Preliminary Report on the Function of the X-zone of the
Mouse Adrenal Cortex.

マウス副腎皮質のX帯の作用についての予報.

Shiichi NISHIDA and Koshi MOCHIZUKI

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After REICHSTEIN (1938) many workers confirmed the androgenic substance
in the mammalian adrenal cortices biochemically and physiologically, but no one
knew from which zone the cortical androgenic substance was secreted. MASUI and
TAMURA (1924, 1926) and other authors suggested the presence of a relationship
between the X-zone of the mouse adrenal cortex and this substance, but no one has
ever proved it clearly. In mice, the present authors (1956, 1957, 1960) found ZINSSER's (1951) ponceau-fuchsinophilia only in the X-zone cells. They (1955) observed
no particular changes in the epithelial cells of the ventral prostate gland of mice
adrenalectomized soon after castration, but noticeable reduction in cell height and
the complete disappearance of light areas in the epithelial cells of this gland of mice
adrenalectomized at the period of appearance of the X-zone in the adrenal cortex
following castration. From these findings, it can be considered that the X-zone cells
may secrete androgenic substance. This is the reason why bioassay is tried on the
androgenic activity of the X-zone. In this paper, only the results obtained from male
mice are reported. Since the nature of fuchsinophilia of the female X-zone cells
differs from that of the male one (NISHIDA and MOCHIZUKI 1960), it requires
further morphological studies to clarify the former.

I. Materials and Methods.

According to SUZUKI et al. (1955), unilateral transplantation of hypophysis or
androgen pellet into the testis of an immature male rat increases the weight of the
epididymis and ductus deferens on the transplanted side, and the rat is very sensible
to this reaction. Therefore, this reaction was used as the bioassay method in the
present study. Immature Wistar rats weighing 22—25g (twenty days old) and castrated immature ones weighing 46—59g (castrated when body weight reached 40—
43g) were used. They received transplantation of pellet of adult male mouse (castrated or intact) adrenals, cholesterol and 5 per cent androgen respectively (Table 1).
The pellet had been made from whole adrenals and sterilized before transplantation.
Those adrenals which were collected from castrated adult male mice on the 51th day
following castration were examined microscopically for the existence of the X-zone
by the random-sampling method. They were cleaned to be free from fatty tissue and weighed. Then they were dried by the freeze-drying method and stored in the electric refrigerator. Pellet were made from these adrenals. All of the mice were of the bc and D strain and derived from the stock of the authors’ laboratory.

The accessory reproductive glands were examined histologically by using BOUIN’s fixative and hematoxylin-eosin double stain. The height of the epithelial cell was measured in the rat ventral prostate gland in the following manner: In the largest section, the first alveolus was chosen randomly. Then the section was moved 0.5 mm to either direction and an alveolus which came at the center of the microscopic field was selected as the second one. In this way, twenty or more alveoli were submitted to measurement in one animal. Each alveolus was divided into four areas and twenty-five epithelial cells per area (or 100 cells per alveolus) were measured for cell height. In such a small alveolus as consisting of less than one hundred epithelial cells, all cells were measured. Individual cell height was measured by the microscopic micrometer.

The capon-comb test was carried out by the conventional method, in which mouse adrenals were transplanted into combs, or by the method of HOSI et al. (1944), in which adrenal material extracted with alcohol was rubbed into combs. In the former, one or two mouse adrenals were transplanted subcutaneously into one comb. In the latter, extract from one or two adrenals was rubbed into one comb daily. In both methods, the comb on test was examined on the fifth day after the beginning of treatment. Its photograph was taken and the area measured by the planimeter.

II. Observations.

a) Macroscopic observation.

In a preliminary experiment, a pellet prepared from 22 adrenals (dry weight, 14 mg) of castrated adult male mice was transplanted into the right testis of an immature rat (body weight, 23 g). On the other hand, a cholesterol pellet weighing 14 mg was transplanted into the right testis of an animal to serve for a control. In the experimental animal, the epididymis and ductus deferens weighed significantly more heavily on the treated side than on the intact side. In the control animal, however, there was no such difference in weight of these organs between the treated and the intact side. Neither ventral prostates nor seminal vesicles showed the same tendency in weight between the experimental and control animals. The ventral prostate weighed lighter and the seminal vesicle significantly more heavily in the experimental animal than in the control.

