be expected to be of particular value in tumours producing large amounts of melanin. Under these circumstances, malignant melanocytes would be likely to sustain more damage than normal melanocytes, particularly if the patient were kept away from direct sunlight.

\(^{32}\text{P}\) was considered to be the most suitable available radionuclide and DL-1-amino-2-(3,4-dihydroxyphenyl)-ethyl-phosphonic acid (ADEP), an analogue of DOPA, was made and investigated as a possible carrier. Preliminary distribution studies were carried out with tritiated ADEP in mice bearing the Harding-Passey melanoma. The highest initial tritium concentration was in the kidneys, adrenal glands and eyes. Radioactivity fell to low levels in all tissues in 8 days or less but the tumour retained the isotope longer than did other tissues.

Structural analogues of ADEP which may be taken up more selectively by melanoma tissues are being considered.

**TRANSPLANTABLE ADENOCARCINOMATA OF THE COLON IN MICE AS POSSIBLE MODELS FOR CHEMOTHERAPY.** C. R. Ball and J. A. Double, Department of Cancer Research, University of Leeds.

Dimethylhydrazine treatment (17 weekly subcutaneous injections) of NMRI mice results in a 100% incidence of tumours of the colon by 22 weeks (Haase et al., *Br. J. Cancer*, 1973, 28, 530). Primary tumours derived in such mice have been transplanted into syngeneic mice and have resulted in 5 transplantable tumour lines from 51 attempts.

The 5 transplant lines (MAC7, MAC10, MAC13, MAC14, MAC15) are all well differentiated adenocarcinomata, some mucin secreting; each has its own characteristic growth rate (3–16 weeks to reach 5 × 5 mm from an implanted fragment) and thymidine labelling index (12–24%); all have 100% take rates; there is no evidence of de-differentiation during successive transplant generations (up to 8 in one case).

Methods have been developed for using the tumours MAC13 and MAC15 for chemotherapy screening. Initial studies of sensitivity to single dose therapy with 5-fluorouracil, cyclophosphamide, BCNU, CCNU, MeCCNU and methotrexate indicate (i) a general insensitivity to chemotherapy; (ii) that each tumour line has its own spectrum of sensitivity each responding to about half the drugs tested; and (iii) that the tumours are amenable to further development as possible screening models for drugs active against colorectal cancer.

**EFFECTS OF AGE AND CARCINOGEN TREATMENT ON CELL GROWTH IN ORGAN CULTURES OF ADULT MOUSE COLON.** E. A. Defries and L. M. Franks, Imperial Cancer Research Fund, London.

As most differentiated epithelial cells cannot be maintained in monolayer culture, most of the work on chemical carcinogenesis *in vitro* is done using cultures of undifferentiated mesenchymal cells.

Although embryonic intestine can be maintained in organ culture for several weeks, previous tissue culture experiments using adult intestinal tissue had been restricted to 24–48 h. We have established an organ culture system by which adult mouse colon can be maintained, in a modified form, for at least 28 days.

After an initial degenerative phase the explants are covered by a layer of well differentiated surface epithelium with a variable number of crypts extending into the lamina propria. Cell division is confined to the crypts and cells move out of the crypts into the surface compartment. These cultures have been used for studies on the effect of donor age and carcinogen pretreatment on subsequent mitotic index *in vitro*. Preliminary experiments appear to show that carcinogen treatment alters the growth capacity of the intestinal epithelial cells though they remain responsive to the growth controlling mechanisms in the intact animal.

**EFFECTS OF PROTEOLYTIC ENZYMES AND A SYSTEMIC CARCINOGEN ON SURFACE STRUCTURE AND GROWTH OF ADULT BLADDER EPITHELIUM IN ORGAN CULTURE.** G. M. Hodges and G. Spacey, Imperial Cancer Research Fund, London and M. D. Muts, Imperial College, London.

The scanning electron microscope (SEM) is a useful tool for studying surface changes in cells *in vivo* and *in vitro* and it can also
be used for growth assessment using SEM autoradiography.

