Effects of Testosterone on Messenger Ribonucleic Acid and Protein Synthesis in Rat Seminal Vesicle

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In a previous report [Higgins *et al.* (1976) *Biochem. J.* **158**, 271–282] we described the effects of alterations in androgen status on the synthesis of two basic secretory proteins of the rat seminal vesicle. In the present paper we examine the effects of testosterone on the activity of mRNA in the seminal vesicle. Total cellular poly(A)-rich RNA was isolated and translated in a cell-free system prepared from wheat germ. Translation products were separated on denaturing polyacrylamide gels and the protein bands corresponding to the two basic secretory proteins were identified immunologically. Incorporation of radioactive methionine into these bands was taken as a measure of the individual mRNA activities. Total mRNA activity was estimated by radioactivity in total acid-precipitable material. The results show that 1 to 2 weeks after castration the activities of mRNA molecules for the basic secretory proteins were decreased 10–20-fold on a tissue basis. Testosterone given *in vivo* rapidly and substantially restores mRNA activity to normal. Since these changes correlate closely with variations in the rates of synthesis of the secretory proteins in whole cells it suggests that androgenic steroids control protein synthesis chiefly via mRNA availability. In this respect their action resembles those of other steroid hormones acting in other systems. However, these effects of testosterone on the mRNA molecules for the major secretory proteins could not be distinguished from those on total mRNA. Thus the proportion of the total mRNA population accounted for by the two specific mRNA molecules showed less than a 2-fold variation with androgen status. Similarly the two secretory proteins always accounted for 25–33% of general protein synthesis. This is in sharp contrast with the markedly differential effects of other steroid hormones controlling synthesis of major proteins in other well-studied systems. We interpret our results as indicating that testosterone regulates the mRNA population of the seminal vesicle as a whole.

Steroid hormones characteristically regulate the synthesis of proteins in responsive tissues. We have recently described some properties of two basic proteins (seminal-vesicle-basic proteins S and F) secreted by rat seminal vesicle (Higgins *et al.*, 1976*a,b*). The synthesis of these proteins, which amounts to 20–30% of the protein output of the tissue, is androgen dependent. After castration the synthesis of these proteins is dramatically decreased and this decrease can be reversed if testosterone (but not other steroid hormones) is given *in vivo* (Higgins *et al.*, 1976*a*). Although the number of epithelial cells, responsible for elaborating the vesicular secretion (Moore *et al.*, 1930; Price & Williams-Ashman, 1961; Deane, 1963), decrease after castration and increase after testosterone therapy (Higgins *et al.*,

1976*b*), androgens change the rate at which individual cells synthesize the basic secretory proteins and do not simply change the number of producer cells (Higgins *et al.*, 1976*b*). We therefore consider these proteins to be useful markers for studying the molecular mechanism of androgenic hormone action.

One of the ways in which androgens could influence protein synthesis is by controlling the level of mRNA available for translation. In the present paper we describe the effect of androgens on the mRNA activity of seminal vesicles, assaying the mRNA by its ability to direct protein synthesis in a cell-free translation system derived from wheat germ (Roberts & Paterson, 1973). We interpret the results as indicating that androgenic steroids control the synthesis of specific proteins in sensitive tissues largely via mRNA availability. In this respect they act like many other steroid hormones (see review by Higgins & Gehring, 1978). When, however, the effects of testosterone on general protein synthesis and total mRNA are taken into account, it seems that testo-

Abbreviations used: SDS, sodium dodecyl sulphate; RNAase, ribonuclease.

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sterone may not act differentially on the individual basic secretory proteins or their mRNA molecules.

Materials and Methods

All procedures and sources of all materials not specifically mentioned below have been described (Higgins *et al.*, 1976a,b).

