Development of Increased Cytoplasmic Binding of Androgen in the Submandibular Gland of the Mouse with Testicular Feminization∗

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SUMMARY

The binding of [1,2-3H]dihydrotestosterone by cytoplasmic extracts of submandibular glands from normal male mice and from mice carrying the X-linked gene for testicular feminization (Tfm/Y) has been studied by density gradient centrifugation and gel filtration. Androgen binding in this tissue was found exclusively in the portion of the gland that is known to contain androgen-dependent elements. No binding was demonstrated in the glands of either genotype immediately after birth, but at 2 weeks of age and after high affinity binding of the hormone was found in the glands of both male and Tfm animals. At all ages studied, the apparent dissociation constant for this binding was similar in the two genotypes (around 10 nM), but the number of binding sites in Tfm animals at 6 and 12 weeks was 3-fold higher. This difference did not appear to be the result of differential degradation during the preparation of the extracts. The observation that normal and Tfm animals have identical binding parameters at 2 weeks of age suggests: (a) that the postnatal development of androgen binding in submandibular gland is not androgen-dependent; (b) that androgen binding develops prior to the onset of sexual dimorphism of the gland; and (c) that the later development of enhanced binding of androgen by submandibular gland cytoplasm of the adult Tfm mouse is not caused by the appearance of an altered androgen binding protein but is the result of the accumulation of a physiologically normal binding protein.

The submandibular (or submaxillary) gland of the male rodent undergoes a complex, androgen-dependent development that commences at the time of sexual maturation of the animal (1, 2). This sexual dimorphism is manifested not only by distinct histological changes in the interlobular striated ducts (1, 2) but in the formation of several androgen-dependent proteins as well, including nerve growth factor (3, 4) and proteases (2, 5). In the submandibular gland of the mouse with the testicular feminization syndrome (Tfm), however, the histological changes of sexual dimorphism do not develop (6, 7), and there is a striking failure in the formation of nerve growth factor even when testosterone is administered to affected animals for long periods (7). The resistance to androgen action in the submandibular gland of the Tfm mouse is poorly understood but is presumed to be the result of the same inherited defect that causes a failure of androgen action during embryogenesis and the development of a complete male pseudohermaphroditism (8-11).

The nuclear uptake of radioactive androgen is deficient in the submandibular glands of the Tfm mouse (10), and cytoplasmic extracts of the submandibular glands of these adult animals exhibit a greater capacity for binding dihydrotestosterone than do similar extracts from male mice (11). However, it has not been possible to determine whether the increased binding was the result of the accumulation of a normal binding protein or was instead due to a structural alteration of the binding protein resulting in an enhanced binding capacity.

To gain insight into the pathogenesis of the enhanced dihydrotestosterone binding in this disorder, a series of studies was undertaken to assess the developmental pattern of androgen binding in the submandibular gland and to measure both the binding affinity and the binding capacity for dihydrotestosterone at various ages.

MATERIALS AND METHODS

Treatment of Animals—Mice were propagated from two females heterozygous for the X-linked Tfm gene that were the gift of Dr. S. Ohno, City of Hope Medical Center, Duarte, Calif. The matings were all between Ta/Tfm females and Ta/Y males, and the genotypes of the offspring (normal male, normal female, heterozygous Tfm female, and Tfm male) were identified by making use of the X-linkage relationship between the Tfm gene and the coat marker gene tabby (Ta) (8). In all experiments littermate male and female animals were used for controls. Previous control studies indicated that the Ta gene had no effect on the binding parameters assessed in this study (11).

Preparation of Homogenates—All procedures were performed in a cold room at 4°. The mice were killed with ether, and the

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1 The trivial names used are: testosterone, 17β-hydroxyandrost-4-en-3-one; dihydrotestosterone, 17β-hydroxy-5α-androstan-3-one.
submandibular glands were promptly removed, dissected free of connective tissue and fat, separated from the major sublingual glands, and placed in 10 mM Tris-Cl buffer (pH 7.4) containing 1.5 mM EDTA. Pooled glands from 4 to 20 animals were diced with a razor blade and homogenized with either 3 or 7 volumes of Tris-EDTA buffer in a micro-Dounce homogenizer using 10 strokes of a loose pestle (clearance 0.15 mm) and 10 strokes of a tight pestle (clearance 0.09 mm). In most experiments the buffer contained Trasylol (3000 kallikrein inactivation units (K.I.U.) per ml). The homogenates were centrifuged at 104,000 × g (100,000 rpm) for 1 hour in a Spino Ap 40 rotor. The supernatant from this centrifugation was decanted and either used immediately or in a few instances stored at −20°C.

