EFFECTS OF MALE SEX HORMONES UPON EXLACRYMAL GLANDS OF RODENTS

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Sexual dimorphism of external lacrymal glands (the glands of Loewenthal) of rats has been described by several authors1-5). Yamada, one of the authors, reported that sex hormones and their unbalances affected apparently the functional state of the glands of rats but they could not induce the prominent proliferation of them, and that a prolonged stimulation with androgens induced a specific histological alteration in the glands. Recently, Quintarelli et al.6) have studied the effects of testosterone propionate upon the exlacrimal glands of young mice. Now to be described on the exlacrymal glands of rodents, sex difference in the functional state was detected histochemically, and excess amounts of androgens caused some specific structure, eosinophilic materials in which were histochemically observed.

MATERIALS and METHODS

Both 28 mature albino rats of Sprague-Dawley strain and 15 two month-old mice of A/Jax strain were used. They were home-grown, kept in air-conditioning room, and fed a commercial ration. Tap water is given ad libitum. Both 6 male and 6 female rats were sacrificed for the controls. Daily injection of 2 mg of testosterone propionate for 4 weeks was performed into the thigh of the other 16 male rats which were sacrificed 30 days after the first injection. Both 5 male and 5 female mice were applied to the controls. The other 5 male mice were received a weekly injection of 0.1 mg of Enarmon depot of Teikoku Zoki Co. (testosterone enantate 11.6 mg + testosterone propionate 2 mg) 3 times and sacrificed 3 weeks after the first injection. All animals were killed by decapitation, and the exlacrymal glands were immediately removed. After weighing of the glands by means of a analytical chemical balance, one was fixed in a 10 % neutral formol solution, and another was applied to the
specific histochemical techniques. Either formol-fixed or alcohol-fixed tissues were embedded in a paraffin in the usual fashion. The sections were stained with hematoxylin and eosin, Masson's trichrome stain method, alcian blue after Mowry's method, toluidine blue after Hempelmann's method or Ono et al. 's method, and colloidal iron after Müller's method. They were also treated with periodic acid-Schiff reaction after McManus. Histochemical activities of DPN-diaphorases and acid phosphatases in the glands were investigated on a cryostat section. The former was determined by means of nitro-BT after Nachlas and the latter by Takamatsu's method.

**EXPERIMENTAL RESULTS**

Gross appearance of the gland of two the rodents was the same each other, and became to be bigger and more tan on the androgenized rodents than the controls. The absolute weight of the exlacrimal glands of rats was $153.3 \pm 11.1$ mg in males, $137.0 \pm 15.4$ mg in females, or $143.7 \pm 34.1$ mg in the androgenized males, respectively. The relative weight of them per 100 g of body weight was $65.4 \pm 2.4$ mg, $72.1 \pm 10.2$ mg, or $62.2 \pm 16.5$ mg, respectively. The absolute weight of the exlacrimal glands of mice was $33.7$ mg in males, $28.5$ mg in females, or $52.7$ mg in the androgenized males, respectively. Relative weight of them per 1 g of body weight was $1.3$ mg, $1.1$ mg, or $1.9$ mg, respectively. The numbers would indicated that the male had somewhat bigger gland than the female, and that big dose of androgen exhibited a decrease in the weight of the gland in male rats but an increase in male mice generally.

Histological appearance of the exlacrimal glands of mice was the same that of rats, and of the tubuloalveolar type resembling the serous salivary gland. In the cytoplasm of acinar cells of the terminal portions, secretory granules clearly stained with Masson's trichrome stain method, DPN-diaphorase and acid phosphatase were distinctly detected. Histochemical reactions for polysaccharide materials were never seen in the terminal portions of the glands on the controls.

Nuclei of the glandular cells of the terminal portions in the male were bigger and more vesicular than in the female. More amounts of secretory granules and enzymes were histochemically detected in the male than in the female. In the other ordinary observations on the exlacrimal glands of the rodents, it was difficult to detect a sex difference.

Prolonged administrations of androgen induced a prominent alteration in the histological structure of the terminal portions of the exlacrimal glands. The nuclei of the
terminal portions underwent a polymorphism accompanied by an over-all increase in size. Basophilia in the cytoplasm of the terminal portions was particularly strong in perinuclear areas while more peripheral cytoplasmic zones were lightly stained, almost colorless. Many vacuoles began to appear in the polyhedral cytoplasms of acinar cells of the glands, which finally changed to a follicular structure with a relative large lumen and with a thin layer of the acinar cells. The stronger activities of enzymes were found only in the peripheric cytoplasmic zones (near the free surface) of the glandular cells in the androgenized rodents. In the androgenized mice, many ovoid vacuoles appeared in the hyperplastic glands histologically as well as the hyperplastic glands of rats, but a follicular structure was very rare. In the androgenized rats, however, a follicular type was not rare, and it was interesting that such follicular type was usually found only in the atrophic glands of androgenized males. Some follicular type had pyknotic nuclei, eosinophilic narrower cytoplasms and strongly eosinophilic materials in the cavity. The same materials were found in the inter- and intra-lobular ducts. The periodic acid-Schiff reaction was sometimes demonstrated in the cytoplasmic vacuoles and it was always exceedingly intense in the eosinophilic materials. When the histochemical test for the detection of acidic radicals, e.g. the stain method with alcian blue or with toluidine blue, was utilized a negative reaction was reported in both the eosinophilic materials and the cytoplasmic vacuoles.

