Androgenic Control of Nucleic Acid and Protein Synthesis in Male Accessory Genital Organs

H. G. WILLIAMS - ASHMAN

Brady Urological Institute, The Johns Hopkins Hospital, and Department of Pharmacology and Experimental Therapeutics, The Johns Hopkins School of Medicine, Baltimore, Maryland

ABSTRACT A survey is given of experimental studies on the influence of treatment with androgenic hormones *in vivo* on various intermediary reactions involved in ribonucleic acid (RNA) and protein synthesis in the prostate gland and seminal vesicle, with particular reference to the control of the growth and functional differentiation of these organs by testosterone and related steroids. Studies on the influence of androgens on RNA metabolism and protein biosynthesis in mouse kidney, certain muscles, and some other extragenital tissues are also considered.

An ever-increasing amount of experimental effort has been expended over the last 5 years toward examining various intermediate reactions involved in ribonucleic acid and protein synthesis in male accessory genital glands. Much of this work has been carried out *vis-a-vis* the all-embracing effects of androgens on the growth and functional differentiation of these organs. The strategy and tactics of these researches have often resembled those employed in comparable investigations on the action of estrogenic hormones on various structures in the female genital tract. (Comparatively little attention has been given to the nature and chronology of molecular events that underlie the biological actions of gestagens.) The remarkable progress in our understanding of the role of various forms of ribonucleic acid in gene expression and protein synthesis, together with the failure of many attempts to explain the action of sex hormones in terms of their direct effects on either isolated enzyme systems or on the permeability of cell or organelle membranes, has naturally focused attention on the sex hormonal control of the synthesis and turnover of specific enzymes and structural proteins.

Before considering some phenomenological details of these processes in the male reproductive tract, it may be well to list certain aspects of the chemical physiology of androgens and estrogens which must ultimately be taken into account by any comprehensive molecular theories of sex hormone action.

(1) It is well established that common pathways exist for the biosynthesis of androgens and estrogens in the ovary, testis, adrenal cortex, and placenta. These two categories of sex hormones can be elaborated in the aforementioned steroid factories in both male and female mammals. Progesterone appears to be a common intermediate in the transformation of cholesterol into both androgenic and estrogenic steroids, as well as adrenocortical hormones.

(2) Whereas estrogenic activity is a property of many types of nonsteroidal molecule, very few substances that are not steroids have been found to be androgenic, and those that have are only feebly active.

(3) The natural ovarian estrogens, and their more potent non-steroidal synthetic congeners (such as diethylstilbestrol), exert their estrogenic effects in doses which are two to three orders of magnitude lower than those at which testosterone displays its major androgenic actions.

(4) Although there is evidence that estradiol-17β can be concentrated in certain female secondary sexual tissues, and can induce uterine growth without undergoing any chemical change (Jensen, '63), metabolic transformation products of testosterone and Δ^4-androstene-3,17-dione are readily detectable in male accessory sexual organs soon after administration of these steroids in physiological doses (Harding

and Samuels, '62; Jensen, '63; Pearlman, '63). Whether selective concentration of androgens in tissues of the male genital tract (cf. Butenandt, Günther, and Turba, '60) is of widespread significance remains controversial.

(5) By and large, the sex genotype has relatively little influence on the reactivity of many mammalian cells to androgens and estrogens.

(6) Some of the physiological actions of estrogens on the female genital tract (*e.g.*, uterine hyperemia and water imbibition) are manifest more quickly than most of the known actions of androgens on male accessory sexual tissues.

(7) It is extremely difficult to distinguish in any rigorous fashion between a "target" and "non-target" tissue for androgens and estrogens. Szirmai ('62) has provided an excellent discussion of variations from one species to another in the sensitivity of different connective tissue, epithelial and muscle cells to sex hormones, with special reference to the embryology and location of these tissues. He points out that only in relatively few instances can androgens and estrogens act in the same way on homologous or identical structures; usually their effects are mutually antagonistic. In this connection, there comes to mind such striking phenomena as the antagonism by estrogens of androgen action on prostatic epithelia, and the uterotrophic action of testosterone (Huggins, '47), as well as the ability of estrogens to induce squamous metaplasia and fibromuscular hypertrophy in some male accessory glands (Price and Williams-Ashman, '61).

