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SOME BIOLOGICAL ASPECTS ON THE PREPUTIAL GLAND OF THE FEMALE RAT

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Masao WAKABAYASHI, Toyomi FUJITA, Hidenori NINOMIYA, Kazushige NAKAMURA and Yoichi SHIRAI. *Some Biological Aspects on the Preputial Gland of the Female Rat.* Kobe J. Med. Sci. **12**, 71-89, June 1966. The preputial gland of rat was very rich in β-glucuronidase as reported first by Beyler and Szego in 1954 (1, 2). Several investigations on the gland itself have been made in connection with steroid (3-6), but any role of the gland or significance of the enzyme in it have not been clarified yet. The present paper deals with correlation of β-glucuronidase with morphology and histochemistry first, then applying the enzyme in the gland to DEAE-cellulose column chromatography, several components of the enzyme have been seperated and properties of these enzymes have been comparatively studied by inhibited attitude for heavy metal ions, Glucaric acid and by heat denaturation.

INTRODUCTION

The existence of the preputial (clitoral) gland of the rodents has been well known since long time ago (7, 8), and concerning the gland several investigations have been reported in connection with steroid hormones (3-6), but a significance of the gland has not so far been clarified yet. Beyler and Szego (1, 2), in 1954, reported that the preputial gland of the female rat had a β-glucuronidase activity of the order of 1,500 phenolphthalein units/mg of moist tissue, making it the richest known source of the enzyme, but any role of such the richest enzyme existence in the gland has not been known at all.

From these information, it was decided that correlative study of the gland with the enzyme would be profitable, and first the morphological and histochemical studies were systematically carried out, then the β-glucuronidase components in the gland were seperated applying the enzyme to DEAE-cellulose column chromatography. Properties of such the seperated enzyme components were compared each other by the attitude for heavy metal inhibitors, heat denaturation and D-glucaro-(1→4)-lactone which was a specific inhibitor for β-glucuronidase.

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MATERIALS AND METHODS

1. *Assays of β-glucuronidase*

The method employing biosynthetic phenolphthalein mono-β-glucuronide as substrate was employed here according to Talalay *et al.* (9).

2. *Protein determination*

Protein was determined by the colorimetric method of Lowry *et al.* (10). Crystalline albumine from the bovine serum (Armour Laboratory) was employed as standard of protein determination.

3. *Histological studies on the preputial gland*

A preputial gland removed from the female rat weighing 120 g was fixed in neutral formalin. The vertical serial sections were first made in parallel to the urethra at 5μ in thickness, and stained with Hematoxylin-eosin. Another block of tissue was embedded in celloidin after fixing in neutral formalin. Next, serial cross sections to the longitudinal axis of the urethra were cut at 20μ in thickness. These sections also were stained with Hematoxylin-eosin.

4. *Localization of β-glucuronidase in the preputial gland by the method of enzymorphology*

A) 8-Hydroxyquinoline glucuronide method

This procedure followed the methods of Fishman and Baker (11), and Kuroda (12) with some modification for the last part.

1) Substrate

a) Preparation of substrate

Biosynthesis of 8-hydroxyquinoline glucuronide was performed as follows: Instead of 8-hydroxyquinoline, 2 g of water soluble 8-hydroxyquinoline sulfate (Wako Pure Chemical Industries Ltd.) recommended by Kuroda (12), was dissolved in 2 ml of water and administered through a glass syringe and stomach tube into rabbit stomach. The urine was filtered through cotton gauze, adjusted to pH 4.0 and stored in the cold room. Next day, crop of greenish crystals of glucosiduronic acid were removed by filtration and dried in the air.

b) Preparation of substrate solution

This step followed Fishman and Baker's method (11).

2) Test procedure

This step was markedly improved. A block of the tissue not more than 2 to 3 mm thickness was fixed in 10% neutral formalin at 4°C for 2 hours and the tissue was rinsed briefly in cold distilled water. The rinsed tissue was cut on the freezing microtone at 10-20μ, receiving the sections into distilled water. These sections were transfered to the incubation solution, reported by Fishman and Baker contained in a small beaker. It was immediately incubated at 37.5° for one and half hour, and it was steeped in 0.5 M oxalic acid, sodium oxalate buffer, pH 5.0, for 2 or

3 minutes and then rinsed in distilled water briefly. It was steeped in mixture of 1% aqueous potassium ferrocyanide added the same volume of N-HCl for 20 minutes. It was stained in basic resorcin-fuchsin which was prepared as follows: 0.2 g of resorcin-fuchsin in 20 ml of 2% HCl-alcohol (1 : 1) placed in a motor was ground to dissolve, then 40 ml of 1% HCl-alcohol was poured in this resorcin-fuchsin solution. Dehydration was performed successively in 70% ethanol, absolute ethanol (2 changes), xylene, toluene and mount in Balsame.

