digestion with spleen exonuclease was consistent with an original sequence of Py-G-G-A-thioU-A-G, thus supporting previous but unpublished deductions of Mr B. G. Barrell obtained by an independent method.

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Chemical Modification of Nucleic Acid Components and Transfer Ribonucleic Acid

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Numerous studies on the reduction of the pyrimidine components of nucleic acids have been carried out, mainly with rhodium as catalyst. Results of these studies are summarized by Schein & Schein (1967). In recent work on the synthesis of oligoribonucleotides (Griffin, Reese, Stephenson & Trentham, 1966) with benzyl ethers as specific hydroxy-protecting groups to ensure the formation of only 3',5'-internucleotidic links, it was discovered that removal of the benzyl groups by reduction in the presence of palladium catalysts could also cause considerable reduction of the pyrimidine bases, unless carefully controlled.

By using this observation it has been possible under very mild conditions in the presence of palladium catalysts to reduce uridylyl-(3'-5')-uridine (UpU) to dihydrouridylyl-(3'-5')-dihydrouridine, uridylyl-(3'-5')-adenosine (UpA) to dihydrouridylyl-(3'-5')-adenosine, and uridylyl-(3'-5')-uridylyl-(3'-5')-uridine (UpUpU) to dihydrouridylyl-(3'-5')-dihydrouridylyl-(3'-5')-dihydrouridine. Under similar conditions, the reduction of uridylyl-(3'-5')-adenylyl-(3'-5')-uridine (UpApU) was incomplete.

In some comparative hydrogenation studies at room temperature, with palladium catalysts under controlled pH conditions (3.5-4.2), the following order of ease of reduction was observed: cytidine $\gg$ uridine $>$ ribosylthymine. For example, cytidine 2',3'-(cyclic)-phosphate could be reduced in the presence of uridine 2',3'-(cyclic)-phosphate, with very little reduction of the latter. Pseudouridine was easily hydrogenolysed (comparable with cytidine) to open the sugar ring, but further reduction of the pyrimidine base was very slow (comparable with ribosylthymine). Pyrimidine nucleosides and dinucleoside phosphates were more easily reduced than the corresponding nucleotides. Adenosine and

guanosine, and derivatives of these bases, were not reduced.

Hydrogenation of transfer RNA (from baker's yeast) under the conditions used above resulted in about 4-10% loss of E_{260} , corresponding to the reduction of only a few of the pyrimidine bases in the macromolecule.

These results, and the potential application of hydrogenation under these very mild conditions to specific modification of transfer RNA molecules, will be discussed.

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A Simple Technique for the Separation and Estimation of ^3H -Labelled Androgen Metabolites

By G. E. NEAL, J. E. BELHAM and D. C. WILLIAMS. (*Marie Curie Memorial Foundation, Caterham, Surrey*)

Unlike the action of oestrogens on female target tissues, the action of testosterone on the male accessory sex organs is associated with metabolism of the hormone. It is therefore possible that metabolism of testosterone is a necessary prerequisite for its hormonal action.

We have therefore studied the metabolism of testosterone in the rat ventral prostate by using [^3H]testosterone of high specific radioactivity. It became apparent during this study that there was a need for a technique by which the radioactive content of the androgen metabolites could be rapidly evaluated. Scanning of t.l.c. plates was found to be inadequate owing to difficulties in separating individual androgen metabolites. It therefore seemed desirable to combine the greater resolution of g.l.c. with the sensitivity of scintillation spectrometry. The system that we have evolved is both simpler and more reproducible than column effluent-splitting techniques.

The tissue extraction was as previously described (Belham, Neal & Williams, 1968). Essentially, 10 μg . of each of a range of unlabelled carrier steroids was added to an ethanolic extract. The androgens were then separated as a group from other lipids by t.l.c. and a sample of the t.l.c. eluate was applied to a Pye 104 Gas Chromatograph. Satisfactory separations were obtained with a 7 ft. column of 3% QF₁ at 215°. The retention times

of the androgen metabolites ranged from 7 to 40 min. as monitored by a flame ionization detector.

