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Sexual Dimorphism of Mouse Submaxillary Glands and Its Relationship to Nerve Growth Stimulating Protein.* (29798)

ELMER D. BUEKER, PEDDRICK WEIS† AND ISAAC SCHENKEIN
Departments of Anatomy and of Biochemistry, College of Dentistry and Graduate School of Arts and Science, New York University

The biological activity of mouse submaxillary gland (MSG) protein from the mature male mouse was shown to be 4 to 7 times more concentrated than from the adult female MSG(1). This sexual difference may in some way be associated with the sexual dimorphism of the MSG. Intercalated ducts connect the acini with the serous tubules. The serous tubules hypertrophy with the administration of testosterone and the diameter, tubular index (T.I.), is a good measure of the degree of masculinization of the gland (2). This dimorphism has also been associated with several physiological differences: concentration of amylase(3), protease(4), iodine(5), and oxygen consumption(6). The structure can be changed to that of the opposite sex by castration of the male, or by testosterone injection of the female(2) and this has been associated with a reversal in the nerve growth protein content(7). The structure of the female gland has been shown to undergo a "spontaneous masculinization" during pregnancy and lactation(8). In this investigation we studied "spontaneous masculinization" as expressed in the dimorphism of the serous tubules of the female MSG and correlated these observations with a concomitant "masculinization" or increase in the production of nerve-growth-stimulating protein.

Materials and methods. Female Swiss white mice were sacrificed by chloroform inhalation at various life stages: prepubescent (3-6 weeks) and mature virgin (6-12 weeks) various stages of pregnancy and lactation, and several months after lactation. Some animals, representing pregnant stages, were in their first pregnancy and some were not. Immediately after sacrificing, their submaxillary glands were excised. One-half of one lobe was fixed in acetic acid-alcohol-formalin or in 10% formalin, sectioned and stained with hematoxylin and eosin. The diameter of the secretory tubules was measured in microns and recorded as the tubular index (T.I.)(8). The remainder of the gland was homogenized in isotonic saline buffered at pH 7.2, and centrifuged at 15,000 $\times$ g for 10 minutes. All steps were done at 4°C. The supernate was decanted, analyzed for total protein by the Lowry method(9), and assayed at 10-fold

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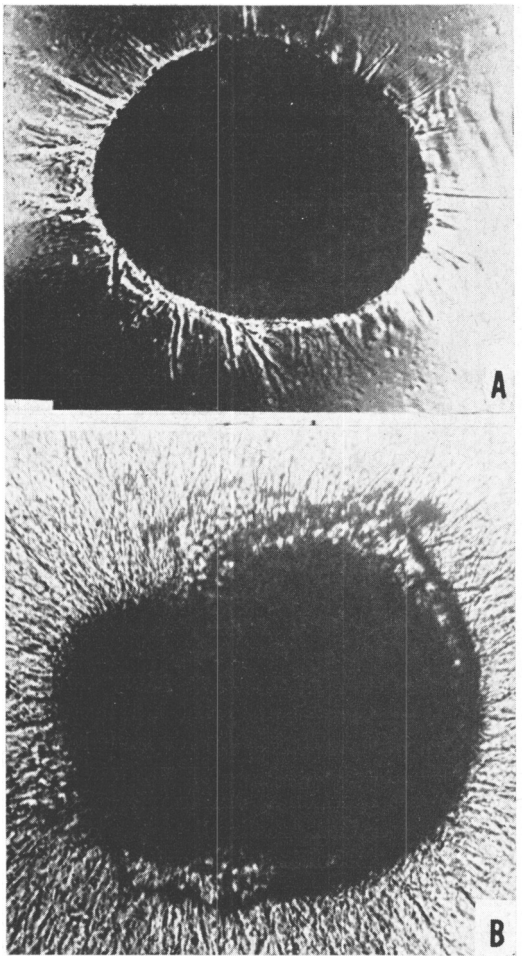


FIG. 1. (A) Spinal ganglion explant of 8-day chick embryo after 16 hr in hanging drop culture (⅓ rooster plasma + ⅔ M199); (B) Similar preparation, except for media, which contained ⅓ M199 with 0.50 μg of NGSP per ml; observe 3+ nerve outgrowth.

dilutions for nerve growth stimulating activity on the same day. Spinal ganglion explants from 8-day chick embryos were grown in hanging drop cultures supplemented with MSG-protein preparations(10). The amount of total protein per ml required to elicit a 3+ nerve outgrowth was determined and recorded in units of biological activity (B.U.) (11)(Fig. 1). This semi-quantitative method is sensitive to 2-fold dilutions(1).

