PANCREATIC ISLET CELL AND OTHER TUMOURS INDUCED IN RATS BY HELIOTRINE-A MONO-ESTER PYRROLIZIDINE ALKALOID; THE EFFECTS OF ADDITIONAL TREATMENT WITH NICOTINAMIDE.

R. Schoental, The Royal Veterinary College, London.

The carcinogenic action of diester pyrrolizidine alkaloids (Schoental, Cancer Res., 1968, 28, 2237) has been confirmed in several laboratories (Harris and Chen, Cancer Res., 1970, 30, 2881; Svoboda and Reddy, ibid., 1972, 32, 908; Newberne and Rogers, Plant Foods for Man, 1973, 1, 23).

The mono-ester alkaloid, heliotine, has been claimed not to be carcinogenic (Bull, Culvenor and Dick, The Pyrrolizidine Alkaloids, 1968, North Holland Publishing Co.).

In experiments to be described, heliotine induced in white rats various chronic lesions and tumours, including adenomata of the pancreatic islet cells. Pancreatic islet cell tumours have already been described among rats treated with pyrrolizidine alkaloids, including the mono-esters from Amsinckia intermedia (Schoental, Fowler and Coady, Cancer Res., 1970, 30, 2127).

TREATMENT OF A METASTASIZING MURINE TUMOUR WITH CORYNEBACTERIUM PARVUM. T. E. Sadler and J. E. Castro, Urology and Transplant Unit, Royal Postgraduate Medical School, London.

Effects of Corynebacterium parvum (C. parvum) on the metastasizing Lewis lung carcinoma were studied in C57/B1 mice. Intravenous or intraperitoneal C. parvum given at the same time as subcutaneous inoculation of tumour significantly reduced the primary tumour mass, and the number of pulmonary metastases observed at 21 days. Subcutaneous C. parvum had no effect on either primary tumour or metastases. Macroscopic pulmonary metastases were not observed 10 days after tumour inoculation. When the primary tumour was excised at that time pulmonary metastases were found at 21 days.

Combined effects of surgical excision and C. parvum on pulmonary metastases were studied. There was minimal protection when C. parvum and surgery were on the same day but protection occurred when C. parvum was given before tumour excision.

In defined conditions surgery and C. parvum prevent pulmonary metastases from the Lewis tumour. (This work is supported by the Cancer Research Campaign.)

MECHANISM OF ACTION OF TUMOUR INHIBITORY NITROSOUREAS. T. A. Connors and J. B. Hare, Chester Beatty Research Institute, London.

1,3-Bis(2-chloroethyl)-1-nitrosourea (BCNU) and related nitrosoureas are highly selective anti-tumour agents. Because of their chemical structure, it has been suggested that they act similarly to the bifunctional alkylating agents. They have been compared with two alkylating agents and shown to be quite distinct in a number of properties: (1) They have a much wider spectrum of action and can cure animals with tumours insensitive to alkylating agents; (2) tumours resistant to BCNU are cross-resistant to other nitrosoureas but are collaterally sensitive to alkylating agents; (3) nitrosoureas rapidly inhibit the incorporation of precursors into both nucleic acids and proteins, unlike the alkylating agents which have a specific effect on thymidine incorporation into DNA; (4) labelled nitrosoureas react predominantly with nuclear protein and only to a small extent with nucleic acids; (5) BCNU interferes specifically with the incorporation of thymidine triphosphate into DNA, and its cytotoxicity can be prevented by low doses of thymidine.

Although the toxicity of the nitrosoureas may be due partly to their alkylating properties, they are quite distinct from the bifunctional alkylating agents and probably act by a different mechanism.

DISTRIBUTION STUDIES WITH AN AMINOPHOSPHONIC ACID ANALOGUE OF DOPA IN MICE BEARING THE HARDING-PASSEY MELANOMA. M. J. Stringer, J. A. Stock and L. M. Cobb, Chester Beatty Research Institute, London.

A compound which is incorporated selectively into melanin during biosynthesis in vivo, and which carries an isotope of adequate radiotoxicity and short half-life, has a potential application in the treatment of malignant melanoma. Such therapy would
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.

$^{32}$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 found 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, MeCNU 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. Muter, 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