**DISCUSSIONS and SUMMARY**

Pathognomonic action of male sex hormone upon the submaxillary gland of mice was found by Lacassagne, who presented at first the sexual dimorphism of the other organs than the reproductive organs. Unexpected effect of sex hormones upon the other organs than the reproductive organs are not very prominent but interesting, and especially the effect of sex hormones upon the metabolic organs should be discussed because of their metabolic actions. Morii et al. suggested that the metabolic action of androgen would induce sexual dimorphism of the submaxillary glands of mice. Aragaki, Nakagawa & Morii pointed out that a prolonged administration of androgen was followed by the distinct histological alterations of the exlacrimal gland of rats, and that this specific effect of androgen was detected even on the hypophysectomized rats. They supposed androgens induced in general a hyperplastic state of the glands. Gabe reported that sex hormones affected apparently the structure and function of the exlacrimal glands, and indicated the cellular vacuolization and the nuclear polymorphism on the glands of androgenized male rats. Minami observed that the gland
of females was heavier than that of males, and that hypophysectomy was followed by an atrophy of the glands. But, male rats in her experiments had heavier exlacrimal glands in absolute number than the females. It was considerable that the body weight of her male rats was too heavy to correct the relative weight of the gland. In nuclei of acinar cells of the exlacrimal glands of rats, specific stimulation with androgens was reported by Italian authors\(^2\). Recently, Yamada\(^5\) has studied on sex difference of the exlacrimal glands of rats. Her experimental results indicated that the sex difference exhibited prominently in the functional state, and that hyperfunctional state seemed to be followed only by slight hypertrophic findings. She has also described a follicular structure of the exlacrimal glands of rats received with a prolonged administration of androgen, and speculated that this type would be an exhaustive appearance, which was a final demonstration of the oversecretion of meso-crine type. Quintarelli et al. have observed the effect of androgen on the exlacrimal gland of mice. Repeated injections of testosterone proponate (1 mg daily, for 50 days) have determined some pronounced modifications of the glands. The modifications were not only characterized by cytoplasmic vacuoles and an appearance of glycoprotein materials, but also 4 or 5 times enlargement in size.

In our experimental results, sexual dimorphism of the exlacrimal glands of rodents was not distinct either on naked eyes or histologically. But, it became to be more apparent by means of the histochemical techniques and was proved more prominently on the mouse than on the rat. It has been suggested that androgen would cause a hyperactive and hyperplastic state of the gland. Actually, moderate stimulation of androgen to mice was followed by distinct hypertrophic and hyperfunctional findings. Excess of androgenic stimulation, however, did induce an atrophic and a follicular structure in the exlacrimal gland of rats. According to our histochemical observations, neutral mucopolysaccharide or mucoprotein was proved intensely in the cavity of the follicular type. Vacuolization of acinar cells has been reported in the protein-secreting glands like the parotid of old rodents and old dogs\(^9\). The formation of vacuoles in the gland parenchyma was explained on the basis of functional modifications due to aging. Therefore, tissues which physiologically are protein-secreting would modify their activity in senescence and apparently begin to synthesize glycoprotein material. Histochemical findings on the follicular structure suggested that this structure was involution type of the acinar cells due to the overwork followed by overstimulation of male sex hormone. Male sex hormone would be a main inducer of sexual dimorphism of the exlacrimal glands as well as the submaxillary gland and induce the male
type, stimulated state. Otherwise, a prolonged stimulation with androgens was followed
by a follicular structure the exhaustive state of the exlacrimal glands like senile
condition of the parotid.

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（和 文 抄 録）

顎顔類外塩腺に対する男性ホルモンの効果

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ラットおよびマウスの外塩腺を組織化学的に検査し、その性的二型性を認めた。それは主に機能的なもので、男性
ホルモンの作用による。男性ホルモンは外塩腺を刺激し、その機能を亢進させるが、同ホルモンの持続的大量投与
は、外塩腺を強く肥大増生させ、ついで疲労萎縮に陥らしめる。疲労に陥った外塩腺は特異な組織構造—渦胞型を
呈し、渦胞内に生理的に認められない澱粉粒が出現し、あたかも老年性変化のような所見を呈する。