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The Control of the Form and Function of the Ribosomes in Androgen-Dependent Tissues by Testosterone

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1. The ribosome content of the rat ventral prostate gland is controlled by the concentrations of circulating androgens and the polyribosomal complement of the total population of ribosomes is acutely dependent on androgenic stimulation. After the administration of testosterone to castrated rats *in vivo*, there is a pronounced increase in the amounts of heavy (150–240S) polyribosomes. 2. These results are consistent with a pronounced increase in the mRNA and rRNA content of the prostate gland after the administration of testosterone *in vivo*. 3. From studies conducted both *in vivo* and *in vitro*, the heavy prostate polyribosomes formed after androgenic stimulation are particularly active in protein synthesis. 4. The androgen-stimulated increase in the formation of prostate polyribosomes has a mandatory requirement for sustained RNA and protein synthesis. 5. Since the androgen-mediated increase in prostate polyribosomes may also be suppressed by the concomitant administration of certain anti-androgenic steroids *in vivo*, the response in polyribosome formation is probably initiated by the binding of a metabolite of testosterone, 5α-dihydrotestosterone, in the prostate gland. 6. The relevance of these findings to the pronounced increase in protein synthesis in androgen-dependent tissues after hormonal stimulation is discussed.

There is abundant evidence to support the view that protein synthesis in the majority of mammalian cells is controlled by both steroids and polypeptide hormones. In keeping with this widely accepted concept, the androgens stimulate the incorporation of amino acids into polypeptide linkage in all the androgen-dependent accessory sexual glands (see review by Williams-Ashman *et al.*, 1964). The rat ventral prostate gland has long been adopted as a convenient experimental system in which to study the androgenic control of protein synthesis, and it was established in earlier studies that the administration of testosterone to castrated animals enhanced the ability of prostate ribosomes to promote protein synthesis *in vitro* (Liao & Williams-Ashman, 1962; Mangan *et al.*, 1967).

The principal objective of the present study was to ascertain whether the complement of polyribosomes in the prostate gland was controlled by androgens. Such an investigation is not only germane to an understanding of the general mechanism of the androgenic control of protein synthesis but should also provide unequivocal evidence that a stimulation of mRNA synthesis was an integral feature of the androgenic response. The latter postulate has previously been based on the rather indirect evidence of differences in the ability of ribosomes derived from animals of various androgenic status to incorporate radioactive amino acids into protein in the presence of synthetic types of mRNA, notably poly(UG) and poly(U) (Liao & Williams-Ashman, 1962; Mangan *et al.*, 1967; Mainwaring & Wilce, 1972). The interpretation of these studies is open to some doubt in view of evidence suggesting that such synthetic mRNA molecules are not necessarily bound to ribosomes in a manner identical with that for naturally occurring mRNA (Gilbert, 1963; Takanami & Okamoto, 1963). The earlier studies on the prostate gland were also based on the necessary assumption that the rate of translation is similar with all samples of mRNA, irrespective of their origin. These difficulties in interpretation can be readily circumvented by more direct studies on the content and function of prostate polyribosomes after hormonal stimulation, for an increase in polyribosome production can occur only with concurrent increases in both rRNA and mRNA.

Materials and Methods

Materials

Chemicals. L-[U-^{14}C]Phenylalanine (sp. radioactivity 405mCi/mmol), L-[^{3}H]phenylalanine (sp. radioactivity 1.2Ci/mmol) and a mixture of ^{14}C-labelled amino acids (54mCi/mg-atom of carbon) were obtained from The Radiochemical Centre, Amersham, Bucks., U.K. Tris base (99.5% pure), ATP, GTP, dithiothreitol, 2-mercaptoethanol, poly(U) and Triton X-100 were obtained from Sigma (London)

Chemical Co., London S.W.6, U.K. Ribonuclease-free sucrose was supplied by Cambrian Chemicals Ltd., Croydon, Surrey, U.K. *Escherichia coli* (ribonuclease-free mutant, M.R.E. 600) was obtained from the Microbiological Research Establishment, Porton Down, Wilts., U.K. Electrophoretically purified ribonuclease (free of protease and deoxyribonuclease) was purchased from Worthington Biochemical Corp., Rahway, N.J., U.S.A. The anti-androgen 6α-bromo-17β-hydroxy-17α-methyl-4-oxa-5α-androstan-3-one was generously given by Hoffmann–La Roche Ltd., Basle, Switzerland. All other chemicals were of the highest available purity and glass-distilled water was used in making up all solutions.

Animals. All experiments were done on adult male Sprague–Dawley rats weighing 240–260g. Bilateral orchidectomy was performed by the scrotal route under fluothane anaesthesia. Testosterone phenylpropionate (2.5mg) was administered subcutaneously into the flank in 0.25ml of arachis oil. Control animals received an injection of oil only. 6α-Bromo-17β-hydroxy-17α-methyl-4-oxa-5α-androstan-3-one (15.0mg) was administered similarly in oil. Actinomycin D and cycloheximide, however, were injected intraperitoneally in 0.9% (w/v) NaCl.

Preparation of subcellular fractions

All preparations were done in a cold laboratory at 4°C, with thoroughly chilled apparatus.