ANDROGENS AND REACTIONS INVOLVED IN RIBONUCLEIC ACID AND PROTEIN METABOLISM IN THE PROSTATE GLAND AND SEMINAL VESICLES

General morphological and biochemical considerations

The most remarkable morphological change in male accessory glands which occurs soon after orchiectomy of postpuberal males is the shrinkage of the cytoplasm of their tall columnar epithelial cells, accompanied by a massive collapse and loss of their endoplasmic reticulum (Brandes and Groth, '63; Deane and Porter, '60; Harkin, '57; Szirmai, '62; Price and Williams-Ashman, '61). There also occurs a marked fall in the oxygen consumption and certain respiration-coupled synthetic activities by slices of these tissues (Huggins, '47; Nyden and Williams-Ashman, '52; Wicks and Villee, '64). Within a few days after excision of the testes, a decline occurs in the level of certain respiratory enzymes and in the mitochondrial population density (Edelman, Brendler, Zorgniotti, and Edelman, '63; Price and Williams-Ashman, '61; Williams-Ashman, '62). Castration also leads to a loss of cytoplasmic basophilia (largely due to RNA). In some male accessory genital glands, such as the mouse seminal vesicle (Deane and Porter, '60), androgen withdrawal does not result in a very marked decline in the number of RNA-rich granules per unit volume of intercisternal cytoplasm, although with the cytoplasmic shrinkage and loss of endoplasmic reticulum, the total number of ribosomes per epithelial cell is profoundly reduced. In other glands, such as the rat ventral prostate, there occurs a more marked post-castrate decrease in the epithelial cell ribosomal population density (Brandes and Groth, '63; Price and Williams-Ashman, '61). All of these morphological and biochemical changes are rapidly reversed by treatment of recently castrated animals with testosterone (cf. Williams-Ashman, Liao, Hancock, Jurkowitz, and Silverman, '64). It is worth noting that "electron dense bodies" appear in the supranuclear region of prostatic epithelial cells as a result of orchiectomy or estrogen treatment (Brandes and Groth, '63). It is conceivable that these structures are lysosomes, and that they may perform a scanvenging function when the prostatic cells dwindle after removal of androgens from the circulation. Since the report of Hertz and Tullner ('53), much too little attention has been given to the biochemistry of the post-castrate regression of the prostate and seminal vesicles, which is obviously a complex and far from passive process.

Amino acid penetration and activation

As yet there are no compelling reasons to believe that the effects of androgens on protein synthesis in male accessory glands are in any large measure dictated by actions of these hormones on either the active transport of amino acids, or on the enzymatic synthesis of transfer RNA-amino acids. Recent reviews by Riggs ('64) and Tomkins and Maxwell ('63) emphasize, however, the paucity of experimental work on the influence of androgens on these processes in mammalian tissues. Kochakian, Tanaka, and Hill ('61) reported that the activity of amino acid-activating enzymes (measured by ^{32}PP-ATP exchange in the absence and presence of amino acid mixtures) in guinea pig prostate and temporal muscle changed in direct proportion to the alterations in mass of the tissues induced by castration or androgen treatment. On the contrary, the specific activity of these enzymes in guinea pig seminal vesicle was markedly diminished by orchiectomy and restored by injection of testosterone. In experiments with isolated rat seminal vesicle slices, Wilson ('62) observed little influence of testosterone *in vivo* on either the penetration of labeled amino acids into the slices or on the formation of transfer RNA-amino acids.

Aminoacyl transfers by ribosomes

From studies on isolated seminal vesicle slices, Wilson ('62) concluded that testosterone affects protein biosynthesis in this organ primarily by enhancing the incorporation into microsomal ribonucleoprotein of aminoacyl residues derived from transfer RNA-amino acids. Recently, a series of investigations have been carried out on the amino acid-incorporating capacities of isolated prostatic ribonucleoprotein particles (Liao and Williams-Ashman, '62; Silverman, Liao, and Williams-Ashman, '63; Williams-Ashman, and Liao, '63; Williams-Ashman *et al.*, '64; Liao, '65a). These experiments disclosed that injection of testosterone into recently castrated rats over periods of 48–72 hours (during which interval the androgen caused the fresh weight of the rat ventral prostate to double) resulted in: (1) an approximately twofold increase in the quantity of ribosomal material extractable by deoxycholate treatment of crude cell particulate preparations (the RNA:protein ratio of the ribosomes and the gross base composition of the ribosomal RNA were unaffected by testosterone administration), and (2) an increase in the capacity of the isolated ribosomes to support the transfer to protein-like material of valine-^{14}C or phenylalanine-^{14}C derived from the corresponding preformed transfer RNA-amino acids.

Evidence in support of the latter was obtained under experimental conditions in which both the rate and extent of the aminoacyl transfers were proportional to the quantity of ribosomal material added to the reaction mixtures, and where the levels of GTP, ATP, Mg^{++} ions, soluble transfer enzymes and sulfhydryl compounds were not rate-limiting. The marked diminution in aminoacyl transfers by ribosomes from control as compared with testosterone-treated castrates was nullified either by addition to the isolated ribosomal systems of appropriate synthetic polyribonucleotides rich in codons for the amino acid tested (poly UG for valine and poly U for phenylalanine) or by addition of prostatic nuclear RNA isolated from testosterone-treated animals (Williams-Ashman *et al.*, '64; Liao, '65a). These findings, together with failure to observe any marked difference in the ability of either ribosomes or soluble protein fractions from the prostates of the untreated or androgen-treated castrates to degrade synthetic template RNA's, suggested that the increased aminoacyl transfer capacity of prostatic ribosomes from the androgen-treated animals was due to increased levels of template RNA associated with these ribonucleoprotein particles. Two aspects of these investigations are worthy of note. First, the ribosomes studied *in vitro* had been detached from the endoplasmic reticulum by treatment with detergents. Further studies on the amino acid-incorporating activity of the ribosomes in association with the lipoprotein membranes of the endoplasmic reticulum may be enlightening. Second, it has not been possible under a variety of circumstances to demonstrate any meaningful *in vitro* effects of testosterone or Δ^4-

androstene-3-17-dione on aminoacyl transfers by the isolated prostatic ribosomal systems.

Experiments of rather similar design (Breuer and Florini, '65; Florini and Breuer, '65) hint that testosterone can increase the number as well as the amino acid-incorporating capacity of ribosomes in the skeletal muscles of orchiectomized rats. The increased protein-synthesizing capacity of the ribosomes was related to the levels of template RNA associated with the particles. Sucrose density gradient analyses revealed that the testosterone-induced elevation in the aminoacyl-transfer activity of the muscle ribosomes was paralleled by an increase in the population of polyribosomes.

