METABOLITES OF BENZO (A) PYRENE (BP) WHICH BIND TO DNA. H. W. S. KING, K. ALEXANDROV, M. R. OSBORNE, M. H. THOMPSON and P. BROOKES, Pollards Wood Research Station, Bucks.

The binding of metabolites of BP to added native or denatured DNA catalysed by rat liver microsomes, and to endogenous DNA catalysed by rat liver nuclei, was studied by hydrolysis of the isolated DNA and column chromatography on LH20 Sephadex. The same 2 major deoxyribonucleoside-hydrocarbon products were obtained from microsomes and from nuclei. That which eluted first was indistinguishable from the single product obtained from mouse embryo cells exposed in culture to BP, and from the single product obtained when microsomes metabolized BP-7, 8-dihydrodiol, or, to a much lesser extent, BP-4,5-diol. Formation of this product by microsomes from BP was prevented by inhibitors of epoxide hydrase such as TCPO. The second product which eluted from the column was not observed in mouse embryo cells. Its formation was not prevented by TCPO. It was also observed when BP-9, 10-diol was added to microsomes.

THE DETECTION OF ACTIVATED CARCINOGENS WITH BACTERIOPHAGE λ. S. M. TOOGOOD and R. C. GARNER, Department of Experimental Pathology and Cancer Research, University of Leeds.

Use has been made of inactivation and clear plaque mutation of bacteriophage λ to study some activated carcinogens. Phage inactivation was found after incubation with a direct-acting carcinogen, 7-bromomethylbenz(a)anthracene and also after incubation with liver mixed function oxidase enzymes and aflatoxin B_1, a carcinogen requiring metabolic activation. In the latter case ultraviolet reactivation of the phage by the host bacterium was also demonstrated. This mutational assay may have certain advantages over bacterial tests in that clear plaque mutagenesis is a forward mutation assay which detects agents causing frameshift, base-pair substitution or deletion mutations. In addition, the slow inactivation of this phage in animals makes it suitable for *in vivo* mutation studies. Viable plaque particles have been recovered in the liver, lung, kidney and spleen up to 24 h after intraperitoneal phage administration. It is hoped that this can be used to measure organ-specific activation of carcinogens.

METABOLIC N-OXIDATION AND THE pKa CONCEPT. J. W. GORROD, Chelsea College, London.

The metabolic oxidation of nitrogen occurring in organic molecules can be achieved *in vitro* with a hepatic microsomal fraction fortified with NADPH in the presence of molecular oxygen. Two enzyme systems are known which can carry out this process, one utilizes a flavoprotein whilst the other is mediated *via* an electron transport chain terminating in cytochrome P-450.

A concept has been developed (Gorrod, *Chem-Biol. Interactions*, 1973, **7**, 289) which differentiates these processes depending upon the pKa of the substrate N-oxidized. Basic amines are oxidized by the flavoprotein system, non-basic nitrogen employs the cytochrome-P450 system and aromatic amines are probably substrates for both systems. The actual system utilized depends upon the pKa of the substrate, the relative affinities towards the enzymes and the ratio of enzyme within a tissue.

It is suggested that this concept may be useful in deciding the correct animal species for use in toxicological testing of nitrogen compounds.

ISOZYMES OF β-N-ACETYLHEXOSAMINIDASES FROM NORMAL COLONIC MUCOSA AND CHEMICALLY INDUCED RAT COLONIC TUMOURS. N. MIAN and C. A. NUTMAN, Department of Experimental Pathology and Cancer Research, University of Leeds.

Isozymes of β-N-acetylglucosaminidase (EC 3.2.1.30) and of β-N-acetylgalactosaminidase (EC 3.2.1.53) from 1, 2-dimethylhydrazine induced rat colonic tumours and from the mucosa of control and tumour bearing animals were separated on polyacrylamide gel electrophoresis. There was no electrophoretic differences between the isozymes of the two β-N-acetylhexosaminidases.

Colonic mucosa from normal and tumour bearing animals contained a fast anodic and preponderant isozyme A, a slow and weak isozyme B and an intermediate isozyme C. On the contrary, in colonic tumours isozyme C was absent and the activity of isozyme A