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ANDROGEN ACTION IN THE RAT VENTRAL PROSTATE: EFFECT OF CASTRATION AND TESTOSTERONE TREATMENT ON POLYRIBOSOMES

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SUMMARY

Two ribosomal fractions were prepared from rat ventral prostate and examined by electron microscopy. One fraction sedimenting at 10,000 *g* contained ribosomes associated with heavy membrane fragments (heavy fraction), and another sedimenting with the 105,000 *g* pellet contained ribosomes in free clusters or associated with light membrane fragments (light fraction). Centrifugation of both fractions on sucrose gradients revealed the presence of various absorbance peaks of polyribosomes which could be dissociated into monoribosomes by treatment with ribonuclease, and into ribosomal subunits by exposure to EDTA. Castration caused the dissociation of polyribosomes of both fractions into monoribosomes within 12–24 h and into ribosomal subunits within 48–72 h. In the heavy fraction, dissociation of polyribosomes was detected as early as 12 h after castration, while in the light fraction these changes were not noted until 24 h after castration. Polyribosomal peaks of both fractions disappeared completely between 48–72 h. Testosterone treatment of the castrate restored polyribosomal peaks at a rate similar to the rate of dissociation after castration. The rate of restoration in the light fraction appeared to be faster than in the heavy fraction. In doses of 1 mg/day, 17β-hydroxy-5α-androstan-3-one was as effective as testosterone, and 5α-androstane-3α,17β-diol was somewhat less effective. Cortisol had a very slight effect, while progesterone and estradiol-17β had no effect on polyribosome reassembly. To determine which components of polyribosomes were most affected by castration, the incorporation of tritiated cytidine into polyribosomes was studied in castrates and testosterone treated castrates. Within periods of 12, 18, or 48 h, testosterone treatment of the castrate caused an increase in cytidine incorporation into all components of polyribosomes.

INTRODUCTION

Evidence has accumulated to indicate that maintenance of polyribosomal structure is closely linked to hormonal stimulation in organs dependent upon sex steroids. Electron microscopic studies of rat ventral prostates have demonstrated that castration causes marked disorganization of the endoplasmic reticulum and gradual loss of ribosomal particles [1, 2]. Similar changes occur in the ultrastructure of estrogen target tissues after interruption of estrogen treatment [3]. Estrogen withdrawal causes a prompt decrease in both polyribosomal concentration and protein synthesis in both the chick oviduct [3] and the rat uterus [4].

Since the growth stimulating action of androgen would appear to involve initially one or more of the steps in the formation of ribosomes [5] or other components of polyribosomes, we have undertaken a series of studies in the rat ventral prostate to determine the action of androgen on maintenance of polyribosomal structure. This paper reports on the earliest effects of castration and testosterone treatment on the maintenance of polyribosomes.

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MATERIALS AND METHODS

Animals

Male Sprague–Dawley rats weighing 300–400g were obtained from Zivic–Miller and fed *ad libitum*. The rats were castrated by bilateral orchidectomy through a ventral midline incision under ether anesthesia. Castration included the epididymis and a portion of the vas deferens. Testosterone injections were given subcutaneously in 0·1 ml ethanol as specified under the legends to the figures. The animals were killed by decapitation under ether anesthesia.

Chemicals

Macaloid was a gift from Baroid Division, National Lead Co., P.O. Box 1675, Houston, Texas. Bentonite was obtained from Fisher Scientific Co., polyvinyl sulfate from Eastman Kodak, bovine pancreatic ribonuclease from Worthington, sucrose (reagent grade) from Baker and Adamson, testosterone, progesterone and cortisol from Steraloids, and estradiol-17β from Mann Research Laboratories. [5-^{3}H]-Cytidine (S.A. 26·5 Ci/mmol) was purchased from Schwarz–Mann. Its radiochemical purity was checked by thin-layer chromatography on silica gel in n-butanol saturated with water.

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Partial grading of bentonite and macaloid

Crude bentonite was graded by the method of Watts and Mathias[6] and suspended in water. Macaloid was graded as described by Stanley and Beck[7]. Ten grams of crude macaloid were suspended in 600 ml boiling water and blended in a Waring blender at medium speed for 10 min. The suspension was centrifuged at 16,000*g* for 20 min, and the supernatant was discarded. This treatment was repeated, and the pellet was suspended in water.

