former, the latter being eluted with 10mM-NAD⁺. The efficiency of elution of the lactate dehydrogenase seemed to increase on addition of the substrate lactate to the coenzyme eluent.

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A Possible Role of Zinc in the Metabolism of Testosterone by the Prostate Gland

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The prostate gland contains a relatively large concentration of zinc. This is influenced by the endocrine status of the animal, decreasing on castration and increasing on androgen administration. Dependence of the prostate on zinc is illustrated by the damaging effects of administering Zn²⁺ ion-chelating agents such as diphenylthiocarbazone (Whitmore, 1963). The role of zinc in the prostate has not, however, been clarified. The normal structure and function of the prostate gland in men and dogs is also dependent on 5 α -dihydrotestosterone (Siiteri & Wilson, 1970), which we have shown, in superfusion experiments, to be concentrated by human prostatic tissue slices (Giorgi, Stewart, Grant & Scott, 1971). The results now reported suggest that the concentration of free Zn²⁺ in prostatic cells may influence androgen metabolism, on which prostatic function depends.

Nuclei were isolated from fresh human hyperplastic prostatic tissue by the method of Sierralta & Minguell (1970). They were checked for freedom from gross cytoplasmic contamination by examination under the microscope, after staining by haematoxylin and eosin, and were incubated with [1,2-³H]testosterone under the conditions described by Bruchovsky & Wilson (1968). In some experiments EDTA was omitted, apparently without effect. ³H-labelled testosterone and 5 α -dihydrotestosterone were isolated from the incubation mixtures after addition of small amounts of carrier steroid, purified by t.l.c. and, after addition of larger amounts of carrier, recrystallized to constant specific radioactivity. Tritium in these two steroids accounted for by far the greater part of the isotope

added before incubation, in keeping with the findings of Bruchovsky & Wilson (1968).

Zinc chloride (Specpure; Johnson and Matthey, London E.C.1, U.K.) was added to some incubation mixtures in concentrations ranging from 0.05 μ M to 0.5mM (i.e. 6.54ng–65.4 μ g/incubation). In separate experiments these concentrations were shown to be without effect on the production of NADPH in the incubations. Concentrations of 50 μ M and greater did, however, almost completely inhibit the NADPH-dependent reduction of testosterone to 5 α -dihydrotestosterone, whereas lower concentrations of Zn²⁺-stimulated formation of 5 α -dihydrotestosterone by 20–30% of the control values. Concentrations of Cd²⁺, Hg²⁺ or Mn²⁺ up to 0.5mM were without effect.

The zinc content of the amounts of nuclei incubated, as determined by atomic adsorption spectrometry, varied from 61.3 to 183.9nmol (i.e. 4–12 μ g). This zinc appeared to be bound in the nuclei and did not leak out during incubation. The reduction of testosterone by microsomal preparations from human prostatic tissue and by preparations from the rat ventral prostate was also inhibited by 50 μ M-zinc chloride.

Since these observations might be explained by possible effects of Zn²⁺ on the binding of steroids to proteins in prostatic cells, such effects are at present under investigation.

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Selective Increases in Type I Hydrogen from Reduced Nicotinamide-Adenine Dinucleotide Phosphate in Liver from Phenobarbitone-Treated Rats

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Phenobarbitone administration to experimental animals is known to increase various microsomal parameters. These include the content of cytochrome P-450 and the activity of the mixed-function oxidase system (Orrenius, Das & Gnosspelius, 1969).

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The majority of such studies have utilized isolated microsomal fractions, and involved either the addition of exogenous NADPH or the addition of an NADPH-generating system. In the present study, intact tissue sections have been used, and the NADPH has been generated *in situ*, by allowing the glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase present in the section to act on their respective substrates. NADPH production was measured by neotetrazolium chloride reduction, the formazan being eluted and measured spectrophotometrically (Altman, 1969a, 1971). The addition of phenazine methosulphate results in an increased production of formazan. This higher activity is identical with that produced in homogenates of the same tissue, and is equivalent to the total hydrogen generated (Altman, 1969b). The hydrogen that reduces the tetrazole in the absence of phenazine methosulphate has been defined as type I hydrogen and probably represents the NADPH used in microsomal reactions; the 'extra' hydrogen, which is only seen in sections in the presence of phenazine methosulphate, has been defined as type II hydrogen and probably represents NADPH that is used biosynthetically (Altman & Chayen, 1970; Altman, 1971; Chayen, Altman & Butcher, 1971). In homogenates no such distinction is possible, since spectrophotometric determination of NADPH production will measure all the NADPH as soon as it is produced (i.e. type I + type II). It is therefore equivalent to the tetrazolium technique in the presence of phenazine methosulphate (Altman, 1969b).

Male Wistar rats (each weighing approx. 200g) were given phenobarbitone (1 mg/ml) in their drinking water and were killed after 4 days (average consumption 35 ml/day). Controls received plain water. Sections (10 μ m) of the liver were incubated, to demonstrate type I and type II hydrogen from the glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase reactions. The capacity to oxidize NADH and NADPH was also tested. The activities, expressed as nmol of hydrogen/10 min per mm³, were as follows for sections from phenobarbitone-treated rats (values for controls are given in parentheses): for the glucose 6-phosphate dehydrogenase reaction, type I hydrogen, 24.40 (8.35), type II hydrogen, 21.60 (19.00), total, 46.00 (27.35); for the 6-phosphogluconate dehydrogenase reaction, type I hydrogen 81.00 (34.40), type II hydrogen, 80.00 (93.50), total, 161.00 (127.90); for capacity to oxidize NADH, 85.00 (64.50); for capacity to oxidize NADPH, 70.00 (43.60). Thus, although the total activity of each enzyme was increased after phenobarbitone treatment, analysis of both types of hydrogen shows that only type I was increased. Moreover, Butcher

(1971) has shown that this increase is confined to the centrilobular region.

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The Effect of Phenobarbitone on the Production of Type I Hydrogen from Reduced Nicotinamide-Adenine Phosphate in Different Regions of the Liver Lobule

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The hydrogen from NADPH generated in the cytosol may be oxidized through the microsomal respiratory chain and used in mixed-function oxidases associated with cytochrome P-450 (type I hydrogen) or used in biosynthetic mechanisms (type II hydrogen). The use of thin tissue sections (10 μ m) makes it possible to differentiate between these two types of hydrogen (Altman & Chayen, 1970). Altman (1971) has shown a selective increase in the production of type I hydrogen generated from the dehydrogenation of glucose 6-phosphate or 6-phosphogluconate after administration of phenobarbitone. The elution methods used in that study give a measure of the activity present in the whole section; they do not show how the activity varies in the different parts of the liver lobule. Such selective measurements can be made by using either a scanning and integrating microdensitometer or a double-beam recording microdensitometer. The results obtained with these instruments (relative absorption with the former; the height of the pen deflexion with the latter) have been converted into absolute extinction by reference to standard filters of known density measured under the same conditions.

In the control liver the rate of production of type I hydrogen generated from the dehydrogenation of 6-phosphogluconate was 2.5 times as