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# Catabolism of [4-$^{14}$C]Testosterone by Subcellular Fractions of Human Prostate

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1. Incubation conditions were established in experiments with human-prostate homogenates for almost complete conversion of [4-$^{14}$C]testosterone into at least ten transformation products. 2. Whole homogenates of tissue with benign hypertrophy were shown to contain 3$\alpha$-, 3$\beta$- and 17$\beta$-hydroxy steroid dehydrogenases, $\Delta^4$-3-oxo steroid 5$\alpha$- and 5$\beta$- reductases and unidentified hydroxylases. 3. Most of the 17$\beta$-hydroxy steroid-dehydrogenase activity was located in the mitochondria, which showed little other activity. 4. The 3$\alpha$- and 3$\beta$-hydroxy steroid dehydrogenases and the 5$\beta$-reductase were located in the high-speed supernatant and required supplementation with NADPH for activity. 5. The 5$\alpha$-reductase was located in both microsomal and high-speed supernatant fractions and also required supplementation with NADPH.

Various Laboratories have reported considerable differences in the metabolism of testosterone and androstenedione (androst-4-ene-3,17-dione) by human prostatic tissue preparations (Wotiz & Lemon, 1954; Ofner, Smakula, Wotiz, Lemon & Mescon, 1957, 1965; Farnsworth, Brown, Lano & Cross, 1962; Farnsworth & Brown, 1963; Farnsworth, 1965; Acevedo & Goldzieher, 1964, 1965). These differences are probably due to a number of factors such as the morphology and pathology of the specimens, the time taken between surgical removal and incubation, the variety of experimental conditions used and superimposed catabolism by microbiological contaminants. Their results have, however, shown clearly that the human prostate is capable of metabolizing these circulating $C_{19}$ steroids that have long been held to be directly responsible for the trophic stimulation and functional differentiation of the gland.

The present investigation was undertaken to establish incubation conditions that would permit the identification and assay of the prostatic enzymes concerned. This paper describes such experimental conditions for homogenates of human prostatic tissue with benign hypertrophy, provides evidence for a number of new enzymes and transformation products in addition to confirming some of those already reported, and localizes these activities in subcellular particulate fractions.

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## EXPERIMENTAL

*Materials.* NAD$^+$ of 83·5% purity assayed with yeast alcohol dehydrogenase (EC 1.1.1.1) and NADP$^+$ of 85% purity assayed with yeast glucose 6-phosphate dehydrogenase (EC 1.1.1.49) were obtained from the Sigma Chemical Co., St Louis, Mo., U.S.A., and NADPH of 81% purity assayed with yeast glutathione reductase (EC 1.6.4.2) was from the Nutritional Biochemicals Corp., Cleveland, Ohio, U.S.A. All solvents were redistilled before use. Silica gel G and alumina G, adsorbents for thin-layer chromatography, were purchased from Brinkman Instruments Inc., Great Neck, Long Island, N.Y., U.S.A. Commercial and given specimens of $C_{19}$ steroids required for reference purposes and carrier addition and [4-$^{14}$C]testosterone (22$\mu$c/$\mu$mole; New England Nuclear Corp., Boston, Mass, U.S.A.) were purified by preparative thin-layer chromatography on ethyl acetate-washed silica gel G as described by Dyer, Gould, Maistrellis, Peng & Ofner (1963). For the present incubation studies prostatic tissue with benign hypertrophy was removed from patients by either perineal or suprapubic prostatectomy. The pathological diagnosis of the prostatic tissue used for the detailed analysis of the products of homogenate incubation in Expt. 9 (Table 1) was one of benign hypertrophy, with no evidence of chronic prostatitis or malignancy. The prostate gland was removed from a 78-year-old man by suprapubic prostatectomy and was firm and rubbery in consistency.