B) Naphtol AS-BI (6-bromo-2 hydroxy-3 naphthoyl-o-anisidine) glucuronide method

Method (A) has been critisized on the specificity for β-glucuronidase, so in order to make sure, the procedures for naphthol AS-BI glucuronide method (13) have been employed here. Substrate, naphthol AS-BI glucuronide, was supplied as generous gift by Dr. S. Suzuki, Laboratory of Chugai Pharmaceutical Co. Ltd.

5. *Fractionation of subcellular components of the preputial gland and liver homogenates*

5 female rats weighing 140 g each were anethsized under ether and rapidly bled by cutting through carotid artery. The preputial glands were removed from rats and minced with scissors into a pyrex homogenizer. As soon as possible, all further manipulations were carried out at 0-4°. 0.25 M sucrose solution was added in a portion 9 ml/g of tissue. The homogenate was very carefully completed in 2 minutes with 30 seconds interval. The homogenate was subjected to differential centrifugation in the Model L Spinko ultracentrifuge equipped with roter No. 40 according to Hegeboom and coworkers' method (14). The liver homogenate was performed by the same method, too. Each step by differential centrifugation was examined under the electron microscope. It was noticed that the first residue by centrifugation was still contaminated with a small amount of particles of the second step, but the sample such obtained at each step was employed for the following experiments as it was. β-Glucuronidase activity in each residue suspended in acetate buffer, pH 4.5 were assayed. The supernatant for the last part dialyzed against same acetate buffer overnight was employed for β-glucuronidase assay.

6. *Seperation of the preputial gland enzyme by DEAE-cellulose column chromatography*

Chromatography was performed by the methods of Moore and Lee (15), and Kawada *et al.* (16) on the enzyme of the preputial glands. DEAE-cellulose (SERVA) had an exchange capacity of 0.5 meq/g. The column was packed 1 g per 10 to 15 ml, as a depth 30 cm in 1.1 cm inside diameter tube with fritted glass bottom (total is 5 g of cellulose). First, the minced preputial gland in isotonic sucrose sotlution was homogenized for 2 minutes and the homogenate was centrifuged at 18,000×g for 3 hours at low temperature. The resulting supernatant containing about 80% of total β-glucuronidase in the gland was absorbed on the DEAE-cellulose in column. The column was first washed by 200 ml of 0.005 M Tris-phosphate buffer, pH 7.8, then eluted by a linear gradient of NaCl concentration from 0.1 to 0.3 M in 0.005 M Tris-phosphate buffer, pH 7.8. This main components obtained

by such elution were concentrated in a dyalyzing tube embedded in Carbowax 1,500 (polyethylen glycol 1,500). The concentrated enzyme fractions were applied to the column again, and eluted stepwisely by 0.13, 0.183 and 0.23 M NaCl in the same buffer mentioned above respectively.

7. *Inhibition experiment*

a) General assay procedure

The incubation mixture consisted of 0.1 ml of enzyme, 0.8 ml of buffer, and 0.1 ml of substrate. These mixtures were incubated at 37.5° for 2 hours at the pH range from 3.5 to 8.0. In the case of the inhibition experiments, 0.1 ml of inhibitor solutions consisted of different concentration was added to 0.7 ml of buffer.

b) Buffers

Two buffers with a series of pH values (3.5 to 8.0) were made up: 0.1 M acetic acid and sodium acetate buffer (3.5 to 5.5) and 0.1 M sodium phosphate monobasic and dibasic buffer (6.0 to 8.0).

c) Enzyme

Most important was the attempt to adjust 5 peaks of β-glucuronidase seperated by the column to dilution at which each barely accomplished 100% hydrolysis of 0.002 M phenolphthalein β-glucuronide at the optimal for each enzyme. Specific activities of these enzymes were as follows: A-fraction, eluted by 0.13 M NaCl concentration, 19,500 (A-1), 101,500 (A-2) and 180,000 (A-3); B fraction (B) eluted by 0.183 M NaCl, 33,900; C fraction (C) eluted by 0.23 M NaCl concentration, 3,680, respecitively.

d) Heat denaturation of the enzymes seperated by DEAE-cellulose column

Each enzyme protein amount was adjusted to 600 μg per ml by adding bovine serum albumin (Armour Laboratory). All glass tubes each of which contained 1 ml of enzyme (in the presence of albumin) was set in a 100 ml beaker half-filled with water maintained at the desired temperature. The tubes were taken out of the beaker at regular intervals up to 15 minutes. This experiment was performed at two temperatures (60 and 65°).

e) Heavy metal ion inhibitors

As effect of inhibitors concentration, 0.63 mM of $CuSO_4$, 0.04 mM of $HgCl_2$ and 0.16 mM of $AgNO_3$ were employed according to Fernley (17).

f) D-Glucaro-(1→4)-lactone

One-third of bipotassium D-glucarate was converted into D-glucaro-(1→4)-lactone by boiling D-glucarate solution for 30 minutes. Effect of the inhibitor was observed in a concentration of 10^{-6} M as D-glucarate.

