

Recent theories of the aetiology of HD have postulated immune reactivity to viral or thymic antigens as fundamental to the disease process (Kaplan, *Hodgkin's Disease*, Harvard University Press, 1972).

HD spleen tissue in short-term culture produces high levels of immunoglobulin G in comparison with control spleen cultures. Preliminary results suggest that this immunoglobulin binds to peripheral blood lymphocytes. In addition, supernatants from HD lymph node and spleen cultures inhibited the migration of guinea-pig peritoneal macrophages *in vitro* in 11 of 15 cases. Control supernatants from carcinomatosis and reactive lymph nodes and normal spleens inhibited significantly in only 1 of 16 cases.

The biological properties of this inhibitor parallel those described for lymphocyte derived Migration Inhibition Factor: MIF (David and David, 1972). Lymphokine production *in vitro* is compatible with elevated levels of immune reactivity in HD tissue.

ATTEMPTS TO CULTURE HUMAN TUMOURS IN VITRO. R. H. WHITEHEAD and L. E. HUGHES, Department of Surgery, Welsh National School of Medicine, Cardiff.

There is a need for tissue culture lines of human tumours for *in vitro* studies. Attempts have been made to establish such lines from 220 tumour specimens. Ninety-six specimens from breast carcinoma patients have been cultured. These comprised 64 tumour specimens and 32 pleural effusions from patients with advanced disease. No cell lines have been obtained from the tumour specimens although epithelial cells proliferated for at least one month in 19 of the 64; 24/32 pleural effusions proliferated and 13 of these yielded cell lines. The nature of the cell is in doubt because mesothelial cells grow very well under the culture conditions used.

Seven of 29 colonic carcinomata specimens and 7/8 ascitic fluids from patients with colon carcinoma have also grown for at least one month. Three cell lines have been obtained from ascitic fluids; however, the cells are considered to be mainly mesothelial in origin. Good growth has been obtained from 12/24 melanomata cultured and two cell lines have been established.

Although numerous supplements have been added to the basic McCoy's 5A medium,

enhanced growth was obtained only after the addition of insulin and galactose.

PROTECTION AGAINST THE CYTOTOXIC AZIRIDINE CB1954 BY AN ARYLIMIDAZOLE WITH ANTI-INFLAMMATORY ACTIVITY. J. A. HICKMAN and D. H. MELZACK, Chester Beatty Research Institute London.

CB1954 (5-[1-aziridinyl]-2, 4-dinitrobenzamide) is a potent and selective cytotoxic compound for the Walker tumour (Cobb *et al.*, *Biochem. Pharmac.*, 1969, **18**, 1519). The cytotoxicity was protected against by purine nucleotides and the purine precursor AIC (4-amino-5-imidazolecarboxamide) (Connors and Melzack, *Int. J. Cancer*, 1971, **7**, 86) but CB1954 had no effect on *de novo* purine biosynthesis (Mandel *et al.*, *Cancer Res.*, 1974, **34**, 275). In the search for the mechanism of action of CB1954 this protection phenomenon has been studied further and the anti-inflammatory agent 2-phenyl AIC (Heyes and Ward, *Br. Appl.*, **41**, 261/70) has been found to be a potent protector which gives a dose reduction factor of 90 in an anti-tumour test of CB1954.

2-Phenyl-AIC inhibits prostaglandin synthesis, as do a number of non-steroidal anti-inflammatory agents such as indomethacin. However, since such agents gave no protection against CB1954, it is considered unlikely that the protection mechanism involves prostaglandin synthesis.

LEUCOCYTOTOXICITY IN MALIGNANT AND NON-MALIGNANT COLONIC DISEASES. B. M. VOSE, M. MOORE, P. F. SCHOFIELD and I. W. DYMCK, Paterson Laboratories, Manchester, Park Hospital and University of South Manchester.