*Preparation of tissue.* Freshly removed human prostatic tissue was brought to the laboratory in beakers immersed in crushed ice. Any adhering blood was removed by several washes with small quantities of ice-cold 0·9% NaCl. All subsequent manipulations were carried out in a cold room at 4°. The tissue was blotted several times on surgical gauze, weighed and sampled for a survey of microbiological con-

taminants. It was sliced by hand, passed twice through a Latapie micro tissue grinder (Arthur H. Thomas Co., Philadelphia, Pa., U.S.A.) and mixed in a Waring Blendor for 30sec. with 0·067M-$KH_2PO_4$–$Na_2HPO_4$, buffer, pH7·4, containing sucrose (0·25M) and nicotinamide (0·04M) (3ml./g. of tissue). The mixture was then homogenized for 1min. in a Potter glass homogenizer fitted with a Teflon pestle. Differential centrifugation of the homogenate in a Sorvall refrigerated centrifuge and a Spinco preparative ultracentrifuge according to the methods of Hogeboom (1955) by using the speeds of Myers & Slater (1957) for rat-liver preparations yielded mitochondrial, microsomal and high-speed supernatant fractions as described by Chamberlain, Jagarinec & Ofner (1965). Electron micrographs of the microsomal pellet (given by Dr Paul Goldhaber and Dr John Nalbandian, Harvard School of Dental Medicine) showed that this fraction was contaminated with mitochondria; separation of the prostatic mitochondria by ultracentrifugation apparently requires higher speeds than for the analogous rat-liver preparation.

*Incubations.* [4-$^{14}$C]Testosterone (140$\mu$g./60000 counts/min. or 14$\mu$g./60000 counts/min. or 0·7$\mu$g./60000 counts/min.; cf. Table 1) in methanol (0·1ml.) was incubated with the equivalent of 1g. wet wt. of tissue (homogenate or subcellular fraction) in 30ml. of a medium consisting of 0·067M-phosphate buffer, pH7·4, containing sucrose (0·025M) and nicotinamide (0·04M). Incubations were for 2hr. in air at 37°. Supplementation with cofactor was with either $NAD^+$ (2mg.) and $NADP^+$ (2mg.) or NADPH (2mg.). Enzyme reaction was terminated by shaking the incubation mixture with chloroform and freezing. After thawing, the aqueous layer was extracted with a further volume of chloroform. The solvent extracts were combined and evaporated to dryness in a stream of $N_2$ to give the crude steroid extract. All the radioactivity was in this fraction.

Bacterial examination of specimens before and after incubation showed either no growth (e.g. Expt. 9 in Table 1) or the presence of less than $10^4$ colonies of *Escherichia coli*, *Klebsiella aerobactor* or *Staphylococcus aureus* after aerobic and anaerobic cultivation for 72hr. at 37°. Plain nutrient agar was used for colony counts; the plates were counted after incubation for 18hr. at 37°. The culture medium used for the attempted isolation of organisms consisted of blood agar, endo agar and thioglycollate broth (given by Miss Ruth Norton, Bacteriology Laboratory, Lemuel Shattuck Hospital).

*Thin-layer chromatography.* Crude steroid extracts were fractionated on silica gel G after application as three spots 0·5cm. apart. After development with chloroform–ether (9:1, v/v), the plates were dried and then developed again in the same direction with the same solvent mixture. One of two procedures was used for evaluating the radiochromatograms. In the first the pattern of radioactive metabolites was determined by eluting successive zones ($\frac{1}{4}$in.) with chloroform and measuring the radioactivities of portions of the eluates. Alternatively, a portion (generally 1%) of the incubation extract was chromatographed separately on the same plate and the radioactivities of successive zones ($\frac{1}{4}$in.) of this guide strip were measured after direct transfer of the silica gel to counting vials containing scintillation fluid; the pattern of radioactive metabolites thus obtained determines which areas of the chromatogram of the bulk of the extract have to be eluted. Marker steroid standards were chromatographed on the same plate and were revealed with an $H_2SO_4$ spray reagent after all the radioactive zones had been removed (for further details see Dyer *et al.* 1963; Chamberlain *et al.* 1965). Chromatography of standards (both unlabelled and labelled) mixed with entire incubation extract demonstrated that the presence of the endogenous lipid did not affect their mobilities. Alumina G was used for the subsequent separation and identification of epimeric diols, with methylene dichloride–ether (9:1, v/v), twice, as the developing solvent.