If these differences in weight of the epididymis and ductus deferens are due to the androgenic action of the transplanted mouse adrenal cortex, it can be thought that the same reaction may also be shown by the castrated immature rat. An experiment on castrated immature rat gave results which are summarized in Table 1-b. These rats were castrated at the age of twenty-four days (body weight, 40–43 g), used for experimental transplantation five days after castration, and slaughtered seven days after transplantation. A pellet of castrated-mouse adrenals (15 mg) was transplanted into the abdominal muscle. A rat castrated but not treated and two
Table 1. Weights of testis, accessory reproductive organs and adrenal in rats.

a) Preliminary experiment.

<table>
<thead>
<tr>
<th>Rat No.</th>
<th>B. W. at autopsy</th>
<th>Testis (mg)</th>
<th>Epididymis (mg)</th>
<th>Duc. def. (mg)</th>
<th>Ventr. Prost. (mg)</th>
<th>Sem. Ves. (mg)</th>
<th>Adrenal (mg)</th>
<th>Remarks</th>
</tr>
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<tbody>
<tr>
<td>R-1</td>
<td>53</td>
<td>229</td>
<td>140</td>
<td>29.0</td>
<td>25.0</td>
<td>15.6</td>
<td>12.9</td>
<td>14.7**</td>
</tr>
<tr>
<td>R-2</td>
<td>59</td>
<td>146</td>
<td>169</td>
<td>24.1</td>
<td>22.9</td>
<td>14.7</td>
<td>14.3</td>
<td>27.0</td>
</tr>
</tbody>
</table>

b) Experiment on castrated rats.

<table>
<thead>
<tr>
<th>Rat No.</th>
<th>B. W.</th>
<th>Testis (mg)</th>
<th>Epididymis (mg)</th>
<th>Duc. def. (mg)</th>
<th>Ventr. Prost. (mg)</th>
<th>Sem. Ves. (mg)</th>
<th>Adrenal (mg)</th>
<th>Remarks</th>
</tr>
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<tbody>
<tr>
<td>R-3</td>
<td>76</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R-4</td>
<td>61</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R-5</td>
<td>71</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R-6</td>
<td>65</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>12.1</td>
<td>6.4</td>
<td></td>
<td></td>
<td>5.0</td>
<td>20.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>10.9</td>
<td>5.9</td>
<td></td>
<td></td>
<td>7.9</td>
<td>19.2</td>
<td>Female mouse adrenals, 15 mg</td>
</tr>
<tr>
<td></td>
<td></td>
<td>40.0</td>
<td>20.4</td>
<td></td>
<td></td>
<td>44.9**</td>
<td>84.0</td>
<td>15.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10.9</td>
<td>6.2</td>
<td></td>
<td></td>
<td>6.6</td>
<td>18.4</td>
<td>Castrated</td>
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</table>

c) Experiment on intact immature rats.

<table>
<thead>
<tr>
<th>Rat No.</th>
<th>B. W.</th>
<th>Testis (mg)</th>
<th>Epididymis (mg)</th>
<th>Duc. def. (mg)</th>
<th>Ventr. Prost. (mg)</th>
<th>Sem. Ves. (mg)</th>
<th>Adrenal (mg)</th>
<th>Remarks</th>
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<tr>
<td>R-7</td>
<td>33</td>
<td>64</td>
<td>80</td>
<td>11.3</td>
<td>11.3</td>
<td>7.9</td>
<td>7.2</td>
<td>5.9</td>
</tr>
<tr>
<td>R-8</td>
<td>28</td>
<td>71</td>
<td>62</td>
<td>10.9</td>
<td>10.7</td>
<td>6.3</td>
<td>6.4</td>
<td>*</td>
</tr>
<tr>
<td>R-9</td>
<td>29</td>
<td>93</td>
<td>70</td>
<td>11.0</td>
<td>10.2</td>
<td>8.3</td>
<td>8.4</td>
<td></td>
</tr>
<tr>
<td>R-10</td>
<td>30</td>
<td>70</td>
<td>60</td>
<td>10.3</td>
<td>9.5</td>
<td>7.2</td>
<td>6.5</td>
<td>6.7</td>
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<tr>
<td>R-11</td>
<td>30</td>
<td>67</td>
<td>61</td>
<td>11.0</td>
<td>10.8</td>
<td>8.2</td>
<td>8.5</td>
<td><em>,</em>*</td>
</tr>
</tbody>
</table>

