

# Effects of Gonadectomy on the Histological Structure of the Mandibular Glands in Male Mice

## I. On the Adult Mice

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### INTRODUCTION

Concerning the mandibular glands of mice, a difference between the histological structure of the male and that of the female was pointed out by LACASSAGNE (1940)<sup>1)</sup>, since then an apparent relationship between the endocrine system and the mandibular glands in rodents has been demonstrated by a number of investigators. It was shown that the atrophy of the granular tubules of the mandibular glands happening after hypophysectomy was to be repaired by the administration of androgenic hormone (KRONMAN and CHAUNCEY (1964)<sup>2)</sup>, LACASSAGNE and CHAMORRO (1940)<sup>3)</sup>, SREEBNY (1960)<sup>4)</sup>). On the other hand, the existence of the protease activity in the extracts of the mandibular glands of mice was reported biochemically by JUNQUEIRA et al. (1949)<sup>5)</sup>. It was ascertained that the mean-protease-activity of the glands of adult male was nearly twice as large as that of adult females.

The effects of gonadectomy on the structure of the mandibular glands were investigated by a number of investigators (LACASSAGNE (1940), JUNQUEIRA et al. (1949)<sup>5)</sup>, SHAFER and MUHLER (1953)<sup>6)</sup>, SREEBNY (1960)<sup>4)</sup>, CARAMIA (1966)<sup>7)</sup>).

In these reports, however, descriptions of the quantitative estimation of the histological structure in the mandibular glands during a long period after gonadectomy are quite few.

This paper reports some changes in the quantitative assessments of the granular tubules and acini in the mandibular glands in mice after gonadectomy. Namely, the purpose of the present study is to reaffirm the relationship between the testes and the mandibular glands in mice.

### MATERIALS AND METHODS

95 male ICR-JCL mice were employed in this study as material-animals. On the 90th day after birth, testes, epididymides and a part of vasa deferens in both sides were ectomized under anesthetization with sodium pentobarbital. As controls, the intact mice 90,

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100, 110 and 120 days old were used. Animals were fed on a commercial laboratory chow (Oriental Yeast Co.) and given water *ad libitum*.

On the 3rd, 5th, 10th, 15th, 20th, 25th and 30th day after operation, the animals were sacrificed and the mandibular glands in both sides were removed. The glands were weighed immediately and the right glands were fixed in Bouin solution, the left ones were in Zenker-formol solution, and embedded in paraffin. Along the long axis the right glands were consecutively cut at 6 microns, and stained with hematoxylin and eosin for the execution of a quantitative estimation, while the left glands were subjected to Azan, PAS, Alcian-Blue staining.

In the course of the quantitative estimation, using the section ocular micrometer, the areas occupied by the granular tubules and acini within a segment 335 microns in square at a lobulus in the central part of the histological section, were counted at 25 sections per one animal respectively; the number of the granular tubules and acini in a square being counted, too. A square was subdivided into 25 small demarcations. The above-mentioned areas were showed by the number of the small demarcations.

Then, the ratio of tubules area to acini area, or the so-called tubular/acinar ratio (T/A), was calculated.

On the other hand, the outer and inner diameters of the granular tubules showing the round-shape in the histological sections were measured with ocular micrometer in 50 tubules per one animal. The inner diameter of the granular tubule was fixed to be equivalent to the diameter of the lumen. The height of epithelia of the granular tubule was obtained by the following formula; (outer diameter of the granular tubule—inner diameter of the granular tubule)/2. Furthermore, the number of the nuclei in the above-mentioned granular tubules were counted. The body-weight of mice examined in