Differences in the luminal surface structure of bladder from normal and MAMA (methylazoxy-methanol-acetate) treated C57 BL mice were observed using the SEM but the relation of these changes to the action of the carcinogen remains to be determined. Certain of the morphological features noted in vivo were maintained in organ cultures of adult bladder tissues and may provide useful markers for normal and experimentally treated bladder epithelium.

In organ culture cellular outgrowth from the bladder epithelium occurred earlier and was more extensive in explants from the carcinogen treated animals. There was also evidence of stimulation of epithelial outgrowth following proteolytic enzyme treatment of bladder explants. This work forms part of a project on stromal–epithelial carcinogenesis in the bladder.

A FREEZE ETCH SCANNING (SEM) AND TRANSMISSION (TEM) ELECTRON MICROSCOPE STUDY OF LANDSCHUTZ ASCITES TUMOUR (LAT) CELL SURFACES. R. G. P. PUGH-HUMPHREYS, Cell Research Unit, Zoology Department, University of Aberdeen.

Microvilli observed by SEM on LAT cells (Pugh-Humphreys and Sinclair, J. cell. Sci., 1970, 6, 477), were enveloped by plasmalemma and possessed a core of 5 nm diameter microfilaments in continuity with subplasmalemmal actin-like microfilaments. Freeze etch and TEM studies revealed that microfilaments inserted into the plasmalemma, possibly linking to membrane components, were also closely associated with cytoplasmic microtubules and 8 nm diameter filaments. 6–10 nm diameter particles, believed to be proteins and/or lipoprotein complexes (Singer and Nicolson, Science, N.Y., 1972, 175, 720) were observed predominantly within, and sometimes spanning the width of the plasmalemma of freeze etched LAT cells.

Discrete patches of electron dense material on LAT cell surfaces observed by TEM after staining with ruthenium red (Lauff, Anat. Rec., 1971, 171, 369) and concanavalin A–peroxidase (Bernhard and Avrameas, Expl cell Res., 1971, 64, 232) indicated the presence of externally located carbohydrates attached to plasmalemma components.

THE METABOLIC FORMATION OF WATER-SOLUBLE DERIVATIVES FROM DIHYDRODIOLS OF POLYCYCLIC HYDROCARBONS. G. R. KEYSELL, J. Booth and P. Smits, Chester Beatty Research Institute, London.

Polycyclic hydrocarbons are initially metabolized by microsomal mono-oxygenases into epoxides that are then converted into dihydrodiols, phenols and glutathione conjugates. The dihydrodiols are themselves further metabolized by the mono-oxygenase to intermediates that yield water soluble products with glutathione.

Studies on the metabolism of the 5,6- and 8,9-dihydrodiols of benz(a)anthracene show that the 5,6-dihydrodiol is converted into one and the 8,9-dihydrodiol into 2 conjugates. We have shown that the 5,6-dihydrodiol is metabolized on the 8,9-bond and the 8,9-dihydrodiol on the 5,6- and 10,11-bonds. The formation of the 8,9-dihydrodiol 10,11-oxide of benz(a)anthracene as a metabolite of the 8,9-dihydrodiol by rat liver microsomal fractions has been demonstrated and this type of diol-epoxide may have a role in the further metabolism of dihydrodiols.

ENVIRONMENT AND TUMOUR GROWTH. P. J. HOUGHTON and D. M. TAYLOR, Institute of Cancer Research, Sutton, Surrey.

Studies using pulmonary Lewis lung carcinomata (1–50 mg) have suggested an altered mode of growth in contrast to that of the intramuscular tumour, as determined by biochemical parameters. DNA, RNA and protein concentrations have been shown to fall over this weight range, whilst marked necrosis has not been recorded. Isotopic tracer studies have suggested an increase in extracellular fluid. Pulmonary colonies have lower macromolecular concentrations than comparable intramuscular tumours. It is suggested that the cellularity of the pulmonary colonies is lower than in those grown in an intramuscular site, and that this difference may be explained in the context of the diffuse nature of the pulmonary environment allowing loose cell packing, in contrast to the restricting influence to expansion found in the intramuscular environment.