RESULTS

1. *Distribution of β-glucuronidase activity in organs of both female and male rats*

Total and specific activities of β-glucuronidase in each moist tissue of both the female and male were determined as shown in Table 1. These activities in the

Table 1 Distribution of total and specific activities of β-glucuronidase in both male and female rats

ORGAN	SEX	β-GLUCURONIDASE	
		Total activity (unit)	Specific activity (unit/mg)
PREPUTIAL GLAND	male	42,900	8,580
	female	52,500	11,290
LIVER	male	350,000	497
	female	343,037	435
KIDNEY	male	8,500	100
	female	9,000	192
SPLEEN	male	29,424	940
	female	20,950	709
ADRENAL	male	1,200	156
	female	1,465	263
INTESTINE	male	9,200	98
	female	8,304	149
STOMACH	male	2,760	41
	female	1,660	77
TESTIS	male	14,456	240
	female	—	—
OVARIUM	male	—	—
	female	456	276
VAGINA	male	—	—
	female	5,060	604
UTERUS	male	—	—
	female	442	23
HEART	male	201	20
	female	150	13
BRAIN	male	825	36
	female	900	41
LUNG	male	1.160	70
	female	1,200	65
THYROID	male	850	130
	female	1,100	121
SERUM (u./ml)	male	60	1.3
	female	67	1.2
URINE	male	0	
	female	0	

Specific activity = activity (units)/protein (mg)

preputial gland in both female and male rats were extremely higher than those in other organs. The enzyme activities in the liver, spleen and vagina were rather strong, whereas their purities were relatively low basing upon their specific activities. Enzyme activity in the peputial gland of female rat surpassed always to that of male rat. Concerning total activity, however, the liver was the strongest and the preputial gland and spleen followed this.

2. *Histology of the normal preputial gland of female rat*

When the section was made in parallel to the longitudinal axis of the urethra, many mucous glandular cells like the sebaceous gland cells were observed. Half the number of these glandular cells is no relation to localization of hair follicles (Plate 1). Fat tissue was very rich and capillaries also were often seen arround the cells in the gland. Several ducts surrounded by basal cells could be often seen (Plate 2). After embedding the gland tissue in celloidin, serial vertical sections to the longitudinal axis of the urethra were made and serial sections on the duct were shown in the next plates. The Plate 3 shows two ducts just risen from the glandular cells and the Plate 4 indicates these two ducts running in parallel with the urethra. The last Plate shows the two ducts just opened into the urethra (Plate 5).

3. *Localization of β-glucuronidase in the normal preputial gland*

As reported by Fishman and Baker (11), intense β-glucuronidase activity was stained in glandular epithelium. The cytoplasma stained deeply. The basal cells lining the duct were strongly reactive (Plate 6).

Another method employing naphol AS-BI glucuronide as substrate also showed intense enzyme activity stained in the basal cells lining duct and also around the urethra (Plate 7).

4. *Seperation of subcellular components from the preputial glands homogenates*

Distribution of β-glucuronidase in the gland cells studied by histochemical method also was confirmed by the differential centrifugation method of Hogeboom

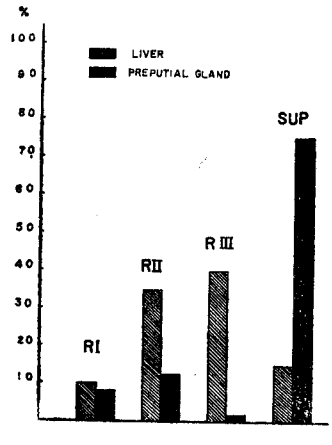

Fig. 1 Separation of subcellular components from the preputial glands and liver homogenates. Shaded bar indicates the liver experiments; black bar, the preputial gland experiments. R I, the precipitate after centrifuging the gland homogenate at 700 × g for 10 minutes; R II, the precipitate at 5,000 × g for 10 minutes; R III, the precipitate at 54,000 × g for one hour and SUP indicates the supernatant after centrifuging at 54.000 × g for one hour. When enzyme activity recovered in all cell fractions were expressed as 100%, enzyme activity in each fraction was calculated as per cent.

et al. (14). When total enzyme activity in the homogenate was calculated as 100%, most of activity was found in particulates in the case of the liver, while more than 75% of the enzyme located in the soluble fraction in the case of the preputial gland (Fig. 1).