Pretreatment of sucrose solutions for density gradients

To insure complete absorbance of nucleases on bentonite and macaloid, the sucrose solutions were prepared one or two days in advance and stored at 0°C. Just before preparation of sucrose gradients, bentonite and macaloid were removed by centrifugation at 15,000*g* for 10 min.

Homogenization and isolation of subcellular fractions

Ventral prostates were quickly removed and chilled in ice-cold saline. Glands were blotted dry, cleaned of extraneous tissue and weighed. All subsequent steps were carried out at 0–2°C. Tissue was minced with scissors and homogenized in 10 vol./(w/v) of 0·25 M sucrose in 0·01 M Tris–HCl, pH 7·8 at 25°C, 0·05 M KCl, 0·15 mM $MgCl_2$ (TKM) buffer containing 0·02% polyvinyl sulfate, 0·1% bentonite [6] and 0·1% macaloid [7] in a Duall homogenizer with 5 strokes of a motor-driven Teflon pestle. Homogenates were centrifuged at 1000*g* (ave) for 10 min to remove nuclei, unbroken cells, and large cellular debris. Postnuclear supernatants were centrifuged at 10,000*g* for 10 min to sediment mitochondria and heavy microsomal particles [8]. Postmitochondrial supernatants were centrifuged at 105,000*g* for 1 h in a Spinco 40 rotor to sediment light microsomes and free ribosomes. Ribosomes of both the 10,000*g* and 105,000*g* pellets were dissociated from membranes of the endoplasmic reticulum by homogenization in 2·5 ml of 1% Triton X-100 in TKM buffer using 5 strokes of a loosely-fitting Dounce homogenizer. Aggregated material was removed by centrifugation at 600*g* for 5 min. Membrane-free ribosomes in the supernatant were either analyzed by centrifugation on sucrose gradients without any further treatment or were first sedimented through discontinuous sucrose gradients of 2 ml of 1·6 M sucrose over 4 ml of 2·0 M sucrose at 105,000*g* for 3 h. The pellets were analyzed on sucrose gradients.

Centrifugation of sucrose gradients

Ribosomes were fractionated on discontinuous sucrose gradients [9] prepared by layering 3 ml of 55% and 4 ml each of 50, 35, 30, 25, 20 and 15% (w/v) sucrose solutions in TKM buffer. Gradients were