Density Gradient Centrifugation—For density gradient centrifugation, aliquots of the supernatant were added to tubes in which radioactive dihydrotestosterone in benzene solution had previously been added and then taken to dryness. The final concentration of the [1,2-3H]dihydrotestosterone was 10 nM. The tubes were digested repeatedly in the cold and allowed to equilibrate for 2 hours at 4°C. Portions of the protein-hormone mixture were then pipetted onto top of continuous 5 to 20% sucrose density gradients in the Tris-EDTA buffer so that the final volume was 5.1 to 5.2 ml. The tubes were centrifuged at 50,000 rpm for 22 hours in a SW 50.1 Beckman-Spinco rotor. At the end of the centrifugation the bottom of the tube was punctured utilizing a Beckman Fraction Recovery system, and 5-drop fractions were collected into counting vials and assessed for radioactivity in a liquid scintillation counter as before (11).

Sephadex G-25 Chromatography—Columns (7 × 0.55 cm) were prepared in Disposo Pipets at 4°C with Sephadex G-25 (medium) that had been equilibrated with Tris-EDTA buffer. A chloroform solution of radioactive steroid in disposable culture tubes (6 × 50 mm) was evaporated to dryness under air at room temperature. The tubes were placed in ice, and submandibular gland cytosol (0.11 ml) diluted to contain approximately 1 mg of protein per ml (range 0.6 to 2.0) was added to each tube. The tubes were digested on a vortex mixer and incubated for 6 to 14 hours in the cold. A portion (0.1 ml) of the incubation mixture was layered on the column and eluted with the Tris-EDTA buffer. The eluant was collected in two fractions, the first containing 1.4 to 1.5 ml (“bound radioactivity”) and the second containing 3 ml (“free radioactivity”). Radioactivity was assessed in each fraction by liquid scintillation counting. In a typical experiment 15 columns were run concurrently in a 20- to 30-min period. The columns were ordinarily washed with 6 ml of Tr-ex-EDTA buffer and used again. The entire chromatography procedure was performed in a cold room at 4°C.

Analytical Procedures and Reagents—Protein was determined by the method of Lowry et al. (12) with bovine serum albumin as the standard. [1,2-3H]Dihydrotestosterone (50 Ci per mmole) was obtained from New England Nuclear Corp., and dihydrotestosterone was obtained from Steraloids, Inc. (Pawling, N.Y.). Ribonuclease-free sucrose was purchased from Schwarz-Mann, and Trasylol was obtained from FBA Pharmaceuticals (New York).

RESULTS

The submandibular gland of the mouse consists of two anatomical divisions, the submandibular gland proper and the major sublingual gland (2). The submandibular portion consists of acini that are connected by intercalated ducts to convoluted granular tubules, whereas the sublingual area contains acini and intercalated ducts but no granular tubules. Only the tubular elements exhibit androgen-dependent sexual dimorphism (1, 2).

To determine whether dihydrotestosterone binding changes with age, submandibular gland supernatants from newborn, 2-week, 6-week, and 12-week male and Tfm animals were equilibrated with [1,2-3H]dihydrotestosterone (10 nM) and subjected to sucrose density gradient centrifugation (Fig. 2). No binding was demonstrable in the preparations from the newborn, whereas a discrete binding peak, approximately equal in male and Tfm animals, was observed in the preparations from 2- and 6-week-old mice. Since the binding is present only in the major sublingual portion of the gland, it is likely that the binding is involved in androgen action. In all subsequent studies, the sublingual portions were dissected from the submandibular glands and discarded prior to homogenizing the tissue.

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old mice. At 12 weeks of age, however, binding in the Tfm was greater than in the male. In previous studies in which more binding was always observed in the Tfm, all animals were 10 weeks of age or greater (11). Three tentative conclusions were drawn from these data. First, since the differentiation of the submandibular gland is not complete at birth, it seems likely that the appearance of this binding is related to maturation of the intralobular ducts (2). Second, the appearance of the binding precedes the development of sexual dimorphism, a relationship that is compatible with a possible role of the binding in this process. Third, the difference in binding between male and Tfm animals is related to the age of the animals.

