exponentially growing cultures (Hahn and Little, *Curr. Top radiat. Res.*, 1972, **8**, 39).

The following results were obtained with Chinese Hamster V79-379A cells grown in suspension to limiting cell density; cells remaining viable for 48 h in a post-mitotic state. Response to treatment with *e.g.* methyl methane sulphonate was compared with the response of similar, exponentially growing cells.

The survival curves have similar slopes but the shoulder is much reduced with stationary cells. Non-semi-conservative synthesis occurs in both cases but the recovery of template-DNA size is slower and less complete in the case of stationary cells. This is consistent with the hypothesis that ligation of DNA is a deficient aspect of repair in these non-dividing cells.

DECREASE IN THE ACCURACY OF DNA POLYMERASE FOLLOWING TREATMENT WITH γ-RAYS AND METHYLNITROSOUREA. R. SAFHILL, Paterson Laboratories, Manchester.

DNA synthesis *in vivo* must be a very accurate process in order that the integrity of the base sequence of the genome be maintained. When using *in vitro* systems, DNA polymerases from both bacterial and mammalian sources have been found to incorporate only one wrong base in several hundred thousand, whilst *in vivo* their accuracy could result in only one wrong base in 10^{10}.

We have been investigating the effect of ionizing radiation and various carcinogenic agents upon DNA polymerases and have found that γ-rays or methylnitrosourea will decrease both the activity and accuracy of *E. coli* DNA polymerase I, as well as the proteolytically cleaved form of the enzyme, by up to 2 orders of magnitude. An error-prone DNA polymerase has recently been observed in human leukaemic cells and several recent reports further indicate that there may be a connection between the accuracy of DNA synthesis and carcinogenesis (Loeb, Springgate and Battula, *Cancer Res.*, 1974, **34**, 2311).

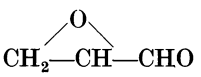
UNSCHEDULED DNA SYNTHESIS STUDIED IN THE INTACT ANIMAL. V. M. CRADDOCK, MRC Laboratories, Carshalton, Surrey.

While much evidence demonstrates the importance of DNA repair polymerase activity in mutagenesis and carcinogenesis, most work has been carried out using cell cultures. To relate repair of DNA to carcinogenesis, information is needed on its occurrence in the intact animal. The methods available, involving autoradiography or use of BUdR, have disadvantages. A new method is suggested which depends on the fact that the DNA and chromosomal protein content of the nuclei double in replicating cells, so that the nuclei become larger and denser and sediment more rapidly in a sucrose gradient. After an injection of ^{3}H-thymidine, non-cycling diploid and tetraploid nuclei can be separated from replicating ^{3}H-labelled nuclei (Haines *et al.*, *FEBS letters*, 1975, **10**, 113). Nuclei in which DNA repair synthesis is taking place do not double their content of genetic material, the ^{3}H-profile following the non-cycling diploids and tetraploids. Liver nuclei isolated from animals treated with DMN or MMS gave a " repair type " profile, which contrasted with that given by nuclei from untreated animals, or from animals in which DNA synthesis had been reduced by treatment with cyclohexamide.

CHROMIUM CARCINOGENESIS AND GLYCIDAL FORMATION. R. SCHOENTAL, Department of Pathology, Royal Veterinary College, London.

The mechanism by which simple inorganic compounds induce tumours is no less obscure than that operating in the case of organic carcinogens. It has been recognized that the latter probably acquire in the course of their metabolism epoxy- and carbonyl, or other functional groups, which allow cross linking of cellular macromolecules in a permanent manner (Schoental, *Br. J. Cancer*, 1973, **28**, 436; *Ibid.*, 1974, **29**, 92).

The tanning ability of chromates depends on the formation of glycidal, derived from neutral fats by hydrolysis and subsequent oxidation of glycerol. This epoxyacraldehyde,



cross-links proteins etc in the hides. Glycidal is known to be carcinogenic for mice and for rats (Van Duuren *et al.*), and I suggest that

chromium salts could induce tumours at sites of cell damage, where release of hydrolytic enzymes and subsequent formation of glycidal can occur.