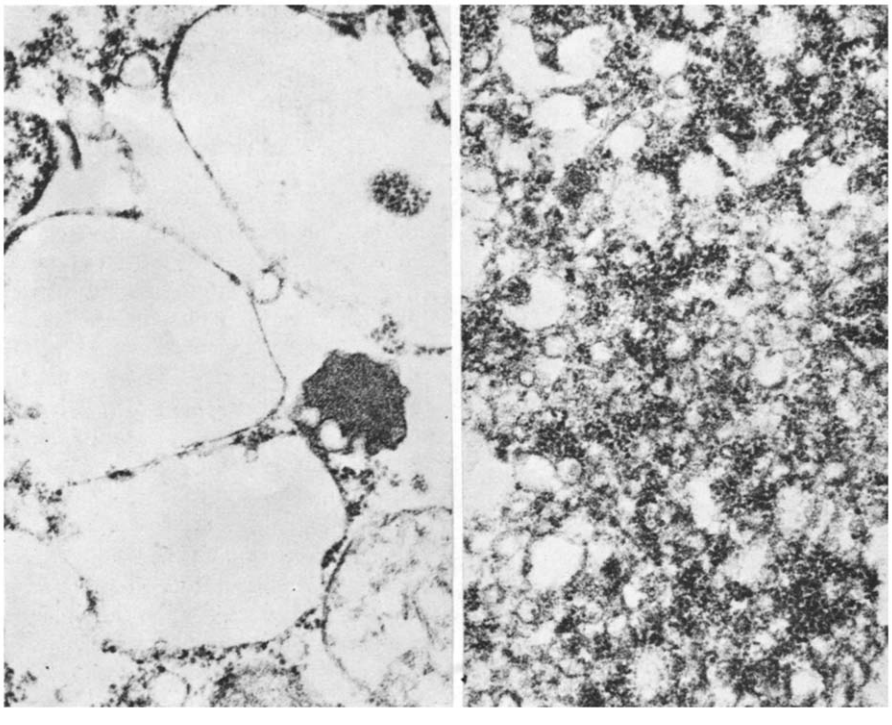

Fig. 1. Electron micrographs of thin sections of the 10,000 *g* and 105,000 *g* pellets. Ventral prostates from intact adult rats were homogenized in 10 vol (w/v) of 0·25 M sucrose in 0·01 M Tris–HCl buffer (pH 7·8 at 25°C) containing 0·05 M KCl, 0·01 M $MgCl_2$, 0·02% polyvinyl sulfate, 0·1% bentonite and 0·1% macaloid, and the 10,000 *g* and 105,000 *g* pellets (shown on the left and right, respectively) were obtained by centrifugation. Electron micrographs (50,000×) were made from thin sections as described in the text.

equilibrated overnight at 5°C. Aliquots of 2 ml of ribosomal suspension were layered on top of the gradients, and the tubes were centrifuged in a Spinco SW 25·1 rotor at 58,600*g* for 2·5 h. Gradients were fractionated from the top by a density-gradient fractionator (Model D), and absorbance at 254 nm was monitored continuously by an U.V. analyzer (Model UA-2) (Instrumentation Specialties Co., Lincoln, Nebraska, U.S.A.).

Ribonuclease treatments

Ribosomes suspended in 1·5 ml of 0·01 M Tris–HCl (pH 7·8 at 25°C), 0·05 M KCl and 0·01 M $MgCl_2$ were incubated at 0°C for 15 min with 1 ml of ribonuclease (0·4 mg/ml of 0·34 M sucrose in the same buffer). The mixture was centrifuged at 15,000*g* for 5 min, and the supernatant was analyzed by centrifugation on sucrose gradients.

Preparation of thin-sections and grids for electron microscopy

Ribosomal pellets were fixed in 2% oxmium tetroxide in 0·1 N veronal acetate buffer (pH 7·4 at 25°C) for 30 min. Blocks of 1 mm^3 were dehydrated in graded alcohols and embedded in Epon 812. Thin sections were cut with a diamond knife, stained with uranyl acetate and lead hydroxide.

After centrifugation of ribosomal preparations on sucrose gradients, 1 or 2 drops from fractions corresponding to absorption peaks were placed on 1 cm^2 blocks of 2% agar. Sucrose solutions were imbibed by the agar and each block was covered with a drop of 0·75% parlodion in amyl acetate. Excess was removed to allow parlodion to dry in thin films containing ribosomal particles. Films were transferred to copper grids and shadowcast with chromium at a 10° angle [10], Thin sections and grids were examined with a JEM T-7 electron microscope.

RESULTS

Isolation and identification of polyribosomes

Two ribosomal fractions were prepared from rat ventral prostate. A heavy fraction sedimented with the 10,000*g* pellet, and a light fraction sedimented with the 105,000*g* pellet. Ribosomes in the 10,000*g* pellet were associated with heavy membrane fragments (Fig. 1, left) and are referred to as the heavy ribosomal fraction. In the 105,000*g* pellet, ribosomes were present as free clusters or light membrane fragments (Fig. 1, right) and are referred to as the light ribosomal fraction.

Centrifugation of these two fractions on 15–55% discontinuous sucrose gradients, after treatment with Triton X-100, yielded absorbance profiles with several peaks (Fig. 2). Heavy and light fractions resulted in similar ribosomal profiles when prepared with buffers containing either 10 mM or 0·15 mM $MgCl_2$, although preparations in low magnesium resulted in more peaks of lighter polyribosomes. When gradient