5. *Separation of the preputial gland enzyme by DEAE-cellulose column chromatography*

The results of chromagraphy for the enzyme in the soluble fraction of the gland by linea gradient was shown in Fig. 2. Three peaks obtained by such elution

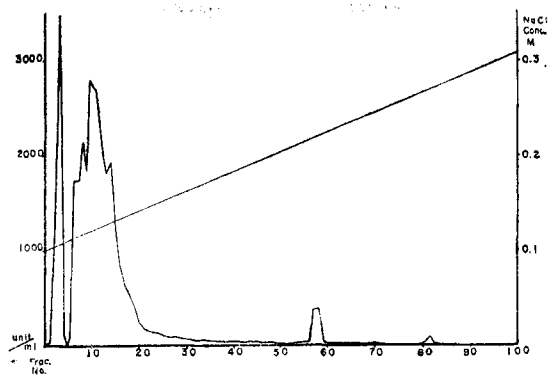

Fig. 2 DEAE-cellulose column chromatography of soluble β-glucuronidase from the normal preputial gland by gradient elution. A heavy line, β-glucuronidase activity (unit/ml). Volume of effluent in each tube was 4 ml. Eluant was 0.005 M Tris-phosphate buffer, pH 7.8 with NaCl concentration from 0.1 to 0.3 M.

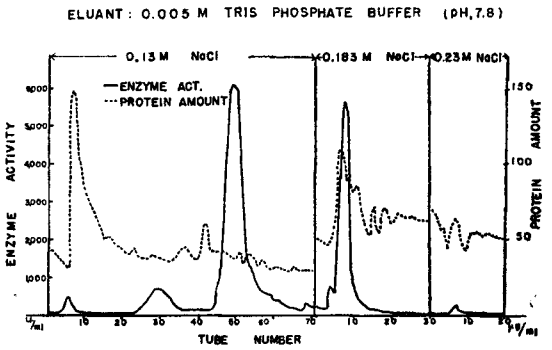

Fig. 3 DEAE-cellulose column chromatography of β-glucuronidase in soluble fraction of the preputial gland homogenate by stepwise elution. First elution was made by 500 ml of 0.005 M Tris-phosphate buffer, pH 7.8, containing 0.13 M NaCl, the second elution was performed by 500 ml of the same buffer with 0.183 M NaCl. The last eluant was 500 ml of the buffer with 0.23 M NaCl. A heavy line, β-glucuronidase activity (unit/ml); broken line, potein amount (mg/ml). Volume of effluent in each tube was 4 ml.

were concentrated at low temperature and then the solution so concentrated was applied to the same type column again. Elution was performed stepwisely with 0.005 M Tirs-phosphate buffer, pH 7.8, consisting different concentration of NaCl, 0.13, 0.183 and 0.23 M respectively. As shown in Fig. 3, the number of the enzyme components was three by 0.13 M of NaCl concentration and one by each other elution, namely five enzyme peaks were obtained in all. When the high concentration of the enzyme protein was adsorbed on the column, the additional peak was often obtained by eluting by 0.183 M NaCl concentration. Enzyme activity in each peak was rather variable in every experiment, even under the same experimental conditions.

6. *Properties of each enzyme seperated by DEAE-cellulose column chromatography*

a) Effects of heavy metal ions

First, 2 mM of phenolphthalein β-glucuronide was hydrolyzed by the five enzyme preparations (A-1, A-2, A-3, B and C) over the pH range from 3.5 to 8.5, and the results are plotted in Fig. 4. Existence of mercuric ion showed great

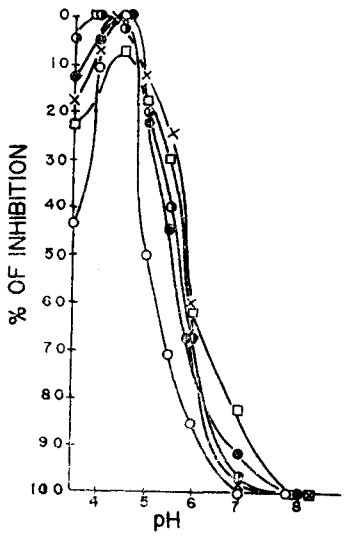

Fig. 4 β-Glucuronidase-catalyzed hydrolysis of 2 mM of phenolphthalein glucuronide as a function of pH. ○——○, A-1 (specific activity, 19,500); ◑——◑, A-2 (specific activity, 101,500); ●——●, A-3 (specific activity, 180,000); ×——×, B (specific activity, 33,900); □——□; C (specific activity, 3.680). Buffer system was 0.1 M acetate buffer (3.5-5.5) and 0.1 M phosphate buffer (6.0-8.0). Values are recorded as percentage of the inhibition (0% presenting complete hydrolysis of 2 mM phenolphthalein glucuronide) Incubated 2 hours.