*Paper chromatography.* The Bush (1952) system A was used for the confirmation of identity of the isolated metabolites [light petroleum (b.p. 90–100°)–methanol–water (5:4:1, by vol.); 37°].

*Gas–liquid chromatography.* Determination of steroids was carried out by means of a Jarrell–Ash chromatograph model 28–700 equipped with a flame ionization detector. A coiled glass column 6ft. long was used with 3% of fluorosilicone QF-1-0065 (FS-1265-10000cs) on 50–60 mesh Anakrom ABS as stationary phase (purchased from Analabs Inc., Hamden, Conn., U.S.A.) and $N_2$ as carrier gas. Samples (1–5$\mu$l.) of ethyl acetate or ethanol solutions containing 0·5–8·0$\mu$g. of steroid were injected for analysis. Flow rates were adjusted to give a retention time of 10min. for each compound and weight analysis was by measurement of peak height after the compilation of a standard mass–peak height curve.

*Radioactivity measurements.* Portions of extracts or of eluates of zones from thin-layer and paper chromatograms were transferred to counting vials, the solvent was evaporated to dryness under a stream of $N_2$ and scintillation fluid [5ml. of toluene–95% ethanol (3:1, v/v) containing 1,4-bis-(4-methyl-5-phenyloxazol-2-yl)benzene (1·12mg.) and 2,5-diphenyloxazone (11·2mg.)] added. Samples were counted in a Packard Tri-Carb liquid-scintillation spectrometer. Quenching due to small quantities of silica gel ($\frac{1}{4}$in. zone) transferred directly to the vials proved negligible.

*Methods of carrier estimation and reverse isotope-dilution analysis.* In reverse isotope-dilution experiments, weight analysis of carrier $\Delta^4$-3-oxo steroid was by measurement of the extinction at 230, 240 and 250m$\mu$, assuming $\epsilon_{240\,m\mu}$ 15500 (in ethanol). With steroids that are transparent to ultraviolet light, determination of added and recovered carrier was by gas–liquid chromatography as described above. In most cases, where the radioactivity of the sample was too low for reverse isotope-dilution analysis by crystallization to constant specific activity, our procedure was as follows: the unknown metabolite was chromatographed (on paper or thin layer of adsorbent) in admixture with authentic carrier (50–100$\mu$g.). The area of the chromatogram containing the carrier was divided into successive zones; weight of the carrier in each was analysed by gas–liquid chromatography and the radioactivity of a corresponding sample determined in the liquid-scintillation spectrometer.

## RESULTS

### *Homogenate incubations*

Table 1 summarizes some of our experiences in incubations of [4-$^{14}$C]testosterone with homogenates of 12 specimens of human prostatic tissue with benign hypertrophy. Correlation of extent of transformation with substrate concentration is apparent.

Table 1. *Metabolism of* [4-$^{14}$C]*testosterone by homogenates of human prostatic tissue with benign hypertrophy*

All incubations were with homogenate equivalent to 1 g. wet wt. of tissue in 30 or 40 ml. of phosphate buffer for 2 hr. as described in the text.

| Expt. no. | Cofactors | Testosterone ($\mu$g.) | % of testosterone metabolized | Polar metabolites |
|---|---|---|---|---|
| 1 | $NAD^+$, $NADP^+$ | 140 | 7 | — |
| 2 | $NAD^+$, $NADP^+$ | 140 | 4·3 | — |
| | $NAD^+$, $NADP^+$ | 14 | 27 | — |
| 3 | $NAD^+$, $NADP^+$ | 14 | 3 | — |
| | $NAD^+$ | 14 | 17 | — |
| | $NADP^+$ | 14 | 30 | — |
| | — | 14 | 37 | — |
| 4 | $NAD^+$, $NADP^+$ | 14 | 14 | — |
| | $NAD^+$ | 14 | 2 | — |
| | $NADP^+$ | 14 | 19 | — |
| 5 | $NADP^+$ | 14 | 9 | — |
| 6 | — | 14 | 6 | — |
| | $NADP^+$ | 14 | 7 | — |
| 7 | $NAD^+$, $NADP^+$ | 14 | 20 | — |
| 8 | $NAD^+$, $NADP^+$ | 14 | 35 | — |
| 9 | NADPH | 0·7 | 82 | 3 |
| | $NAD^+$, $NADP^+$ | 0·7 | 100 | 4 |
| 10 | $NAD^+$, $NADP^+$ | 14 | 39 | 7 |
| | $NAD^+$, $NADP^+$ | 0·7 | 100 | 10 |
| | $NAD^+$, $NADP^+$, NADPH, NADH, ATP | 0·7 | 100 | 5 |
| 11 | NADPH | 0·7 | 100 | — |
| | $NAD^+$, $NADP^+$ | 0·7 | 100 | 16 |
| | NADPH-regenerating system | 0·7 | 100 | — |
| 12 | $NAD^+$, $NADP^+$ | 0·7 | 100 | — |
| | NADPH | 0·7 | 100 | — |

