Androgen Uptake by Rat Ventral Prostate

By G. E. NEAL. (*Marie Curie Memorial Foundation, Limpsfield Chart, Surrey, U.K.*)

After injections of [^3H]testosterone *in vivo* into castrated rats, high-speed supernatant fractions of tris or TMK (tris-MgCl₂-KCl-pH 7.4)-buffer homogenates of rat ventral prostate contain radioactivity bound to high-molecular-weight substances (Unhjem, Treter & Aakvaag, 1969; Belham, Neal & Williams, 1969). Supernatants from iso-osmotic-sucrose homogenates contain negligible amounts of labelled complex. Cyproterone acetate *in vivo* decreases the amount of radioactivity associated with the TMK supernatant but not with the sucrose supernatant.

Nuclei isolated from intact prostates (from rats castrated 2 days before), incubated at 2°C or 25°C in 0.88% NaCl containing [^3H]testosterone, showed considerable nuclear labelling. Chromatography on Sephadex G-25 of 0.6%-NaCl extracts of the nuclei indicated the presence of both free and bound radioactivity at both temperatures. At 25°C more labelled dihydrotestosterone than testosterone was found in the bound fraction; at 2°C this was reversed. Nuclei from incubations at 25°C, isolated in TMK buffer, contained considerably decreased amounts of bound radioactivity compared with the nuclei isolated in sucrose medium, but approximately the same amount of free radioactivity. Treatment of rats with cyproterone acetate *in vivo*, before incubation at 25°C, decreased the radioactivity in both free and bound peaks, although the labelled dihydrotestosterone/testosterone ratio was similar to that in the controls.

Sucrose-density-gradient centrifugation of nuclei labelled *in vivo*, isolated in sucrose, showed that radioactivity was associated with material sedimenting at 4S and less. When these nuclei were extracted with TMK buffer, the extract contained considerable amounts of radioactivity sedimenting at above 4S.

Part, at least, of the high-molecular-weight labelled complex found in cytosol fractions isolated in buffer TMK is apparently due to extraction from the nuclei. The lack of effect of cyproterone acetate on the labelling *in vivo* of the sucrose-isolated cytosol suggests that little specific uptake of radioactivity takes place in this fraction. The incubation studies *in vitro* demonstrate the solubilization of bound label by TMK buffer, and also show that solubilization is accompanied by changes in sedimentation characteristics. The conversion of testosterone into dihydrotestosterone, and not entry of label into the nucleus or formation of the bound complex, appears to be temperature-sensitive. It appears that, in contrast with the action of oestradiol on oestrogen-

sensitive tissues (Gorski, Toft, Shyamala, Smith & Notides, 1968), androgens may enter the prostate nucleus without the necessity for specific transporting substances in the cytosol. This is supported by the findings of Bashirelaki, Chader & Villee (1969).

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The Partial Purification of a Soluble Androgen Receptor

By W. I. P. MAINWARING and R. IRVING. (*Endocrinology Group, Imperial Cancer Research Fund, London W.C.2, U.K.*)

The presence of a 5 α -dihydrotestosterone-binding protein in the 105 000g supernatant fraction of rat prostate has been reported by several investigators (Mainwaring, 1969; Unhjem, Tveter & Aakvaag, 1969; E.-E. Beaulieu & I. Jung, personal communication). The steroid-receptor complex has a sedimentation coefficient of 8.0S and a Stokes radius of 84Å. Preliminary attempts at the purification of the receptor were negated by the ready aggregation of the 8S protein into complexes of high sedimentation coefficient and varying degrees of solubility. However, the 8S protein may be dissociated in 0.5M-KCl into a 4S form (46Å radius), which may be stabilized by the presence of low concentrations of Ca²⁺ ions, even on removal of the KCl (de Sombre, Puca & Jensen, 1969).

Pooled tissue from 18–24 Wistar rats was homogenized in 50mM-tris-HCl buffer, pH 7.4, containing 0.1mM-EDTA and 0.25mM-dithiothreitol, and the 105 000g supernatant fraction was labelled with 0.05 μM -[^3H]5 α -dihydrotestosterone. Purification of the receptor was accomplished by the following procedures: gel-exclusion chromatography on Sephadex G-200, fractionation with ammonium sulphate (in the presence of 0.5M-KCl and 2mM-CaCl₂), ion-exchange chromatography on DEAE-Sephadex and, finally, preparative polyacrylamide-gel electrophoresis (Jovin, Chrambach & Naughton, 1964). The overall purification was 800–1000-fold. Work on the determination of criteria of purity is in progress.