RNA extraction

To minimize degradation by nucleases all glassware and solutions were autoclaved before use. Seminal vesicles from 20–40 adult male Sprague–Dawley rats were dissected free of connective tissue and coagulating glands and then frozen on solid CO₂. Tissue was used at once or stored at –70°C for up to 3 months. A percussion tissue disintegrator (Thermovac Industries Corp., Copiague, N.Y., U.S.A.) cooled with a mixture of ethanol and solid CO₂ was used to pulverize the frozen glands. Powdered tissue (5–6g) was resuspended in 10 vol. of ice-cold homogenization buffer (0.01M-Tris/HCl, pH 7.4, 0.01M-NaCl and 0.01M-EDTA) containing 0.5% (w/v) SDS (Merck, West Point, PA, U.S.A.), 0.5mg/ml heparin (Sigma, London SW6, U.K.; type II, Na⁺), 10μg/ml cycloheximide (Sigma) and 0.1% (v/v) diethyl pyrocarbonate (Sigma). Then 10 vol. of fresh phenol (saturated with 0.5M-Tris/HCl, pH 7.4, 0.01M-NaCl and 0.01M-EDTA) was added and the mixture homogenized with a laboratory blender (Silverson Machines, Chesham, Bucks., U.K.) for 1 min at slow speed and 30 s at top speed. The emulsion was shaken at room temperature for 10 min and the phases were separated (4000g for 10 min). The aqueous layer was removed and kept on ice while the interphase plus phenol were re-extracted with 0.5 vol. of homogenization buffer plus 0.5% SDS. The pooled aqueous phases were extracted twice with equal volumes of phenol/CHCl₃ (1:1, v/v). The aqueous phase was adjusted to 0.1M-NaCl and 0.02M-potassium acetate (pH 5) and the nucleic acids were precipitated overnight at –20°C with 2 vol. of ethanol. The precipitate was collected (20000g for 30 min), washed with 2 × 10 vol. of 66% ethanol/0.1M-NaCl/0.02M-potassium acetate, redissolved (>1mg/ml) in sterile 0.01M-Tris/HCl, pH 7.4, and stored at –70°C until required. Since up to 15% of the RNA remains at the interphase of the first phenol extraction, the interphase material was washed with 3 × 10 vol. of 66% ethanol/0.1M-NaCl/0.02M-potassium acetate (pH 5) at –20°C to remove phenol and resuspended in 10 vol. of homogenization buffer. Proteinase K (500μg/ml; Merck) that had been autodigested overnight at room temperature was added (final concentration 50μg/ml) and the mixture was incubated at 20–25°C for 1 h. Nucleic acids were recovered by using the phenol/CHCl₃ procedure and ethanol precipitation (as above)

and added to the bulk nucleic acid. The proportion of RNA in the extracted nucleic acids and the total RNA content of the tissue was determined by hydrolysis with 0.3M-NaOH (Higgins *et al.*, 1976b).

Separation of polyadenylated RNA

Poly(A)-rich RNA was separated from other RNA by affinity chromatography (Aviv & Leder, 1972). Briefly, 5–15mg of RNA at a concentration of 1mg/ml in 0.01M-Tris/HCl, pH 7.4/0.5M-KCl was applied to columns containing about 1g of (dT)_{12–18} cellulose (Collaborative Research, Waltham, MA, U.S.A.; polyadenylate-binding capacity 25.5A₂₆₀ units/g of cellulose). Poly(A)-rich RNA was eluted with 0.01M-Tris/HCl, pH 7.4, and was precipitated with ethanol as above. It was redissolved (>250μg/ml) in sterile 0.01M-Tris/HCl, pH 7.4, and stored at –70°C. Between 1% and 3.5% of total RNA was retained by the oligo(dT) cellulose whereas, on the basis of hybridization of [³H]polyuridylylate (see below), between 11 and 16% of the poly(A) in total RNA failed to bind.