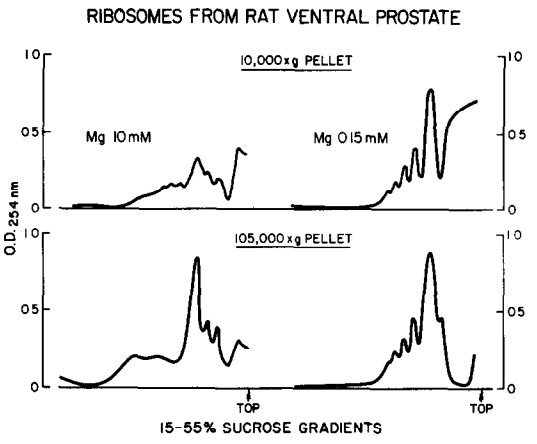

Fig. 2. Sucrose gradient profiles of ribosomes in the 10,000*g* and 105,000*g* pellets from rat ventral prostate. Ventral prostates were homogenized in 10 vol. of 0·25 M sucrose in the same buffer described under Fig. 1, but containing either 0·01 M or 0·15 mM $MgCl_2$. The 10,000*g* and 105,000*g* pellets were resuspended in 0·25 M sucrose in 0·01 M Tris–HCl buffer, containing 0·01 M or 0·15 mM $MgCl_2$, 0·05 M KCl, and 0·02% polyvinyl sulfate. The two fractions were layered over 15–55% discontinuous sucrose gradients and centrifuged in a Spinco SW 25·1 rotor at 25,000 rev./min for 2·5 h at 4°C. Gradients were fractionated, and the absorbance at 254 nm was measured. In each gradient, monoribosomes form the major absprotion peak.

fractions were examined by electron microscopy, peak 1 was shown to contain largely monoribosomes, while peaks 2–4 contained aggregates of various sizes (Fig. 3). Preliminary purification of the ribosomal fractions on 1·6–2·0 M discontinuous sucrose gradients yielded polyribosomal peaks similar to those obtained with the crude ribosomal preparation. Ribonuclease treatment of ribosomes from either the heavy or light fraction caused a shift in the absorbance peaks from those of heavy polyribosomes to monoribosomes. Treatment with EDTA caused a shift to slower moving peaks characteristic of ribosomal subunits.

Effect of castration on polyribosomal patterns

The heavy and light ribosomal fractions were prepared from equal amounts of tissue, and sedimentation profiles were determined by centrifugation on sucrose gradients as described above [9]. Ribosomal aggregation was shown to be greatly influenced by Mg^{2+} over a concentration range of 0·15 mM to 10 mM. A concentration of 0·15 mM was compatible with stability of the polyribosomes and yet sufficiently low to reduce aggregation. Although there was improved resolution of polyribosomal peaks at the lower Mg^{2+} concentration, the effect of castration was similar.

Typical profiles of polyribosomes at various periods after castration are shown in Fig. 4. A decrease in the heavy polyribosomal peaks of the heavy fraction was detectable as early as 12 h after castration, while in the light fraction the effect of castration was not detected until after 24 h. Complete dis-