Extensive investigations by Kochakian on mouse kidney (Kochakian, '62; Kochakian, Hill, and Aonuma, '63) and guinea pig male accessory glands (Kochakian, '64) are also consistent with the view that the effects of testosterone on protein biosynthesis in these organs are contingent upon more primary changes in the synthesis or intracellular translocation of ribosomal and messenger RNA's. The possibility that male sex hormones also influence protein synthesis at the level of translation of messenger RNA's cannot, however, be overruled by the limited experimental evidence available at present.

Most studies on the effects of androgens on protein biosynthesis have dealt with the incorporation of radioactive amino acids into ill-defined mixtures of polypeptide material. It is well known that testosterone treatment *in vivo* can differentially influence the activity of many enzymes in male accessory sexual tissues (Mann, '64; Price and Williams-Ashman, '61; Williams-Ashman, '62, '64, '65a, b). Particularly striking increases in the levels of D-amino acid oxidase and β-glucuronidase in mouse kidney are known to follow injection of androgens (Frieden, Harper, Chin, and Fishman, '64). But most of these reported androgen-induced changes in tissue protein levels are quantitative rather than qualitative in nature. However, an interesting qualitative action of testosterone on an isozyme of esterase in mouse kidney was recently described by Shaw and Koen ('63). They showed that this isozyme was present only in the kidney of sexually mature males; the enzyme could not be detected in renal extracts of females or prepuberal males. But if testosterone was injected for 7 days, then the esterase isozyme was present in kidney extracts from both female and juvenile male mice. Other kidney esterases separable by starch electrophoresis were not affected by sex hormones. Shaw and Koen ('63) concluded that testosterone induced the synthesis of the mouse kidney esterase isozyme, which was not found in other organs.

Further investigations on the control of protein biosynthesis by testosterone would be greatly facilitated if methods could be developed for measurement of incorporation of amino acids into specific proteins by cell-free enzyme preparations from male accessory genital organs.

Synthesis and template activity of ribonucleic acids

The ability of androgens to increase the total RNA levels and RNA:DNA ratio in male accessory glands and some other susceptible organs has been widely documented (Frieden, '64; Kochakian, and Harrison, '62; Williams-Ashman *et al.*, '64). Excessive doses of testosterone can increase the RNA:DNA ratio in these tissues to well above the normal values. A careful study by Liao ('65a) demonstrated that in adult rats sacrificed 70 hours after orchiectomy, the injection of testosterone over this interval increased the levels of total and ribosomal RNA, but did not alter the content of nuclear RNA per unit amount of DNA, in the rat ventral prostate. The base composition of the nuclear RNA (A:U:C:G = 16:22:26:36) and of the ribosomal RNA (A:U:C:G = 18:20:24:38) was not affected by the androgen treatment, nor was the sedimentation profile of the prostatic nuclear RNA in a sucrose density gradient (the latter being very similar to the sedimentation profile of isolated prostatic ribosomal RNA). It was shown, however, that the template activity of the refined prostatic nuclear RNA, measured with both bacterial and prostatic ribosomal amino acid-incorporating systems, was markedly increased within as little as 24 hours after treatment of recently castrated rats with testosterone. Similar increases

in the relative template activity of RNA isolated from prostatic ribosomes were also demonstrated in the *E. coli* system, although the inherent template activity of the ribosomal RNA from both groups of animals was much less than that of the corresponding preparations of nuclear RNA.

Much more rapid effects of androgens on RNA synthesis in rat seminal vesicles were recently discovered by Wicks and Kenney ('64). They show that within an hour or so after injection of testosterone into rats castrated 12–15 hours previously, the rate of incorporation of ^{32}P into vesicular RNA was increased by 50%, and continued to rise until an approximately 2- to 3-fold increase was attained. The base composition of the pulse-labeled RNA was intermediate between that of total seminal vesicle RNA and "DNA-like RNA." Wicks and Kenney ('65) have also reported that in rat seminal vesicle, little turnover of the phosphorus of the -CCA termini of transfer RNA's occurred after pulse labeling with ^{32}P. But 90 minutes after injection of testosterone, there occurred a 2- to 3-fold increase in the synthesis of transfer RNA in this organ.