Estimation of polyadenylic acid content of poly(A)-rich RNA

Poly(A) was estimated by hybridization to poly-[5-³H]uridylylate. Stock poly[5-³H](U) (Miles, Slough, U.K.; sp. radioactivity 54.2μCi/μmol of phosphorus, 6.8μg of polymer/μCi) was diluted in 0.06M-Tris/HCl, pH 7.2/0.9M-NaCl/0.09M-sodium citrate to a final concentration of 4μCi/ml. Each assay contained 50μl of poly[5-³H](U), 50μl of RNA sample plus 200μl of formamide buffer (3 vol. of formamide:1 vol. of 0.12M-Tris/HCl, pH 7.2, 1.8M-NaCl and 0.18M-sodium citrate) and was incubated at 45°C for 30 min, which control experiments showed was ample for complete hybridization to occur. Unhybridized RNA was digested for 1 h at 37°C with 4ml of 0.5M-NaCl/0.01M-magnesium acetate/0.01M-Tris/HCl, pH 7.2, containing 10μg of pancreatic RNAase/ml (Boehringer, Mannheim, Germany). Bovine serum albumin (Sigma fraction V; 50μg) and trichloroacetic acid (0.5ml; 50%, w/v) were added and the samples cooled at 0°C for 30 min. Precipitated material was collected on Millipore filters (0.45μm), washed with 5% trichloroacetic acid and dried. Polyadenylic acid (Miles; K⁺ salt) was used as a standard, hybridization being linear up to 1μg of poly(A).

Cell-free protein synthesis

Poly(A)-rich RNA was translated in a cell-free system from wheat germ (Roberts & Paterson, 1973) containing L-[³⁵S]methionine (The Radiochemical Centre, Amersham, Bucks., U.K.; sp. radioactivity 200–600Ci/mmol). Full details of the reaction mixture are given by Wheeler *et al.* (1977). Samples (25μl) were

incubated at 20–25°C for 2 h. Duplicate 2 μ l samples were used to measure total incorporation of radioactivity into acid-precipitable material (Wheeler *et al.*, 1977). Incorporation into specific secretory proteins was measured with the remainder of the translation sample. First total proteins were precipitated (0.5 ml of 5% trichloroacetic acid), washed twice with 5% trichloroacetic acid and redissolved in 50–100 μ l of dispersion buffer (1% SDS, 6M-urea, 0.7M-2-mercaptoethanol) at 100°C (for 1–2 min). Proteins were then separated on polyacrylamide gels and their radioactivity estimated (see below).

Polyacrylamide gel electrophoresis

This was based on the methods of Laemmli (1970) and Studier (1972). Running gels (16 cm $\times$ 10 cm $\times$ 0.2 cm) contained 20% acrylamide, 0.13% *NN'*-methylenebisacrylamide, 0.375M-Tris/HCl, pH 8.9, and 0.36% SDS with 0.29 μ l of *NNN'*-tetramethylethylenediamine. Polymerization was with 0.8 mg/ml of (NH₄)₂S₂O₈. Spacer gels (16 cm $\times$ 2.5 cm $\times$ 0.2 cm) contained 5% acrylamide, 0.4% *NN'*-methylenebisacrylamide, 0.06M-Tris/HCl, pH 6.7, 0.1% SDS with 0.58 μ l of *NNN'*-tetramethylethylenediamine/ml and 5 μ g of riboflavin/ml, photopolymerized (320 nm) at room temperature. Tray buffer contained (per l) 3 g of Tris, 14.4 g of glycine and 1 g of SDS. Urea (3M) was present in all gels and buffers. Electrophoresis was at constant current (50 mA/gel) until the tracking dye (Bromophenol Blue) was within 1 cm of the bottom of the running gel. Routinely the gels were fixed and eluted for 24 h in 2 litres of acetic acid/methanol/water (7:40:53, by vol.), and then dried on Whatman 3MM paper. Radioactive protein bands were located by radioautography or fluorography with 2,5-diphenyloxazole (Laskey & Mills, 1975). Kodak X-Omat H film was used for 3–4 days at room temperature (radioautographs) or 12–24 h at –70°C (fluorographs). There was no noticeable difference between the methods but fluorography is quicker. Radioactive bands were cut out and the polyacrylamide was hydrolysed with 1 ml of H₂O₂ (100 vol.) for 2–4 h at 60°C. Radioactivity was estimated as below. In some experiments the gels were cut transversely into 1.5 mm slices and the acrylamide was digested as above.

Protein synthesis in tissues

Incorporation of [³⁵S]methionine into total acid-precipitable protein and specific secretory proteins were measured in minced seminal vesicles at 37°C for 45 min (Higgins *et al.*, 1976a).