In Expts. 1–8 only metabolites of higher chromatographic mobility than testosterone were formed. In Expts. 9–12, at the highest tissue/substrate ratio of the series, hydroxylated products (polar metabolites) and diols were also produced. The following detailed analysis of the metabolites produced in Expt. 9 reveals the nature of changes due to changes in supplementation with cofactor.

*Experiment* 9

*Homogenates.* Fig. 1 shows the distribution of radioactivity after silica gel G chromatography of the products obtained on incubation of [4-$^{14}$C]-testosterone with prostate homogenates. In Expt. 9A the medium was supplemented with NADPH and in Expt. 9B with $NAD^+$ and $NADP^+$. The metabolite patterns shown in Fig. 1 were derived by chromatographic analysis of small portions (1%) of the incubation extracts and served only as guides for the elution of the radioactive zones I–IV. The incubation extracts were further analysed as follows.

*Experiment 9A: zone I.* This zone corresponded to the position of reference 5$\alpha$-androstane-3$\alpha$,17$\beta$-diol on the silica gel G plate. Chromatography on alumina G revealed two main peaks of radioactivity as shown in Fig. 2. Material with the $R_F$ of 5$\alpha$-androstane-3$\alpha$,17$\beta$-diol in both thin-layer-chromatography systems was oxidized with chromium trioxide (cf. Chamberlain *et al.* 1965) to give material identical with 5$\alpha$-androstane-3,17-dione on co-chromatography with a mixture of androst-4-ene-3,17-dione, 5$\beta$-androstane-3,17-dione and 5$\alpha$-androstane-3,17-dione carriers. The metabolite was therefore assigned the structure 5$\alpha$-androstane-3$\alpha$,17$\beta$-diol. This conclusion was confirmed by a reverse isotope-dilution experiment in which authentic 5$\alpha$-androstane-3$\alpha$,17$\beta$-diol was added; specific activity of crystals and residues from mother liquors remained unchanged after successive recrystallizations from acetone–light petroleum (b.p. 40–60°), methanol–chloroform and ethanol–water. The minor zone of radioactivity on the alumina G plate (Fig. 2) had the $R_F$ of 5$\alpha$-androstane-3$\beta$,17$\beta$-diol. After oxidation this material gave both 5$\beta$- and 5$\alpha$-androstane-3,17-dione. No standard for 5$\beta$-androstane-3$\beta$,17$\beta$-diol was available; since we showed that 5$\beta$-androstane-3$\alpha$,17$\beta$-diol could not be in this zone, the minor peak was tentatively identified as a mixture of 5$\alpha$-androstane-3$\beta$,17$\beta$-diol and 5$\beta$-androstane-3$\beta$,17$\beta$-diol.