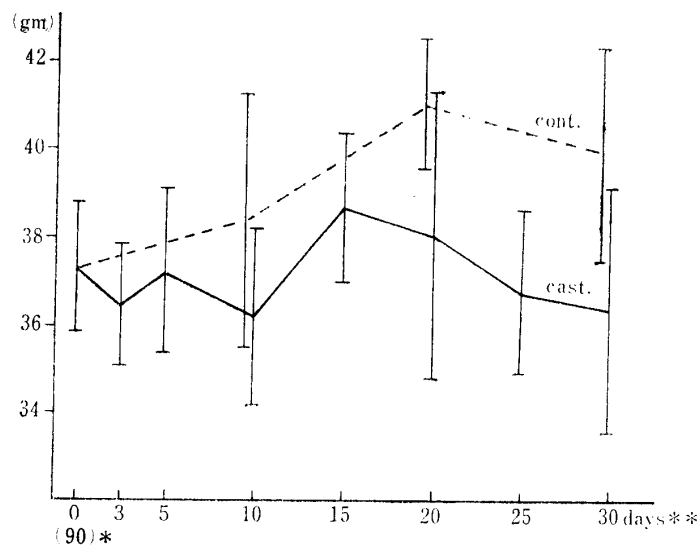


Fig. 1. Body-weight

\* the day when gonadectomy was carried out  
 \*\* days after gonadectomy  
 cont. control group  
 cast. gonadectomized group  
 means  $\pm$  standard deviation

this experiment is shown in figure 1.

### OBSERVATIONS

Gonadectomy resulted in a complete success. In the gonadectomized mice, both the seminal vesicles and the coagulating glands showed distinguished atrophies.

#### 1) gland-weight (Fig. 2, 3)

The mandibular gland-weight in the control mice increased as the mice grew older, the mean-weights of the mandibular glands in both sides being about 200 mg on the 120th day after birth. On the other hand, the gland-weights in the gonadectomized animals significantly decreased on the 3rd day after gonadectomy, showing a marked reduction on the 5th day. From this time on to the 30th day after gonadectomy, the curve of reduction showed a gentle slope, and on the 30th day the mean-gland-weight was fixed to be about 130 mg, showing the difference of 50 mg from that of 90 days old control mice.

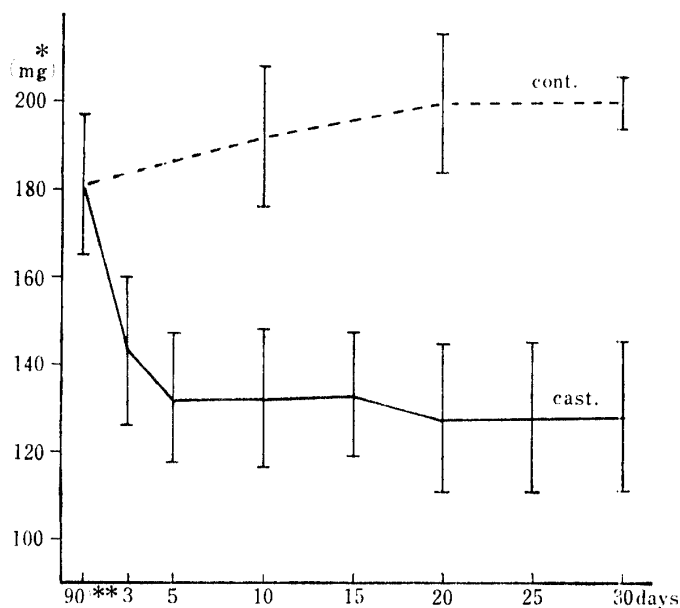


Fig. 2. Gland-weight

The form of the graphs in Fig. 2~Fig. 9, is the same as that in Fig. 1.

\* The value shows a total of weights of the mandibular glands in both sides.

\*\* days of age when gonadectomy was carried out.

The gland-weight-percentage per the body-weight in the control group was about 0.50%, being almost constant during the period from 90~120 days, while that in the gonadectomized mice showed a distinguished decrease until the 5th day after gonadectomy, becoming constant at the later period as shown in figure 3.

