporated in the pancreas is compared with age, the changes in the rate of tryptophan incorporation do not correspond with the changes in the submaxillary gland in the prepuberal, puberal or postpuberal period. Protein and tryptophan incorporation in the pancreas does not follow the growth pattern of the accessory sex organs in which growth increases during puberty. The pancreas does not exhibit sex dimorphism as determined by protein or tryptophan incorporation into the gland (Table 4,5).

Submaxillary gland weight, amount of protein, quantity of tryptophan and numbers of terminal tubules with granules containing tryptophan

have different values in intact males and female mice of different ages. The greatest degree of difference is manifested at some point during the puberal period. The influence of gonadal hormones on the development of sex-dimorphism of the submaxillary glands is better understood by comparing normal relative submaxillary gland weight, protein and tryptophan incorporation, and number of terminal tubules to the changes which occur as a consequence of castration and gonadal hormone injections.

Castration prevents the attainment of normal relative submaxillary gland weight in both prepuberally castrated males and prepuberally castrated females. Furthermore, the relative gland weight of these males is not significantly different from the females. Castration of adult males and adult females also results in a loss of relative submaxillary gland weight. The loss of weight in males is greater than the loss of relative submaxillary gland weight in females (Table 15). Atkinson et. al. (1959) has also reported a significant decrease in relative submaxillary gland weight in adult males and females, castrated for 10 to 12 days.

The reduction in relative submaxillary gland weight is accompanied by a decrease in protein incorporation per mg of gland in prepuberally castrated and adult castrated males. The degree of submaxillary gland weight loss and of protein reduction is approximately the same, implying an equal loss of protein and non-protein components of the gland. This is in disagreement with Atkinson et. al. (1959) who reported that the reduction in submaxillary weight in castrated males is accompanied by a decrease in the absolute weight of water and in the non-lipid fraction of the submaxillary gland.

Reduced protein incorporation per mg in the submaxillary glands of castrated males is accompanied by reduced incorporation of tryptophan in

the gland. In prepuberally castrated males, tryptophan incorporation is reduced to the amounts observed in immature males. Incorporation in adult castrated males is not reduced to this extent. In both groups, the reduction in tryptophan incorporation is greater than the reduction in protein incorporation (Table 15). This suggests that a deficiency of gonadal androgens decreases the synthesis of proteins containing tryptophan to a greater extent than non-tryptophan proteins.

The number of terminal tubules with granules containing tryptophan are also reduced and the extent of reduction parallels the reduction of tryptophan in both the prepuberally castrated and adult castrated males (Table 15).

Raynaud (1944) noted that, although reduced, granules continue to develop in the terminal tubules of the submaxillary glands of males castrated at birth. Harvey (1952) reported that granules do not fail to form in prepuberally castrated males and do not disappear after long castration in adult males. It was concluded that androgens of testicular origin do not appear to be necessary for the synthesis and maintenance of a minimal number of granules. Furthermore, the submaxillary gland of castrated males is similar to that of the normal young female with respect to relative gland weight, tubular diameter and density of granules in the terminal tubules.

Castration of both prepuberal and adult males reduces the relative weights of accessory sex organs to weights similar to those of immature males.

In contrast to the effects of castration in males, in females the incorporation of protein per mg of submaxillary gland is normal. The reduction of relative submaxillary gland weight in these females is not

accompanied by a reduced protein incorporation (Table 15).

In their analysis of the submaxillary glands, Atkinson et. al. (1959) found that castration of adult females reduced the absolute weight and relative weight of water in the submaxillary glands but increased the relative weight of the non-lipid fraction. The reduction of absolute submaxillary gland weight in castrated females, thus, is related to the loss of water rather than the decrease in non-lipid portion of the gland. In a similar way, reduction of relative submaxillary gland weight in castrated females is related to the reduced non-protein components of the gland rather than a decrease in the incorporation of protein by the gland.

Although the incorporation of protein per mg of submaxillary gland in prepuberally castrated females is normal, tryptophan incorporation decreases to a quantity equal to that of normal prepuberal females. The reduction in tryptophan is accompanied by a reduction in the number of terminal tubules with granules containing tryptophan. The response to castration is different in prepuberally and adult castrated females. In adult castrated females, both the incorporation of tryptophan and the number of terminal tubules with granules containing tryptophan are normal. These observations agree with those of Harvey (1952) who noted that ovariectomy tends to reduce submaxillary weight and decrease the size of the acini. The loss of granular inclusions and reduction of tubule diameter is apparent in prepuberally castrated females, but not significant in adult castrated females.

There is no apparent relation between the changes which occur in the amount of protein and tryptophan in the pancreas regardless of sex and time of castration. Baker and Pliske (1957) report no significant reduction in absolute and relative weights of the pancreas as a result of castration.

