1969; Patterson & Allcroft, 1970; Patterson & Roberts, 1970). Mouse, rabbit and rat livers demethylate aflatoxins (pathway 2) (Bassir & Emafo, 1970; Patterson & Roberts, 1971a), and aflatoxin hemiacetal is formed *in vitro* by guinea-pig, mouse and avian (chick, duck, quail and turkey) livers (pathway 3) (Patterson & Roberts, 1970, and unpublished work). Pathway (4), involving NADPH-dependent reduction, has recently been identified in avian and rabbit liver (Patterson & Roberts, 1971b). Transformations by pathways (1), (2) and (3) occur in crude or isolated microsomal preparations, but enzymes of pathway (4) are located in a soluble liver fraction (105 000g supernatant).

One-day-old ducklings are extremely susceptible to acute aflatoxin poisoning, and the toxin is probably taken up rapidly by the liver. However, there may be structural requirements for transport of aflatoxin-like molecules, because several closely related compounds are almost non-toxic compared with aflatoxin itself. Two such compounds are aflatoxin B_{2a} (Dutton & Heathcote, 1968) and aflatoxicol (Detroy & Hesseltine, 1968), and both are duckling liver metabolites. On the basis of results obtained *in vitro*, it may be calculated that the total metabolizing capacity of the liver is such that an LD₅₀ dose of aflatoxin B₁ would be completely transformed in under 50s. Consequently it is possible that, once formed intracellularly, primary toxic damage in the duckling is caused by one or both metabolites.

Rat liver has a smaller metabolic capacity, and it would take 64h to convert an LD₅₀ dose into aflatoxin M₁ and the demethylated toxin. Here the parent toxin must be regarded as the active molecule and so it appears that, depending on metabolic patterns, the toxic mechanism varies from species to species.

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Purification of Nicotinamide-Adenine Dinucleotide-Dependent Dehydrogenases by Affinity Chromatography

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We report the chemical binding of (a) NAD⁺ and (b) an AMP analogue to separate gel matrices, and the application of these functionalized gels to the separation of dehydrogenases by affinity chromatography (Mosbach, 1971).

For preparation (a) ϵ -aminohexanoic acid has been coupled to Sepharose 4B by the cyanogen bromide method (Axén, Porath & Ernback, 1967) and subsequently dicyclohexylcarbodi-imide was used to attach NAD⁺, probably via ester linkages (Larsson & Mosbach, 1971). For preparation (b) N⁶-(6-aminohexyl)-AMP (H. Guilford, P.-O. Larsson & K. Mosbach, unpublished work) has been linked directly to activated Sepharose 4B. In both cases a hexacarbon 'spacer' of about 7 Å length increased the steric availability of the cofactor and inhibitor moieties to the dehydrogenases studied, namely D-glyceraldehyde 3-phosphate-NAD oxidoreductase (EC 1.2.1.12) and L-lactate-NAD oxidoreductase (EC 1.1.1.27). Both enzymes were retained by columns packed with gel matrix, but could be eluted by solutions of NAD⁺.

It seems probable that the affinity of the bound AMP analogue for the dehydrogenases parallels the known action of free AMP as a competitive inhibitor (McPherson, 1970, and references cited therein). Further, the coenzyme NAD⁺ retains its binding capacity for NAD⁺-dependent dehydrogenases even when bound.

A 0.1 M-phosphate buffer solution, pH 7.0 (200 μ l), containing D-glyceraldehyde 3-phosphate-NAD oxidoreductase (0.4 mg, 45 U) and bovine serum albumin (1 mg) was applied to a column (11 cm $\times$ 1 cm) packed with preparation (a) containing nucleotide (17.5 μ mol). Albumin appeared in the early fractions whereas the enzyme was eluted only after the application of 10 mM-NAD⁺ (50 μ l). Of the total activity about 90% was recovered in a sharp peak. Similarly, with the same protein solution but preparation (b) containing nucleotide (37 μ mol) as column material and 0.15 mM-NAD⁺ as eluent the same elution pattern was observed. In blanks run with gels lacking nucleotides the dehydrogenases and albumin were eluted together.

Mixtures of D-glyceraldehyde 3-phosphate-NAD oxidoreductase and L-lactate-NAD oxidoreductase could also be resolved. Thus, when both were applied to a column containing preparation (b), elution with 0.15 mM-NAD⁺ afforded only the

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 diphenylthiocarbazon (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.

We thank the Cancer Research Campaign for a grant and Dr G. S. Fell for zinc measurements.

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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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