The latter experiments and the aforementioned studies on prostatic ribosomes suggest, then, that one of the earliest known effects of androgens on male accessory reproductive organs is to increase the synthesis of messenger, ribosomal, and transfer RNA's. A detailed examination of enzymatic pathways for the incorporation of nucleotides into RNA in the rat ventral prostate (Hancock, Zelis, Shaw, and Williams-Ashman, '62; Hancock, Jurkowitz, and Jurkowitz, '65; Williams-Ashman and Liao, '63; Williams-Ashman *et al.*, '64) revealed the presence of only two distinct enzyme systems: (1) a DNA-dependent RNA polymerase associated solely with the cell nuclei, and (2) enzyme(s) which were associated with both nuclear and cytoplasmic fractions that catalyzed the addition of cytidylate and adenylate residues to the termini of preexisting transfer RNA chains (the activity of the latter enzyme(s) was orders of magnitude greater than that of the nuclear RNA polymerase in the prostates of normal animals). Both of these enzyme systems utilized ribonucleoside triphosphate as precursors, but only the nuclear RNA polymerase system was inhibited by low levels of actinomycin D *in vitro*. Initial experiments (Hancock, Zelis, Shaw, and Williams-Ashman, '62) showed that the RNA polymerase activity of crude prostatic nuclear "aggregate" enzyme preparations of recently orchiectomized rats was increased after administration of testosterone over periods of 4–5 days. The effects of the androgen were, however, much more pronounced when the polymerase reactions were studied in media of low ionic strengths, the control activities being markedly enhanced by raising the salt concentration. More recently, Liao ('65b) found that the RNA polymerase activity of prostatic nuclear extracts was significantly increased within 1 hour after injection of testosterone into castrates; no effect of testosterone on the RNA polymerase of crude nuclear extracts of liver or thymus was demonstrable. Again, the effects of testosterone on prostatic RNA polymerase activity were much more pronounced in media of low ionic strengths. Although the RNA polymerase activity of such crude prostatic nuclear preparations is inhibited by actinomycin D and by exposure to DNase(and requires the simultaneous presence of all four major ribonucleoside triphosphates), it has not been possible, despite repeated attempts (Hancock, Jurkowitz, and Jurkowitz, '65), to rid the enzyme preparations of DNA in such a fashion that their ability to catalyze RNA synthesis can be clearly stimulated and directed by exogenous DNA. There is considerable evidence that the RNA polymerase activity of the prostatic nuclear preparations is limited not by the catalytic capabilities of the activating protein, but rather by the priming ability of the DNA that is firmly bound to the extract. The fact that increasing the ionic strength of the reaction mixtures increases the baseline activity of such preparations and diminishes the effects of testosterone treatment hints that androgen *in vivo* affects the levels or activity of substances associated with prostatic DNA (conceivably histones) that limit its priming activity in the RNA polymerase reaction. In marked contrast to the prostatic RNA polymerase system, it has proved relatively easy to isolate

the RNA polymerase of rat testis free from DNA, and to purify the enzyme as a polynucleotide-free protein (Ballard and Williams-Ashman, '64). As yet, it has not been possible to influence the catalytic activity of this resolved testicular RNA polymerase by addition of testosterone or other sex hormones *in vitro*.

Research on the influence of androgens and other hormones on the synthesis and utilization of ribonucleic acids has been severely impeded by lack of precise methods for estimation of discrete RNA's, particularly with respect to the template function of various messenger RNA's (or polycistronic messengers) in protein biosynthesis. Kidson and Kirby ('64) have recently developed countercurrent distribution methods for separation of rapidly labeled RNA's in rat liver. Complex and reproducible patterns were observed under well-controlled conditions. Testosterone was among the hormones that induced rapid, selective, and reversible changes in the rapidly labeled RNA profiles. Kidson and Kirby ('64) are of the opinion that the various polyribonucleotide fractions separable by their techniques are largely messenger RNA's, and application of such methods to the problem of the nature of the androgenic control of RNA synthesis in male accessory organs might be of great value.

INHIBITORS OF RNA AND PROTEIN SYNTHESIS AS ANTAGONISTS OF TESTOSTERONE ACTION

In comparison with studies on the actions of estrogens, comparatively little work has been published on the influence of inhibitors of RNA and protein synthesis on the action of testosterone on the accessory glands. The ability of puromycin to depress aminoacyl transfers by isolated ribosomes, and of actinomycin D to depress the DNA-dependent synthesis of RNA by nuclear RNA polymerase preparations in male accessory glands, is well documented (Williams-Ashman, Liao, Hancock, Jurkowitz, and Silverman, '64). As might be expected, the author has observed in unpublished experiments that intraperitoneal injection of actinomycin D (25 μg per 100 g of body weight) into recently castrated rats largely prevents the striking increases in prostatic and seminal vesicle weight which occur 72 hours after daily subcutaneous injections of testosterone propionate (1 mg per 100 g of body weight). Angeletti, Salvi, and Tacchini ('64) reported that over a period of 5 days, concurrent administration of actinomycin D almost completely blocked the testosterone-induced increase in the weight, soluble protein level, and activities of protease, α-amylase, and nerve growth factor of mouse submaxillary glands.

Frieden *et al.* ('64) have studied some effects of actinomycin D administration on certain biochemical concomitants of the renotropic action of testosterone in mice. They reported that in experiments of 2–3 days' duration, the injection of actinomycin D (total of four doses of 200 μg per kg body weight) injected 1 day prior to and concurrently with testosterone completely inhibited the increase in the kidney β-glucuronidase activity resulting from the androgen treatment. The testosterone-induced incorporation of leucine-^{14}C into isolated kidney slices was, however, not affected by actinomycin D over this time period, although the antibiotic depressed the base-line leucine incorporation values in the controls that did not receive testosterone. Similar findings were obtained when glycine-^{14}C and arginine-^{14}C were used as the labeled amino acids. These experiments on the influence of actinomycin D on the effect of testosterone on amino acid-incorporation by kidney slices are somewhat difficult to interpret, because the rate-limiting steps for entry of the amino acids into protein-like material have not been precisely clarified in such isolated kidney preparations. Frieden *et al.* ('64) also found that actinomycin D had little effect upon the increase in renal β-glucuronidase activity due to testosterone administration if actinomycin treatment was begun after the first injection of the androgen.

Breuer and Florini ('65) state in a preliminary communication that actinomycin D blocks the testosterone-induced increase in the ribosomal and messenger RNA content of skeletal muscle of castrated rats.