To compare and evaluate our findings on spontaneous masculinization with the experimental masculinization of other investigators, 11 immature females (4 weeks old) were treated for 2 weeks with subcutaneous injections of testosterone propionate in mineral oil, in total weekly dosages of 3, 6, and 12 mg. Two of each dosage level were sacrificed and analyzed after the treatment period; the remainder were treated similarly 2 months after cessation of the testosterone treatment. Controls consisted of females aged 4 and 6 weeks, representing the beginning and end of the treatment period. Five normal untreated males were included for comparison.

Results. The normal young adult male MSG has a T.I. of 50.4 μ and the crude homogenate of submaxillary glands required 1.5 μg/ml protein to evoke a 3+ response in nerve growth. The virgin female MSG has a T.I. of 22 μ (at puberty) to 30-35 μ (at maturity), and required from 1 mg (at puberty) to 20 μg (at full maturity) of protein for a 3+ response. The spontaneous masculinization occurring during pregnancy and lactation becomes obvious as early as the fifth day of pregnancy. Both the T.I. and the nerve growth stimulating protein concentration begin to increase, the trend being more easily followed in the morphological change than in the physiological change (Fig. 2). The greater increase is in the nerve growth stimulating protein. This reaches the normal level of the male during the period of lactation (Fig. 3). The T.I. of the gland during lactation is greater than

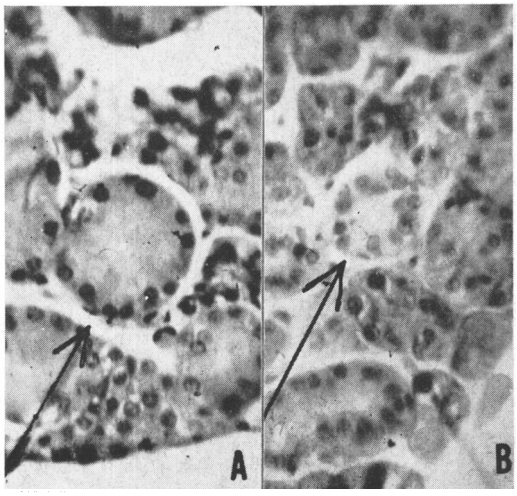
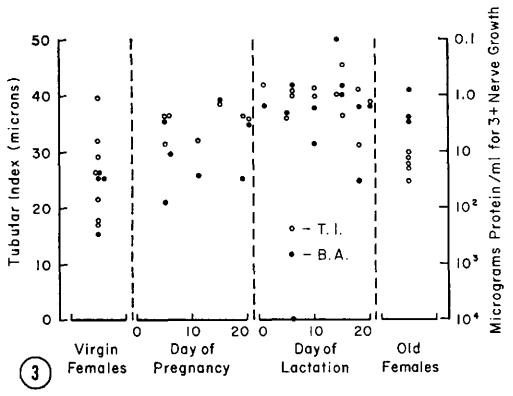


FIG. 2. (A) Serous tubule (50 μ) of submaxillary gland of male mouse (arrow); (B) Similar tubule (35 μ) from female mouse.



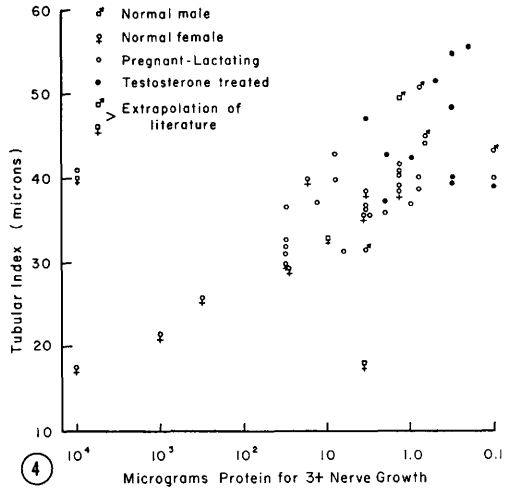


FIG. 3. Diameters of female serous tubules (T.I.) as related to biological activity (B.A.); or amount of submaxillary gland protein in μ per ml required for 3+ nerve growth during period of pregnancy and lactation.

FIG. 4. Tubular indices (T.I.) in μ as related to biological activity (B.A.); B.A., μg of NGSP per ml required for 3+ nerve outgrowth.

40 μ. The 5 females which were isolated 4-6 months after weaning their young had maintained their T.I. at 38.2 μ and their nerve growth activity at 3.0 μg, approximately one-half the concentration of the normal male.