inhibition for hydrolysis of phenolphthalein β-glucuronide by all enzyme preparations, especially at a pH above the optimum up to 8.0. Curves are very steep. Actions of mercury ions on A-1 enzyme appears to be most efficient and hydrolysis of substrate is inhibited even at a pH more acid than 4.5 (Fig. 5).

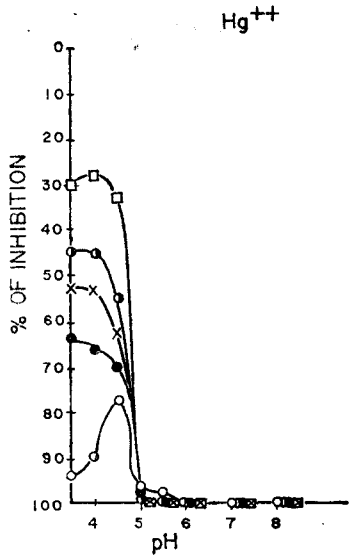

Fig. 5 Inhibition of β-glucuronidases by $HgCl_2$. A function of inhibition concentration is 0.04 mM.

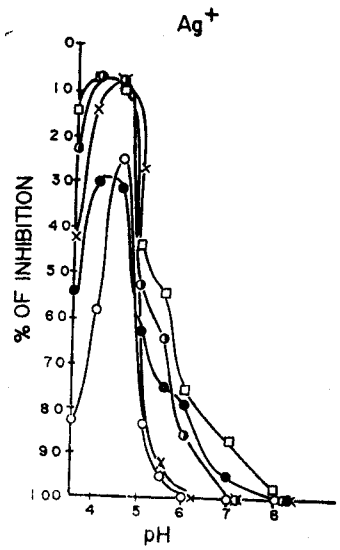

Fig. 6 Inhibition of β-glucuronidases by $AgNO_3$. A function of inhibition concentration is 0.16 mM.

Effect of silver ion on these enzyme preparations is shown in Fig. 6. Hydrolysis of substrate by A-2, B and C preparations is not affected by Ag^+ at its concentration. On the other hand, A-1 and A-3 enzymes are inhibited to some extent and the curve by A-1 is very steep.

Cupric ion is very weak inhibitor at pH 4.5 and is more potent at pH 5.5 up to 8.0 (Fig. 7). Each inhibition percentage for five enzyme preparations at pH 4.5 has been arranged in Table 2.

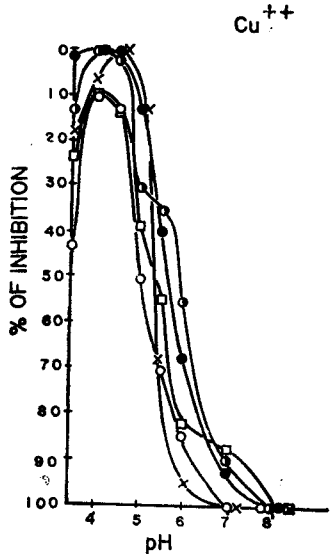

Fig. 7 Inhibition of β-glucuronidases by $CuSO_4$. A function of hibition concentration is 0.63 mM.

Table 2 Effect of metal ions on β-glucuronidases seperated by DEAE-cellulose column chromatography

ION	CONCENTRATION (mM)	INHIBITION % OF A-1	A-2	A-3	B	C
Hg^{++}	0.04	76.0	55.0	69.0	62.5	33.0
Ag^+	0.16	25.0	7.0	32.0	6.0	10.0
Cu^{++}	0.63	13.0	0.0	0.0	0.0	0.0

b) Heat danaturation of the enzyme

At 60°, 50 to 60% of all enzyme activities disappeared after heating for 15 minutes, and at 65°, most of β-gllcuronidase activities suffered 80 to 100%

loss. Also, it should be noted that C enzyme was resistant to heating at 60° and 65° (Fig. 8, 9). At both of these temperatures, B enzyme was rapidly denatured by heating at 60° for 5 minutes. Others are quite difficult to be distingushed each other by heating.