The cytotoxicity of peripheral blood leucocytes from healthy donors and from patients with disorders of the large bowel, including ulcerative colitis, colon carcinoma, Crohn's disease, irritable colon and diverticular disease for cells cultured from malignant, normal adult and foetal colonic tissues has been compared. Leucocytotoxicity was defined in this study as a statistically significant reduction of target cell survival in microplate wells treated with leucocyte preparations compared with the survival of cells in wells exposed to growth medium

alone. Cytotoxicity was detectable in preparations from donors in all categories examined, including those from individuals without any known abnormality, so that differences between groups were quantitative rather than qualitative.

Leucocytes from 14 patients with ulcerative colitis showed significantly greater reactivity against cells cultured from malignant, normal and foetal colonic tissue than did those from the 22 healthy donors. Similarly, leucocytes from the 13 patients with colonic neoplasia displayed marked ability to kill cells, particularly those originating from the foetal gut. However, there was no evidence that the leucocytes from these patients were cytotoxic for colon cancer derived target cells. The leucocytotoxicity for the cells from malignant, normal and foetal colonic tissue in patients with other colonic disorders including Crohn's disease and spastic colon syndrome (7 patients each), and non-colonic tumours (20 patients) did not differ significantly from healthy controls.

SYNERGISM BETWEEN THE ANTI-TUMOUR AGENT CIS PLATINUM (II) DIAMMINEDICHLORIDE AND CAFFEINE: TOXICOLOGICAL AND MOLECULAR STUDIES. H. W. VAN DEN BERG and J. J. ROBERTS, Pollards Wood Research Station, Bucks.

The toxicity of the anti-tumour agent *cis* Platinum (II) diamminedichloride, (*cis* Pt (II)), towards Chinese Hamster V79-379A cells was enhanced by post-treatment incubation in the presence of a non-toxic concentration of the purine analogue caffeine. *Cis* Pt (II) also induced chromosome aberrations in this cell line, and the frequency and severity of this damage were increased by caffeine. A time-course study of the appearance of chromosome aberrations suggested

that these aberrations arise as a result of DNA synthesis on a damaged template. The ability of caffeine to enhance the toxic and chromosome-damaging effect of *cis* Pt (II) may be attributable to its ability to interfere with a post-replicative NDA repair mechanism, and molecular studies using alkaline sucrose gradient techniques have shown that DNA is made in smaller segments in *cis* Pt (II) treated cells in the presence of caffeine.

CIS-DICHLOROBIS (CYCLOPENTYLAMINE) PLATINUM II: ACTION ON CHO CELLS *IN VITRO* AND INTERACTION WITH IONIZING RADIATION. I. SZUMIEL and A. H. W. NIAS, Department of Radiobiology and Health Protection, Institute of Nuclear Research, Warsaw, Poland. (IAEA Fellow) and Glasgow Institute of Radiotherapeutics, Belvidere Hospital, Glasgow.

Cytotoxic activity of *cis*-dischlorobis (cyclopentylamine) platinum II (DBCP) has been studied using CHO cells. The half-life of DBCP was determined as well as the dose-survival relationship in medium containing Na⁺ and Cl⁻ at two different concentrations. The data obtained are summarized in the Table.

From experiments performed on synchronized CHO cells, it is concluded that DBCP is not a phase-specific drug at a dose level reducing survival to about 25%. Among early effects of DBCP treatment are inhibition of ³H-TdR incorporation into DNA and inhibition of cell division. Clone-size analysis performed for the first 5 days after treatment revealed the presence of non-lethal damage. DBCP was found to be dose-modifying for ionizing radiation (a factor of 1.2) when applied 1 h before or 1 h after irradiation.

Concentration in the medium*

Na ⁺ (mmol/l)	Cl ⁻ (mmol/l)
106	108
119	120

Half-life (min)
ca 160
ca 45

Dose-survival curve parameters†

D ₀ (μg/ml)	D _Q (μg/ml)	n
9.3	17.5	6.7
14.0	27.5	7.3

* HEPES-buffered (20 mmol/l) Ham's F12 supplemented with 16% calf serum;

† treatment for 1 h at 37°C.