* Mitotic figures and ** colloid substance were observed. The animals of each experimental group are litter mates.
rats given a cholesterol pellet (14.6 mg) and a 5 per cent androgen pellet (15 mg),
respectively, by intramuscular transplantation served as controls. The epididymis,
ductus deferens, ventral prostate, and seminal vesicle weighed more heavily in the
androgen-administered animal than in the others. There were no significant differ-
ences among the other three castrated animals (one administered with mouse adren-
als, one with a cholesterol pellet, and one not treated). In these animals, the
ventral prostate gland was pale in color and too small to be weighed exactly. There-
fore, these animals were discarded from the weight data of this gland. On the other
hand, the adrenal weight was the lightest in the androgen-administered rat. This
result was inconsistent with that of the preliminary experiment. Then, in order to
confirm the result of the latter, an experiment was performed with intact immature
male rats (Table 1-c). Pellets of castrated-male-mouse adrenals weighing 5.6 to 13.2 mg
were transplanted. Sham operation and transplantation of adrenal pellet (10.7 mg)
derived from adult male mice (having no X-zone) were carried out in the control ex-
periment. The ventral prostate glands were discarded from the weight data in this
experiment for the same reason as in the preceding experiment. The table shows no
significant differences between the treated and intact sides of the animals. From
these weight data, it cannot be considered that the X-zone secretes any androgenic
substance.

b) Histological observation.

Further histological examination was performed on the ventral prostate glands
and seminal vesicles of the rats. The data on epithelial cell height are given in
Table 2 and Fig. 1. No epithelial cell height was measured in the seminal vesicle.
The epithelial cells of the ventral prostate were significantly higher in the experi-
mental animals than in the controls, including those given adult-male-mouse adrenals.
Among those animals to which had been transplanted pellets of castrated-adult-male
mouse adrenals, there were no significant differences in the height of the epithelial
cells of the ventral prostate. In the androgen-administered animal (positive control),
the epithelial cells of this gland were higher than in any other animal on experi-

<table>
<thead>
<tr>
<th>Rat No.</th>
<th>Mean (μ)</th>
<th>Lower limit (μ)</th>
<th>Upper limit (μ)</th>
<th>Remarks</th>
</tr>
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<tbody>
<tr>
<td>R-1</td>
<td>16.86</td>
<td>15.94</td>
<td>17.80</td>
<td>$\oplus$ mouse adrenals, 14 mg</td>
</tr>
<tr>
<td>R-2</td>
<td>9.46</td>
<td>9.06</td>
<td>9.86</td>
<td>Cholesterol, 14 mg</td>
</tr>
<tr>
<td>R-3</td>
<td>6.64</td>
<td>6.00</td>
<td>7.22</td>
<td>$\ominus$, 14.6 mg</td>
</tr>
<tr>
<td>R-4</td>
<td>6.80</td>
<td>6.44</td>
<td>7.14</td>
<td>$\oplus$ mouse adrenals, 15 mg</td>
</tr>
<tr>
<td>R-5</td>
<td>21.94</td>
<td>20.12</td>
<td>23.76</td>
<td>5% androgen, 15 mg</td>
</tr>
<tr>
<td>R-6</td>
<td></td>
<td></td>
<td></td>
<td>Not measured</td>
</tr>
<tr>
<td>R-7</td>
<td>9.98</td>
<td>9.28</td>
<td>10.72</td>
<td>Sham operation</td>
</tr>
<tr>
<td>R-8</td>
<td>9.44</td>
<td>8.96</td>
<td>10.30</td>
<td>$\oplus$ mouse adrenals, 10.7 mg</td>
</tr>
<tr>
<td>R-9</td>
<td>16.64</td>
<td>15.46</td>
<td>17.82</td>
<td>$\oplus$ mouse adrenals, 13.2 mg</td>
</tr>
<tr>
<td>R-10</td>
<td>15.56</td>
<td>14.38</td>
<td>16.68</td>
<td>$\ominus$, 8.8 mg</td>
</tr>
<tr>
<td>R-11</td>
<td>18.14</td>
<td>16.12</td>
<td>20.16</td>
<td>$\ominus$, 5.6 mg</td>
</tr>
</tbody>
</table>

The results of the experiment on castrated rats showed no increase in cell height by the X-zone pellet (compare R-3 and R-4 in Table 2 and Fig. 1). These results agreed with the weight data. In all the animals to which had been transplanted X-zone or androgen pellets, some epithelial cells of the ventral prostate contained light areas in them. Such light areas were not seen in all the negative control animals, including one to which a pellet from adult-male-mouse adrenals had been transplanted (Figs. 3, 4, 7, and 8). The alveoli of the ventral prostate were longer in diameter in the animals administered with X-zone or androgen pellets than in the negative control animals. The epithelial cells of the ventral prostate were low and cuboidal in shape and had no such light areas as mentioned above in the control animals, but contained a few mitotic figures in the animal administered with an adult male-mouse adrenal pellet (Fig. 8). Colloid substance was observed in the glandular lumen in some experimental animals (Table 1 and Fig. 2).