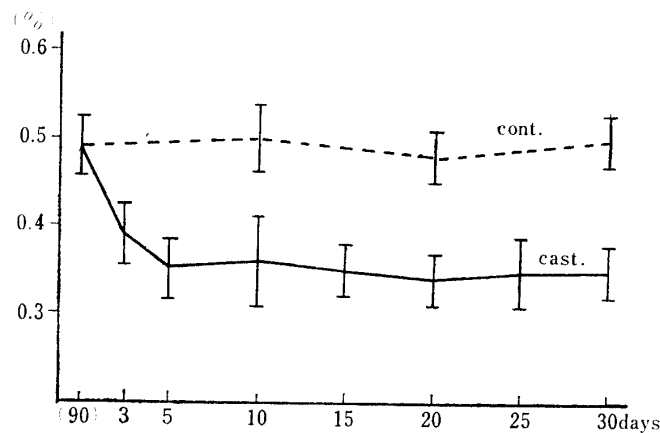


Fig. 3. Gland-weight-percentage per body-weight.

## 2) histological observations

According to not a few literatures, a lobulus in the mandibular glands of the rodents consists of four main parts, following the flow of the secretion; (1) acini, (2) intercalated ducts, (3) secretory ducts, and (4) intralobular striated ducts. Owing to the result of this experiment, in the control mice, it became possible for us to divide the secretory duct into two parts; namely, the striated tubule and the granular tubule.

The former was very short, neighbouring the intercalated duct, its columnar epithelial cells showing many acidophil basal striations below the nuclei. The latter occupied a large portion of the secretory duct and its epithelial cells containing abundant granules showed no basal striations.

Owing to the ascertainment that the epithelia lining the lumen of the granular tubules consisted of the high columnar epithelial cells containing numerous acidophil granules, the boundary line between the granular tubules and the intralobular striated ducts became to be noted distinctly.

The area of the granular tubules in a lobulus was about twice as large as that of the acini in the control mice of 90 days of age. In the control group, the granular tubules developed further as the days advanced, reaching the 120th day after birth. The acini were laid between the exceedingly developed granular tubules. The intercalated ducts and striated tubules were visible, uncommonly.

In the gonadectomized mice, the structure of the acini was left unchanged, nevertheless the granular tubules showed distinguished atrophies. The diameter of the granular tubules became smaller after gonadectomy. The epithelial cells of the granular tubules showed a decrease of the acidophil granules, and on the 15th day after gonadectomy the area of the granular tubules in a lobulus became nearly the same as that of the acini.

In the decreasing-degree of the granules, individual variations were to be noted, from the one losing all the granules on the 3rd day and to the one containing yet considerable amount of granules on the 30th day after gonadectomy. The first disappearance of the granules in the granular tubules arose in the epithelial cells lying adjacent to the striated tubules. Thus with the increasing lapse of days after gonadectomy the

striated tubules became longer than those in the control mice.

Moreover, in the gonadectomized mice, in the granular tubules near the intralobular striated ducts the content of granules decreased, letting the basal striation become visible. Consequently the boundary lines between the granular tubules and the intralobular-striated ducts were made not to be recognized, clearly.

### 3) quantitative assessments

#### a) acini (Fig. 4, 5)

The changes in the number of the acini observable at 335 square microns in a lobulus in the mandibular gland sections are shown in figure 4. In the control mice, a curve of the number of the acini when the mice were 90~120 days of age showed a slow decent, but the degree of the decent was not significant statistically. In the gonadectomized mice, the number of the acini increased abruptly up to the 15th day after gonadectomy. The differences on and after the 10th day, between the control and gonadectomized mice, were significant.