The most significant data appeared when the biological activity and tubular index of each animal is plotted on a semi-log scale (Fig. 4); yielding a straight-line resultant. The experimental animals which reached the highest levels included normal males, testosterone-treated young females, and a few lactating females (Fig. 5). The testosterone-treated females which were sacrificed 2 months after treatment still had masculine levels of nerve growth stimulating protein and a T.I. equal to that of a post-weaning female. There was a wide variation in the secretory activity of the tubules, but this showed no correlation to the T.I. or biological activity.

Discussion. The existence of many hormones, or hormone-like substances, which are responsible for the growth and maintenance of specific tissues has been postulated in many areas of biology. Gradually, evidence has accumulated to prove some of these hypotheses. The salivary gland has been implicated in these roles, having been shown to have not only exocrine but also endocrine functions, *e.g.*, parotin(12), the "tooth-lid" factor(13), and nerve growth stimulating protein. The salivary gland is in turn, influenced by several hormones. Originally, Lacassagne(2) showed the necessity for androgens in maintaining the secretory tubules. Later, Grad and LeBlond showed that only the size of serous tubules depended upon androgens, while thyroid hormone was necessary for the function of the tubule cells(14). Soon, investigators realized that the MSG could serve as an index of androgen secre-

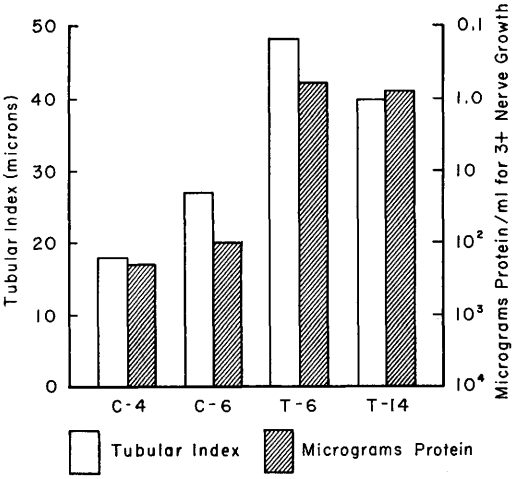


FIG. 5. Tubular indices (T.I.) as related to bioassay of nerve growth activity of the same glands; 4-week female controls (C4); 6-week female controls (C6); 6-week females treated during 5th and 6th weeks with testosteron (T-6); 14-week females testosterone treated 5th and 6th week and not sacrificed until end of 14th week (T-14).

tion of the adrenal cortex(15). The adrenal cortex as well as tumors, have been shown to secrete sufficient androgen to affect the secretory tubules(8). Now, it appears that the production and/or storage, and possibly the release into the circulation, of nerve growth stimulating proteins is directly influenced by androgens(7).

To investigate the mechanism of the effect of androgens on the secretory tubule, we have started a series of experiments designed to find any direct effect of testosterone upon the production of nerve growth stimulating protein (NGSP) and its activity *in vitro*. As for the purpose of the increase of NGSP in the pregnant or lactating mouse, we can only hypothesize. Mouse placenta has shown only traces of nerve growth activity(16), and we have been unable to isolate NGSP from 14-20-day mouse embryos. We have observed that anti-NGSP rabbit serum has no effect upon mouse fetuses when injected into the mother. Apparently antisera for this protein from a treated mother *via* the mammary glands inhibited the growth of the sympathetic nervous system in nursing mice (17). Since the active components of NGSP consist of relatively low molecular weight compounds (8400 and 4000)(18), this material could also pass through the mammary glands. This would serve a useful function, since the sympathetic nervous system, which is most responsive to NGSP, is rapidly developing at that time. If this is the case, then the masculinization of the female MSG during pregnancy and lactation may be essential for the development of parts of the newborn's nervous system, and is, in part, the beneficiary of the increased androgen secretion by the adrenal cortex and the corpus luteum, which was shown to occur during pregnancy and lactation(19).

Summary. A protein obtained from the mouse submaxillary gland, which enhances the growth of sympathetic and spinal ganglia and their processes, is 4-30 times more concentrated in the male than in the female submaxillary gland, an organ which shows a sexual dimorphism in histological appearance and in several physiological differences. The masculinization of the gland which occurs during pregnancy and lactation, as a result of increased androgen secretion during that time, has been shown to be correlated to a logarithmic increase in concentration of nerve growth protein, to the point where it may equal that of the male. It is suggested that this increase may serve the function of aiding the development of parts of the nervous system of the suckling mouse.

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