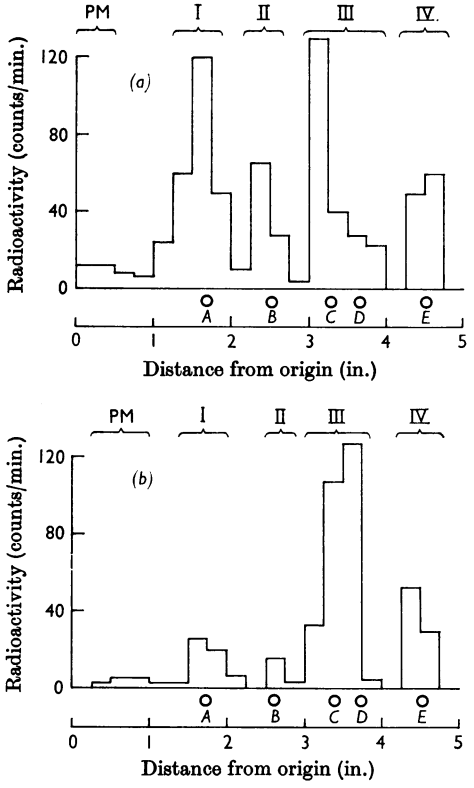


Fig. 1. Thin-layer chromatography of radioactive metabolites from incubation of [4-$^{14}$C]testosterone (0·7 $\mu$g.) with prostate homogenate (1 g. wet wt.). Chromatography was on silica gel G with chloroform–ether (9:1, v/v), twice, as developing solvent. Homogenates were supplemented with (*a*) NADPH and (*b*) $NAD^+$ and $NADP^+$ (see the text). Marker steroids used as standards were: *A*, 5$\alpha$-androstane-3$\alpha$,17$\beta$-diol; *B*, testosterone; *C*, 17$\beta$-hydroxy-5$\alpha$-androstan-3-one; *D*, androst-4-ene-3,17-dione; *E*, 5$\alpha$-androstane-3,17-dione. Zone PM, polar metabolites, probably hydroxylated products (Table 1).

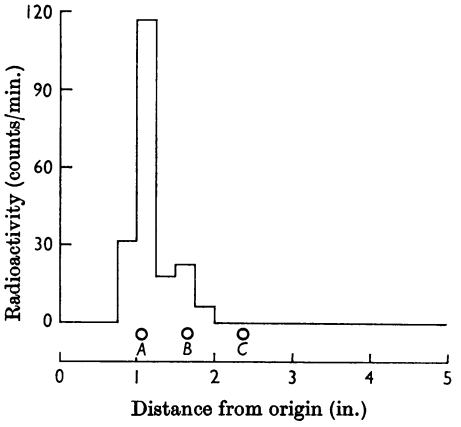


Fig. 2. Thin-layer chromatography of androstanediols from incubation of [4-$^{14}$C]testosterone (0·7 $\mu$g.) with prostate homogenate (1 g. wet. wt.) supplemented with NADPH. Chromatography was on alumina G with methylene dichloride–ether (9:1, v/v), twice, as developing solvent. Marker steroids used as standards were: *A*, 5$\alpha$-androstane-3$\alpha$,17$\beta$-diol; *B*, 5$\alpha$-androstane-3$\beta$,17$\beta$-diol; *C*, androst-4-ene-3$\beta$,17$\beta$-diol.

*Experiment* 9*A*: *zone II*. This material had the $R_F$ of testosterone on silica gel G. Co-chromatography with carrier testosterone in the Bush system A gave one peak of radioactivity coinciding exactly with the carrier testosterone.

*Experiment* 9*A*: *zone III*. Co-chromatography of this material in the Bush system A with carrier 17$\beta$-hydroxy-5$\alpha$-androstan-3-one and androsterone showed radioactivity corresponding to both carriers as well as a small amount of androst-4-ene-3,17-dione. Oxidation of the ring-A-reduced metabolites gave 5$\alpha$-androstane-3,17-dione only.

*Experiment* 9*A*: *zone IV*. This material was co-chromatographed with carrier 5$\alpha$-androstane-3,17-dione and 5$\beta$-androstane-3,17-dione. Most of the material moved with the 5$\alpha$-isomer when chromatographed on silica gel G (chloroform–ether, 95:5, v/v; twice); approx. 10% of the fraction moved with 5$\beta$-androstane-3,17-dione (Fig. 3*a*).