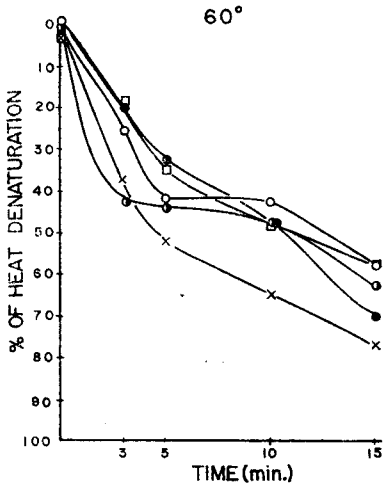

Fig. 8 Heat denaturation of β-glucuronidases at 60°. Values are percentage of heat denturation.

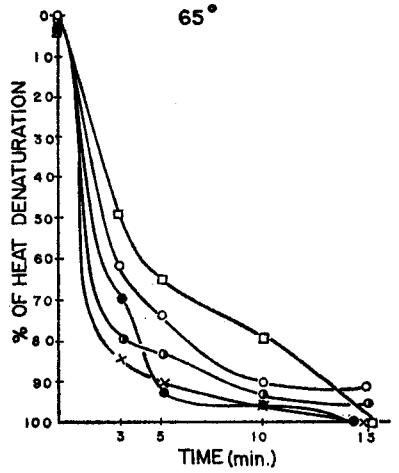

Fig. 9 Heat denaturation of β-glucuronidases at 65°.

c) Inhibition by D-glucaro-(1→4)-lactone

All enzymes were inhibited greatly by this inhibitor in a concentration of 10^{-6} M in the similar order of magnetitude (Table 3).

Table 3 Effect of D-glucaro-(1→4)-lactone on β-glucuronidases seperated by DEAE-cellulose column chromatography

	PREPARATIONS OF				
	A-1	A-2	A-3	B	C
INHIBITION (%)	98	95	97	93	100

DISCUSSION

The existence of the preputial gland of the rat has been known since long time ago (7, 8), but any role on this gland has been obscured. According to Iwasaki (4), the histological studies on the mouse preputial glands showed that the cells with glandular-like structure were divided into a large number of lobes by intracellular cells and an extra-secretory duct originated from the gland cells opened into the prepuce enclosed the urethra. In the case of rat, we also recognized that the ducts risen from gland cells opened into orifice of the urethra. In 1935, Korenchevsky *et al.* (3) reported that the development of the preputial gland was accelated by treating with exogenous androgens, whereas treatment with estrogens did not cause any significant change. In 1954, Beyler and Szego (2) found that a β-glucurnidase activity in this gland was extremely high and variable with the variation of hormone level in blood during pregancy of rats. β-Glucuronidase is apt to respond by estrogen treatment and the enzyme level of the gland increased 4 hours after injecting with 17β-estradiol into castrated female rats. The response was blocked by cortisone (Compond E and Cortisol F).

Iwasaki (4), Nakao *et al.* (5, 6) made correlative experments between steroid hormone and the function of this gland of mice of various strains. In the early stage of pregnant rats, the weight of the gland increased 2-fold and then returned to the normal weight in the last stage. β-Glucuronidase of the gland is very sensitive to various kinds of steroids.

Table 4 Effect of adrenalectomy on β-glucuronidase of the preputial gland

	NORMAL	ADRENALECTOMIZED
Total activity	71,020	42,775
Specific activity	12,921	6,031

The adrenalectomized rats were injested with the solution consisting of 6% glucose and 1% NaCl instead of ordinary tap water for 6 days after operation.

As shown in Table 4, however, β-glucuronidase activity in the gland is not only depressed by 40% a week after adrenalectomizing the female rats, but its specific activity also decreased by 50%. This suggests that the adrenal hormone would not affect directly depression of the enzyme level, but it seems to cause decrease of the enzyme activity as secondary phenomenon after protein has decreased first. From such a view point, it would seem overhasty to state that the enzyme itself was sensitive to hormones directly.

As regards the histochemical study of β-glucuronidase in this gland of rat, the enzyme, according to Fishman and Baker (11), was strongly reactive in basal cells lining duct. Our method improved also supports this result. The method using 8-hydroxyquinoline glucuronide as substrate, however, had been criticized in the repects of specificity for β-glucuronidase and of difficulty of technique (17-19). Recently Hayashi *et al.* (13) reported a new method identifying β-glucuronidase activity. A principle of this method using Naphthol AS-BI (6-bromo-2-hydroxy-3-naphthoyl-o-anisidine) glucuronide was first originated by Pugh and Walker (20). This method also confirmed histochemical localization of β-glucuronidase in the preputial gland.