In the detector hydrogen is burned in a stream of air, producing water. The metabolites emerging from the column pass into the flame and are oxidized during detection. If steroid oxidation is complete the only products are water and carbon dioxide. It is therefore possible by condensing the water vapour and absorbing the carbon dioxide emerging from the detector to monitor the ^3H or ^{14}C associated with each detected peak.

The 'dead space' of the detector was reduced to a minimum by a polytetrafluoroethylene insert. Effluent gases emerged via a narrow thin-walled polytetrafluoroethylene tube, which passed through an ice bath in order to condense the water vapour. The condensate was collected in scintillation-counting vials containing 7 ml. of scintillator (Bray, 1960). Radioactivity recoveries of $81 \pm 5\%$ have been obtained, indicating that oxidation is complete and that within this closed system trapping is efficient. During eight replicate experiments radioactivity determinations, of individual metabolites, were within $\pm 5\%$.

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The Reception of Androgens in the Rat Ventral Prostate

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Adult castrated rats were injected intraperitoneally with [^3H]testosterone *in vivo*. After 40 min. the animals were killed and prostatic nuclear and cytoplasmic fractions were prepared. Androgens were isolated by coupled t.l.c. and g.l.c. Labelling was predominantly associated with dihydrotestosterone. Anderson & Liao (1968) reported similar findings for prostatic nuclei, but in their experiments labelling of dihydrotestosterone in the cytoplasmic fraction was less predominant. The administration *in vivo* of cyproterone acetate, an anti-androgen whose action has been shown to be on the target organ (Neumann & Berswordt-Wallrabe, 1966), resulted in marked decrease in the amounts of labelled dihydrotestosterone recovered from both cytoplasmic and nuclear fractions after injection of [^3H]testosterone *in vivo*.

Studies *in vitro* with isolated prostatic nuclear and cytoplasmic fractions showed that [^3H]testosterone is converted into a range of metabolites, given in decreasing order of labelling: androstenediol, dihydrotestosterone, androstenedione, andro-

sterone and androstenedione. Addition of cyproterone acetate to the incubations indicated that the anti-androgen does not fundamentally alter the pattern of metabolism of testosterone.

These results could indicate that, of the metabolites formed *in vivo*, dihydrotestosterone is selectively retained within the prostate in both the nucleus and the cytoplasm.

Experiments *in vitro*, in which [^3H]testosterone and dihydrotestosterone were added to nuclear and high-speed-supernatant fractions obtained from castrated or cyproterone-treated castrated rats, indicated that cyproterone antagonizes the uptake of label *in vitro* by both these subcellular fractions. The high-speed-supernatant fractions obtained from prostates after injection of [^3H]testosterone *in vivo* were subjected to Sephadex G-100 column chromatography. High labelling in a substance tentatively identified as dihydrotestosterone was observed in the column-excluded fraction. Treatment of the rat with cyproterone acetate *in vivo* was found to decrease labelling in this high-molecular-weight fraction.

These results suggest that cytoplasmic receptors for androgens are present in the rat ventral prostate, and they are similar to those observed by Toft & Gorski (1966) for oestrogens in the rat uterus. In contrast with the uterine oestrogen receptors, however, it appears that in the rat ventral prostate metabolism of testosterone to dihydrotestosterone is a necessary prerequisite for attachment to the receptors.

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The Properties of Fractions of Streptolysin S

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Three biological activities have been attributed to streptolysin S. Originally described as a haemolytic toxin (Todd, 1938), it has since been shown to release enzymes from lysosomes (Weissmann, Keiser & Bernheimer, 1963) and also to induce blast transformation in a high percentage of cells when added to lymphocyte cultures (Hirschhorn, Schreiber, Verbo & Gruskin, 1964). It has been suggested by Taranta, Cuppari & Quagliata (1968),