Ribosomes and polyribosomes. These were isolated by a procedure based essentially on method II described by Liao & Williams-Ashman (1962) but with the non-ionic detergent, Triton X-100 (Hunter & Korner, 1966) replacing sodium deoxycholate. Prostate glands were thoroughly chilled in ice-cold medium A (0.1M-KCl–10mM-$MgCl_2$–40mM-NaCl–6mM-2-mercaptoethanol in 25mM-Tris–HCl buffer, pH7.5; Korner, 1969). A 30% (w/v) tissue homogenate was prepared as described by Mainwaring & Wilce (1972) and a total particulate fraction was collected by sedimentation at 105000*g* for 1h in the A60 rotor of a Christ ultracentrifuge. After careful decantation of the supernatant, the sediment was gently resuspended by hand homogenization in 10ml of medium A, by using a loose-fitting Potter–Elvehjem homogenizer. A one-ninth volume of 10% (v/v) Triton X-100 in medium A was added dropwise with constant stirring, and the mixture was centrifuged at 10000*g* for 15min. The supernatant fraction was used as a source of both unfractionated ribosomes and a polyribosome-enriched fraction. The former were prepared by centrifugation at 105000*g* for 1.5h in the A60 rotor. Polyribosomes were prepared by layering the 10000*g* supernatant fraction (4.0ml) over an equal volume of 1.5M-sucrose in medium A and centrifugation at 150000*g* for 1.5h. Both sediments were resuspended by gentle homogenization in sufficient medium A to give a concentration of rRNA of approx. 2mg/ml. After thorough mixing with an equal volume of glycerol, the suspensions were stored at −20°C. Under these conditions, no visible aggregation or loss in synthetic activity was found during storage for up to 4 weeks. When fractions were to be analysed in sucrose-density gradients, they were not stored in 50% (v/v) glycerol but were analysed directly after preparation. Concentrations of ribosomes and polyribosomes were estimated by extinction analysis at 254nm, an extinction of 1.0 being termed 1 unit of particles (approx. 30μg of RNA). Ribosomes prepared in the standard manner described above, without centrifugation in 1.5M-sucrose, are synonymously described as total or unfractionated ribosomes.

One aspect of this method of preparation (Liao & Williams-Ashman, 1962) warrants further comment. Inhibitors of ribonuclease, such as cell sap, were not present during the latter part of the procedure and some degradation of the ribosomes or polyribosomes during the course of their preparation cannot be rigorously excluded. The method was adopted to ensure a strict uniformity between the present study and earlier work (Liao & Williams-Ashman, 1962; Mangan *et al.*, 1967).

Translocase fraction. A partially purified preparation of translocase factors was isolated essentially as described by Moldave (1963). The initial extract was prepared from the 105000*g* supernatant fraction of normal (non-castrated) animals, after the removal of considerable protein by precipitation at pH5.2 (Mainwaring & Wilce, 1972). The preparations were stored in 50% (v/v) glycerol–25mM-KCl–10mM-$MgCl_2$–2mM-dithiothreitol in 20mM-Tris–HCl buffer, pH7.8, at −20°C for at least 2 months without detectable loss in activity.

tRNA. This was isolated from *E. coli* by the method of von Ehrenstein & Lipmann (1961), extensively dialysed against deionized water, freeze-dried and stored at −20°C. Only a single band migrating at 4S, relative to markers of *E. coli* 18S and 23S rRNA, was identified after electrophoresis in agarose-supported polyacrylamide gels (Dingman & Peacock, 1968). A pH5 enzyme fraction (Mainwaring & Wilce, 1972) was used as a source of rat liver tRNA. As described in detail below, no attempt was made to purify the tRNA of the pH5 enzyme fraction until it had first been charged with [^{3}H]phenylalanine.

Charging of tRNA with [^{14}C]- and [^3H]-phenylalanine

Phenylalanine aminoacyl synthetase was prepared from *E. coli* up to stage IV of the procedure devised by Stulberg (1967). The enzyme was purified further by ion-exchange chromatography in columns (2.1cm×21cm) of DEAE-cellulose (Whatman DE

11). Elution was performed with a linear gradient developed from 300ml volumes of 50mM-K_2HPO_4–KH_2PO_4 buffer, pH7.5, and 0.2M-potassium phosphate buffer, pH6.5; both buffers contained 1mM-EDTA and 10mM-2-mercaptoethanol. Active fractions eluted between 0.18 and 0.23M concentrations with respect to phosphate were precipitated at a 70% saturation of $(NH_4)_2SO_4$ and stored in 50% (v/v) glycerol in 25mM-potassium phosphate buffer, pH7.5, at −20°C for up to 4 months without deleterious change. *E. coli* tRNA (25.0mg) was charged with 25μCi of [^{14}C]phenylalanine under the conditions recommended by Stulberg (1967). The labelled tRNA (15000c.p.m./100μg of tRNA) was extracted as described above and stored at −20°C. This was not a suitable means for charging rat liver tRNA to a high specific radioactivity with [^{14}C]phenylalanine. For the labelling of rat liver tRNA, liver pH5 enzyme fraction (45mg of protein) was incubated as described for *E. coli* tRNA but bacterial aminoacyl synthetase was omitted and the [^{14}C]phenylalanine was replaced by 30μCi of [^{3}H]phenylalanine. The labelled tRNA was released by extraction with phenol (von Ehrenstein & Lipmann, 1961). The specific radioactivity of the final product, after extraction and purification, was 20000c.p.m./100μg of tRNA.

Fractionation of polyribosomes

Linear 15–50% (w/v) sucrose gradients, volume 5.0ml, were prepared in medium B (25mM-KCl–10mM-$MgCl_2$ in 35mM-Tris–HCl buffer, pH7.5) with the aid of a model 570 gradient former (Instrument Specialities Co., Lincoln, Nebraska, U.S.A.). Freshly sedimented polyribosomes were resuspended in medium B and 0.2ml samples (10–20E_{254} units) were layered over the gradients and centrifugation was conducted at 20000$g_{av.}$ for 4h at 4°C in the STi60 rotor of a Christ ultracentrifuge. The contents of each gradient were subdivided mechanically into 25 fractions, volume 0.20ml, with monitoring of E_{254} by using a model 182 gradient analyser (Instrument Specialities Co.). It was absolutely essential to use ribonuclease-free sucrose in making up the gradients. Other sources of sucrose led to instability of the polyribosomes. In certain experiments polyribosomes were incubated with ribonuclease (0.1μg/ml) for 10min at 0°C in medium B immediately before analysis.