Immunoprecipitation of seminal-vesicle protein

The secretory proteins, seminal-vesicle-basic proteins S and F, were separated from other seminal

vesicle proteins by Sephadex G-200 and preparative gel electrophoresis (Higgins *et al.*, 1976a). About 100 μ g of protein in 1 ml of sterile 0.14M-NaCl was emulsified with 2 ml of Freund's adjuvant (Difco, Detroit, MI, U.S.A.) and injected subcutaneously at multiple sites (thorax and limbs) into adult male New Zealand White rabbits. Injections were repeated twice at 2 week intervals and the animals were bled 2 weeks later. Serum was separated and stored at –70°C. Immunoprecipitation of proteins synthesized in the wheat-germ system was carried out by the procedure of Kessler (1975). Each translation sample received 0.25 ml of buffer (0.01M-Tris/HCl, pH 7.4, 0.15M-NaCl, 0.01M-EDTA, 0.5% Nonidet 40) plus 0.25 ml of the appropriate serum. After 10 min at room temperature formaldehyde-treated *Staphylococcus aureus* Cowan I strain (10⁹ cells in 0.25 ml of water) were added. Ten minutes later, the cells were recovered (1000g for 5 min), washed three times in buffer (0.5 ml) and heated at 100°C in 50–100 μ l of dispersion buffer (see above). Debris was removed by centrifugation and the solubilized proteins were separated on polyacrylamide gels (see above).

Radioactivity

Dried Millipore filters were immersed in 10 ml of toluene phosphor [6 g of 5-(biphenyl-4-yl)-2-(4-t-butylphenyl)-1-oxa-3,4-diazole/litre of toluene] and counted in a Nuclear-Chicago II scintillation counter at 30% efficiency for ³H and 75% for ³⁵S. Digested gel samples were mixed with 1.0 ml of aq. scintillant [750 ml of toluene, 250 ml of Triton X100 and 6 g of 5-(biphenyl-4-yl)-2-(4-t-butylphenyl)-1-oxa-3,4-diazole]. Efficiency was also 75% for ³⁵S.

Results

In the wheat germ cell-free translation system, poly(A)-rich RNA from seminal vesicles of normal rats promotes synthesis of several polypeptides of various sizes, whereas little or no incorporation of [³⁵S]methionine is seen in the absence of exogenous RNA (Fig. 1a). Prominent among the translation products are two rapidly migrating species S (*R_F* 0.52) and F (*R_F* 0.60) (Fig. 1a). The basic secretory proteins of the rat seminal vesicle, seminal-vesicle-basic proteins S and F, described in an earlier report (1) migrate slightly more rapidly (*R_F* 0.57 and 0.67 respectively) on 20% polyacrylamide/sodium dodecyl sulphate gels than the translation products S and F (results not shown). However, serum from rabbits immunized with the two seminal-vesicle-basic proteins precipitates both translation products S and F in contrast to pre-immune serum, which is ineffective (Fig. 1b). So far we have not successfully raised antisera to seminal-vesicle-basic protein F alone, but antiserum from rabbits immunized with seminal-

Fig. 1. Synthesis of seminal-vesicle-basic proteins by cell-free system and their immunological identification

In (a) poly(A)-rich RNA ($0.5 \mu\text{g}$) from seminal vesicles of normal rats was used to direct incorporation of [^{35}S]methionine in the wheat germ system. Translation products were analysed on denaturing polyacrylamide gels and the gels were cut into transverse slices (1.5 mm). Radioactivity was determined in each slice as described in the Methods section ($\bullet$). Control translations received buffer in place of RNA ($\circ$). The position of basic proteins S and F is indicated. In (b) antiserum for seminal-vesicle-basic proteins S and F ($\bullet$) or pre-immune serum ($\circ$) and in (c) antiserum for seminal-vesicle-basic protein (S) ($\bullet$) were added to translation samples that received RNA. Immune precipitates (see the Methods section) were analysed on gels as in (a). Electrophoresis is from left to right.

vesicle-basic protein S precipitates only the more slowly-migrating component S (Fig. 1c). Of the radioactivity in areas S and F about 8 and 12% respectively appear to resist successive precipitations with

Fig. 2. Relationship between amount of RNA added and specific protein synthesis in wheat germ system

Poly(A)-rich RNA from seminal vesicles of normal rats was added as indicated to duplicate samples of wheat germ system. Incorporation of [^{35}S]methionine into translation products basic proteins S ($\bullet$) and F ($\circ$) was determined (see the Methods section).

antiserum to seminal-vesicle-basic proteins S and F (data not shown).