Since the epithelial cell height of the seminal vesicle was not measured, nothing can be stated about it. The development of the folds of the mucosa and epithelial cell height, however, were observed in the experimental animals. The development was quite significant in the positive control animal (Fig. 15). Neither secretion granules nor halo-like areas were present even in the glandular cells in all the experimental animals, except the positive control. These findings should be noted. In this study, the cytoplasm of epithelial cells of the seminal vesicle was pale, as compared with that of an adult male rat.

The capon comb test for androgenic substance of the X-zone was performed by both transplanting and rubbing methods with only negative results (Table 3).
Fig. 2. No. R-1, preliminary experiment. Note light areas and colloid substance. Pellet: castrated mouse adrenals, 14 mg.

Fig. 3. No. R-2, preliminary experiment. Note low epithelial cells. No light areas are seen. Pellet: cholesterol, 14 mg.

Fig. 4. No. R-3, castrated immature rat. Epithelial cells are low and pale. Pellet: cholesterol, 14.6 mg.

Fig. 5. No. R-4, castrated immature rat. No significant changes are seen. Pellet: castrated mouse adrenals, 15 mg.

Figs. 2–19. All sections were fixed with BOUIN’s fixative and stained by the hematoxylin-eosin double staining method. Figs. 2–10: Ventral prostate glands, Figs. 11–19: Seminal vesicles. ×420
Fig. 6. No. R-5, castrated immature rat. Note tall epithelial cells, light areas and colloid substance. Pellet: 5% androgen, 15 mg.

Figs. 7 and 8. No. R-8. Low epithelial cells and no light areas are observed. Tall cells are shown locally (Figs. 7 and 8). Mitotic figure is present in these cells (Fig. 8). Pellet: male mouse adrenals, 10.7 mg.

Fig. 8

Fig. 9. No. R-10. Tall epithelial cells and light areas are shown. Pellet: castrated mouse adrenals, 8.8 mg.
Fig. 10. No. R-11. Tall epithelial cells, a mitotic figure and a few light areas are seen. Compare this case with No. R-10. Pellet: castrated mouse adrenals, 5.6 mg.

Fig. 11. No. R-1. Note tall epithelial cells. Mucosal folds have developed, forming alveolar structure.

Fig. 12. No. R-2. Note low epithelial cells and simple mucosal folds.

Fig. 13. No. R-3. Note low epithelial cells and simple folds.
Fig. 14. No. R-4. Note epithelial cells a little taller than those of rat No. R-3 and their pale cytoplasm.

Fig. 15. No. R-5. Note the tallest epithelial cells, complete alveolar structure, and a small amount of colloid substance. Secretion granules and halolike areas are not clearly seen at this magnification.

Fig. 16. No. R-7, subjected to sham operation. Note low epithelial cells and simple folds.

Fig. 17. No. R-8. No development is shown in epithelial cells and folds.
Fig. 18. No. R-9. Note a little taller epithelial cells and a little developed folds. Pellet: castrated mouse adrenals, 13.2 mg.

Fig. 19. No. R-11. There are no significant differences between rat No. R-9 and this case, except that the development of folds is a little less conspicuous in this case.

Table 3. Results of capon comb test for X-zone (areas, not actually measured but determined by planimeter).