Incorporation of ^{14}C-labelled amino acids into protein

Incorporation in vitro. Each assay (total volume 1.0ml) contained 50μmol of Tris–HCl buffer, pH7.4, 80μmol of NH_4Cl, 2μmol of dithiothreitol, 10μmol of $MgCl_2$, 0.2μmol of GTP, 100μg of [^{14}C]phenylalanyl-tRNA from *E. coli*, 500μg of translocase factors and ribosomes or polyribosomes equivalent to 50μg of rRNA. Samples were incubated for 5min at 37°C to achieve temperature equilibration and reactions were initiated by the addition of the ribosomes or polyribosomes. Where indicated, 100μg of poly(U) was added to the standard assay system. The total incubation was for 30min at 37°C. The acid-insoluble radioactivity incorporated into protein was collected on Whatman GF/A glass-fibre discs and processed as described in detail by Mainwaring & Wilce (1972).

Incorporation in vivo. Animals were anaesthetized and a ^{14}C-labelled amino acid mixture was injected directly into the prostate gland in 0.1ml of 0.9% (w/v) NaCl under aseptic conditions (Mainwaring & Wilce, 1972). When administered by other routes, the uptake of radioactive amino acids into prostate proteins was exceedingly low.

General procedures

Measurement of radioactivity. Acid-insoluble precipitates, retained and washed on glass-fibre discs, were assayed for radioactivity in a toluene-based scintillation fluid (5g of 2,5-diphenyloxazole/litre) in a Nuclear–Chicago mark II liquid-scintillation spectrometer. Counting efficiency for ^{14}C was 75% and 48% for ^{3}H.

Other procedures. Protein was determined by the method of Lowry *et al.* (1951) with bovine serum albumin (Sigma Chemical Co.) as standard. Concentrations of ribosomes and polyribosomes used in analyses in sucrose-density gradients were calculated on their extinction at 254nm, one unit of particles being equivalent to an extinction of 1.0. Where more accurate determinations were needed, especially in determinations of the yields of particles, RNA was estimated by the more precise spectrophotometric procedure of Munro & Fleck (1966), with yeast RNA as standard. Proteins were dissociated from polyribosomes and ribosomes and separated by electrophoresis in polyacrylamide gels as described by Gestland & Staehelin (1967). After staining with Coomassie Brilliant Blue R, gels were photographed and the negatives scanned in a Joyce-Loebl microdensitometer.

Results

Specific effect of androgens on the amounts of total ribosomes and polyribosomes in the prostate gland

The administration of testosterone to castrated animals *in vivo* had a profound effect on the contents of ribosomes and polyribosomes of the prostate gland in addition to promoting a marked increase in the rate of growth of the gland. The results of these experiments are summarized in Table 1. Androgenic stimulation for 2 days restored the size of the prostate gland to that present in normal (non-castrated) animals and effected a pronounced increase in the

Table 1. *Effect of androgens on the amounts of ribosomes and polyribosomes in the rat ventral prostate gland*

Complete details of the preparation of the total ribosomal fraction and polyribosomes are given in the Materials and Methods section. The results are the means of estimations from three experiments, with preparations derived from the pooled glands (approx. 1.0–1.62g wet wt. of tissue) of four animals per experimental group. Values from individual experiments were within ±6%. Animals were castrated and used 48h later. Testosterone (2.5mg/animal) was administered to one group at the time of castration and another group was given testosterone additionally 24h after castration. The average weight of the prostate gland is given in mg wet wt.; the yields of total ribosomes and polyribosomes are quoted in two ways: mg of rRNA/gland or mg of rRNA/g wet wt. of tissue. The recovery of rRNA in the total ribosome preparations was within the range 60–70% in all experimental groups.

	Wt. of prostate gland	Total ribosomes			Polyribosomes		
		Yield		RNA:protein ratio	Yield		RNA:protein ratio
		Per gland	Per g of tissue		Per gland	Per g of tissue	
Castrated animals (controls)	276	0.62	2.52	1.68	0.23	0.91	0.78
Castrated animals: 1 day of testosterone treatment	379	0.94	3.04	1.71	0.46	1.04	0.80
Castrated animals: 2 days of testosterone treatment	483	1.42	3.59	1.72	0.63	1.52	0.85
Normal animals	492	1.60	3.80	1.75	0.65	1.62	0.85

amounts of both total ribosomes and polyribosomes. The RNA:protein ratio in these particulate fractions was not significantly changed after hormonal stimulation. For reasons not understood, the RNA:protein ratio was higher for ribosomes than polyribosomes. It remains a possibility that certain cellular components of high absorbance at 254nm were removed by sedimentation in 1.5M-sucrose, as employed in the preparation of polyribosomes. With the reasonable assumption that 90–92% of the total RNA present in prostate homogenates was rRNA, then only 60–70% of this was recovered in the preparations of total ribosomes. This relatively low recovery was found in all experimental groups, and is explained by the necessary inclusion of the bivalent cation Mg^{2+} in all preparative media. High recoveries of prostate fractions containing rRNA can be obtained only in the presence of EDTA (Harding & Samuels, 1961) and the use of this chelating agent was precluded in the present investigation. The adverse effects of the presence of Mg^{2+} in the recovery of prostate microsomal fractions, for example, was reported by Mainwaring & Wilce (1972).