ANDROGENS AND DNA SYNTHESIS

Relatively little attention has been paid to the biochemistry of androgen-induced changes in DNA synthesis in accessory reproductive organs. The classical experiments of Burkhart ('42) using the colchicine technique showed that following injection of a single dose into castrate rats, cell hypertrophy and nuclear enlargement were observable at 23 hours, whereas mitotic activity began at 35 hours and reached a maximum at 43 hours. Wicks and Villee ('64) observed that ^{14}C derived from glycine-2-^{14}C was hardly incorporated into DNA by seminal vesicle slices prepared from castrated rats, but that detectable incorporation of the radioisotope into DNA was manifest with slices obtained from animals 36 and 48 hours after injection of testosterone propionate, but not before that time (marked changes in oxygen consumption, uptake of glycine-^{14}C into acid-soluble material, and incorporation of glycine-^{14}C into RNA were observed in seminal vesicle slices within 18–24 hours after administration of the androgen). Very recently, Sheppard, Tsien, Mayer, and Howie ('65) reported that treatment of orchiectomized rats with methandrostenolone caused an increased uptake of thymidine-^{3}H into the DNA of the levator ani, ventral prostate, and seminal vesicles; no effect was observed in adrenal, thymus, or leg muscle, and a decreased uptake of thymidine-^{3}H in the kidney was noted. The effects of this anabolic agent were observable only after 2 days of treatment.

Of related interest is the finding of Cantarow and Zagerman ('64) that the testosterone-induced growth of seminal vesicles in castrated rats is inhibited by simultaneous treatment of the animals with 5-fluorouracil. However, the extent of growth inhibition by the pyrimidine analogue could be overcome by high doses of the androgen at all tolerated levels of fluorouracil administration. Cantarow and Zagerman ('64) point out that the principal effect of fluorouracil in animal tissues is to inhibit DNA synthesis, via the formation of fluorodeoxyuridylic acid, a potent inhibitor of thymidylate synthetase. However, it is also known that fluorouracil can be incorporated into RNA, and it is possible, as these investigators conjecture, that testosterone may increase the entry of fluorouracil into RNA at the expense of its conversion into fluorodeoxyuridylic acid. Dorfman ('63) has described the anti-androgenic action of 5-fluorouracil in the cock's comb.

Much interest attaches to further investigations on the interrelationships between RNA and DNA synthesis in male accessory genital organs. In this regard, it would be particularly interesting to examine various reactants in the DNA polymerase system, and also enzyme systems responsible for the production of deoxyribonucleoside triphosphates. Very recently, Weiss, Zagerman, and Kokolis ('65) reported that testosterone induces large increases in the activity of thymidine kinase and thymidylate synthetase in mouse seminal vesicle. Any comprehensive biochemical explanation for the stimulation by androgens of cell division in accessory reproductive organs must account for the ultimate curtailment of the steroid-induced growth of these organs.

CONCLUSIONS

The experimental evidence available today is by and large consistent with the view that androgenic hormones initiate and maintain the functional differentiation of the prostate gland and seminal vesicles — and their elaboration of all sorts of secretory products (Mann, '64; Price and Williams-Ashman, '61) — by regulating the biosynthesis of rate-limiting enzymes and structural proteins. Furthermore, certain of the effects of testosterone on the incorporation of amino acid into proteins by cell-free extracts of these tissues seem to be contingent upon more primary changes in the ribosomal population density and in the levels of template RNA's. It is fast becoming apparent that an increased synthesis of transfer, ribosomal, and messenger-like RNA's is one of the earliest detectable metabolic events in intact male accessory glands of castrates following the injection of testosterone. In accord with this are the early increases in RNA polymerase activity of crude nuclear extracts of the prostate after stimulation by testosterone *in vivo*. Such changes in nuclear RNA polymerase activity, however, are in all probability a

reflection of ill-defined perturbations in the priming ability of the DNA bound to the preparations, and it simply is not known how far these effects are biochemically removed from the primary receptors for androgenic hormones. Nor is it possible, from the very limited data at hand, to come to any sure conclusions as to the degree of selectivity of activation by testosterone of the synthesis of complementary RNA copies of specific genes. Certainly there are many reasons to suppose (Williams-Ashman *et al.*, '64; Williams-Ashman, '62, '64, '65a, b) that the receptors for androgenic and other steroid hormones are probably proteinaceous in nature. As a guide to further experimentation, it still seems reasonable to surmise that such proteins may be in some way connected with regulation of synthesis of specific polyribonucleotides, or conceivably with the translocation of various RNA's from the cell nucleus to the cytoplasmic sites of protein biosynthesis. Progress along these lines will undoubtedly be contingent, *inter alia*, upon development of much better understanding of the biochemical intimacies of RNA synthesis and intracellular transport in mammalian cells, and of the interrelationships between RNA- and DNA-synthesizing systems.

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OPEN DISCUSSION

BONNER [1]: Well, first of all, Professor Williams-Ashman, I would like to discuss this elegant experiment concerning what you refer to as RNA polymerase from prostate. You show that at different times, after the administration of testosterone, the ability of this crude system to make RNA increases, provided only that the preparation was isolated at low ionic strength.

WILLIAMS-ASHMAN: No, it was not tested in this way. The nuclear preparations were isolated from both castrated animals and castrated animals treated with testosterone in the same fashion. The RNA polymerase *activities* of both types of preparation were, however, measured at both low and high ionic strengths.

BONNER: Pardon me. When you test at high ionic strength, then at all times after testosterone administration the activity of the system is higher than that of the system tested at low ionic strength, and is constant throughout the experiment. It would seem to me again that this implies that we are here testing something that is a combination of RNA polymerase plus template activity. The fact that RNA-making activity is constant in the high salt concentration medium would seem to me to imply that it is differences in template activity of the DNA that are being manifested.