Whenever the gland was homogenized, the great attention was paid not to damage the fragile grnules. Even so, the enzyme activity showed that always constant level, 75 to 80% of total enzyme activities in the gland, was found in the soluble fraction. This distribution in the gland cells may support histochemical localization of the enzyme.

When the enzyme in the soluble fraction was applied to DEAE-cellulose column chromatography, the enzymes consisting of 5 or 6 peaks were eluted. This result was quite differed from three peaks of the enzymes in the liver demonstrated by Moore and Lee (15), and Kawada *et al.* (16). It must be added that enzyme activities in each peak were variable in every experiment. It may indicate that β-glucuronidase in the gland exist as a kind of secretory fluid, and the enzyme may be liberated from the lysosomes in which the enzyme has probably been synthesized.

In the field of the biochemistry, β-glucuronidase of the gland is a good source for purification. Levvy *et al.* (22) purified β-glucuronidase 25-fold from the gland to give a specific activity of 455,000 on assay in the presence of albumin. They assumed that 7.5% of the protein in the gland is β-glucuronidase. This enzyme has not so far been crystallized, though its activity was at least four times that of crystalline specimen from Helix *pomatia* purified by Alfsen and Jayle (23). On the properties of the enzyme, the enzyme catalyzes not only hydrolysis of the various substrates of glucuronide, but also it could hydrolyze β-galactronide.

Fernley (21) investigated on the effect of heavy metals (copper, silver, mercury, p-chloromercuribenzoate and phenylmercuric hydroxide) on the purified β-glucuronidase from the gland. From one of these results one can indicate that both SH and SS groups are involved in the active center of β-glucuronidase. On the enzymes seperated by the column chromatography as mentioned above, inhibition attitudes by heavy meatal ions under concentration conditions set up by Fernley (21) were comparatively studied, but these results didn't support that the seperated enzymes were definitely isozymes.

From these experiments, a role of the gland has not been clarified so far, but β-glucuronidase may exist as a secretory enzyme, if one presumes that the enzyme has its major application at the present time in the hydrolysis of urinary steroid glucuronide.

SUMMARY

1. Histological and histochemical studies on the preputial gland of the female rat were conducted in the connection with β-glucuronidase.

2. By centrifugal seperation of subcellular components from the preputial gland, more than 75% of the enzyme was found in the soluble fraction.

3. 5 Components of the enzyme were eluted, applying the enzyme of the preputial gland to the DEAE-cellulose column chromatography.

4. Properties of each enzyme such obtained were comperatively studied by inhibitors of heavy metal ions (Ag^{+}, Cu^{++} and Hg^{++}), of D-glucaro-(1→4)-lactone, and heat denaturation.

5. A role of β-glucuronidase in the preputial gland and the function of the gland were duscussed.

We wish to express our gratitude to Dr. K. Yamane who was a medical student in those days and to Miss K. Ando for help in some experiments.

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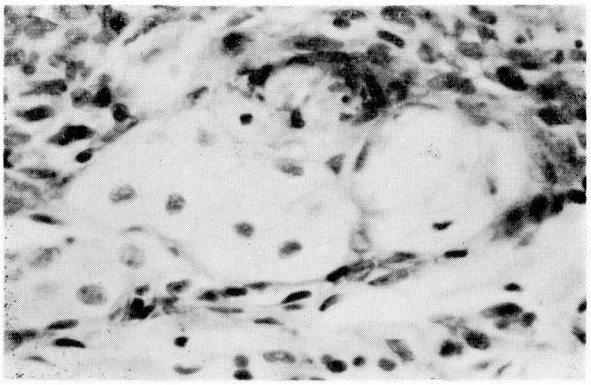

Plate 1 A vertical section of the preputial gland to the longitudinal axix of urethra. Glandullar cell. (×500)

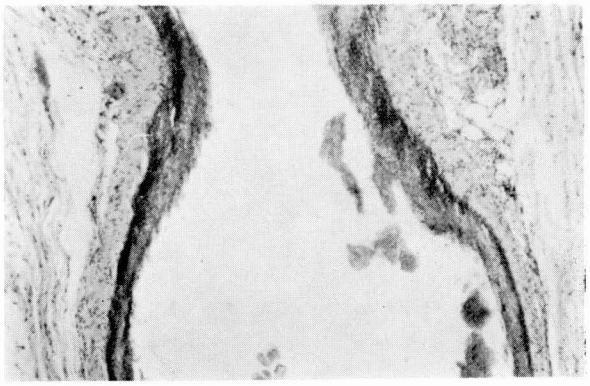

Plate 2 A vertical section of the gland to the logitudinal axis of urethra. Duct. (×50)