In additional experiments the specificity of the androgenic stimulation of polyribosome formation in the prostate gland was established. First, the schedule of injections of testosterone given in Table 1 did not promote the formation of polyribosomes to any appreciable extent in liver or pancreas taken from the animals used in the studies on the prostate gland. Secondly, the formation of prostate polyribosomes in castrated animals was not restored by the administration of corticosterone (2.5mg/animal) or aldosterone (100μg/animal). At these doses, both hormones induce maximal physiological and biochemical changes in their target organs, particularly with respect to stimulation of RNA synthesis (see, e.g., Yu & Fiegelson, 1971; Majumdar & Trachewsky, 1971).

Development of an assay system for the determination of protein synthesis in vitro

Ample justification for using translocase factors derived from liver may be found in our earlier report (Mainwaring & Wilce, 1972). Considerable quantities of tRNA can be isolated with practical facility by phenol extraction of intact *E. coli* cells, but in view of certain reports that bacterial tRNA does not support protein synthesis with eukaryotic ribosomes very efficiently (Mathews, 1970; Aviv *et al.*, 1971) the use of bacterial tRNA in the heterologous cell-free system was critically examined.

The characteristic features of the assay system are summarized in Table 2. When *E. coli* tRNA charged with [^{14}C]phenylalanine was used, the system did not require supplementation with ATP, but the incorporation of radioactivity into peptide linkage required the

Table 2. *Incorporation of [^{14}C]phenylalanine into protein in a heterologous, cell-free system*

Complete details of the assay system are given in the Materials and Methods section. In this experiment, prostate ribosomes and polyribosomes and liver translocase factors were prepared from normal animals. Each assay contained 100μg of *E. coli* tRNA (15000c.p.m. of [^{14}C]phenylalanine), 500μg of liver translocase factors and prostate particulate preparations equivalent to 50μg of rRNA. Where specifically indicated, 100μg of liver tRNA (20700c.p.m. of [^{3}H]phenylalanine) replaced the bacterial tRNA. The results are the mean of duplicate determinations.

	Incorporation of [^{14}C]phenylalanine (c.p.m./100μg of particulate RNA)	
	Total ribosomes	Polyribosome
Complete system	3750	4760
Minus particulate rRNA	54	56
Minus GTP	1210	993
Minus translocase factors	478	306
Plus 2mM-ATP	3840	4820
Plus 100μg excess of *E. coli* tRNA	3640	4440
Plus 100μg of poly(U)	9060	10720
Plus 50μg of puromycin	561	302
Plus 50μg of cycloheximide	600	340
With liver tRNA	7490	9710
With liver tRNA plus poly(U)	15500	17200

presence of GTP. Inhibitors of protein synthesis, such as puromycin and cycloheximide, dramatically suppressed the incorporation of [^{14}C]phenylalanine. Taking into account the difference in the specific radioactivity of the input tRNA, the system did respond somewhat better to liver tRNA than to *E. coli* tRNA, in both the presence and the absence of poly(U). The difference, however, did not warrant the discontinuation of the use of *E. coli* tRNA for the routine determination of the synthetic activity of prostate ribosomes and polyribosomes. The system was saturated with tRNA at 100μg/assay. Further studies established that the reaction was complete after 30min of incubation and that the optimum concentration of $MgCl_2$ was 10mM. Incorporation of [^{14}C]phenylalanine was strictly proportional to the concentration of added particulate fractions in the range 5–75μg of rRNA and to the amount of translocase factors up to a maximum of 500μg of protein. The overall incorporation was much higher with translocase factors than with the pH5 enzymes used for measuring protein synthesis in prostate microsomal fraction (Mainwaring & Wilce, 1972). Finally, the pattern of [^{14}C]phenylalanine incorporation was identical in qualitative terms, but less extensive, when homologous (prostate) translocase factors replaced the heterologous (liver) preparations. The acute specificity present in the translocase (or soluble) factors reported by Clemens & Tata (1972) and Uenoyama & Ono (1972) was not evident in this system. Under optimum conditions of assay, polyribosomes had a higher protein-synthetic activity than the preparations of unfractionated ribosomes.

Stimulation of protein synthesis in prostate ribosomes and polyribosomes after the administration of testosterone in vivo

The effects of the administration of testosterone *in vivo* on the incorporation of phenylalanine into protein by unfractionated prostate ribosome fractions are presented in Table 3. As reported by previous investigators (Liao & Williams-Ashman, 1962; Mangan *et al.*, 1967), ribosomes from testosterone-treated animals supported a significantly higher incorporation of [^{14}C]phenylalanine into protein than did ribosomes isolated from castrated animal controls. Conversely, the influence of poly(U) on the incorporation of [^{14}C]phenylalanine was more significant with preparations from castrated animals, lending additional support to the inference that prostate ribosomes derived from androgen-depleted animals have a lower endogenous content of mRNA. The difference in the activity of the ribosome preparations cannot be attributed to the selection of a 30min period of incubation; the differences in the activities of ribosomes from castrated controls and androgen-treated animals were maintained at all times of incubation at 37°C, and both reactions were complete within 30min.