*Experiment* 9*B*: *zone I*. Thin-layer chromatography on alumina G was used as above to resolve the diol fraction. Only 5$\alpha$-androstane-3$\alpha$,17$\beta$-diol and 5$\alpha$-androstane-3$\beta$,17$\beta$-diol could be identified. By the method of oxidation ring-A-saturated 5$\beta$-steroids were shown to be absent; 40% of this fraction was found to consist of hydroxylated products that are oxidized to triones (Chamberlain *et al.* 1965).

*Experiment* 9*B*: *zone II*. This material had the $R_F$ of testosterone on silica gel G. Co-chromatography with carrier testosterone in the Bush system A gave one peak of radioactivity coinciding exactly with carrier. However, unchanged substrate accounted for less than 0·2% of the incubated testosterone.

*Experiment* 9*B*: *zone III*. This zone was analysed in the same way as zone III of Expt. 9A. The Bush chromatogram showed androst-4-ene-3,17-dione, 17$\beta$-hydroxy-5$\alpha$-androstan-3-one, androsterone and an unidentified metabolite, which was not 17$\beta$-hydroxy-5$\alpha$-androst-1-en-3-one, with $R_F$ slightly less than that of 17$\beta$-hydroxy-5$\alpha$-androstan-3-one. No 5$\beta$-reduced steroids were detected by the method of oxidation.

*Experiment* 9*B*: *zone IV*. Co-chromatography of this material with carrier 5$\alpha$-androstane-3,17-dione and 5$\beta$-androstane-3,17-dione confirmed identity

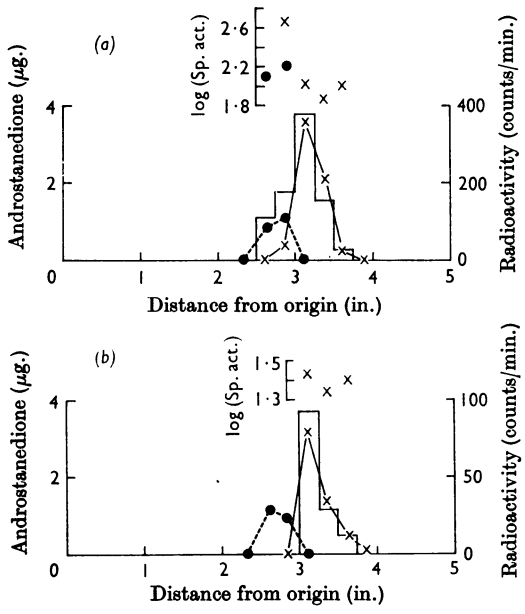


Fig. 3. Thin-layer chromatography of androstanediones from incubation of [4-$^{14}$C]testosterone (0·7 μg.) with prostate homogenate (1 g. wet wt.). Chromatography was on silica gel G with chloroform–ether (95:5, v/v), twice, as developing solvent, and the radioactivity in the zones was measured (cf. histogram). Added carrier steroids, 5α-androstane-3,17-dione (×) and 5β-androstane-3,17-dione (●), were determined quantitatively by gas–liquid chromatography (see the text). Homogenates were supplemented with (*a*) NAPDH and (*b*) NAD$^+$ and NADP$^+$. The logarithms of the specific activities (counts/min./μg.) of the eluates of successive zones in the peak region of the thin-layer chromatograms are plotted above the elution histograms.

of the radioactive material with the former and the absence of the latter (Fig. 3*b*).

*Particulate fractions.* Products obtained from the incubation of testosterone with mitochondrial, microsomal or high-speed supernatant fractions were identified and measured by the methods described above. The results of these experiments are summarized in Table 2. Individual enzyme activities were collated by summation of the amounts of the appropriate metabolites and are expressed as μμmoles of substrate converted under the conditions of the experiment. Maximum possible activity is 2400 μμmoles/g. wet wt. of tissue/2 hr. These enzyme activities are recorded in Table 3.