Thus the experiments shown in Fig. 1 indicate that in the wheat-germ-system poly(A)-rich RNA from seminal vesicles directs the synthesis of polypeptides with antigenic determinants in common with the secretory proteins, seminal-vesicle-basic proteins S and F. We presume that the immediate translation products S and F are precursors of the authentic secretory proteins, though this has not been verified directly by, for instance, peptide mapping. Therefore, to examine the effects of testosterone on the mRNA activity of RNA from seminal vesicles we can use the wheat germ system to translate poly(A)-rich RNA isolated from the glands of rats in different androgenic states. However, before this can be carried out it is essential that incorporation of [^{35}S]methionine be linearly related to added RNA. Although the absolute incorporation varied somewhat with different wheat germ preparations, total incorporation of methionine was always linear with up to $1 \mu\text{g}$ of poly(A)-rich RNA per assay and usually up to $2 \mu\text{g}$ (Fig. 2). There was no significant difference between poly(A)-rich RNA from normal rats or rats castrated 7 days previously. Routinely therefore about $0.5 \mu\text{g}$ of poly(A)-rich RNA was used in each translation sample. Since K^+ and Mg^{2+} are necessary for translation of mRNA (Roberts & Paterson, 1973), we also investigated the effects of varying the concentrations of KCl and MgCl_2 on translation of seminal-vesicle mRNA (Fig. 3). Optimal translation of mRNA molecules for seminal-vesicle-basic proteins occurred with about 1.65 mM-MgCl_2 and between 55 mM- and 90 mM-KCl and, despite slight variations in these optima for different wheat germ

Fig. 3. Ionic requirements for translation of seminal-vesicle RNA

Poly(A)-rich RNA from normal rats (—) or from rats castrated 14 days before (----) was used in the wheat germ system. In (a) the concentration of MgCl_2 was varied with 85 mM-KCl; in (b) the concentration of KCl was varied with 1.65 mM- MgCl_2 . The products were separated on gels and bands S (●) and F (○) (see Fig. 1) were cut out and assayed for radioactivity.

preparations, RNA from normal and castrated rats behaved identically (Fig. 3). Routinely we used 1.65 M- MgCl_2 and 85 mM-KCl in our cell-free system.

In the experiments described below, mRNA activity per gland was calculated as follows. A weighed sample of tissue from a known number of rats was used to isolate total RNA and poly(A)-rich RNA (see the Methods section). The efficiency of isolation was determined each time. Between 41% and 75% of total RNA was extracted with no reproducible difference between normal, castrated and testosterone-induced rats. On the basis of polyadenylic acid measurements, 80–90% of the poly(A)-rich RNA was isolated by oligo(dT)–cellulose affinity chromatography.

Fig. 4. Effect of androgenic status on specific mRNA and protein synthesis in seminal vesicles

Rats were castrated on day zero and at the intervals shown groups were killed and seminal vesicles removed. After 14 days remaining groups received daily injections of testosterone propionate. Total poly(A)-rich RNA was isolated and mRNA for seminal-vesicle-basic proteins S (●) and F (○) was estimated (for details see the Methods section). Results are means $\pm$ S.E.M. for three separate determinations of between two and seven RNA preparations at each time point. Synthesis of seminal-vesicle-basic proteins S (■) and F (□) was determined in minced tissue at 37°C (see the Methods section).

graph. It therefore seems that hormonal status does not influence recovery of the poly(A)-rich RNA. The mRNA activity of the poly(A)-rich RNA was then determined by measuring the incorporation of radioactive amino acid into general and specific proteins in the wheat germ system supplied with a known amount of poly(A)-rich RNA (details in the Methods section).