MUTAGENICITY OF HAIR COLOURANTS IN BACTERIA: POSSIBLE LINK WITH CARCINOGENICITY. S. VENITT and C. T. BUSHELL, Pollards Wood Research Station, Bucks.

Preliminary results (C. E. Searle, unpublished data) obtained in mice suggest a possible carcinogenic hazard associated with certain hair colourants. Eleven such products were tested for mutagenicity in bacteria, since there is a reasonable correlation between carcinogenicity and mutagenicity. Six colourants were strongly mutagenic in *Salmonella typhimurium* TA 1538 (frameshift): of these, one required metabolic activation with liver supernatant (LS), 5 did not. Two of these were also mutagenic in another frameshift mutant, TA 1537. We obtained negative results in all strains which revert by base-pair substitution (*S. typhimurium* TA 1535 and *E. coli* WP2). 2-nitro-p-phenylenediamine (2NP) and 4-nitro-o-phenylenediamine (4NO), both dyes known to be present in one of the colourants tested, were mutagenic (without LS) in TA 1537 and TA 1538. A combination of TLC and spot testing showed that 5 of the 6 mutagenic colourants contained one or both of these dyes, together with other unidentified mutagenic components. It is suggested that products containing these dyes should be withdrawn from use pending further studies of their biological activity.

TESTS OF TWO HAIR COLOURANTS FOR CARCINOGENICITY BY REPEATED APPLICATION TO MOUSE SKIN. C. E. SEARLE and D. G. HARNDEN, Department of Cancer Studies, University of Birmingham Medical School and O.H.B. GYDE, Department of Haematology, East Birmingham Hospital.

Routine questioning of a patient with neutropenia revealed that she frequently used 2 "semi-permanent" hair colourants. When she subsequently developed myeloproliferative disease it was decided to initiate preliminary tests on these materials by topical application to mice since no information was available on their long-term effects.

One preparation contains 2-nitrophenylenediamine, the other an azo-dye metal derivative and an aminonitrophenol, in addition to detergent, perfume and a clay base. They are being applied twice weekly in aqueous acetone to the clipped skin of A and DBA mice. Some of each preparation is probably absorbed orally through licking.

So far, malignant lymphomata have developed after 26–57 weeks in 6–9% of treated mice and 0–3% of controls. Only low concentrations of dyes themselves are present in the solutions applied and tests using single constituents are now needed.

2-Nitro-p-phenylenediamine and 4-nitro-o-phenylenediamine are present in a number of proprietary hair colourants. When added to cultures of human blood lymphocytes, a small increase in chromatid aberrations was observed at 48 h.

THE NITROSATION OF FOOD AMINES UNDER STOMACH CONDITIONS. C. S. DYKE and C. L. WALTERS, B.F.M.I.R.A., Leatherhead, Surrey.

Nitrosamines can be formed by the action of nitrous acid on secondary and tertiary amines and quarternary compounds. Nitrosation is catalysed by thiocyanate which is secreted in the saliva and particularly that of smokers (Boyland, *Nature, Lond.*, 1974, **248**, 601). Nitrosation of food amines at high levels of nitrate (0·14 mol/l) atypical of the stomach of the consumer has led to the formation of up to 80 μg/kg N-nitrosopiperidine (N Pip). At 0·145 mmol/l nitrite, a level considered to be the maximum likely to occur normally in the stomach, nitrosation occurred but to a much reduced extent with the formation of volatile nitrosamines, particularly in the presence of thiocyanate. Studies in which volunteers were given a meal including cured meat containing nitrite within the legal limit have so far been negative, following recovery of the meal after 30 min, whilst corresponding *in vitro* studies have revealed 1·7 μg/kg N Pip after 1 h and 3·4 μg/kg after 3 h. The possibility therefore exists that nitrosamines could be formed in the stomach after a longer residence time, unless this is precluded by an inhibitory physiological factor such as ascorbate.