## DISCUSSION

The most striking feature of our homogenate experiments is the consistent and almost complete transformation of testosterone at a substrate concentration 0·08 μM and a tissue (dry wt.)/substrate ratio of 270000:1, in the presence of either NAD$^+$ and NADP$^+$ or NADPH. We have confirmed the presence of 17β-hydroxy steroid dehydrogenase (Wotiz & Lemon, 1954; Kinson, 1962), 3α-hydroxy steroid dehydrogenase (Farnsworth & Brown, 1963; Acevedo & Goldzieher, 1965) and Δ$^4$-3-oxo steroid 5α-reductase (Farnsworth & Brown, 1963) in human prostate tissue with benign hypertrophy. In addition, evidence for the presence of prostatic 3β-hydroxy steroid dehydrogenase and Δ$^4$-3-oxo steroid 5β-reductase has now been obtained. Transformation into at least ten $C_{19}O_2$ metabolites has been demonstrated. Since the formation of many of these products had already been reported from

Table 2. *Metabolites of testosterone after incubation with homogenates and subcellular fractions of human prostatic tissue with benign hypertrophy*

All values are expressed as percentages of incubated [4-$^{14}$C]testosterone (0·7 μg.) incubated with the equivalent of 1 g. wet wt. of tissue in 30 ml. of phosphate buffer, pH 7·4, as described in the text. Hom., Whole homogenate; Mit., mitochondrial fraction; Mic., microsomal fraction; Hss, high-speed supernatant.

| | % of testosterone metabolized | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | NADPH | | | | NAD$^+$+NADP$^+$ | | | |
| Metabolite | Hom. | Mit. | Mic. | Hss | Hom. | Mit. | Mic. | Hss |
| Unchanged testosterone | 18 | 91 | 70 | 10 | Trace | 90 | 98 | 85 |
| Androst-4-ene-3,17-dione | 3 | 8 | — | 1·4 | 12 | 2·5 | 2 | 0·5 |
| 5α-Androstane-3,17-dione | 3·6 | 1 | 1·5 | 0·1 | 28 | Trace | — | 0·6 |
| 5β-Androstane-3,17-dione | 0·4 | — | — | — | — | — | — | — |
| 17β-Hydroxy-5α-androstan-3-one | 25 | — | 22 | 3·4 | 32 | — | — | 1 |
| 17β-Hydroxy-5β-androstan-3-one | — | — | — | 15 | — | — | — | — |
| Androsterone | 14 | — | — | — | 16 | — | — | — |
| 5α-Androstane-3α,17β-diol | 30 | — | — | 42 | 3·4 | — | — | 0·5 |
| 5α-Androstane-3β,17β-diol | 1·5 | — | — | 3 | 2·0 | — | — | — |
| 5β-Androstane-3α,17β-diol | — | — | — | 6·8 | — | — | — | — |
| 5β-Androstane-3β,17β-diol | 1·5 | — | — | 5 | — | — | — | — |

Table 3. *Enzyme activities of homogenates and particulate fractions of human prostatic tissue with benign hypertrophy*

Enzyme activities are expressed as $\mu\mu$moles of substrate converted/1 g. wet wt. of tissue/2 hr. under the experimental conditions described in the text.

| | Enzyme activities | | | | |
|---|---|---|---|---|---|
| | Hydroxy steroid dehydrogenases | | | $\Delta^4$-3-Oxo steroid reductases | |
| | 3$\alpha$ | 3$\beta$ | 17$\beta$ | 5$\alpha$ | 5$\beta$ |
| With NADPH | | | | | |
| Homogenate | 1060 | 65 | 450 | 1800 | 45 |
| Mitochondrial fraction | 0 | 0 | 200 | 25 | 0 |
| Microsomal fraction | 0 | 0 | 35 | 560 | 0 |
| High-speed supernatant | 1200 | 180 | 35 | 1160 | 640 |
| With $NAD^+ + NADP^+$ | | | | | |
| Homogenate | 460 | 48 | 1360 | 1990 | 0 |
| Mitochondrial fraction | 0 | 0 | 75 | 6 | 0 |
| Microsomal fraction | 0 | 0 | 40 | 0 | 0 |
| High-speed supernatant | 25 | 0 | 25 | 50 | 0 |

other Laboratories, only partial identification was undertaken in the present study, which was chiefly concerned with the subcellular localization of the human-prostate enzymes concerned in these transformations. At this time our identification of the new 3$\beta$-hydroxy and 5$\beta$-reduced radioactive metabolites must be regarded as tentative and is based on the identity of their chromatographic mobilities in the free and oxidized state with those of authentic standards.