Having established that the methods of assay used in the present study afforded experimental results

consistent with earlier investigations (Liao & Williams-Ashman, 1962; Mangan *et al.*, 1967), the influence of androgenic stimulation of the prostate gland on the form and function of the polyribosomes was investigated. In the first series of experiments, polyribosomes derived from the prostate glands of castrated and testosterone-treated castrated animals were fractionated in sucrose-density gradients, and the ability of individual fractions to support the incorporation of [^{14}C]phenylalanine into protein was compared both in the presence and absence of poly(U) *in vitro* (Fig. 1). Androgenic treatment of

Table 3. *Influence of androgens in vivo on the incorporation of* [^{14}C]*phenylalanine into protein by preparations of unfractionated prostate ribosomes*

Preparations of ribosomes were prepared from the prostate glands pooled from six animals and assayed for their ability to promote the incorporation of [^{14}C]phenylalanine into protein in the system described in the Materials and Methods section. The results are the means of triplicate determinations; where indicated, 100μg of poly(U) was added to the standard assay system. The schedule of the injections of testosterone is given in the legend to Table 1.

	Incorporation of [^{14}C]phenylalanine (c.p.m./100μg of rRNA)	
	Without poly(U)	With poly(U)
Castrated animals (controls)	2050	6870
Castrated animals: 1 day of testosterone treatment	3000	6950
Castrated animals: 2 days of testosterone treatment	3510	7500
Normal animals	3960	8490

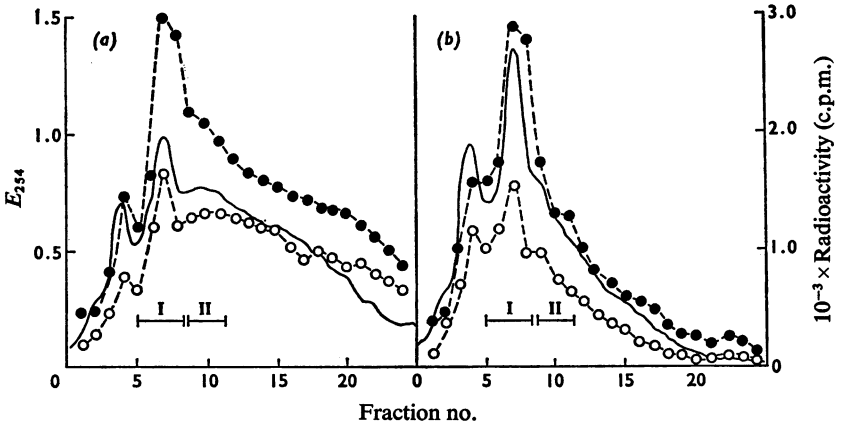

Fig. 1. *Effect of androgens on the form and function of prostate polyribosomes measured in vitro*

Two groups of castrated animals (six animals per group) were given two daily injections of either testosterone or oil as described in the legend to Table 1. Animals were killed 48h after castration and polyribosomes (20E_{254} units) were fractionated in 5ml linear sucrose-density gradients. The direction of centrifugation is from left to right. After fractionation, 40μl samples of each 0.2ml fraction were added to the standard assay system for protein synthesis in the presence and absence of poly(U). See the Materials and Methods section for complete details. (*a*) Testosterone-treated animals. (*b*) Castrated animals (controls). ——, E_{254}. Incorporation of [^{14}C]phenylalanine into protein was measured in the presence (●) or absence (○) of poly(U). Areas of the gradient profiles marked I and II were later analysed in the analytical ultracentrifuge (see Fig. 2).

castrated animals provoked a significant change in the sedimentation behaviour of prostate polyribosomes. Whereas the vast majority of the polyribosomes from androgen-depleted animals was recovered in two large peaks in the upper region of the gradients, the polyribosomes from androgen-stimulated animals were largely recovered as a series of heterodisperse peaks sedimenting to the lower regions of the gradient. As a consequence of hormonal treatment *in vivo*, there was also a pronounced fall in the amount of lighter polyribosome fractions that was the predominant feature of the polyribosome profile of preparations derived from castrated animals. Equally important changes in the synthetic activity of prostate polyribosomes ensued from androgenic stimulation. Two peaks of radioactivity, precisely coincident with regions of maximum extinction at 254nm, were evident in the polyribosomes derived from castrated animals. A conspicuous feature of these gradients was the virtual absence of protein synthesis in the fractions from the lower region of the gradients.

In sharp contrast, the polyribosomes from androgen-treated animals were far more active in supporting protein synthesis *in vitro*, and this was particularly apparent in the area corresponding to heavy or high-molecular-weight ribosomal aggregates.

When the incorporation of [^{14}C]phenylalanine into protein was measured in the presence of poly(U), maximum stimulation occurred in areas of both gradient profiles corresponding to the maximal amounts of rRNA monitored by extinction analysis at 254nm. However, the influence of the synthetic mRNA on protein synthesis was consistently more noticeable with the prostate polyribosomes isolated from castrated animals. These results indicate that testosterone promotes an increase in the amount of heavy polyribosomes in the prostate gland and that these are particularly active in protein synthesis. Bearing in mind that the quantity of polyribosomes is increased about threefold after androgenic stimulation (Table 1), the increase in protein synthesis in the prostate gland that is due to the stimulation of polyribosome formation must be very significant indeed.

To determine the sedimentation coefficients of the various forms of prostate polyribosomes revealed by sucrose-density-gradient centrifugation, samples of polyribosomes were examined in the analytical ultracentrifuge (Fig. 2). Accurate determinations of the sedimentation coefficients could only be achieved with two major but slower-sedimenting components of unfractionated preparations of polyribosomes, one of sedimentation coefficient 81.0S and the other of sedimentation coefficient 124.5S. These values correspond to ribosome monomers and dimers respectively and they are in excellent agreement with previously published values of the sedimentation properties of the major ribosome components in the prostate gland (Mangan *et al.*, 1967). The extremely rapid rate of sedimentation of other constituent polyribosomes prevented an accurate determination of their sedimentation coefficients. When samples of partially purified polyribosomes from the sucrose gradients (Fig. 1) were run in the analytical ultracentrifuge, area I contained mainly 81S ribosome monomers and area II was particularly rich in 124.5S ribosome dimers. By using the principal u.v.-absorbing peak, especially prominent in Fig. 1(*b*), as an internal sedimentation marker, the heavy polyribosomes formed after androgenic stimulation have sedimentation coefficients in the broad range 150–240S.