The results of translating poly(A)-rich RNA from rats of different hormonal states show that after castration there is a pronounced decrease in the activity per gland of mRNA for seminal-vesicle-basic proteins; within 7–14 days the activity for seminal-vesicle-basic protein S is decreased by 10-fold (Fig. 4a), whereas that for seminal-vesicle-basic protein F is decreased by about 20-fold (Fig. 4b).

Fig. 5. Effect of androgenic status on general protein synthesis, total RNA and total mRNA in seminal vesicles (a) In the experiment of Fig. 4 total mRNA activity (●) was assayed in the wheat germ system by measuring total incorporation of [35 S]methionine (see the Methods section). Results are means $\pm$ S.E.M. as described in Fig. 4. Incorporation of methionine into general cellular proteins (■) was determined in minced tissue at 37°C (see the Methods section). (b) Seminal vesicles were assayed for total RNA (▲), polyadenylic acid (△) and poly(A)-rich RNA (□) as in the Methods section.

Testosterone given *in vivo* rapidly and substantially restores the activities per gland of these specific mRNA molecules (Fig. 4). In each case the changes in mRNA activity correlate quite well with the changes in the rates of synthesis of these two secretory proteins in whole tissue (Fig. 4), suggesting that the major factor controlling specific protein synthesis is the mRNA rather than some other component involved in protein synthesis.

It is possible that these changes in the mRNA molecules for the basic secretory proteins are confined to these particular mRNA molecules. Alternatively, they may merely be part of a more

general change in the total mRNA population. We therefore looked at the effects of castration and androgen therapy on total mRNA.

Fig. 5(a) shows that hormonal manipulation also results in pronounced changes in the total mRNA activity per gland and here too the changes correlate closely with changes in general protein synthesis in whole tissue. As with specific secretory proteins, therefore, the overall capacity of the tissue to synthesize protein seems to be determined largely by its mRNA. The data of Fig. 5(b) show that the effects of hormonal manipulation are not confined to mRNA, there being quantitatively similar changes in the total RNA (largely ribosomal), total poly(A)-rich RNA and total polyadenylic acid content of the seminal vesicle.

Since manipulation of the androgenic status of the rat affects, on a tissue basis, the specific mRNA molecules and total mRNA in quantitatively similar ways, it is clear that the concentrations of mRNA molecules for two major androgen-responsive proteins change very little within the total mRNA population. This is shown in Table 1 where the amounts of [35 S]methionine incorporated by the wheat germ system into seminal-vesicle-basic proteins S and F are expressed as a percentage of the total incorporation. On average seminal-vesicle-basic protein S accounts for 11–16% of the total incorporation and seminal-vesicle-basic protein F for 4–10%. Fig. 6 illustrates this in another way. Here the activities of mRNA molecules for the basic proteins and for total mRNA are expressed in terms of the total RNA content of the tissue. Variation is no more than about two-fold for either total or individual mRNA molecules.

Histological investigations (Moore *et al.*, 1930; Price & Williams-Ashman, 1961; Deane, 1963) indicate that the epithelium of the seminal vesicle synthesizes secretory proteins. Castration and subsequent androgen treatment affect epithelial-cell numbers (Higgins *et al.*, 1976b), there being a 60% decrease during the 2 weeks after castration and marked hyperplasia 1 to 2 days after testosterone therapy is started. Thus variation in mRNA activity per gland with the androgenic status may merely result from changes in epithelial-cell numbers rather than an effect on the mRNA activity of individual cells. We have tested this proposition by re-expressing the data of Fig. 4 in terms of epithelial-cell numbers from our previous report (Higgins *et al.*, 1976b). In doing so we have assumed that the mRNA molecules for seminal-vesicle-basic proteins S and F are confined to the epithelium, but that total mRNA and polyadenylic acid are evenly distributed between all cell types. The results of such calculations show that substantial changes occur in the mRNA content of individual cells in response to the amount of circulating testosterone (Fig. 7).