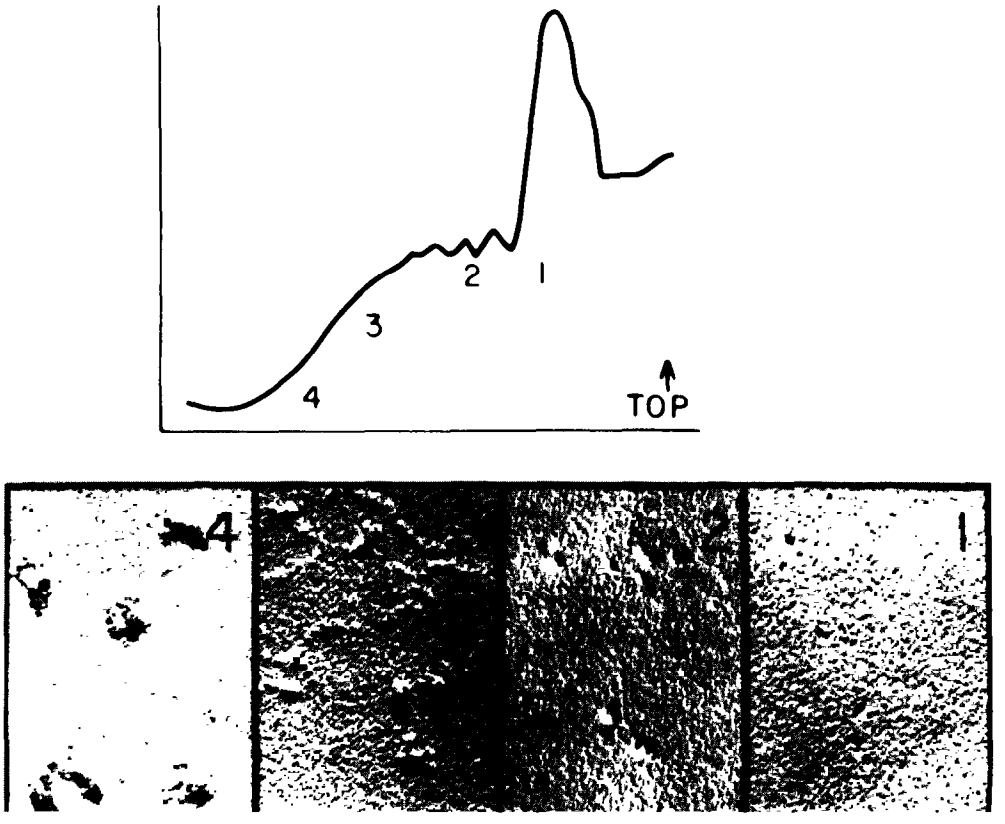

Fig. 3. Electron micrographs of ribosomal material corresponding to absorbance peaks on sucrose gradients. The 105,000 *g* pellet was prepared and fractionated on 15–55% sucrose gradients. Aliquots of gradient fractions corresponding to absorbance peaks 1–4 were transferred to specimen grids and photographed as described in the text. The reverse negatives were used for printing the pictures (30,000×).

appearance of polyribosomes in both fractions required 48 to 72 h. Early breakdown of polyribosomes was associated primarily with an increase in monoribosomes, while at a later stage after castration (72 h), polyribosomes dissociated further into ribosomal subunits.

Effect of testosterone on the reappearance of polyribosomes in the ventral prostate of the castrated rat

Testosterone (1 mg/day) maintained a normal polyribosomal profile when given immediately after castration. If testosterone treatment was delayed until after castration changes had developed, the rate at which the atrophic process was reversed was similar to the rate of dissociation after castration. As shown in Figs. 5 and 6, testosterone treatment of castrated rats stimulated the formation of polyribosomes in both the heavy and light fractions. The partial dissociation of polyribosomes seen 24 h after castration was reversed completely by 12–24 h after treatment with 1 mg of testosterone, however, after 72 h had elapsed following castration, complete reassembly of polyribosomes required a longer period of testosterone treatment. In the heavy fraction, restoration was not complete before 72 h of treatment (Fig. 5). In the light fraction of the 72 h castrate, a greater restoration of polysomes appeared to occur within 48 h after treatment (Fig. 6). To determine the minimal dose of testosterone required for reassembly of polyribosomes in the heavy and light fractions, 72 h castrates were treated with varous amounts of testosterone, and the area under the absorbance peaks was measured (Table 1). It was apparent that 1 mg/day testosterone was well above the minimum dose required to bring about maximum restoration of polyribosomes in both the heavy and light fractions. The results suggested also that the testosterone requirement for reassembly of polyribosomes in the light fraction might be somewhat lower than for the heavy fraction.

Organ and steroid specificity of polyribosomal response

In addition to its action on accessory sex glands, testosterone also stimulates metabolic processes in various other tissues which are not totally dependent on androgen. To test whether a polyribosomal response to testosterone withdrawal is also found in another androgen responsive tissue, the effects of castration and testosterone treatment in kidney were also studied. Kidney polyribosomes remained intact 72 h after castration and were not influenced by testosterone treatment of the castrate. In the same rats, polyri-