WILLIAMS-ASHMAN: That's precisely what I tried to imply in my talk.

BONNER: I haven't come to my question! O.K. So, fine; we are at one! Now we go to this purification of the soluble RNA polymerase. You can get a nice RNA polymerase. Others have also shown that the capability of the organ to synthesize RNA's rises 3-fold over a period of 60 minutes after hormone treatment.

WILLIAMS-ASHMAN: The increased RNA polymerase activity following testosterone treatment *in vivo* was measured in the prostate, not in the testis.

BONNER: What I wanted to find out was whether in the case of the organ from which you purified the RNA polymerase, it is now possible to determine if the RNA polymerase itself varies, or if it is template activity.

WILLIAMS-ASHMAN: We have not examined the effects of testosterone *in vivo* on the RNA polymerase activity of *testis*. Testosterone does not have any effect on the purified testicular enzyme.

TOMKINS [2]: Have you looked into any other circumstances except the one that you showed? Does the total nuclear RNA stay the same?

WILLIAMS - ASHMAN: The RNA : DNA ratio of isolated nuclei remains at the value of 0.25.

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[1] James Bonner, Division of Biology, California Institute of Technology, Pasadena, California.

[2] Gordon M. Tomkins, National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda, Maryland.

WOOL [3]: There is one puzzling aspect to the results. Incorporation of radioactivity from sRNA charged with ^{14}C-phenylalanine into protein is decreased when ribosomes from the prostate of castrate animals are used to catalyze protein synthesis. If that defect is to be accounted for by a deficiency of template RNA, as you suggest, then I should have expected that addition of artificial template RNA (that is to say, polyuridylic acid) would not merely restore synthesis to normal, as you found, but would actually result in an amount of synthesis that was apparently greater than that observed with ribosomes from the prostate of hormone-treated animals to which polyuridylic acid was also added. The basis of my prediction is that ribosomes from the prostate of castrate animals have less natural template RNA; therefore, they should bind greater amounts of artificial template RNA (polyuridylic acid). The artificial template RNA contains only codons for phenylalanine; in natural template RNA the codon for phenylalanine appears, on the average, only once in each 20 triplets. Since the only radioactive amino acid is phenylalanine, there should be far greater incorporation of radioactivity into protein when ribosomes from the prostate of castrate animals are used. Can you account for the discrepancy between observation and prediction?

WILLIAMS-ASHMAN: I think that one can still conclude from these experiments that the *capacity* of unprogrammed prostatic ribosomes to react with the synthetic template RNA is not diminished by androgen withdrawal under these experimental conditions. It seems to me that a possible explanation for the increased amino acid incorporation in the absence of poly U by ribosomes from testosterone-treated castrates is that relatively higher levels of template RNA's are associated with the total ribosomal population.

Ts'o [4]: I would like to make a comment on the very elegant research you have done on this soluble, purified mammalian polymerase. You mentioned the fact that when you added testosterone and tried to examine the effect of the hormone on various expressions of the enzyme, you did not find any effect. I would like to know whether you have done this experiment in conditions where the system was strictly DNA-dependent, and concentration of enzyme was not limiting. This is a very difficult experiment to do. I think that you have demonstrated that steroids have no effect on the enzymes. I just wonder whether you have examined the effect of the steroid on the template activity of the DNA. This can be done only when DNA is limiting in the system.

WILLIAMS-ASHMAN: We have not examined the effect of testosterone under the conditions you mention. But we have performed a number of different types of experiment, including examination of the effect of preincubating the enzyme with testosterone under conditions where considerable polymerase activity is lost, the assays being initiated by later addition of DNA. I should emphasize, however, that our inability as yet to demonstrate any *in vitro* action of testosterone on the soluble testicular enzyme preparations does not necessarily mean that the enzyme cannot be influenced by testosterone under other experimental conditions.

NOVELLI [5]: I would like to make a comment on this poly U experiment. If you do a very brief incubation at reduced temperature, and then take your reaction mixture and put it on a sucrose gradient, so you can see where the incorporation is taking place, you will see that with rat liver all of the endogenous incorporation is in the polysomal region. The monomers do not incorporate the amino acid. When the same experiment is repeated with poly U, the incorporation in the polysomal region remains unchanged, but the bulk of the poly U-stimulated incorporation is associated with the monomers.

So I would interpret your experiment to indicate that both preparations have a comparable amount of competent monomers.

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[3] Ira G. Wool, Department of Physiology, University of Chicago, Chicago, Illinois.

[4] Paul O. P. Ts'o, Department of Radiological Science, Johns Hopkins University, Baltimore, Maryland.

[5] G. David Novelli, Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee.

