IN VITRO SENSITIVITY SCREENING SYSTEM FOR HUMAN CANCERS TO DRUGS AND HYPERTHERMIA (42°C). J. A. Dickson and M. Suzangar. Cancer Research Unit, Department of Clinical Biochemistry, University of Newcastle upon Tyne.

Approximately 75% of a series of over 200 human solid neoplasms were sensitive to either hyperthermia (42°C) or cytotoxic drug(s), or a combination of these, as defined by a 30% or greater inhibition of respiration and/or anaerobic glycolysis brought about by the agent in fresh and/or cultured tumour.

The inhibition was paralleled by decreased radioactive precursor incorporation into DNA, RNA and protein by the tumour slices. In a solid Yoshida rat tumour there was close correlation between the biochemical response in vitro to heat and drugs and the in vivo response of the tumour. Sensitivity in this system is taken to indicate a drug and/or heat responsive cell population within the tumour. The extent to which such a result predicts patient response will depend upon the existence or emergence of resistant cells in the tumour in vivo, and on the sensitivity of metastatic cells.

THE NATURE OF THE RESISTANCE TO METHYLENE DIMETHANE SULPHONATE IN THE YOSHIDA SARCOMA. B. W. Fox and M. Fox. Paterson Laboratories, Christie Hospital, Manchester.

In order to define the biochemical change responsible for the induction of resistance towards a potent antitumour agent methylene dimethane sulphonate, tumours in vivo have been transferred to in vitro conditions in which they grow continuously as single cell suspension cultures. Both resistant and sensitive cells are maintained in vitro and cross-resistant studies indicate that the two cell lines show a similar wide difference in sensitivity to ultraviolet radiation and sulphur mustard, but no difference to x-rays and methyl methane sulphonate. This pattern of sensitivity implies a deficiency on the part of the sensitive cells of a step in the repair of damage produced in the DNA by u.v. or MDMS but not by x-rays and MMS. This has been shown to be the case and the Yoshida sensitive line thus resembles the human repair deficiency disease of xeroderma pigmentosum.

EVIDENCE FOR THE ACTIVE INTERMEDIATE IN CYCLOPHOSPHAMIDE METABOLISM. P. J. Cox (introduced by T. A. Connors). Chester Beatty Research Institute, London.

Two ethyl derivatives of an intermediate of cyclophosphamide metabolism, 4-hydroxy-cyclophosphamide, have now been isolated and identified, by T.L.C. and mass spectrometry, in extracts of microsomal incubations with [32P]-cyclophosphamide. By the use of a cell culture bioassay technique, these derivatives have been shown to be highly toxic to Walker ascites cells. The toxicities of various previously identified metabolites were assessed in an in vitro bioassay system. The results were consistent with the view that the formation of 4-hydroxycyclophosphamide is the important step in the activation of cyclophosphamide, as this metabolite can break down spontaneously to the active alkylating agent, phosphoramide mustard [N,N-bis(2-chloroethyl) phosphorodiamidic acid], and acrolein. 4-hydroxycyclophosphamide has been tentatively inferred as the hydrolysis product of both ethyl derivatives isolated, but attempts to extract more than minute quantities of the free metabolite from in vitro microsomal incubations have been unsuccessful.

ENZYME ACTIVATED ALKYLATED AGENTS. C. R. Ball, J. A. Double and J. Goodban. Department of Cancer Research, University of Leeds.

Recently Ross and co-workers (Bukhari, Everett and Ross, Biochem. Pharmac., 1971, 21, 963) published the syntheses of 3 conjugates of p-hydroxyaniline mustard which they suggested might be selective for tumours with high levels of β-glucuronidase, phosphatase and sulphatase. The O-glucuronide, O-phosphate and O-sulphate respectively were expected to be deconjugated in vivo by the appropriate enzymes. High enzyme activity in a particular tumour could lead to selectivity of action due to the greater release of the rapidly reacting p-hydroxyaniline mustard in the tumour than elsewhere.