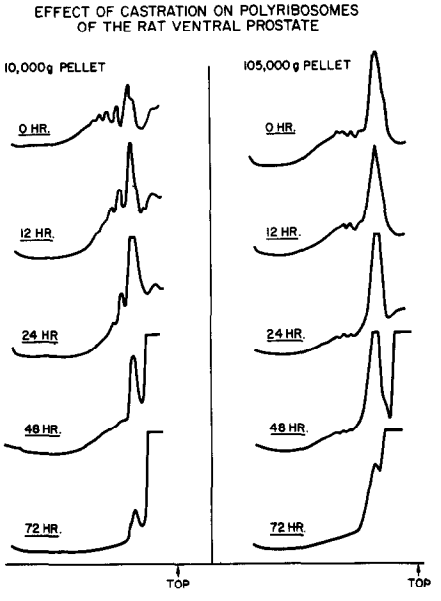

Fig. 4. Sucrose gradient profiles of ribosomes in the 10,000*g* and 105,000*g* pellets obtained from ventral prostates at different intervals after castration. Adult male rats were castrated for the periods indicated. Ventral prostates were homogenized in 0·25 M sucrose-0·15 mM $MgCl_2$ in the same buffer described under Fig. 2 and fractionated by differential centrifugation. Ribosomes from the 10,000*g* and 105,000*g* pellets were centrifuged on 15–55% discontinuous sucrose gradients in the same buffer but without the macaloid and bentonite, and the absorbance was measured continuously at 254 nm. 0, 12, etc., refer to the hours after castration. The baseline corresponds to the lowest point on each tracing ($O.D._{254\ nm} = 0$).

bosomes isolated from ventral prostate and epididymis were completely degraded in the castrate and restored to normal by testosterone treatment of the castrate.

In order to evaluate the steroid specificity of the polyribosomal response, 72 h castrate rats were treated daily for 3 days with several other steroids. Ventral prostate polyribosomal fractions were prepared and analyzed on sucrose gradients. In doses of 1 mg/day, 17β-hydroxy-5α-androstan-3-one was as effective as testosterone, and 5α-androstane-3α, 17β-diol was somewhat less effective. Cortisol had only a slight effect, while progesterone and estradiol-17β had no effect on polyribosome reassembly.

Possible role of ribonuclease in the dissociation of polyribosomes of the prostate of castrated rats

Alpers and Isselbacker[11] have shown that breakdown of polyribosomes during isolation is directly related to ribonuclease activity in the homogenate. Since ribonuclease levels in target tissues may increase following hormonal withdrawal [3, 12], it was necessary to ascertain that the absence of polyribosomes in prostate of the castrate was not due to degradation by endogenous ribonuclease during tissue fractionation as previously suggested by Parsons *et al.*[12]. To test this possibility, homogenates of in-

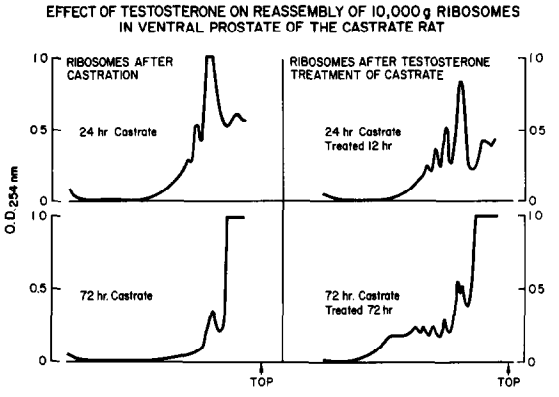

Fig. 5. Sucrose gradient profiles showing restoration of polyribosomes in the heavy fraction after testosterone treatment of rats castrated for various periods. Adult rats were castrated and following the intervals of time shown on the left, treatment with 1 mg of testosterone daily was started and continued for the periods indicated to the right of the vertical line. Ventral prostates were removed, and the 10,000*g* pellets were prepared and analyzed after centrifugation on 15–55% discontinuous sucrose gradients.

tact rat prostates were prepared using as the homogenizing solution the 10,000*g* supernatants from 72 h castrate rats. Ribosomal fractions were isolated and analyzed on sucrose gradients. No effect on polyribosomes of intact rats was observed.

Degradation of polyribosomes during tissue fractionation might also result from the absence of ribonuclease inhibitors in prostates of the castrates. Roth[13] first recognized the presence of specific ribonuclease inhibitors in 105,000*g* supernatant fraction from rat liver. To determine whether the breakdown of prostate polyribosomes isolated after castration might be prevented by ribonuclease inhibitor, prostates from 72 h castrates were homogenized in the presence of supernatant fractions from liver, and the polyribosomes were analyzed on sucrose gradients. The gradient profile of the 72 h castrate again lacked any polyribosomal peaks.