The increased conversion of testosterone into 17-oxo steroids by $NAD^+$ and $NADP^+$ over that by NADPH-supplemented homogenates is not surprising in view of the cofactor addition. However, the extent of reductive aerobic metabolism in the presence of the oxidized forms of added nicotinamide nucleotides is certainly remarkable as is the high degree of stereospecificity in the reduction of the $\Delta^4$-3-oxo group. Evidence for the transformation of testosterone into 17$\beta$-hydroxy-5$\alpha$-androst-1-en-3-one by minces of human prostatic tissue with benign hypertrophy has been published (Ofner *et al.* 1957, 1965); this compound is not formed in either of the present cofactor-supplemented homogenate incubations. A recent correlation of chemical structure with androgenic activity, based on the weight of the ventral prostate in the castrated male rat (Hilgar & Hummel, 1964), suggests that the major transformation products of our homogenate experiments may exert a more potent biological effect on the rat prostate than does the male sex hormone itself. Farnsworth & Brown (1961) made the significant observation that 0·1 $\mu$M-testosterone stimulates the synthesis of fatty acids and protein *in vitro* in the rat prostate.

After completion of our work Farnsworth (1965) reported the 10-demethylation and subsequent aromatization of testosterone by slices of prostatic tissue with benign hypertrophy. The present detailed analysis of one homogenate provided no evidence for the formation of 19-nortestosterone, oestrone, oestradiol or oestriol. These compounds, if present in our thin-layer-chromatography fractions, would have been readily separated from other metabolites by subsequent paper partition chromatography.

The five enzymes detected in the prostate homogenate were localized in subcellular fractions with, in most cases, decreased activity. In both series of experiments the principal enzyme associated with the mitochondrial fraction was the 17$\beta$-hydroxy steroid dehydrogenase. The low concentration of 5$\alpha$-reductase present in this fraction may be due to contamination by microsomal particles. The principal enzyme of the microsomal preparation was the 5$\alpha$-reductase, which required NADPH for activity; the presence of some 17$\beta$-hydroxy steroid-dehydrogenase activity in this preparation may be readily explained as being due to contamination with mitochondria, as documented by electron micrographs. The 3$\alpha$- and 3$\beta$-hydroxy steroid dehydrogenases were found only in the high-speed supernatant fraction and required supplementation with NADPH.

The finding that the 5$\beta$-reductase was present in the high-speed supernatant fraction and active only on supplementation with NADPH agrees with its known occurrence in an analogous fraction isolated from male rat liver (Forchielli & Dorfman, 1956). Surprisingly, however, the 5$\alpha$-reductase was also found in this fraction; its activity was increased 20-fold when NADPH was substituted for $NAD^+$

and NADP+. The evidence therefore points to the presence of both microsomal and soluble 5α-reductase activities, although a measure of fragmentation and solubilization of the microsomal enzyme by treatment of the tissue homogenate in the Waring Blendor must be taken into consideration. The increased activity of the 5β-reductase in the high-speed supernatant fraction over the whole homogenate may be due to the removal of an inhibitor during the subcellular fractionation.

It is not known why the prostate of aging men is the site of both benign and malignant growths, but it is known that endocrine factors are of prime importance. Benign hypertrophy of the human gland can arise in either the median or in the two lateral lobes, whereas carcinoma originates in the posterior or dorsal lobe (Price, 1963); benign prostatic hypertrophy can remain confined or spread to either region. Huggins & Webster (1948) mapped the limits of these structurally distinct entities by their response to sex hormone. The homogenates of some of our prostate specimens were much more active in transforming added testosterone into highly polar hydroxylated metabolites than others. Such differences in the patterns of prostatic androgen metabolism *in vitro* may be related to morphological characteristics of the diseased gland such as predominant localization of the benign hypertrophy in either the median or lateral lobes.

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