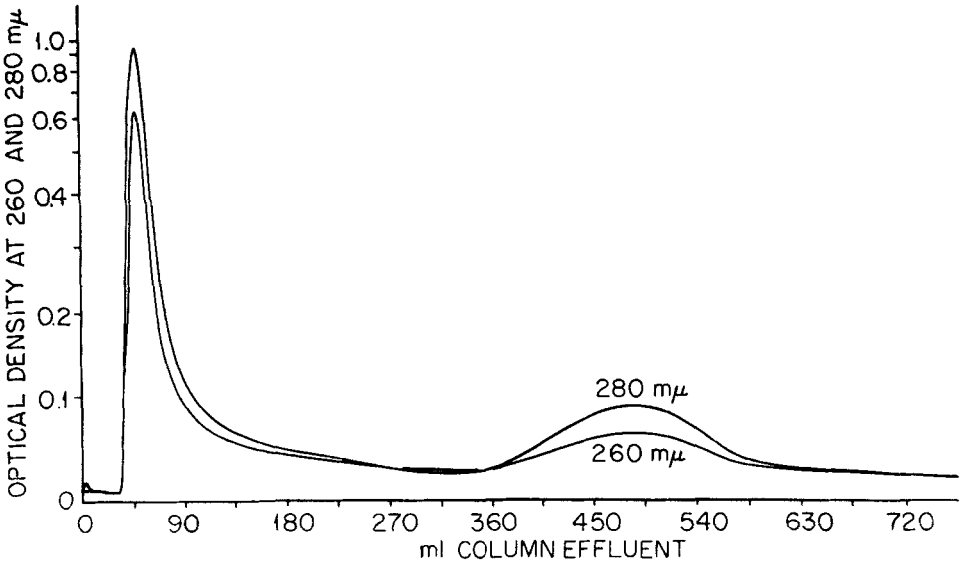
HANSON [6]: In line with Dr. Bonner's comments, there was one point which I did not understand. You were using the purified polymerase, with DNA of an unspecified kind. Down in the table you showed an item which said "minus ammonium sulphate," and the activity fell by half. If this were "clean" DNA, one would think there was an ionic strength effect in addition to the removal of the histones.

WILLIAMS-ASHMAN: Phillip Ballard has demonstrated a small (about 50%) activating effect of relatively low ionic strengths (about 0.2) on the RNA-polymerase activity of soluble testicular enzyme preparations, which exhibit an absolute requirement for exogenous DNA. I think that it is very likely that this is an effect of ionic strength on interaction of the RNA polymerase protein with the DNA, since "clean" DNA preparations were used in these experiments. The effects of high ionic strengths (approximately 1.0) on the RNA polymerase activity of prostatic nuclear extracts are, I believe, of quite a different nature. In the latter situation, the RNA polymerase activity is dependent on DNA firmly bound to the prostatic nuclear extracts. Here the effects of high salt concentrations could well be due to displacement of inhibiting substances such as histones, or to inactivation of inhibiting factors associated with the aggregates, such as RNase.

BOND [7]: I have been looking at a liver protein component, and its response to both testosterone and estradiol which provides an interesting experimental model. The protein which I have designated as a sex-associated protein is present in large amount (about 3% of the soluble or supernatant protein) in the liver of the male rat and appears by similar evaluating criteria to be absent from the liver of the female. I will return to this point momentarily.

Discussion Figure A shows the sex-associated protein chromatographically. The chromatogram was developed on DEAE-cellulose by equilibrium elution with a single buffer. Consequently, the vast majority of the cellular proteins remained on the column at the time that the chromatogram was completed. The first large peak is a complex consisting of basic and relatively uncharged proteins, the so-called fall-through components. The second smaller peak represents the sex-associated protein. This component cannot be de-

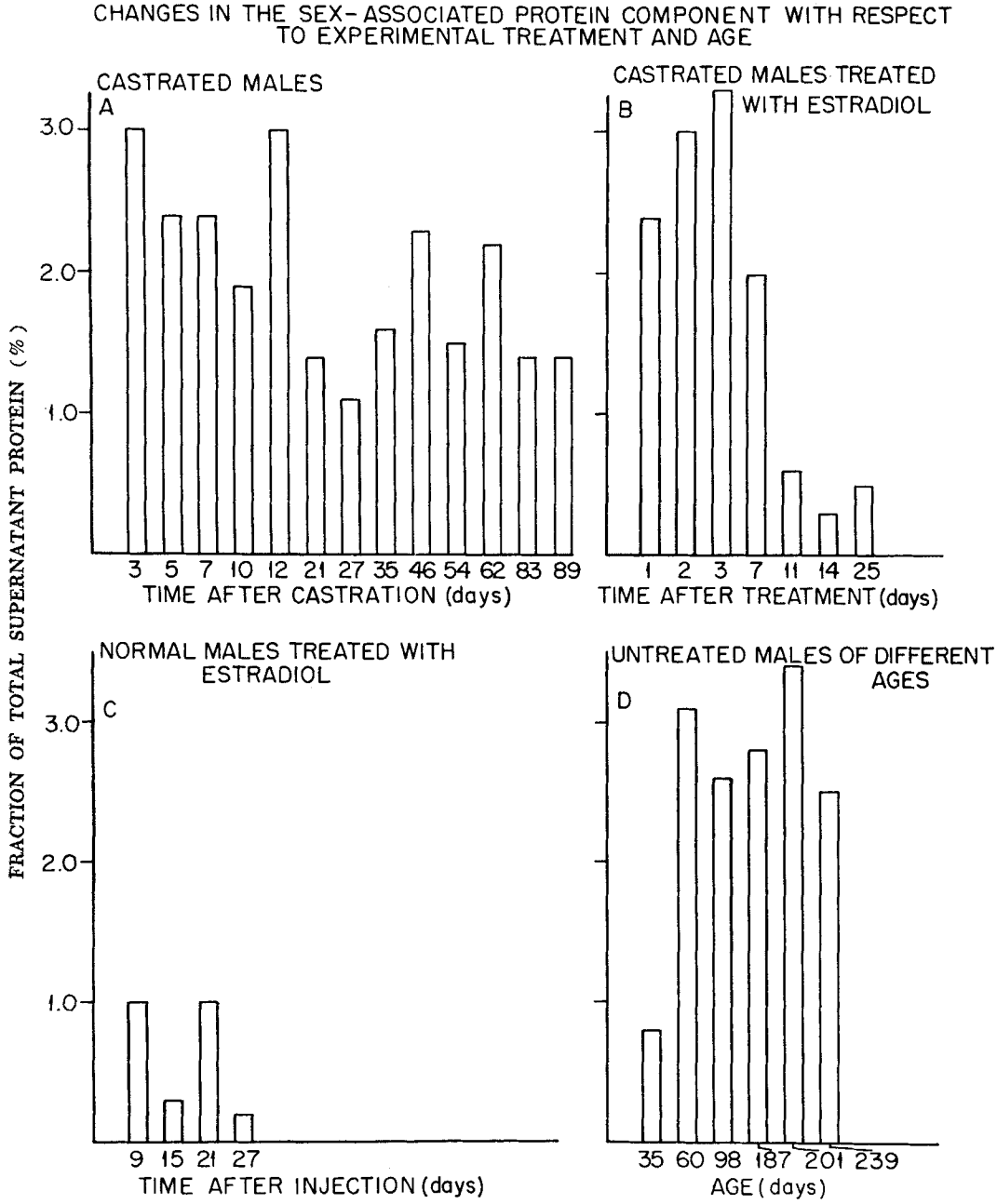
[6] John B. Hanson, Department of Agronomy, College of Agriculture, University of Illinois, Urbana, Illinois.
[7] Howard E. Bond, Carcinogenesis Studies Branch, National Cancer Institute, Bethesda, Maryland.