Since the submandibular gland of the male mouse is known to contain several androgen dependent proteases (2, 5), some of which are deficient in the Tfm animal,3 the apparent decrease in binding in the normal male might represent destruction of the moiety by proteases released during the homogenization process. In several studies the addition of male supernatant to Tfm cytosol that had been preincubated with dihydrotestosterone in the cold resulted in no loss of binding so long as the mixture was kept at \(4{\circ}\) during the entire experiment (results not shown). However, if after the Tfm supernatant was equilibrated in the cold with \([1,2-3H]dihydrotestosterone\), male supernatant was added and the mixture was incubated at \(37{\circ}\) for 20 min before density gradient centrifugation, the results were considerably different (Fig. 3). Supernatant from the 2- and 6-week male animals did not appear to affect binding in the Tfm, and as before, binding was easily demonstrable in the male at these two ages even after incubation at \(37{\circ}\). On the other hand, at 12 weeks of age supernatant from the male partially destroyed the binding in Tfm supernatant that had been prelabeled with radioactive dihydrotestosterone under identical incubation conditions.

Although this destruction was not observed in the cold, other means of preventing it were sought. These included treatment with dithiothreitol (1 mM) and mercaptoethanol (1 mM and 10 mM), the latter having been observed to inhibit some protease activities in the submandibular gland (5). Of the means examined, however, only the protease inhibitor Trasylol appeared to protect partially against the destruction at \(37{\circ}\) (13). Such protection is demonstrated in Fig. 4, where it can be seen that the destruction of Tfm binding shown in Fig. 3 (no Trasylol) was partially prevented by Trasylol at 5000 or 10,000 k.i.u. per ml. In all subsequent experiments Trasylol was included in the homogenization media at a concentration of 5000 k.i.u. per ml.

To determine whether destruction of the binding by membrane bound proteases might occur during homogenization, an experiment was performed in which 12-week-old male and Tfm submandibular glands were combined and homogenized together. The binding in the mixed sample was compared with that of littermate male and Tfm samples prepared under identical conditions (Fig. 5). In contrast to the previous studies, the samples were exposed to the [1,2-3H]dihydrotestosterone after mixing the male and Tfm glands, and the aliquots of the three preparations applied to the gradients contained similar rather than additive amounts of protein (male 2.75 mg, Tfm 1.92 mg, and male plus Tfm 2.33 mg). In this study the binding observed in the mixed sample was approximately intermediate between that of the male and Tfm preparations homogenized separately, indicating that in the presence of Trasylol and when the samples are kept at \(4{\circ}\) throughout the entire experiment, destruction of the binding was prevented. In separate studies (results not shown) Trasylol was found to have no effect on binding in the Tfm preparations. Thus, the differences in binding between male and Tfm submandibular glands at 12 weeks of age are not likely the result of degradation during the homogenization and assay.

It was not clear whether the differences observed were solely quantitative in nature or whether the binding proteins in male...
Fig. 3. Effect of incubation at 37° on [1,2-3H]dihydrotestosterone binding by supernatant from submandibular glands from mice of varying ages. 100,000 X g supernatant was prepared from submandibular glands from 5 to 20 Ta/Y and Tfm/Y animals of each age group. Aliquots of the supernatant were then equilibrated with 10 nM [1,2-3H]dihydrotestosterone (1 pmole = 37,600 cpm) for 2 hours at 4°. The samples (male equilibrated with [3H]dihydrotestosterone, Tfm equilibrated with [3H]dihydrotestosterone, and a second sample of the Tfm supernatant equilibrated with [3H]dihydrotestosterone to which an equal volume of unlabeled male supernatant was added) were then incubated at 37° for 20 min. Aliquots, 0.1-ml, of the male (containing 1.08 to 2.31 mg of protein) and 0.2-ml aliquots of the mixed sample (containing 2.31 to 4.03 mg of protein) were then added to 5 to 20% sucrose gradients and centrifuged for 22 hours at 50,000 rpm in a Spinco 50.1 rotor.

