MAINTENANCE OF HUMAN TUMOURS IN IMMUNE DEFICIENT MICE. C. R. FRANKS, Imperial Cancer Research Fund Breast Cancer Unit, Guy's Hospital, London. F. T. PERKINS, L. R. BOULGER and J. THORNTON HOLMES, National Institute of Biological Standards and Control.

In a preliminary study (Franks, Perkins and Holmes, Nature, Lond., 1973, 243, 5402; 91) it was shown that human tumours could be grown subcutaneously in thymectomized, irradiated mice (900 rad), which had been reconstituted using $1 \times 10^7$ syngeneic bone marrow cells. This method has justified its earlier promise, and tumour survivals of $4\frac{1}{2}$ months have now been experienced (Franks, Perkins and Holmes, 1974, Communication, Surgeons Sections, Royal Society of Medicine).

The tumours were obtained at operation from patients with primary cancer and transported to the laboratory in Eagle's MEM containing 2% calf serum, 200 u penicillin, and 100 $\mu$g ml$^{-1}$ streptomycin. To date, 13 different histological types have been studied. The growth of tumours was initially assessed by direct palpation and the histology confirmed by microscopical examination. Metastatic growth was observed at post-mortem examination in a melanoma, and HeLa, rectal and colonic tumour types.

INFLUENCE OF TRANSPLANTATION SITE ON THE ESTABLISHMENT OF HUMAN TUMOUR XENOGRAFTS IN IMMUNOSUPPRESSED MICE. J. A. DOUBLE and C. R. BALL, Department of Experimental Pathology and Cancer Research, University of Leeds.

We have transplanted a series of 42 human tumours in immunosuppressed CBA mice (Davies et al., Transplantation, 1966, 4, 438). The take rate achieved has been 5/12 for colon tumours, 8/18 for rectum and 2/12 for bladder. Tumour fragments have been transplanted either under the renal capsule or subcutaneously, and a positive “take” scored as a visible nodule at laparotomy, or in subcutaneous transplants as a palpable nodule, 8 weeks after transplantation.

Our results indicate that the transplantation site may be an important factor in determining a “take” in this system. The total success rate for colorectal tumours (13/30, 43%) compares unfavourably with smaller published series using similar techniques (Castro, Nature, New Biol., 1972, 239, 83; Detre and Gazet, Br. J. Cancer, 1973, 28, 412). However, our success rate (7/11, 64%) in renal capsule transplants is significantly higher than in subcutaneous ones (6/19, 31%). Subcutaneous tumours arise in few animals of the transplanted group, have a tendency to regress, and in our hands are not transplantable. Renal capsule tumours occur in the majority of transplanted animals and further transplantation (3/4 attempted) is frequently possible. The reasons for these differences are not apparent at present.

IMMUNIZATION AGAINST MAREK'S DISEASE USING VIRUS SPECIFIC ANTIGENS FREE FROM INFECTIOUS VIRUS. F. LESSIK and L. J. N. Ross, Houghton Poultry Research Station, Huntingdon.

Previous studies have established that immunization against Marek’s disease (MD) can be achieved using live attenuated viruses serologically related to Marek’s disease virus (MDV). However, the role of viral antigens in immunity is unknown.

We have shown in this study that immunity can be conferred in the absence of vaccine virus replication using detergent soluble antigens extracted from cells infected with attenuated MDV. Extracts were centrifuged at 100,000 $g$ for 2 h and both sedimentable and non-sedimentable (soluble) materials were used to immunize MD susceptible chickens. These were inoculated i.m. at 7 days of age with antigens emulsified in Freund’s complete adjuvant and were given a second inoculation without adjuvant after 10 days. When compared with controls it was noted that immunization with soluble antigens extracted with the non-ionic detergent NP40 reduced mortality from 91% to 35% ($P < 0.01$) during a period of 19 weeks following exposure to MD. A similar degree of protection was obtained.
by immunization with sedimentable materials inactivated with formalin. Further data will be presented on the effect of immunization on other parameters of infection such as viraemia and changes in antibody titres during the course of the disease.

**SURFACE IMMUNOLOGICAL MARKERS IN ACUTE MYELOBLASTIC LEUKAEMIA.** G. M. TAYLOR, C. B. FREEMAN, J. ESCUDER and R. HARRIS, Department of Medical Genetics, St Mary’s Hospital, Manchester.

Before treatment, peripheral blood leucocytes from patients with acute myeloblastic leukaemia (AML) form few T and B cell rosettes compared with normal individuals, and AML blasts do not themselves form rosettes, unlike a minority of cases of acute lymphoblastic leukaemia in which T rosette forming cells are known to occur. A large but variable proportion of peripheral leucocytes from patients with untreated AML possess surface immunoglobulin detected by both direct and indirect immunofluorescence. The pattern of fluorescent staining on AML blasts differs from that seen on CLL cells, and also in contrast in vitro with anti-human immunoglobulin serum. Surface immunoglobulin on AML blasts may represent tumour associated antibody or immune complex.

Our results suggest that the proportion of rosette forming cells, and of cells with surface immunoglobulin can be used as a diagnostic aid for patients with AML.

**TISSUE CULTURE OF MALIGNANT EFFUSIONS AND THEIR USEFULNESS AS TARGET CELLS IN CYTOTOXICITY CELLS.** R. H. WHITEHEAD, University Department of Surgery, Welsh National School of Medicine, Cardiff.

Breast tumours pose special problems for those seeking to study the immune responsiveness of patients to their tumours. It is difficult, if not impossible, to culture breast cancer cells in vitro. This has led previous workers studying lymphocyte cytotoxicity to use cells derived from effusions from patients with advanced breast cancer. This was done because of the belief that pleural effusion cells were free of fibroblasts and were most probably tumour cells.

Comparative cytotoxicity tests have been performed using cells derived from pleural effusions from breast cancer patients and cells derived from ascites from colon carcinoma patients. Cells derived from a malignant melanoma have also been used as target cells. Lymphocytes from patients with breast cancer, colon cancer and melanoma have been tested against their cells. These tests failed to show any tumour specific cytotoxicity (except in the case of melanoma), suggesting that the cells derived from these effusions are of normal origin.

**CELL MEDITATED IMMUNOREACTIVITY IN HUMAN LUNG NEOPLASIA.** B. M. VOSE and M. MOORE, Immunology Department, Paterson Laboratories, Manchester.

The relative susceptibilities of various tissue culture cells derived principally from malignant, normal and foetal lung tissues, to cytolysis by leucocytes from patients with different histological types of lung cancer were investigated using an in vitro microcytotoxicity assay for cell mediated immunity.

Target cells derived from 12- to 17-week old embryo lungs and from pulmonary tumours of different histological types were most susceptible to the cytotoxic action of lung cancer patients’ leucocytes, while a lower but significant frequency of positive reactions has also been observed against cells originating from non-malignant pulmonary tissue.

It is concluded that lung tumour cells may express tumour associated antigens but their nature and specificity remain to be elucidated.

**AN ATTEMPT TO IDENTIFY STIMULATORY SUBSTANCES INTERFERING WITH A TWO STAGE MACROPHAGE MIGRATION INHIBITION (MMI) ASSAY AND TO ASSESS IMMUNOCOMPETENCE.** J. G. AASKOV and H. M. ANTHONY, Department of Experimental Pathology and Cancer Research, University of Leeds.

An improved two-stage MMI test has been developed to measure the primary