Administration of testosterone to prepuberally castrated and adult castrated males results in normal relative submaxillary gland weights. This is accompanied by normal incorporation of protein by the submaxillary gland. Tryptophan incorporation per 10 mg and the number of terminal tubules with granules containing tryptophan are normal (Table 15). Harvey (1952) wrote that injections of androgens into prepuberally and adult castrated males results in increased relative gland weight, tubular diameter, and peripheral granulation of the submaxillary gland over that of the castrated male. Atkinson et. al. (1959) has shown that androgen injections increase both the water and the non-lipid fraction of the submaxillary gland in castrated adult males. This further emphasizes the influence of testosterone on the submaxillary gland.

The submaxillary glands of castrated females injected with androgens show typical masculine appearance. Relative gland weight, protein incorporation, tryptophan incorporation and terminal tubules with granules containing tryptophan surpass those of normal females of similar ages. Furthermore, they equal the androgen treated males in submaxillary gland response to testosterone. The action of androgen upon the submaxillary glands of prepuberally castrated and adult castrated females and its influence on submaxillary gland weight, tubule diameter and density of granulation has been reported by Harvey (1952).

Androgen is further implicated as influencing the sex-dimorphism of the submaxillary gland when immature mice are given testosterone prior to the time of normal puberty. In males, given 0.05 mg testosterone daily from 10 to 29 days of age, the relative submaxillary gland weight is equal to that of adult males. Protein incorporation is normal but tryptophan incorporation and the number of terminal tubules with granules containing

tryptophan are equivalent to those of males in late puberty. Females, treated in a similar manner respond to testosterone injection in the same direction. Although relative submaxillary gland weight is normal, protein incorporation and tryptophan incorporation equal those of adult males. Terminal tubules with granules containing tryptophan equal those of males during early puberty.

When the same amount, 1 mg, of testosterone is given over a shorter period of time, 10 to 19 days of age, relative submaxillary weight decreases in both males and females. Protein incorporation is normal in males but decreases in females. The incorporation of tryptophan is enhanced and is equal to that of early puberal males. Terminal tubules with granules containing tryptophan in these injected immature males equal those of males in late puberty and in injected females equal those of prepuberal males. Testosterone stimulates growth of the accessory sex organs resulting in organs weights equal to those found in puberal males. Harvey (1952) wrote that administration of 0.1 mg of testosterone during 10-29 days of age, can cause the submaxillary glands of 30-day old males and females to appear essentially like those of adult males. An equal amount of testosterone given at a higher rate does not evoke an equal response. This difference in response of animals injected from day 10 to day 29 and those injected from day 10 to day 19 may involve the inability of the younger animals to utilize the larger amounts of testosterone and the excess may be excreted, or other wise metabolized without stimulating the target organ.

The administration of testosterone has no significant effect on the incorporation of protein or the incorporation of tryptophan in the pancreas of immature males and females, prepuberally castrated or adult castrated animals.

When prepuberally castrated males are given estrogen, the relative submaxillary weight, protein incorporation, tryptophan incorporation and terminal tubules with granules containing tryptophan fail to attain normal values. Protein incorporation and the number of terminal tubules with granules containing tryptophan increase and are equal to those of males in early puberty. The relative weight of the accessory sex organs decreases.

When estrogen is given to adult castrated males, there is a loss of relative submaxillary gland weight, protein incorporation, tryptophan incorporation, and numbers of terminal tubules with granules containing tryptophan. When these males are compared with non-injected adult castrated males, the rates of protein incorporation and tryptophan incorporation increase significantly. Estrogen has a limited effect upon these components of the submaxillary glands of males.

Estrogen injection in both prepuberally and adult castrated females results in normal relative submaxillary gland weight and protein incorporation. In prepuberally castrated females, incorporation of tryptophan per mg of gland and the numbers of terminal tubules with granules containing tryptophan are normal whereas in adult castrated females they are above normal values. Relative uterine weight in prepuberally castrated females, injected with estrogen, is within the range of values for normal females and exceeds normal weight in adult castrated females.

Increases in tubular diameter in submaxillary glands of castrated males and females after injection of estradiol was reported by Lacassagne (1940b). Harvey (1952) found that estrogen evokes the repair of all changes resulting from ovariectomy in both young and adult females with increased tubular diameter, weight, granule production and acinar size. In addition, submaxillary glands of gravid females contain granules equal to those of

adult males.

The administration of estrogen to prepuberally castrated and adult castrated females and males has no apparent effect on the incorporation of protein and tryptophan in the pancreas.