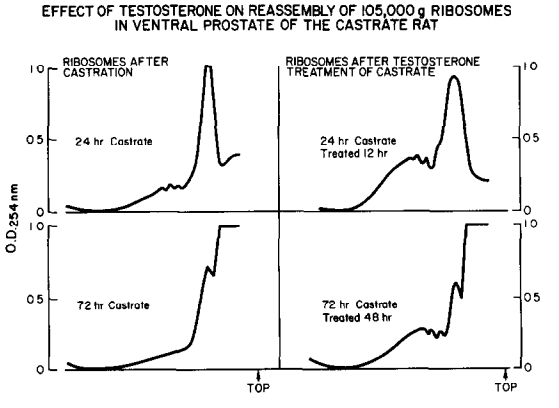

Fig. 6. Sucrose gradient profiles showing restoration of polyribosomes in the light fraction after testosterone treatment of rats castrated for various periods. Rats were treated with testosterone, and ribosomes in the 105,000*g* pellet were analyzed as described under Fig. 5.

Table 1. Per cent of total ribosomal area under the profile attributed to polyribosomes after treatment of the 3-day castrate rat with various doses of testosterone

Testosterone μg/day for 3 days	Heavy fraction (%)	Light fraction (%)
500	78·3	59·9
100	47·6	65·4
50	74·7	70·7
25	72·7	65·6
10	41·0	66·6
5	50·8	69·2
2·5	38·8	59·7
1	16·2	65·3
none	14·3	5·5

The area under the curve was measured with a compensating polar planimeter.

Effect of castration and testosterone treatment on the incorporation of [5-^{3}H]-cytidine into ribosomes

To characterize the polyribosomal components most affected during the early stages of castration, incorporation of tritiated cytidine into polyribosomes of rat ventral prostate was studied in castrates and testosterone-treated castrates. Immediately after castration, all rats were labeled by injection of [5-^{3}H]-cytidine (5 μCi in 5 μl of normal saline solution) directly into each lobe of the ventral prostate. At the same time, one group of rats was given 1 mg of testosterone, subcutaneously, while the other group was used as a control. Twelve and 18 h later, the rats were killed and prostate polyribosomes (heavy and light combined) were prepared and centrifuged on 15–30% sucrose gradients. Gradients were fractionated, monitored for U.V. absorbance at 254 nm, and the radioactivity in each fraction from the gradients was counted. Total RNA was measured in aliquots of each ribosomal suspension and the radioactivity in each fraction was adjusted to reflect equal amounts of total RNA. The absorbance profile consisted of areas, from top to bottom, corresponding to 10–20S, 30–40S, 50–60S and 80S plus light polyribosomes. The majority of heavy polyribosomes were pelleted. The distribution of radioactivity superimposed on the A_{254} absorbance profile is shown in Fig. 7. A major portion of the radioactivity was located near the bottom of the gradient and in the pellet. Increased incorporation of tritiated cytidine during periods of 12 and 18 hours following testosterone treatment could be seen in all regions of the gradient but was greatest in the heavier fractions. When testosterone treatment of the castrate was withheld until 48 h after castration (Fig. 8). Stimulation was greatest in the monosome and polyribosome region.