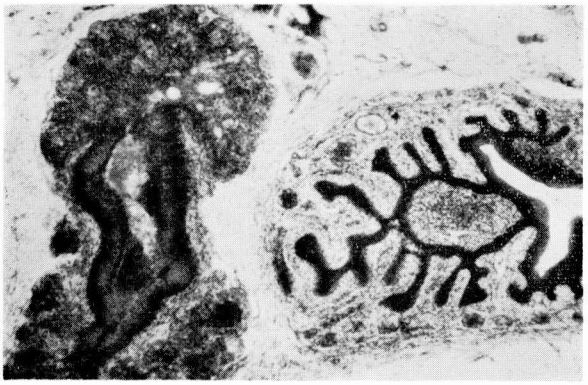

Plate 3 A cross section of the gland to the longitudinal axis of urethra. Two ducts risen from the glandular cells (left), and urethra (right). (×50)

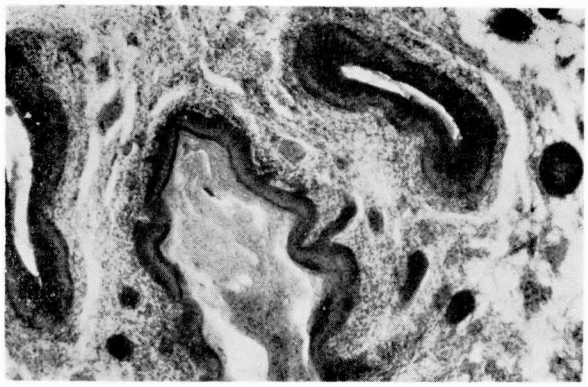

Plate 4 A cross section of the gland to the longitudinal axis of urethra. Two ducts (right and left) and urethra (center). (×50)

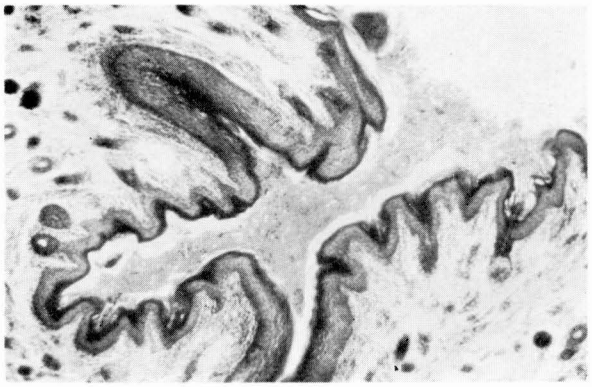

Plate 5 Two ducts and urethra (center). (×50)

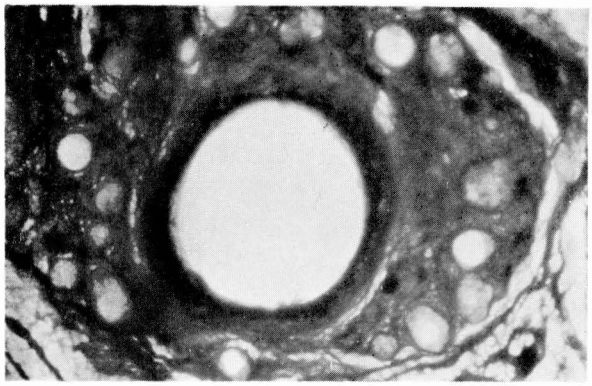

Plate 7 Histochemistry of β-glucuronidase around the duct in the preputial gland stained by the method using 8-hydroxyquinoline glucuronide as substrate. Black stain, β-glucuronidase activity. (×50) Incubated one and half hour.

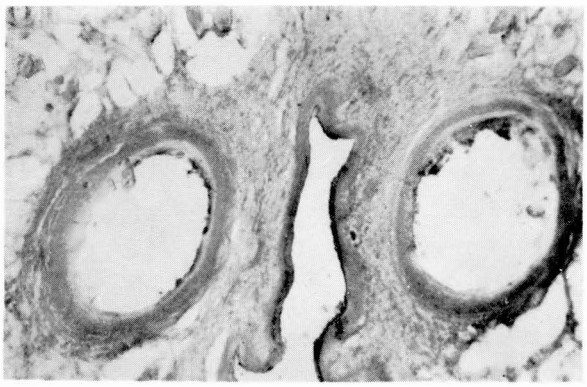

Plate 7 Histochemistry of β-glucuronidase around the duct and the urethra in the preputial gland stained by the method using Naphthol AS-BI glucuronide as substrate. Incubated 7 minutes. Ducts (left and right right), urethra (center). (×50)