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Separation of Δ^4 -3-Oxo Steroids and Δ^5 -3 β -Hydroxy Steroids by Chromatography on Digitonin-Silica Thin Layers

By T. TAYLOR. (*University Department of Child Health, Royal Hospital for Sick Children, Yorkhill, Glasgow C.3, U.K.*)

In an investigation of Δ^5 -3 β -hydroxy steroid dehydrogenase (EC 1.1.1.51) activity in foetal adrenal glands, a method was required for the complete separation of nanogram amounts of Δ^4 -3-oxo steroids from a mixture also containing Δ^5 -3 β -hydroxy steroids. Digitonin precipitation of Δ^5 -3 β -hydroxy steroids is well known (Dorfman, 1962), but the present author has found that near-quantitative precipitation requires relatively large amounts of Δ^5 -3 β -hydroxy steroids (e.g. quantities of 3 β ,17 α -dihydroxypregn-5-en-20-one greater than 100 μ g), and even after multiple precipitation traces of Δ^5 -3 β -hydroxy steroid may remain in the supernatant.

A chromatographic method has been developed that enables complete separation of the two steroid groups on thin layers of silica gel impregnated with digitonin.

A layer (0.3mm thick) is spread on t.l.c. plates from a slurry containing: silica gel (Chromalux; May and Baker Ltd., Dagenham, Essex, U.K.), 30g; digitonin (Koch-Light Laboratories Ltd., Colnbrook, Bucks., U.K.), 3g; water, 48ml; ethanol, 2ml. The spread plates are dried at room temperature and then heated for 1 h at 100°C. After application of the steroid mixture, the plates are developed in the solvent system 2-methylpropan-2-ol-water-ethyl acetate (5:2:5, by vol.) for 4.5 h.

Δ^4 -3-Oxo steroids run as a group (R_F 0.86) whereas 1 μ g amounts of Δ^5 -3 β -hydroxy steroids exhibit R_F less than 0.65. The precise R_F value of individual Δ^5 -3 β -hydroxy steroids depends both on structure and the amounts present (e.g. for 10 ng of cholesterol, R_F 0; for 0.1 μ g and 10.0 μ g of 3 β ,17 α -dihydroxypregn-5-en-20-one, R_F 0.5 and 0.05 respectively). A co-operative effect exists whereby the total mass of Δ^5 -3 β -hydroxy steroids present determines the R_F value of each component Δ^5 -3 β -hydroxy steroid and of the group as a whole (e.g. R_F of 0.1 μ g of 3 β ,17 α -dihydroxypregn-5-en-20-one in the presence of 10 μ g of 3 β -hydroxypregn-5-en-20-one is 0.05). With 1 μ g of Δ^5 -3 β -hydroxy steroid present it is normally possible to obtain uncontaminated re-

covery (94–98%) of Δ^4 -3-oxo steroids from a complex mixture.

Elution of the Δ^4 -3-oxo steroid zone of the plate with ethyl acetate yields (digitonin-free) steroids. Digitonides from the Δ^5 -3 β -hydroxy steroid zone are eluted with methanol, dried and subjected to pyridine hydrolysis (Frame, 1944), yielding the free steroids.

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Effect of Oestradiol 3-Benzate on the Bio-synthesis of Egg Proteins by Cod

By P. A. PLACK and N. W. FRASER. (*Natural Environment Research Council Fisheries Biochemical Research Unit, University of Aberdeen, Aberdeen AB1 3RA, U.K.*)

The major protein constituents of cod eggs were found to be two similar lipoproteins, of mol.wt. about 400 000 and containing 79% protein and 21% lipid. From its phosphorus content, the lipid consisted of about 65% phospholipid. These two lipoproteins were not separated by gel filtration on Sephadex G-200 but were partly resolved by chromatography on triethylaminoethyl-cellulose (Jared & Wallace, 1968). Antiserum to cod eggs was prepared in rabbits and both immunodiffusion and immunoelectrophoresis gave two precipitin arcs close together with extracts of cod eggs.

For a fish of 4 kg wet wt., containing about 800 g of dry matter, some 100 g of egg proteins are produced during maturation of the ovaries over a period of 3–4 months. Egg proteins were detected in the serum of maturing female cod by immunochemical methods and reached a concentration of 32 mg/ml, but they were not present in the serum of immature fish or of male fish with maturing testes.

Oestradiol-17 β 3-benzoate in oil was injected intramuscularly into immature cod to give a slow release of the hormone. Serum samples taken 10 days after injection were tested for egg proteins, and the minimum dose of oestradiol to give a positive result was 30 μ g/kg; with doses of 300 μ g or 1 mg/kg the concentration of egg proteins was 32 mg/ml. Similar results were obtained with both male and female fish. Egg proteins were also present in the livers of injected fish but not in their ovaries, whereas immature control fish had no egg proteins in their livers. These findings suggested that the egg