Harvey (1952) reported that the adrenal glands increased in weight following estrogen injection and suggested that this observation coupled with the fact that adrenal weight decreases following castration implies that the change in submaxillary weight and histology might be mediated by the adrenal gland and secondarily affected by estrogen. Raynaud (1954) reported a more complete atrophy of the terminal tubules with adrenalectomy coupled with castration than with castration alone. My studies indicate that granules containing tryptophan appear prior to puberty in males and slightly later in females. The incorporation of tryptophan-bound protein occurs at similar rates in males and females before puberty and decreases to approximately these rates following castration. Synthesis of granules containing protein-bound tryptophan in the terminal tubules of the submaxillary glands continues at minimal rates in the absence of gonadal hormones. Adrenal cortical hormones, estrogens, and androgens are steroidal in nature. This system in the submaxillary glands responds to both estrogen and testosterone but is especially responsive to androgenic steroids.

Kochakian et al. (1963) investigated the role of various androgens on protein synthesis. The uptake of certain labeled amino acids, especially leucine, was decreased in the kidney following castration and increased after implantation of testosterone propionate pellets. Uptake of tryptophan however was not affected. The changes in amino acid uptake was correlated with changes in the microsomal RNA and protein biosynthesis both

after castration and during growth of the kidney by androgen stimulation. Kochakian postulated the presence of hormone dependant enzymes in the protein biosynthetic systems of certain tissues. Similar work has not been done on the submaxillary gland but hormone-dependent enzymes could be postulated for the synthesis of proteins containing tryptophan in the granules of the terminal tubules. This is a problem for future investigation.

Summary and Conclusions

The submaxillary glands of adult male and female mice exhibit a sex dimorphism which is characterized by greater relative submaxillary gland weight, greater rate of incorporation of total protein and of protein-bound tryptophan, and more numerous terminal tubules with granules containing tryptophan in males than in females.

Histochemical studies of sections of the submaxillary glands show that the large refractory granules of the terminal tubules are protein in nature. This granule protein contains the major portion of the tryptophan which can be detected by p-DMAB staining. In addition to tryptophan, dibasic amino acids and tyrosine are also present in the granules.

The pattern of submaxillary gland growth in males is different from that of females. In males, the submaxillary gland grows faster than the body with a subsequent increase in relative submaxillary gland weight as the animals get older. The greatest increase in relative submaxillary gland weight is observed during puberty. In females, although the gland continues to grow, it is slower than body growth. Relative submaxillary gland weight tends to decrease with age in females.

The submaxillary gland incorporates more non-protein than protein components during the prepuberal period. During puberty, this trend continues in females but changes in males. The increase in submaxillary

gland weight in males results from the increase in protein incorporated by the gland. In females, increase in submaxillary gland weight results from the relatively greater incorporation of non-protein components.

Measurement of total protein includes measurement of protein-bound tryptophan. Tryptophan incorporation and terminal tubules with granules containing tryptophan, after their initial appearance, increase with age. The rate of tryptophan incorporation roughly parallels the rate of the appearance of terminal tubules with granules containing tryptophan. Increased rates of tryptophan incorporation per mg of gland and granule production are observed at puberty in both sexes, however these rates are always greater in males than in females.

In castrated males, the decrease in relative submaxillary gland weight is accompanied by a comparable decrease in total protein. The major portion of the decrease in total protein can be accounted for by the decrease in tryptophan, as measured histochemically and histometrically. Both the reduction in tryptophan incorporation and the appearance of terminal tubules containing tryptophan in prepuberally castrated males approximate that of immature males. The extent of reduction is less in adult castrated males than in prepuberally castrated males.

In adult castrated females, protein incorporation, tryptophan incorporation and numbers of terminal tubules are normal. In prepuberally castrated females protein incorporation is normal but tryptophan incorporation and terminal tubules with granules containing tryptophan decrease.

The most notable effect of testosterone injection in castrated males and females and in normal immature mice is the increase in tryptophan incorporation and in the appearance of terminal tubules containing tryptophan. The submaxillary glands of females respond to testosterone injection

in the same manner and in the same direction as the submaxillary glands of males.

Administration of estrogen to castrated males has a limited effect. Relative submaxillary gland weight, protein incorporation, tryptophan incorporation and the number of terminal tubules with granules containing tryptophan is for the most part slightly greater than in non-injected castrated males but are below normal values.

Estrogen injection is effective in castrated females, and its administration results in normal protein, tryptophan and numbers of terminal tubules with granules containing tryptophan.

It seems well established by castration and testosterone injection that androgens play an important role in the incorporation of protein-bound tryptophan and the synthesis of granules in the terminal tubules of the submaxillary glands of males. The role of estrogen is not as clear although the influence is in the same direction. These experiments do not exclude the influence of other endocrine glands following castration and gonadal hormone treatment.

Protein and tryptophan incorporation by the pancreas is not significantly affected by age, castration or gonadal hormone injection in either males or females. The pancreas does not exhibit sex dimorphism on the basis of protein incorporation or tryptophan incorporation.

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