The changes of the area of the acini measured by the number of small demarcations are shown in figure 5. The area of the acini in the control mice showed a moderate decrease with increasing age, on the other hand, that in the gonadectomized mice increased abruptly after gonadectomy. The size of an acinus (area of acini/number of acini) was constant in both groups. Therefore, it, happened that no change was brou-

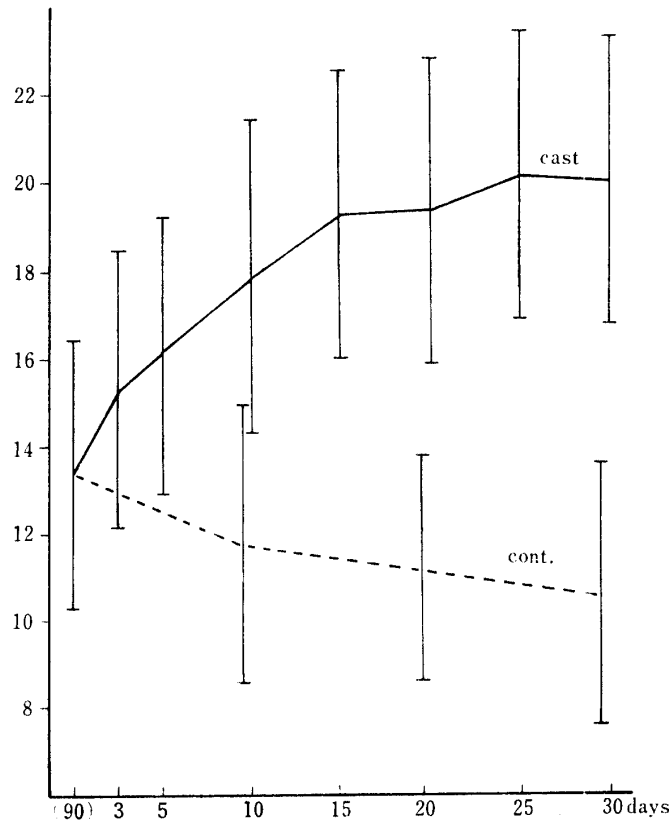


Fig. 4. Number of acini in 335  $\mu^2$ .

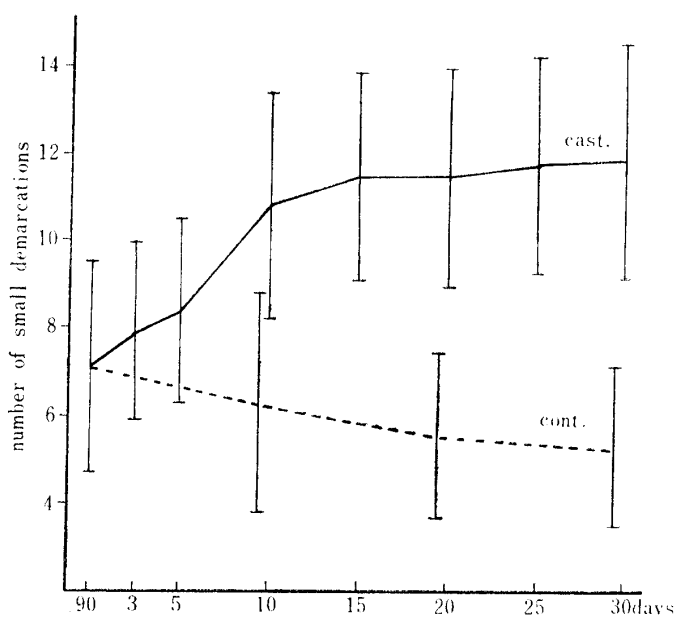


Fig. 5. Area of acini in 335  $\mu^2$ .

ght forth in size of an acinus even after gonadectomy.

b) granular tubules (Fig. 6, 7, 8, 9)

As shown in figure 6, the number of the granular tubules in 335 square microns in a lobulus in the histological sections was left unchanged during 30 days in the control and gonadectomized mice.