Fig. 4. Effect of Trasylol on the destruction of [1,2-3H]dihydrotestosterone binding by supernatant from male submandibular gland. Submandibular gland supernatant was prepared from 31 Ta/Y and 40 Tfm/Y animals 12 weeks of age. Homogenates (4:1 by volume) were prepared using Tris-EDTA buffer that contained Trasylol at a concentration of either 6000 k.i.u. or 10,000 k.i.u. per ml. Supernatants from the male and Tfm glands were then incubated with 10 nM [1,2-3H]dihydrotestosterone (1 pmole = 32,800 cpm) for 2 hours at 4°. The samples (male equilibrated with [3H]dihydrotestosterone, Tfm equilibrated with [3H]dihydrotestosterone, and a second sample of the Tfm supernatant equilibrated with [3H]dihydrotestosterone to which an equal amount of unlabeled male supernatant was added) were then incubated at 37° for 20 min. Aliquots, 0.1-ml, of the male (containing 2.76 mg of protein) and Tfm supernatant (containing 1.52 mg of protein) and 0.2-ml aliquots of the mixed sample (containing 4.28 mg of protein) were then added to 5 to 20% sucrose gradients and centrifuged for 22 hours at 50,000 rpm in a Spinco 50.1 rotor.

Fig. 5. Prevention of the destruction of [1,2-3H]dihydrotestosterone binding by Trasylol in the cold. Submandibular glands from 16 Tfm/Y mice, 7 Ta/Y mice and from a combination of 4 Ta/Y and 4 Tfm/Y mice 12 weeks of age were homogenized in Tris-EDTA buffer containing Trasylol (6000 k.i.u. per ml) and centrifuged at 104,000 X g. The supernatants were equilibrated in 10 nM [1,2-3H]dihydrotestosterone (1 pmole = 32,800 cpm) for 2 hours, and 0.2-ml aliquots containing 1.92 to 2.75 mg of protein were applied to 5 to 20% sucrose gradients and centrifuged at 50,000 rpm in a Spinco 50.1 rotor for 22 hours.
Fig. 6. Quantitation of [1,2-3H]dihydrotestosterone binding by gel filtration on Sephadex G-25. Supernatants, 100,000 X g, from submandibular glands of 6-week old Tfm/Y animals were equilibrated with [1,2-3H]dihydrotestosterone at a concentration of 10 nM (1 pmole = 29,950 cpm) or 10 µM (1 pmole = 28 cpm) overnight in ice. Aliquots, 0.1 ml, containing 1.10 mg of protein were then applied in a cold room at 4° to columns (0.55 X 7 cm) of Sephadex G-25 that had previously been equilibrated in the Tris-EDTA buffer. Five-drop fractions (0.14 ml) were collected and assayed for radioactivity. Routinely, the first 1.5 ml (labeled Sample 1) was collected as the bound fraction, and the next 3.0 ml (labeled Sample 2) was collected as the free or unbound fraction.

Fig. 7. [1,2-3H]Dihydrotestosterone binding by supernatant from Tfm submandibular glands as a function of time. Supernatant prepared from 6-week old Tfm animals was diluted to a concentration of 1.13 mg of protein per ml and aliquots were incubated with [1,2-3H]dihydrotestosterone at a concentration either of 10 nM (20,300 cpm per pmole) or 1 µM (215 cpm per pmole) for varying periods of time. Dihydrotestosterone binding was then assessed by the gel filtration procedure described in Fig. 6.

Fig. 8. [1,2-3H]Dihydrotestosterone-binding by Tfm supernatant as a function of concentration of the hormone. Supernatant was prepared from 14 Tfm/Y animals 6 weeks of age and diluted to a concentration of 1.13 mg per ml (a 64-fold dilution of the starting glands). Aliquots were incubated with varying amounts of dihydrotestosterone at 4° for 3 hours. Aliquots, 0.1 ml, were then assayed for [3H]dihydrotestosterone binding by the gel filtration method described in Fig. 6. A, dihydrotestosterone binding as a function of hormone concentration. B, Scatchard analysis of the data shown in A. For the analysis of the high affinity binding, the projected curve from the flat portion of the experimental curve (-----) was subtracted from the initial portion of the experimental curve to allow delineation of a separate high affinity curve (X---X).

specific binding, i.e. binding that is competed by the cold hormone, has been graphed.