Table 1. *Effect of androgen status on incorporation of [35 S]methionine into seminal-vesicle proteins by wheat germ system*
Poly(A)-rich RNA was translated in the wheat germ system and the incorporation of [35 S]methionine into total proteins was determined after acid precipitation. Incorporation into seminal-vesicle-basic proteins was determined after separation of translation samples on denaturing gels. Details are in the Methods section. Results are mean $\pm$ s.e.m. as in Figs. 4 and 5. Incorporation of methionine into seminal-vesicle-basic proteins is expressed as a percentage of the total incorporation.

State of rat	Total protein (pmol of methionine/gland)	Seminal-vesicle-basic protein S		Seminal-vesicle-basic protein F	
		(pmol of methionine/gland)	(% of total)	(pmol of methionine/gland)	(% of total)
Normal	119.0 $\pm$ 15.5	19.0 $\pm$ 1.2	16	9.6 $\pm$ 1.7	8
Castrated (7 days)	16.0 $\pm$ 4.0	2.4 $\pm$ 0.2	15	0.6 $\pm$ 0.2	4
Castrated (14 days)	10.0 $\pm$ 2.5	1.6 $\pm$ 0.6	16	0.4 $\pm$ 0.2	4
Induced (1 day)	20.5 $\pm$ 5.5	2.7 $\pm$ 0.5	13	1.2 $\pm$ 0.9	6
Induced (2 days)	76.0 $\pm$ 16.0	9.2 $\pm$ 0.8	12	7.8 $\pm$ 1.7	10
Induced (3 days)	91.0 $\pm$ 25.0	10.0 $\pm$ 1.9	11	9.0 $\pm$ 1.1	10

Fig. 6. *Effect of androgenic status on specific activity of individual mRNA species*

Data from Figs. 4 and 5 were used to calculate the specific activities of mRNA for seminal-vesicle-basic proteins S (●) and F (○) and total mRNA (▲) as a function of total seminal-vesicle RNA.

Discussion

In the present study the effects of testosterone on seminal-vesicle mRNA have been investigated by using a cell-free protein-synthesizing system. After castration the mRNA molecules for the major secretory proteins, seminal-vesicle-basic proteins S and F, decrease dramatically both on a tissue basis and at the level of the responsive epithelial cells. Testosterone given *in vivo* rapidly and substantially reverses these effects of castration. Regardless of hormonal status, a close correlation exists between the rates of synthesis of the secretory proteins in minced tissue and their mRNA activities. When the total mRNA activity of poly(A)-rich RNA is examined, here too there is a close correlation between mRNA activity and the rate of general protein synthesis and, furthermore, these parameters change in a quantitatively similar manner to those for the specific proteins. Thus the proportion of the total mRNA accounted for by the two specific

Fig. 7. *Effect of testosterone on RNA content of individual cells*

Cell numbers from an identical experiment (Higgins *et al.*, 1976b) were used to recalculate the data of Figs. 4 and 5. In (a) it was assumed that mRNA molecules coding for seminal-vesicle-basic proteins S (●) and F (○) are confined to epithelial cells, whereas in (b) total RNA (▲) and polyadenylic acid (Δ) were assumed to be in all cell types.

mRNA molecules showed less than a 2-fold variation with androgen status and the two secretory proteins always accounted for 25–33% of general protein

synthesis in the tissue. These findings suggest two consequences of testosterone action. First, the steroid regulates protein synthesis largely through mRNA availability and not *via* any other element of the protein-synthesizing machinery. Secondly, testosterone does not act differentially, i.e. it does not regulate the synthesis of the major secretory proteins independently of general protein synthesis.

Of course only polysomal mRNA is actively engaged in protein synthesis so that by using total cellular poly(A)-rich RNA we have neglected the contribution of nonadenylated mRNA, but have included polyadenylated mRNA not actually in polysomes. Extra-polysomal and poly(A)-deficient mRNA can be quantitatively important in other systems, including the steroid-responsive chick oviduct (Jacobs-Lorena & Baglioni, 1972; Palmiter, 1973; Cox *et al.*, 1974; Rhoads *et al.*, 1973; Scherrer, 1973; Milcarek *et al.*, 1974; Nemer *et al.*, 1974; Herman *et al.*, 1976; Greenberg, 1977), so we have no reason to exclude these possibilities in the seminal vesicle. However what is important is whether testosterone affects the extent of polyadenylation or the subcellular distribution of mRNA. Oestradiol does not affect the proportion of ovalbumin mRNA that is located in oviduct polyribosomes (McKnight *et al.*, 1975). In fact, the close correlation during hormonal manipulation between our estimates of mRNA and protein synthesis suggests that our use of total cellular poly(A)-rich RNA gives us an accurate assessment of relative mRNA activities.