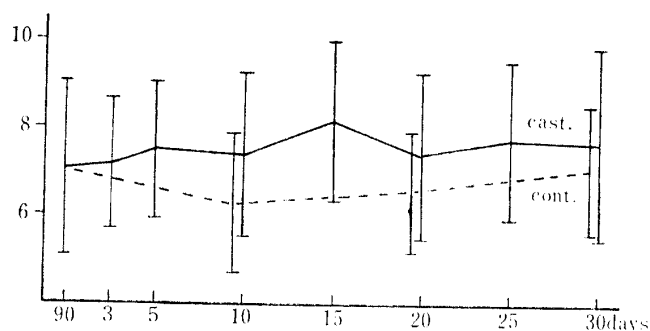


Fig. 6. Number of granular tubules in 335  $\mu^2$ .

On the changes of the area of the granular tubules, however, distinguished differences were noted between the two groups. As shown in figure 7, the area in the control group increased, while that in the gonadectomized group decreased distinctly during the 30 days. Therefore, the size of a granular tubule in the histological section (area of granular tubules/number of granular tubules) showed a distinguished decrease in the gonadectomized mice. Namely, as a result of gonadectomy the granular tubules showed marked atrophy.

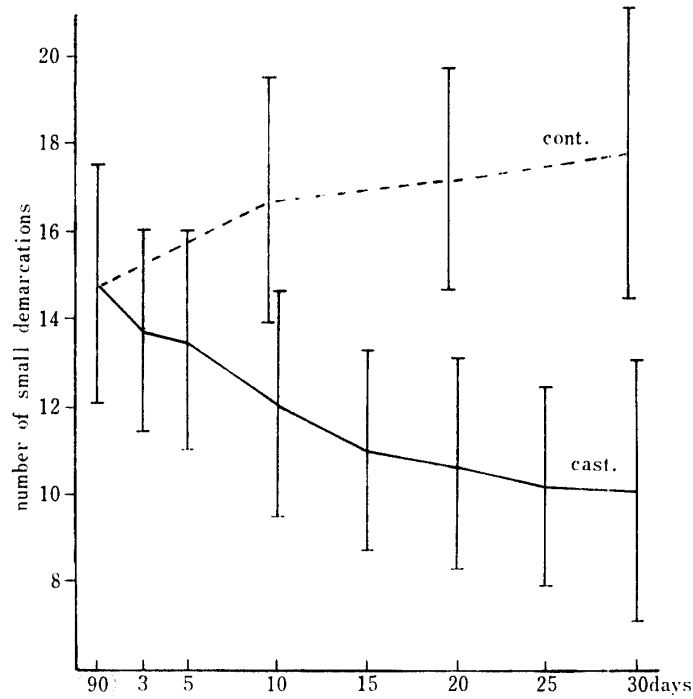


Fig. 7. Area of granular tubules in  $335 \mu^2$ .

The ratio of the area of the granular tubules to the area of the acini, the so-called T/A ratio (area of granular tubules/area of acini) is shown in figure 8. In the control mice 90 days old the mean of T/A was 2.2, and it developed to 3.5 in those of 120 days old, on the contrary, on the 30th day after gonadectomy it decreased to 0.5. Consequently, a great difference between the two groups was recognized.

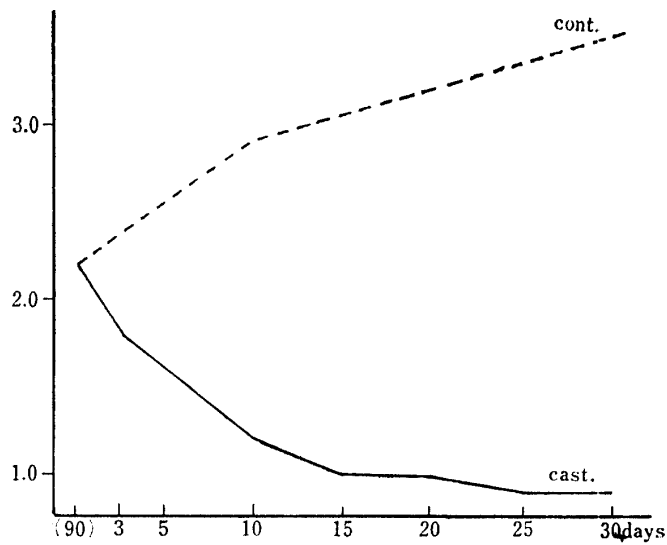


Fig. 8. Tubular/acinar ratio (T/A).