We have determined the ability of the appropriate enzymes to utilize these drugs (kindly supplied by Professor Ross) as sub-
The O-phosphate was readily de-conjugated by all acid and alkaline phosphatases tested but no evidence was obtained for cleavage of the O-sulphate by limpet or rat liver microsomal or lysosomal sulphotases. The O-glucuronide was a good substrate for mammalian liver lysosomal glucuronidases. These results appear to eliminate the O-sulphate as a suitable drug for enzyme activation but substantiate the possible usefulness of the O-phosphate and O-glucuronide.

Friday 6 April

IN VITRO EFFECTS OF ICRF 159. K. HELLMANN and R. C. HALLOWES. Departments of Cancer Chemotherapy and Pathology, Imperial Cancer Research Fund, London.

The effects of ICRF 159 were studied on transformed hamster cells in culture. 5 cm dishes were each plated with $10^4$ cells and cultured at 37°C for up to 72 hours in Eagle's medium containing a range of concentrations of ICRF 159. The cells were either harvested at the completion of the culture period or were transferred to drug-free medium.

Cell numbers increased to $2.5 \times 10^4$ during the first 24 hours in various concentrations of ICRF 159 up to 20 $\mu$g/ml, but the rate of increase diminished with increasing drug concentrations during the next 48 hours. The rate of increase returned towards control values when cells were transferred to drug-free medium, provided the change occurred before 72 hours and only in drug concentrations of less than 10 $\mu$g/ml.

Specific morphological changes occurred in cells exposed to the drug which may enable the site of action of the drug to be determined.

EFFECTS OF THE ANTICOAGULANT, DEGRADED CARRAGEEunan, ON EXPERIMENTAL TUMOUR GROWTH. B. JOLLES, R. G. HARRISON and E. A. MOORE. Cancer and Radiobiology Research Laboratories, General Hospital, Northampton.

As the survival of an experimental tumour graft depends largely on the formation by the host of a new tumour stroma to replace that of the graft which is absorbed within 48–72 hours of implant, the study of substances with “anticoagulant” and fibrinolytic properties is of importance (O'Meara, Irish J. Med. Sci., 1958, 474; Jolles, Lancet, 1963, iii, 1234).

In previous work, the effects of interference with some fundamental events in connective tissue by heparin (Jolles and Greening, Acta Un. int. Cancer., 1960, 16, 682) and of laminarin, a mucopolysaccharide derived from the seaweed Laminaria cloustoni (Jolles, Remington and Andrews, Br. J. Cancer, 1963, 17, 109) have been shown to reduce the rate of growth of Sarcoma S.180 in mice.

In the present series, in which the design of the experiments was along the same lines as those followed in the heparin and laminarin studies, a degraded Carrageenan derived from red seaweeds injected subcutaneously (0.05 ml in a 1:0, 1:5 or 2% concentration) 3 times weekly for 2 weeks, or twice weekly for 4 weeks at a site adjacent to the implanted tumour or intraperitoneally (0-1 ml/animal) reduces the rate of tumour growth.

PROLACTIN AND BREAST CANCER. P. G. SALUJA, J. M. HAMILTON and M. GRONOW. Department of Experimental Pathology and Cancer Research, University of Leeds.

Although prolactin is of supreme importance in the aetiology and genesis of rodent mammary tumours (Muhlböck and Boot, Cancer Res., 1959, 19, 402; Pearson et al., Trans. Ass. Am. Physns, 1969, 82, 225), it is not known whether it is implicated in mammary carcinogenesis in other species. In view of the many similarities that exist between human and canine breast cancer (Misdorp, 1964, Thesis, Utrecht; Schneider, Cancer, N.Y., 1970, 26, 419), an investigation was carried out of the prolactin concentration in the adenohipophysis of dogs afflicted with breast tumours.

Baseline values were established for normal dogs in which pituitary prolactin concentration was found to vary according to reproductive state (e.g. low in dioestrus, high in lactation). In bitches with mammary carcinoma, prolactin levels were significantly higher than in normal subjects of comparable endocrine state. This finding indicates that prolactin imbalance may be involved in canine mammary neoplasia.