In addition to these considerations, our indirect measurements of mRNA activity in the wheat germ system might not be simply related to the actual amount of mRNA present if testosterone altered some feature of mRNA necessary for its translation in this heterologous system. This seems unlikely since the ionic requirements for the translation of seminal-vesicle mRNA are unaffected by hormonal status. Furthermore, several groups have examined the effects of steroid hormones on specific mRNA molecules by using both indirect translational assays and direct estimation by nucleic acid hybridization methods and found close agreement between the two (Palmiter, 1975; Rosen & O'Malley, 1975; Palmiter *et al.*, 1976; Martial *et al.*, 1977). Thus, although we know only that testosterone controls mRNA activity in the seminal vesicle, it seems reasonable to assume that it also controls the amount of mRNA.

In regulating specific protein synthesis chiefly *via* mRNA availability testosterone behaves like glucocorticoids, oestrogens and progestins in other systems (O'Malley *et al.*, 1972; Chan *et al.*, 1973; Harris *et al.*, 1975; McKnight *et al.*, 1975; Ringold *et al.*, 1975, 1977; Schutz *et al.*, 1975; Steinberg *et al.*, 1975; Kurtz *et al.*, 1976; Mullinix *et al.*, 1976; Palmiter *et al.*, 1976; Martial *et al.*, 1977; Tushinski *et al.*, 1977). However, in acting non-specifically

and not differentially, testosterone action in the seminal vesicle differs quite sharply from the markedly differential action of these other steroids. Thus glucocorticoids increase the synthesis of a few specific proteins without noticeable effects on the total mRNA population or general RNA and protein metabolism (Tomkins *et al.*, 1966; Kohler *et al.*, 1969; Bancroft & Tashjian, 1971; Dannies & Tashjian, 1973; Dickson *et al.*, 1974; Ivarie & O'Farrell, 1978). Even in oviduct where the egg-white protein ovalbumin can account for over 60% of the protein output of tubular-gland cells of oestrogen-stimulated chicks, its contribution to overall protein synthesis is negligible in hormone-withdrawn animals (Palmiter, 1975; Rosen & O'Malley, 1975). Not surprisingly therefore the concentration of ovalbumin mRNA within the mRNA population shows huge variations depending on the hormonal state of the animal (Axel *et al.*, 1976; Monahan *et al.*, 1976; Cox, 1977; Hynes *et al.*, 1977). However, it should be emphasized that testosterone may not act generally in all its target tissues. Experiments almost identical to those reported here showed that testosterone acts differentially in the rat ventral prostate (Heyns *et al.*, 1977). In addition, analyses of sequence complexity in the poly(A)-rich RNA fraction of the prostate also show that testosterone markedly affects the specific activities of a few major mRNA species within the total mRNA population (Parker & Mainwaring, 1977; Parker & Scrace, 1978).

Although our results support the view that testosterone regulates the mRNA population of the seminal vesicle as a whole, it could still be that the hormone acts directly and differentially on a small group of proteins, including seminal-vesicle-basic proteins S and F, which together account for most of the protein output of the gland. If so, their mRNA would also account for most of the total mRNA and we should see the disappearance of these few very abundant sequences following castration and their reappearance on induction. Nucleic acid hybridization methods are available (Bishop *et al.*, 1974) to test this possibility.

Finally in suggesting a non-differential action for testosterone in the seminal vesicle, we do not wish to imply necessarily that the primary locus of testosterone action is fundamentally different from that of other steroid hormones in other situations. Nevertheless our data also show that total RNA varies in parallel with the mRNA, so that conceivably testosterone affects some basic aspect of the metabolism of all RNA species.

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