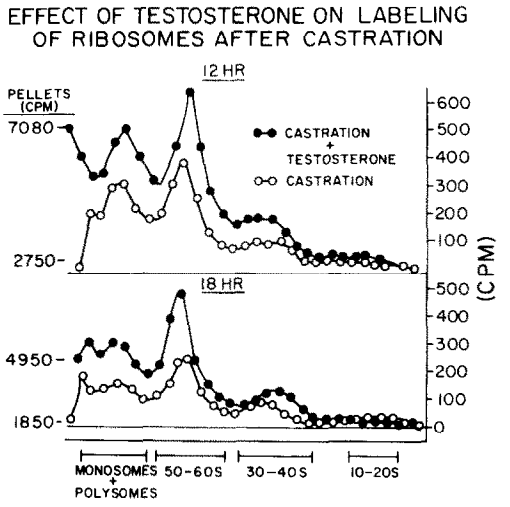

Fig. 7. Effect of testosterone on [5-^{3}H]-cytidine incorporation into polyribosomes of rat ventral prostate during periods of 12 or 18 h following castration. Rats were castrated and 5 μCi of [5-^{3}H]-cytidine were injected immediately into each lobe of the ventral prostate. Two groups of animals received 1 mg of testosterone at the time of castration, and two groups were used as untreated controls. After 12 or 18 h, the animals were killed and the ventral prostates homogenized in 10 vol. of 0·25 M sucrose in 0·01 M Tris–HCl buffer (pH 7·8 at 25°C), containing 0·05 M KCl and 0·15 mM $MgCl_2$. The homogenate was centrifuged at 600*g* for 10 min, and the postnuclear supernatant centrifuged at 105,000*g* for 1 h. The combined mitochondrial and microsomal pellet was treated with 1% Triton X-100 in the Tris buffer and centrifuged through a 1·6/2·0 M sucrose gradient at 105,000*g* for 3 h. The purified ribosomal pellet was resuspended in the same Tris buffer, and the radioactivity counted in 15 ml of Triton X-100–toluene mixture (1:1 v/v), containing 0·01% POPOP and 59,000 × *g* for 15 h. The gradients were fractionated into 1 ml fractions using an ISCO model D density gradient fractionator each fraction was diluted to 2·5 ml with water, and the radioactivity counted in 15 ml of Triton X-100–toluene mixture (1:1 v/v), containing 0·01% POPOP and 0·4% PPO. The pellets refer to ribosomal material that pelleted at the bottom of the gradient tubes.

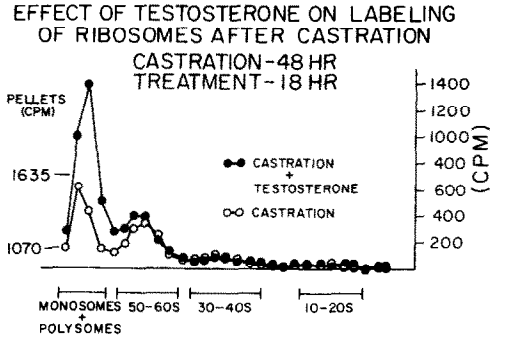

Fig. 8. Effect of testosterone on [5-^{3}H]-cytidine incorporation into polyribosomes of rat ventral prostate 48 h following castration. Mature male rats were castrated and after 48 h the ventral prostates were labeled by direct injection of [5-^{3}H]-cytidine (5 μCi per lobe). One half the animals were given testosterone 1 mg sc and ventral prostates were removed 18 h later. Polyribosomes were fractionated and the radioactivity measured as in Fig. 7.

DISCUSSION

Dissociation of polyribosomes in both the heavy and light fractions was observed to occur earlier than the striking ultrastructural effects of castration on

ergastoplasm and rough endoplasmic reticulum. Helminen and Ericsson[14] reported that changes in ultrastructure were not observed before 48 h after castration. In our studies, however, dissociation of polyribosomes was noted consistently between 12–24 h after castration. Disintegration of the polyribosomes did not appear to result from degradation during fractionation occurring either from an increase in ribonuclease activity or from a loss of endogenous inhibitors of ribonuclease. Alteration of polyribosomes in the heavy fraction appeared to occur at a somewhat shorter time after castration than in the light fraction, suggesting that ribosomes of the heavy endoplasmic reticulum may be more sensitive to hormonal withdrawal than those of the light fraction. The earliest effect of testosterone treatment was on the formation of polyribosomes of the light fraction, suggesting that polyribosomes may be assembled in free form before they attach to membranes of the endoplasmic reticulum.

Although the mechanism of testosterone action on the assembly of ribosomes into polyribosomes is not yet known, our results suggest that testosterone maintains polyribosome structure in part by stimulating the formation of RNA associated with ribosomal subunits, monosomes and polyribosomes. Studies carried out 48 h after castration suggest that testosterone may initially stimulate an RNA species other than ribosomal RNA. In more recent studies on the effect of testosterone on the incorporation of [5-^{3}H]-cytidine into RNA components of polyribosomes (Johnsonbaugh, R. E., French, F. S., and Nayfeh, S. N., manuscript in preparation), we have shown that testosterone stimulates the labeling of an RNA species resembling messenger RNA. Within the first few hours after testosterone treatment, the majority of newly formed polyribosomes are probably derived from preexisting single ribosomes and newly formed messenger RNA. These results are in agreement with the previous finding of Palmitter *et al.*[3] who showed that response to hormonal stimulation involves mainly the formation of polyribosomes from pre-existing ribosomes.

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