The changes of the diameter of the granular tubules, the height of their epithelia and the diameter of their lumens are recorded in figure 9. The diameter of the granular tubules and the height of the epithelial cells suddenly decreased on the 3rd day after gonadectomy.

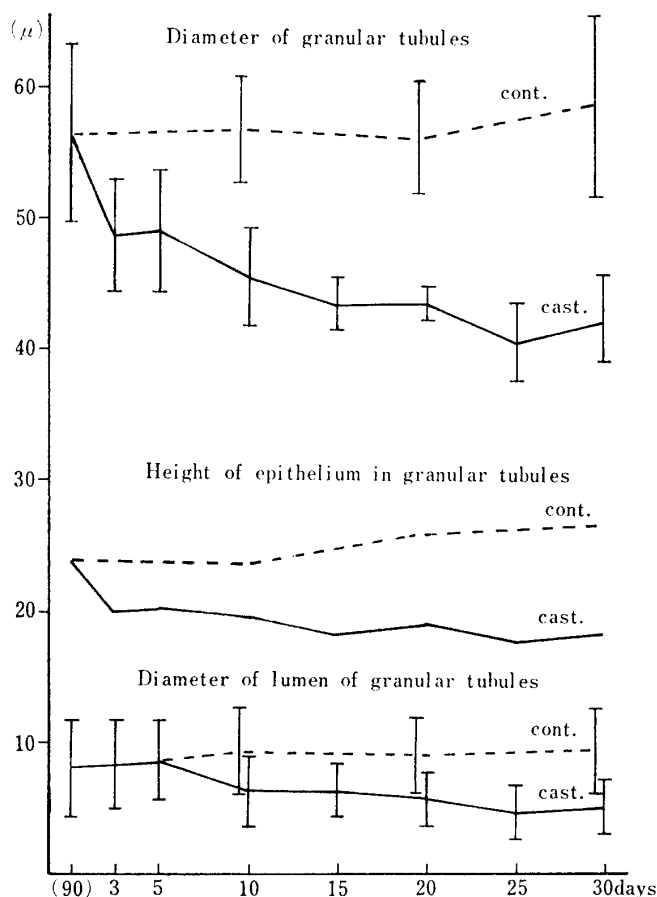


Fig. 9. Diameter of granular tubules

\* Height of epithelium in granular tubules

Diameter of lumen of granular tubules

\* The value is  $\frac{\text{Diameter of granular tubules} - \text{Diameter of lumen of granular tubules}}{2}$

The number of the nuclei in the granular tubules used for the measurement of the diameter had a tendency to be increasing in the control mice, while it had a tendency to be decreasing during 30 days after gonadectomy. The difference between the two groups, however, showed no significance, statistically.

## DISCUSSION

LACASSAGNE (1940)<sup>1)</sup>, RAYNAUD (1950)<sup>8)</sup> and SREEBNY (1960)<sup>4)</sup> reported that the mandibular gland-weight in mice showed decreasing tendency after the gonadectomy. However, they made no observations on the post operation changes covering a long



period.

In the present experiment, the gland-weight and the gland-weight-percentage per body-weight decreased suddenly up to the 5th day after gonadectomy carried out in the mice 90 days old, but after that time, their decreasing was not considerable. On the other hand, the gland-weight in the control group had a tendency to be increasing in those 90~120 days old. Consequently, it may be concluded that the decreasing of the gland-weight occurred during the five days after gonadectomy.

JACOBY (1959)<sup>9)</sup> and CARAMIA (1966)<sup>7)</sup> recorded that acinar development was practically complete between the 4th and 5th week after birth and the duct-transformation was well advanced in the 4th week. In the present experiment, during the period from 90 to 120 days after birth, the granular tubules were still developing slowly, in spite of the fact that the structure of the acini was constant.