As shown in Fig. 7, the time course of the binding was investigated at two concentrations of [1,2-3H]dihydrotestosterone (10 nM and 1 µM). Binding did not plateau until after 4 hours and changed little thereafter over a 24-hour period. Therefore, in the routine gel filtration, samples were left overnight (6 to 11 hours) in the cold prior to assay. The routine assay was performed at dihydrotestosterone concentrations that varied from 1 nM to 1.4 µM (Fig. 8, Panel A) and at protein concentrations near 1 mg per ml. Scatchard plots of such binding curves (Fig. 8, Panel B) reveal, as had previously been shown by equilibrium dialysis (11), that both low and high affinity binding were present. No attempt was made to characterize the low affinity binding, but for the analysis of the high affinity binding, the low affinity line was projected to the ordinate (y axis), and the values from this projected line were subtracted from the high affinity binding for an expression of the high affinity binding line (Fig. 8, Panel B). Since the low affinity binding appeared flat (nonsaturable) under these assay conditions, this correction had no effect on the apparent dissociation constant determined for the high affinity binding (the slope of the line) but did influence the analysis of the number of binding sites per mg of protein (the intercept of the line on the x axis). Because the dissociation constants and number of binding sites are measured in unpurified preparations and because the analysis of each depends on several unproved assumptions, the values obtained are probably valid for comparison between different cytosols but cannot be assumed to have absolute meaning.

Utilizing as a standard procedure the gel filtration of 0.1-ml aliquots of samples that had been incubated at 4° overnight with varying amounts of [1,2-3H]dihydrotestosterone in the presence and absence of excess cold dihydrotestosterone and at a protein concentration of approximately 1 mg per ml, the high affinity binding of dihydrotestosterone was compared between supernatants of male and Tfm submandibular glands obtained from 2-week, 6-week, and 12-week-old mice (Table I). During this period of time both the weight of the glands and the amount of supernatant protein increased 3-fold. High affinity binding was demonstrated in the male and Tfm supernatant at every age studied. Furthermore, the apparent dissociation constant for male and Tfm animals was approximately the same, varying from
The dissociation constants and the number of binding sites were all determined at protein concentrations of 0.6 to 2 mg per ml. Values are given as the mean ± S.E.M. of four to six experiments, each of which was performed on pooled tissues from 6 to 20 littermate TJm/Y and Ta/Y animals.

<table>
<thead>
<tr>
<th>Age</th>
<th>Genotype</th>
<th>No. of experiments</th>
<th>Average weight of glands mg</th>
<th>Supernatant protein mg/gland</th>
<th>Average KD nM</th>
<th>Average binding sites per mg of protein pmoles</th>
<th>Average binding sites per gland pmoles</th>
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<tbody>
<tr>
<td>2 weeks</td>
<td>Ta/Y</td>
<td>4</td>
<td>29 ± 2</td>
<td>1.9 ± 0.3</td>
<td>10 ± 1</td>
<td>4.2 ± 1.0</td>
<td>7.3 ± 0.9</td>
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<tr>
<td></td>
<td>TJm/Y</td>
<td>4</td>
<td>38 ± 4</td>
<td>2.5 ± 0.6</td>
<td>10 ± 2</td>
<td>3.5 ± 0.5</td>
<td>8.5 ± 1.7</td>
</tr>
<tr>
<td>6 weeks</td>
<td>Ta/Y</td>
<td>4</td>
<td>63 ± 4</td>
<td>6.2 ± 0.6</td>
<td>12 ± 3</td>
<td>1.7 ± 0.5</td>
<td>10.1 ± 3.4</td>
</tr>
<tr>
<td></td>
<td>TJm/Y</td>
<td>6</td>
<td>71 ± 3</td>
<td>5.9 ± 0.5</td>
<td>8 ± 1</td>
<td>5.2 ± 1.4</td>
<td>33.1 ± 12.5</td>
</tr>
<tr>
<td>12 weeks</td>
<td>Ta/Y</td>
<td>4</td>
<td>79 ± 4</td>
<td>7.3 ± 0.3</td>
<td>10 ± 3</td>
<td>1.4 ± 0.4</td>
<td>10.0 ± 2.5</td>
</tr>
<tr>
<td></td>
<td>TJm/Y</td>
<td>4</td>
<td>88 ± 3</td>
<td>6.5 ± 0.3</td>
<td>9 ± 2</td>
<td>4.6 ± 1.5</td>
<td>30.2 ± 10.7</td>
</tr>
</tbody>
</table>