To ensure that the results obtained from experiments conducted entirely *in vitro* were a valid reflection of the protein synthesis occurring in prostate polyribosomes *in vivo*, radioactive amino acids were injected directly into the prostate gland and the labelled polyribosomes were fractionated in sucrose gradients (Fig. 3). Both in terms of the profile of the polyribosomes obtained by monitoring the gradients at 254nm and the pattern of the incorporation of ^{14}C-labelled amino acids into polypeptide linkage,

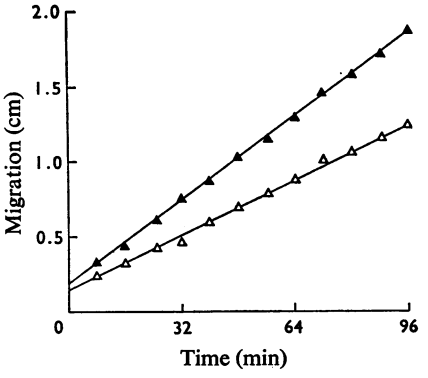

Fig. 2. *Examination of prostate polyribosomes in the analytical ultracentrifuge*

Samples of unfractionated polyribosomes (0.8E_{254} unit) in 10mM-Tris–HCl buffer, pH7.5, containing 10mM-$MgCl_2$, were analysed in the An-D rotor of a Spinco model E ultracentrifuge, by using the u.v. optical system. Centrifugation was conducted at 7°C and 17299$g_{av.}$; photographs were taken at 8min intervals. Calculations of sedimentation coefficients were made on two components, 81S ribosome monomer (△) and 124.5S ribosome dimer (▲). These were predominantly present in areas I and II respectively when samples from the sucrose gradients (Fig. 1) were examined in the analytical ultracentrifuge.

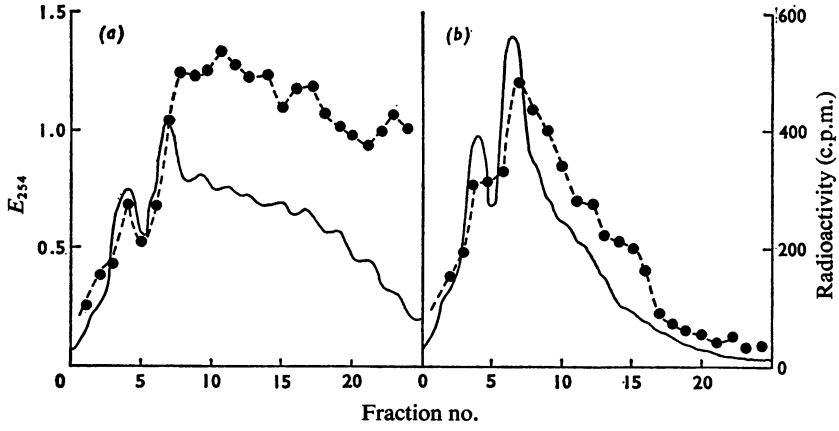

Fig. 3. *Effect of androgens on the form and function of prostate polyribosomes revealed by labelling in vivo*

Two groups of castrated animals (six animals per group) were given injections of either testosterone or oil as indicated in the legend to Table 1. At 48h after castration, the animals were anaesthetized and 5μCi of a mixture of ^{14}C-labelled amino acids was injected directly into the prostate gland. When incorporation of radioactivity was maximal, 15min after the injection of radioactivity (Mainwaring & Wilce, 1972), animals were killed and prostate polyribosomes (20E_{254} units) were separated in 5ml linear sucrose-density gradients. The direction of centrifugation was from left to right. (*a*) Testosterone-treated animals. (*b*) Castrated animals (controls). ——, E_{254}. ●, Radioactivity retained by precipitation and washing with cold 0.5M-$HClO_4$ on glass-fibre discs.

these additional experiments entirely corroborated the findings obtained *in vitro* (Fig. 1). The administration of androgens to castrated animals *in vivo* enhanced the formation of dense 150–240S ribosomal aggregates that contained conspicuous amounts of ^{14}C-labelled nascent polypeptide chains.

Requirement for continued protein and rRNA synthesis in the androgen-mediated increase in prostate polyribosome formation

Since polyribosomes are constructed of proteins as well as rRNA it was considered highly likely that the accelerated rate of synthesis of prostate polyribosomes induced by androgens would be ablated by certain metabolic inhibitors, including actinomycin D and cycloheximide. Earlier studies had demonstrated that low doses of actinomycin D (approx. 25μg/100g body wt.) preferentially suppress synthesis of rRNA rather than mRNA (Perry, 1963; Georgiev *et al.*, 1963; Harel *et al.*, 1964). Liao & Lin (1967) later demonstrated that low concentrations of actinomycin D selectively inhibited the androgen-stimulated nucleolar RNA polymerase that is responsible for the synthesis of prostate rRNA. Additional studies have shown that the structural proteins of the ribosomes are synthesized concurrently with rRNA (Brown, 1967; Perry & Kelley, 1968), predominantly in the cytoplasm of mammalian cells (Heady & McConkey, 1970; Craig & Perry, 1971). For these reasons, if androgens increase the rate of synthesis of prostate polyribosomes *de novo* rather than accelerating the reassembly of an existing pool of ribosome monomers, then the androgen-mediated response should be particularly sensitive to inhibition by actinomycin D and cycloheximide. Further, the increase in polyribosome formation should also be countered by the administration of synthetic anti-androgens *in vivo*. One such steroid, 6α-bromo-17β-hydroxy-17α-methyl-4-oxa-5α-androstan-3-one, suppresses the high-affinity binding of testosterone metabolites in the nucleus of the prostate gland and prevents the androgenic stimulation of the nucleolar form I (amanitin-insensitive) RNA polymerase (Mangan & Mainwaring, 1972).