<table>
<thead>
<tr>
<th>Capon No.</th>
<th>Before test (A)</th>
<th>After test (B)</th>
<th>B - A</th>
<th>Remarks</th>
</tr>
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<tr>
<td></td>
<td></td>
<td></td>
<td>B ×100</td>
<td></td>
</tr>
<tr>
<td>650</td>
<td>21.0</td>
<td>22.1</td>
<td>5.2</td>
<td>Transplanting</td>
</tr>
<tr>
<td>651</td>
<td>24.4</td>
<td>24.9</td>
<td>6.4</td>
<td></td>
</tr>
<tr>
<td>653</td>
<td>20.3</td>
<td>21.2</td>
<td>4.4</td>
<td></td>
</tr>
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<tr>
<td>661</td>
<td>20.1</td>
<td>21.2</td>
<td>5.5</td>
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<tr>
<td>673</td>
<td>21.5</td>
<td>22.4</td>
<td>4.2</td>
<td>Rubbing</td>
</tr>
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<td>686</td>
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<td>36.7</td>
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<td>688</td>
<td>18.5</td>
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<td>698</td>
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<td>24.5</td>
<td>17.8</td>
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<td>699</td>
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<td>41</td>
<td>17.0</td>
<td>23.8</td>
<td>40.0</td>
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</tr>
<tr>
<td>163</td>
<td>17.3</td>
<td>26.9</td>
<td>55.5</td>
<td></td>
</tr>
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<td>687</td>
<td>19.1</td>
<td>27.4</td>
<td>43.5</td>
<td></td>
</tr>
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<td>154</td>
<td>28.4</td>
<td>28.4</td>
<td>0</td>
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<td>677</td>
<td>26.7</td>
<td>26.9</td>
<td>0.7</td>
<td></td>
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III. Discussion.

It has been reported that the X-zone is a reserved zone and has no specific functions (GERSH and GROLLMAN 1939a). However, from the results of the cytological observations in general (MASUI and TAMURA 1924, 1926, HOWARD-MILLER 1927, DEANESLY 1928, and others), and on RNA (MOCHIZUKI and NISHIDA 1952) and vitamin C (NISHIDA and MURAMATSU 1949, ASAKURA and NISHIDA 1955) in the X-zone of the mouse adrenal cortex, it can be considered that the X-zone may have some functions. It has been well known physiologically that there is some relationship between the X-zone and androgenic substance, or that androgen eliminates the X-zone (MARTIN 1930, POLL 1933 a, b, TAKEWAKI 1935, 1938, NISHIDA 1938). On the other hand, it has not been clarified whether the X-zone actually secretes androgenic substance or not. The authors (1956, 1957, 1960) found ZINSSER's ponceau-fuchsinophilia only in the X-zone cells. ZINSSER and ZINSSER (1951) regarded this fuchsinophilic substance as ketosteroid or its precursor. Besides, in adult male mice, adrenalectomy performed soon after castration had no effect on the epithelial cell height of the ventral prostate gland, while that carried out at the period of appearance of the X-zone following castration caused a decrease in epithelial cell height and the complete disappearance of light area from the epithelial cells (NISHIDA and MOCHIZUKI 1955). These facts support the view that androgenic substance is secreted from the X-zone. On the contrary, there are some investigators who could not recognize physiologically the presence of androgenic substance in the X-zone (HOWARD 1946, GERSH and GROLLMAN 1939b).

SUZUKI et al. (1955) reported that unilateral transplantation of androgen pellet into the immature rat testis increased the weight of the epididymis on the treated side. The results of the present study, except the preliminary experiment, showed no significant difference in organ weight between experimental and control animals. Histologically, however, the transplantation of adrenal pellets from castrated adult male mice caused a definite increase in cell height and development of light areas in the epithelial cells, and enlargement of the alveolar lumen and appearance of colloid substance in the ventral prostate gland of immature rats. Since MOORE et al. (1930 a, b), PRICE (1936), and others proved that these histological changes were produced by androgen, it can be considered that the changes observed in the present study were caused by androgenic substance in the X-zone and that androgenic reaction was recognized histologically even when it could not be demonstrated by organ weight.

From the results of the present study, it may be considered that the mouse adrenal cortex exercises a trophic activity directly or indirectly on the immature rat testis and increases the amount of androgen secreted from the rat testis. Taking these results and those obtained from adrenalectomized mice (NISHIDA and MOCHIZUKI 1955), it may be more appropriate to conclude that the X-zone secretes androgenic substance than that it does trophin.

Contrary to the results reported by MOORE et al. (1930 b), the present study made it clear that the changes of the seminal vesicle were less significant than those of the ventral prostate gland in the experimental animals. These changes, however,
were undoubtedly androgenic, for the epithelial cells were higher and the folds of the mucosa greater in the animals administered with pellets from the X-zone and androgen than in the negative control. Neither secretion granules nor halo-like areas could be observed in the epithelial cells of all the animals on experiment, except a positive control. In the present study, only negative results were given by the tests on castrated rats and the capon comb test and the majority of the transplantation experiments performed to get weight data. It has been well known that the sensitivity of the prostate gland to androgen is reduced in castrated rats. CARNES (1940) obtained negative results from his assay for the human fetal cortex, using 1 day old chick comb test. It can be considered that these negative results were obtained because the test material had been used in small doses. These facts indicate that the androgenic substance in the X-zone is small in amount, even if it is actually secreted from this zone. It can be concluded that the secretion of androgenic substance is one of the functions, if not the only function, of the X-zone.