JUNQUEIRA et al. (1949)<sup>5)</sup> and many investigators described that by the gonadectomy the granular tubules of the rodent mandibular glands showed atrophy except for the acini. However, no successive quantitative estimation was carried out by them. In the present experiment, the number and area of the acini, in a definite field of the histological section, increased up to the 15th day after gonadectomy, while the size of each acinus remained unchanged. Whereas, the area occupied by the granular tubules decreased in inverse proportion to the area of the acini, while each tubule in histological section showed atrophy in its size. The change appeared very strongly with the advancing lapse of days after gonadectomy. The so-called tubular/acinar ratio employed for the expression of the sex difference in the rodent mandibular glands showed distinctly the feature of the histological change arising on account of gonadectomy.

It was described by JACOBY and LEESON (1959)<sup>10,11)</sup> that during the early stage of post-natal development, the granular tubules in the rat were the same as the intralobular striated duct, shortly after the granules were produced in the epithelial cells near the intercalated ducts, and, after a time, the number of the granules in the cells increased so much that the basal striations became invisible. The present experiment showed that after gonadectomy the granules in the granular tubules decreased gradually and the regions in which the granules decreased markedly were composed of cells lying near the striated tubules and the intralobular striated ducts, and as the result of the decreasing of the granules the basal striations became recognizable.

Considering this together with the fact that the striated tubules became longer after gonadectomy, we may suppose not unreasonably that as the result of the gonadectomy the granular tubules are to be transformed into the striated tubules. It seems that this sort of the atrophic process of the granular tubules after gonadectomy is a reverse change contrasting to the developmental process of the granular tubules in the early post-natal stage.

As the result of the present quantitative experiment, it is re-affirmed that there is a close connection between the testes and the development of the granular tubules of the mandibular glands in mice.

### SUMMARY

The male mice 90 days old were gonadectomized, and the histological changes of the mandibular glands were examined using the quantitative estimation methods on the 3rd, 5th, 10th, 15th, 25th and 30th day after gonadectomy.

1) The mandibular gland-weight and the gland-weight-percentage per body-weight decreased suddenly up to the 5th day after gonadectomy, but after that no decreasing was observable.

2) The number and the area of the acini in a definite field of the histological section increased distinctly up to the 15th day after gonadectomy, the degree of increasing becoming slight after that. On the other hand, the area of the granular tubules decreased clearly after gonadectomy.

Each acinus remained unchanged in its size, while each granular tubule decreased in its size significantly as the result of the gonadectomy.

3) The diameter of the granular tubule and the height of its epithelial cell decreased strikingly on the 3rd day after gonadectomy, diminishing very slowly after that.

4) The content of the granules in the granular tubules decreased gradually after gonadectomy, but wide individual variations were noted in the extent of the decreasing.

5) The decrease of the granules and the appearance of the basal striations in the granular tubular epithelium arose out the region near the striated tubule as well as out of the intralobular striated duct. Therefore, the length of the granular tubule became shorter after gonadectomy.

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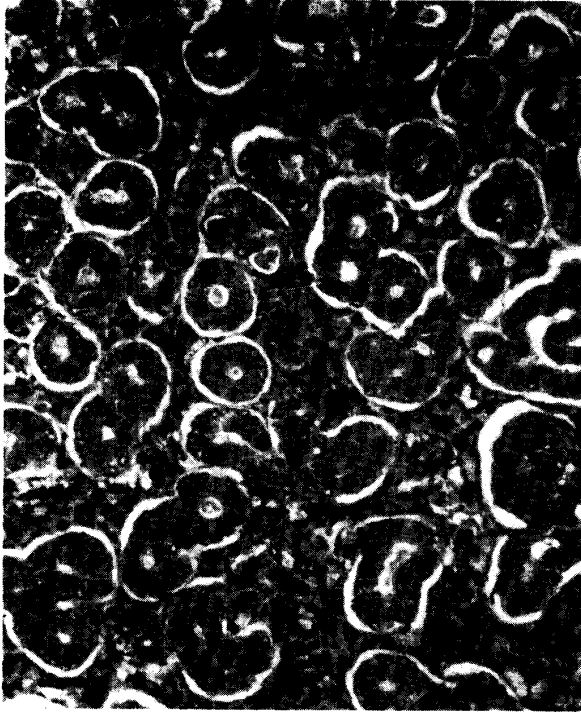


Plate 1.