The effects of the administration of various compounds *in vivo* on the synthesis and activity of prostate polyribosomes are summarized in Table 4. Actinomycin D or cycloheximide did not elicit a major change in the weight of the prostate gland or in the yield and activity of polyribosomes when administered to castrated animals. These parameters were depressed in these animals and the metabolic inhibitors simply enhanced the effects of androgenic deprivation. The administration of testosterone *in vivo* promoted a marked increase in the rate of growth

Table 4. *Requirement for RNA and protein synthesis for the stimulation of polyribosome formation in the prostate gland by the administration of testosterone in vivo*

Animals were castrated and injected with oil (control groups) or testosterone according to the schedule of injections given in the legend to Table 1. Where indicated, actinomycin D or cycloheximide (25 μg/100g body wt.) or other steroids (15mg/animal) was administered concomitantly with the arachis oil and testosterone. All animals were killed 48h after castration and there were five animals per experimental group. Polyribosomes were isolated from the prostate glands and their ability to promote protein synthesis *in vitro* was measured in the standard assay system. The average weight of the prostate gland is given as mg wet wt.; the yield of polyribosomes is quoted as mg of rRNA/g wet wt. of tissue; polyribosome activity is quoted as c.p.m. of [^{14}C]phenylalanine incorporated/100μg of particulate RNA in the absence of poly(U).

	Wt. of prostate gland	Yield of polyribosomes	Activity of polyribosomes
Castrated animals (controls)			
Without additional compounds	274	0.80	2450
With actinomycin D	243	0.70	2340
With cycloheximide	313	0.78	1960
Castrated animals: 2 days of testosterone treatment			
Without additional compounds	511	1.48	4250
With actinomycin D	343	0.73	2680
With cycloheximide	370	0.74	2750
With anti-androgen*	400	0.94	3050
With corticosterone	501	1.49	4120

* 6α-Bromo-17β-hydroxy-17α-methyl-4-oxa-5α-androstan-3-one.

of the prostate gland, together with an increase in the yield and activity of the polyribosomes. These changes were obviated by the concomitant administration of metabolic inhibitors and 6α-bromo-17β-hydroxy-17α-methyl-4-oxa-5α-androstan-3-one. The suppression of the rate of growth of the gland was reflected in a drastic decrease in the yield of polyribosomes and a marked fall in their ability to promote protein synthesis *in vitro*. When samples of polyribosomes were examined in sucrose-density gradients, the administration of the metabolic inhibitors curtailed the formation of the heavy polyribosomes observed after androgenic stimulation (see Figs. 1 and 3). Overall, the results suggest that the formation of prostate polyribosomes requires continued RNA and protein synthesis and that these biosynthetic processes are ultimately set in train by the nuclear binding of metabolites of testosterone. The specificity of antagonistic effect of 6α-bromo-17β-hydroxy-17α-methyl-4-oxa-5α-androstan-3-one is illustrated by the failure to mimic its action by the administration of corticosterone *in vivo*.

Despite the importance of protein synthesis in the formation of prostate polyribosomes, no qualitative or quantitative difference in the structural proteins associated with the polyribosomes could be detected after hormonal stimulation. Androgens clearly stimulate the synthesis of these polyribosomal proteins in a general manner, and the low synthetic activity of polyribosomes in castrated animals cannot be attributed to the deletion of a restricted number of protein components whose synthesis is uniquely under androgenic control.

Time-course of the androgen-induced changes in the form of prostate polyribosomes

In a concluding series of experiments an investigation was conducted into the rate of the stimulation of heavy-polyribosome formation in the prostate gland after the administration of a single injection of testosterone *in vivo*. The results in Fig. 4 show that the increase in heavy polyribosomes is a relatively slow process, requiring approx. 16–20h to attain maximal values. At time-intervals greater than 24h after the administration of androgens, however, the polyribosomes begin to disaggregate. This is consistent with the retention time of androgens in the prostate gland of between 16 and 24h (Tveter & Attramadal, 1968; Mainwaring & Peterken, 1971). With such limited retention times, 24h after the administration of androgenic hormones to castrated animals the effects of hormonal depletion should become apparent again. The complete breakdown of the heavy polyribosomes after incubation with trace amounts of ribonuclease (Fig. 4*f*) is a necessary control, indicating that the ribosome complexes are maintained by small quantities of mRNA (Staehelin *et al.*, 1964). The relative slowness of the response in polyribosome formation to androgenic stimulation is not surprising in the light of the complex sequence of events required for polyribosome synthesis *de novo*,

