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

was markedly reduced compared with isozyme A of normal mucosa. Heating of enzyme extracts at 50 °C for 30 min before electrophoresis caused no change in the electrophoretic mobilities of any of the isozymes but slightly reduced their staining intensities. On the other hand, after heating at 55 °C for 30 min, isozymes A and C were hardly detectable on subsequent staining. Incubation of enzyme extracts with neuraminidase at 37 °C for 18 h caused no significant changes either in the mobility or in the staining reaction of any of these isozymes from normal and experimental tissuse. Isozymes A and C appeared to be soluble enzymes whereas isozyme B, due to its extraction in 0·2% Triton X-100, seemed to be bound to lysosomes.

IS ALKYLATION OF NICOTINAMIDE THE CAUSE OF LIVER NECROSIS THAT FOLLOWS LARGE DOSES OF HEPATOCARCINOGENS? R. SCHOENTAL, Department of Pathology, Royal Veterinary College, London.

Large doses of hepatocarcinogens cause liver necrosis, not seen after doses which can induce tumours. In the course of their metabolism, many hepatocarcinogens yield alkylating entities, known to alkylate nucleic acids, proteins and other cell constituents, including nicotinamide (Schein *et al.*, *Biochem. Pharmac.*, 1973, **22**, 2625; Chu and Lawley, *Chem. Biol. Interactions*, 1974, **8**, 65). Alkylated derivatives of nicotinamide cannot be utilized for the biosynthesis of NAD cofactors, essential for cell survival. Depletion of NAD in the liver occurs after pyrrolizidine alkaloids, dimethylnitrosamine, streptozotocin etc., and can be prevented by pretreatment with nicotinamide, which in some cases prevents also the cytotoxic effects.

More information is needed as regards the role of alkylated derivatives of nicotinamide in cell death and in relation to tumour induction.

PART II: INDUSTRIAL CARCINOGENESIS

ABSTRACTS OF SYMPOSIUM PAPERS

Tuesday 8 April

CHEMICAL BASIS FOR THE RELATIONSHIP BETWEEN MUTAGENESIS AND CARCINOGENESIS. P. BROOKES, Pollards Wood Research Station, Bucks.

A relationship between carcinogenesis and mutagenesis was first suggested by T. Boveri in 1917 and K. H. Bauer in 1928 proposed the somatic mutation theory of cancer, although at that time the nature of the genetic material was unknown. The recognition of the biological role of DNA, following the elucidation of its structure by Crick and Watson in 1953, did not immediately revive the support for the mutation theory of cancer which had been under strong attack. This was mainly the result of the repeated failure to demonstrate mutation with such classic carcinogens as the polycyclic hydrocarbons and aromatic amines. Prior to 1940 polycyclic hydrocarbons were almost the only well studied group of carcinogens. During the subsequent 20 years, however, the number and variety of chemical substances both synthetic and naturally occurring, which were shown to induce cancer in animals, became so great that it seemed impossible to conceive of any common mechanism of tumour induction.

The breakthrough came with the recognition of the importance of metabolism in converting the applied carcinogen to the biologically active form. One class of carcinogen, namely the biological alkylating agents, did not require metabolic activation and a study of their mechanism of action had implicated DNA reaction. It seemed possible therefore that all carcinogens might act *via* electrophilic intermediates similar to those involved in alkylation and that these "ultimate carcinogens" might modify cellular DNA.

A great deal of evidence is now available which supports both the above postulates. In the case of the aromatic amines the identification of N-hydroxy metabolites by the Millers led to an understanding of