8 to 12 nm in the various groups. However, an apparent age-dependent difference in the number of binding sites was demonstrated, whether the results are expressed as the average binding sites per mg of supernatant protein or per gland. At 2 weeks of age, the amount of high affinity binding was approximately the same in the male and TJm glands (7.3 and 5.8 pmoles per gland). By 6 weeks of age, however, binding in the TJm gland increased in keeping with the size of the gland to 33 pmoles per gland, whereas in the male the total amount of binding did not change so that the actual concentration of binding sites per mg of supernatant protein decreased in this 4-week interval. The reason that differences between male and TJm are evident by gel filtration at 6 weeks of age, whereas in most instances no striking changes were found by density gradient centrifugation until 12 weeks of age, is not immediately apparent. Since the density gradient method, as utilized in these studies, measures both low and high affinity binding and since the flatness of the curves precluded the quantification of the low affinity binding by the Scatchard method, it is possible that the apparent discrepancy between the two procedures is the result of age related differences in low as well as high affinity dihydrotestosterone binding.

**Discussion**

Considered together, the inability to demonstrate any difference in the mobility of the binding moieties by ultracentrifugation between the TJm and male animals in this or previous studies (11), the similarity of the apparent dissociation constant for the high affinity binding in the male and TJm animals, the fact that the binding phenomenon appears at a similar age in the two animals, and the demonstration that quantitative differences in the binding are not present at 2 weeks and only become manifest at older ages all suggest that the enhanced dihydrotestosterone binding in the TJm submandibular gland is due to the accumulation of increased quantities of a normal binding protein. It is not possible to deduce whether the enhanced binding in the TJm is due to an increased production or defective consumption or turnover of the binding moiety.

If the enhanced binding of dihydrotestosterone in the TJm submandibular gland supernatant is causally linked to the diminished uptake of hormone in the nucleus that has been described in the TJm submandibular gland (10), it is attractive to speculate that a binding protein may accumulate in the cell cytoplasm because it cannot undergo translocation into the nucleus or binding to the nuclear sites where it is presumed to have its ultimate effect. However, it is impossible to exclude the alternate possibility that the fundamental defect in androgen metabolism lies elsewhere, that as a result of this defect androgen-dependent proteases are not synthesized, and that as a consequence the catabolism of this protein and others is retarded nonspecifically. The evidence is reasonably strong that proteolytic degradation during the homogenization and assay procedures cannot explain the differences reported for the 12-week animals. Nor was it possible to show any temperature-dependent destruction of binding in either male or TJm supernatants at 6 weeks of age when quantitative differences in the high affinity binding are already clear-cut. However, it remains possible that in the intact cell differences in degradation rate might be operative by 6 weeks. Should this be true, another cause for the defective androgen binding in the nuclei would have to be sought.

These findings in the submandibular gland of the TJm mouse are at variance with reports by Gehrig et al. (15) and by Dullock and Bardin (16) that cytoplasmic androgen binding is diminished in the kidney of the adult TJm animal. The kidney and the submandibular gland of the mouse are similar in that both tissues are androgen-independent during embryogenesis but exhibit androgen-dependent sexual dimorphism in the postnatal state. Known androgen-dependent functions in these tissues include the formation of β-glucuronidase (9) and alcohol dehydrogenase by the kidney (17) and the formation of nerve growth factor (3, 4) and proteases by the submandibular gland (2, 5). The reasons for differences in androgen binding between the kidney and the submandibular gland of the TJm animal are not apparent. It is possible that the cytoplasmic androgen binding proteins themselves differ from tissue to tissue or that the concentration of identical proteins is regulated differently.

Whatever the relation between the phenomena reported here and the pathogenesis in the TJm mouse of androgen resistance with its characteristic male pseudohermaphroditism, at least two implications can be drawn about the normal physiology of the submandibular gland. First, since the binding phenomenon in the submandibular gland is not demonstrable at birth but appears by 2 weeks of age in all genotypes examined, it can be concluded that the development of the binding capacity is not androgen-dependent but is the result of other differentiative processes. Second, the appearance of the binding protein prior to the onset of sexual dimorphism in the gland suggests that it may play a role in this process. A careful elucidation of the time sequence relating the appearance of the binding protein, the onset of androgen secretion by the testis, and the development of the capacity for androgen responsiveness in the tissue might provide fundamental insights into the mechanism of sexual differentiation.
insight into the role of cytoplasmic androgen binding in the initiation of androgen action in this tissue.

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REFERENCES