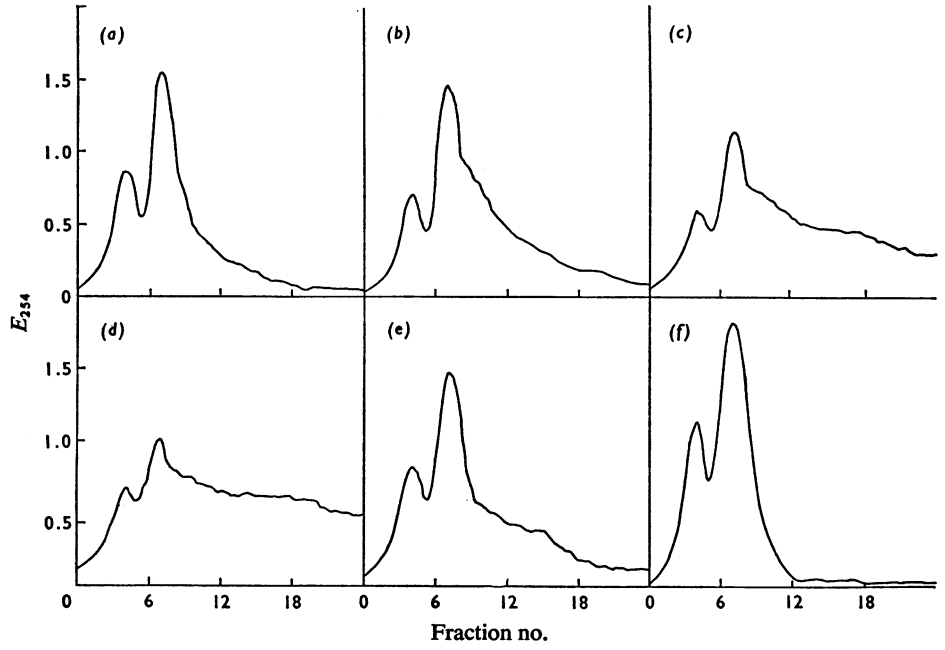

Fig. 4. *Rate of synthesis of heavy prostate polyribosomes after a single injection of testosterone in vivo*

A group of 24 rats, 48h after castration, was given a single subcutaneous injection of testosterone (2.5mg/animal). At various times thereafter, prostate glands from four animals were pooled and polyribosomes ($16E_{254}$ units) were separated in sucrose-density gradients with the direction of centrifugation from left to right. (*a*)–(*f*) represent the polyribosomal profiles established by extinction analysis at 254nm: (*a*) untreated castrated animals (controls); testosterone-treated animals, after (*b*) 3h, (*c*) 9h, (*d*) 18h and (*e*) 30h of androgenic stimulation; (*f*) experimental control, in which polyribosomes from (*d*) were treated with ribonuclease (0.1μg/ml) just before analysis. The fraction size was 0.25ml.

including synthesis in the nucleus, transfer to the cytoplasm and the final assembly process (Girard *et al.*, 1964; Henshaw *et al.*, 1965; Perry, 1965). On the other hand, the slowness of the response argues strongly against the alternative possibility, that polyribosomes are formed by the reassembly of ribosome monomers already present in the cells.

Discussion

The results presented here are harmonious with the findings from other hormone-sensitive systems (see the excellent review by Williams-Ashman & Reddi, 1971). It seems unlikely, however, that the rapid hormonal restoration of muscle polyribosomes by translational rather than transcriptional processes (Wool *et al.*, 1968) can be applied to the prostate gland. First, the hormonal induction of prostate polyribosome synthesis is slow, and secondly, the response is suppressed by actinomycin D and 6α-bromo-17β-hydroxy-17α-methyl-4-oxa-5α-androstan-3-one. The antagonistic effects of this anti-androgen are particularly important. Based on the pioneering work of Bruchovsky & Wilson (1968), among others, the metabolism of testosterone to 5α-dihydrotestosterone (17β-hydroxy-5α-androstan-3-one) is uniquely important in the mechanism of action of androgens in the prostate gland, and the nuclear binding of this metabolite occurs rapidly *in vivo* (Mainwaring, 1969). 6α-Bromo-17β-hydroxy-17α-methyl-4-oxa-5α-androstan-3-one does not influence the pattern of testosterone metabolism in the prostate gland, but prevents the high-affinity binding of 5α-dihydrotestosterone in the nucleus and the subsequent stimulation of nucleolar (form I) RNA polymerase (Mangan & Mainwaring, 1972). The decreased yield of polyribosomes follows logically from these findings, since this form of prostate RNA polymerase is responsible for the formation of rRNA (Liao & Lin, 1967) as in other mammalian cells (Perry, 1967).

Although the response in polyribosome production after the administration of androgens requires 16–20h to reach a maximum, this coincides precisely with the hormonal stimulation of protein synthesis

in the gland and the synthesis of membranes of the endoplasmic reticulum (Mainwaring & Wilce, 1972). It would appear on the strength of these overall findings that all the major components of the cellular machinery for synthesizing proteins are increased in a fairly co-ordinated manner after androgenic stimulation. This co-ordination is consistent with the concept that proteins for secretion or export are generally synthesized on membrane-associated ribosomes and polyribosomes (Sargent & Campbell, 1965; Brew & Campbell, 1967; Askonas & Williamson, 1968), for the prostate is actively engaged in the production of proteins for the prostatic secretion. However, the correlation between protein secretion and membrane-bound ribosomes and polyribosomes is not necessarily upheld in all tissues; certain 'non-secretory' cells (Andrews & Tata, 1971; Rosbach & Penman, 1971) also contain membrane-bound ribosomes and this is especially evident at times of maximal rates of growth.

Such considerations aside, the increase in protein synthesis due to polyribosomes in the prostate gland after hormonal stimulation must be very significant indeed. The polyribosome content of the gland increases nearly threefold and the newly synthesized particles have a particularly high protein-synthetic activity measured both *in vivo* and *in vitro*.

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