It is of great interest to note that a mitotic figure was observed in a few epithelial cells of the ventral prostate gland of a rat to which had been transplanted a pellet of adult male mouse adrenals. Further studies are required to give satisfactory explanation to this finding.

IV. Summary.

Bioassay for the androgenic activity of the X-zone of the adrenal cortex of the castrated adult male mouse was performed by a method in which a pellet was transplanted into the testis of an immature rat. As controls, a rat subjected to sham operation and these administered with pellets of cholesterol, adrenals of intact adult male mice, or androgen (positive control) were used in this study. Weighing and histological examinations were performed. The results obtained are summarized as follows.

The weight data collected from the epididymis, ductus deferens, ventral prostate gland, and seminal vesicle showed no significant differences between the experimental and the control rats, except one used in a preliminary experiment.

In histological observation, the epithelial cells of the ventral prostate gland were higher in the experimental animals than in the control. They were significantly higher even in a rat administered with a pellet weighing 5.6mg than in the control. The highest epithelial cells were found in an animal to which had been transplanted an androgen pellet (positive control). Light areas were observed in the epithelial cells of this gland in all experimental animals, but not in all control animals, except a positive control. Colloid substance was present in the alveolar lumen of the ventral prostate gland in some of the experimental animals, and more abundantly in the positive control than these animals. The alveoli of the gland were larger in diameter in the positive control than in the experimental animals.

In the seminal vesicle there was not so significant a histological difference as in the ventral prostate gland. After the transplantation of X-zone and androgen pellets, the epithelial cells became higher and the folds of the mucosa greater, but no secretion granules nor halo-like areas appeared, except in the positive control.
The results of capon comb test on the X-zone were negative. From these findings, it can be considered that the X-zone secretes androgenic substance, though small in amount.

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であることによって否定される。

輪冠テストの結果も陰性であったが、使用量の不足のためと考えられる。

以上の結果を総合すれば、X帯投与によって、まだ重量的変化を示さない前に
組織学的変化を生じたものであろう、X帯からは男性ホルモン様物質が分泌され
るが、その量は微量であると考えられる。

References.

Asakura, S. and S. Nishida: Morphological studies on the distribution of vitamin C in
the adrenal cortices of mice. II. Relationship between estrous cycle and the distribution type in
X-zone of the adrenal gland of the mouse. Anat. Rec. 75 (1939 a) P. 131-153. — The relation of
the adrenal cortex to the male reproductive system. Amer. J. Physiol. 126 (1939 b). P. 368-
374. — Hosi, T., Y. Suzuki and T. Kasiwabara: Studien über die Hodenfunktion beim
Pferde unter besonderer Berücksichtigung der jahreszeitlichen Schwankungen der in Harne aus-
transitory zone in the adrenal cortex which shows age and sex relationship. Amer. J. Anat.
40 (1927). P. 251-293. — Howard, E.: The effect of adrenalectomy on the accessory reproduc-
S. J.: Effect of certain endocrine secretion on the X-zone of the adrenal cortex of the mouse.
gonadectomy on the structure of the suprarenal glands of mice, with special reference to the
Nishida: The cytoplasmic basophilia (ribonucleic acid) of the X-zone of the mouse adrenal
ences of hypophysectomy in mice. III. Effects upon the ovary, uterus, vaginal epithelium and
Gallagher: Rat-prostate cytology as a testis-hormone indicator and the prevention of castra-
tion changes by testis extract injection. Amer. J. Anat. 45 (1930 a). P. 71-107. — Moore, C. R.,
W. Hughes and T. F. Gallagher: Rat seminal-vesicle cytology as a testis-hormone indicator and
the prevention of castration changes by testis extract injection. ibid. 45 (1930 b). P. 109-
136. — Nishida, S.: Effects of testosterone upon the adrenal cortex of male mouse. J. Chosen
on the distribution of vitamin C in the adrenal cortices of mice. I. Reduced type vitamin C. Jap.
my upon the ventral prostate glands in mice. Endocrinol. Jap. 2 (1955). P. 289-296. — In-
influences of hypophysectomy in mice. II. Effects upon testis, ventral prostate, seminal vesicle
77 (1933 b). S. 113-123. — Price, D.: Normal development of the prostate and seminal vesicle
of the rat with a study of experimental postnatal modifications. Amer. J. Anat. 60 (1936).