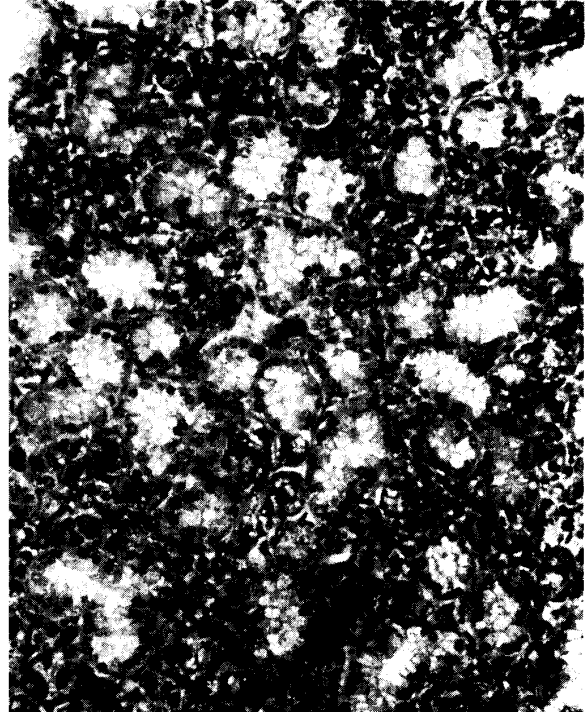


Plate 2.

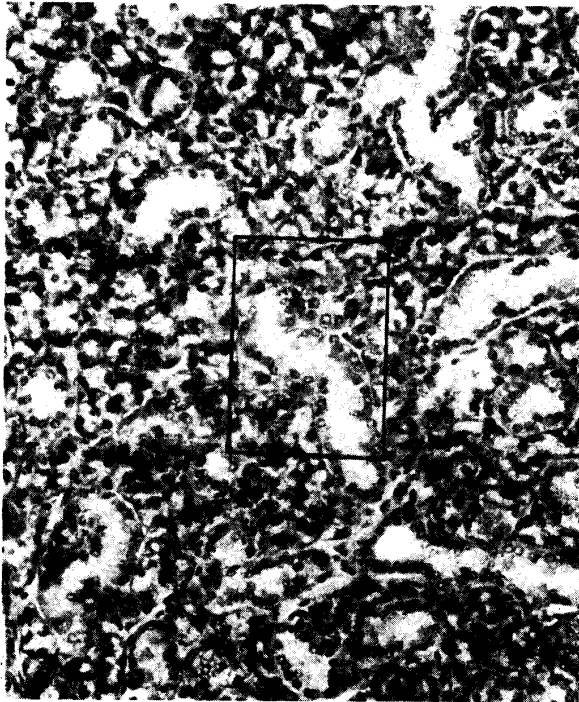


Plate 3.



Plate 4.

**Explanation of Plates**

- Plate 1. Mandibular gland of the 90 days old intact control mouse, showing well developed granular tubules.  $\times 100$ .
- Plate 2. Mandibular gland of mouse on the 10th day after gonadectomy, showing a decrease of granules in the granular tubules.  $\times 100$ .
- Plate 3. Mandibular gland of mouse on the 30th day after gonadectomy.  $\times 100$ .
- Plate 4. Portion of the striated and granular tubules enlarged from the rectangle in Plate 3.  $\times 400$ .
- s : basal striation in the epithelial cell in the striated tubule.
- g : granules in the apical part of the epithelial cell in the granular tubule.