Discussion Figure A

tected in the chromatogram of the female rat liver.

Discussion Figure B describes some of the responses of the sex-associated protein to steroid treatment. The castrated male shows a tendency toward a slow and variable loss of the material with time following castration. The normal male treated with estradiol loses the protein rapidly; in the experiment shown here,



Discussion Figure B

most of the material had been lost by 9 days after treatment. The last figure on this slide shows the relationship of the sex-associated protein to the age of the animals. As you can see, there is only a small amount of the material present at 35 days, whereas the normal complement of the protein is present at 60 days of age. A point at 43 days of age was omitted from this slide. It has a value about intermediate between the other two. Clearly, there is an increase in amount of the protein associated with sexual maturity of the animal.

Corresponding experiments with testosterone-treated females have given opposite effects. Both the normal and castrated female responded by producing the protein in amounts approaching that seen in males. The protein did not appear in the untreated female castrate.

These studies recently have been confirmed by [D.] Barzilai and [G.] Pincus (Proc. Soc. Exptl. Biol. Med., *118:* 57–59, 1965). They also extended the investigations to show a response from progesterone treatment which caused the appearance of the protein in the female and an increase in the level found in the castrated male. It is conjectural, though, as to whether this is from a direct response to progesterone or from a metabolite of progesterone with androgen activity.

The sex-associated protein has been purified to the extent that a highly specific antiserum has been produced. The reagent produces a single precipitin band when diffused in agar against the liver soluble proteins. The sensitivity of the agar diffusion techniques is such that it has been possible to detect the protein in livers of normal female rats. The concentrations are about 32 times less in the female than in the male. Both single and double diffusion quantitative agar techniques give good agreement on this figure which, nevertheless, lacks precision because of its logarithmic nature. Stability problems associated with preparing the highly purified protein have prevented the accumulation of sufficient material to use more sophisticated quantitative methods and, thereby, to arrive at the absolute amounts present in tissue.

It would be of fundamental concern to know if the sex-associated protein exists as a circulating entity in blood. Thus far it has not been detected there, either chromatographically or by the much more sensitive immunochemical techniques.

There does not appear to be a necessary adrenal involvement in the sex-associated phenomenon. The adrenalectomized female still responds to testosterone treatment with an increase in the protein, although the response is rather poor compared to that in the intact animal. Adrenalectomy appears to cause no significant change in the amount of protein found in the male. The adrenalectomized male responds similarly to the intact male when treated with estradiol; the protein disappears rapidly from the liver.

An interesting response occurred when the hormones were administered to animals simultaneously. Estradiol was given in oil, whereas the testosterone was given as a combination of testosterone propionate and testosterone phenylacetate. The hormones were administered in a single treatment with the result that the testosterone was a longer-lasting repository. Adrenalectomized females showed no response until about 20 days after hormone treatment, when the sex-associated protein began appearing in substantial amounts. In similarly treated males, the protein disappeared rapidly after hormone treatment, but at about 20 days it returned in appreciable amounts.

Generally, it appears that I have described a protein that is sex hormone-dependent. There appears to be a direct-acting antagonism between the hormones and a reciprocal-dose relationship. I believe that the phenomenon offers the possibility of studying a highly specific response to hormone action at the molecular level, in addition to studying the physiological consequences of that action.

WILLIAMS-ASHMAN: I appreciate an opportunity to hear about this excellent work. This is another example of an almost qualitative effect of a sex hormone on formation of a specific protein in an extragenital tissue. It brings to mind the work of [C. R.] Shaw and [A. L.] Koen on the induction by testosterone of a specific esterase isozyme in mouse kidney, and Olga

Greengard's studies on the influence of estrogens on plasma phosphoprotein formation in male chickens.

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