after drug administration is much too low to be compatible with the observed delay in tumour growth. If, however, assay of surviving fraction is delayed until 48 hours, then the value obtained for surviving fraction may be up to 50–100 times higher than that measured at 2 hours (depending on the drug dose and tumour size) and perfectly compatible with the delay in tumour growth. Data on cell loss from treated tumours and the proliferation rate of surviving clonogenic cells indicate that ' recovery from potentially lethal damage ' is the main factor operating to produce these results. The increase in measured surviving fraction between 2 and 48 hours appears to be considerably less with MeCCNU than with cyclophosphamide or BCNU, and this is probably the reason why MeCCNU is by far the most effective of these three agents in terms of growth delay and tumour cure for the EMT6 tumour. Very low surviving fractions measured at short times after administration of bleomycin appear to be artefactual, owing to drug carry-over and high drug sensitivity during the making of cell suspensions.

Fractionated irradiations of a rat tumour and foot at different dose-rates

1605A

H. B. Kal and J. F. Gaiser
Radiobiological Institute TNO, Rijswijk, The Netherlands

TNO
20742

Experiments were performed to investigate the effectiveness of X-rays applied at dose-rates of 4 Gy h^{-1} and 204 Gy h^{-1} for the induction of tumour growth delay and normal tissue damage. The aim was to determine whether the therapeutic ratio for doses applied at a dose-rate of 4 Gy h^{-1} is equivalent to that obtained at a dose-rate of 1 Gy h^{-1} presently used in some clinical trials, in order to shorten the irradiation time for the patient.

A rat rhabdomyosarcoma was used to evaluate the effectiveness of four daily doses of 300 kV X-rays administered at a dose-rate of 4 Gy h^{-1} as compared with four daily doses at a dose-rate of 204 Gy h^{-1}. Skin of the rat foot was used as a normal tissue. Four daily doses at dose-rates of 4 and 204 Gy h^{-1} were given; these were followed 1 or 3 days later with an acute dose of 15 Gy. The results indicate that the treatment of the tumour at 4 Gy h^{-1} is less effective per unit dose than the treatment at 204 Gy h^{-1}. The greater amount of sublethal damage repaired during the low-dose-rate irradiation may account for the relative decrease in effectiveness of the treatment at 4 Gy h^{-1}.

However, fractionated irradiation of the rat foot at 4 Gy h^{-1} seems to be more effective per unit dose than fractionated irradiation at 204 Gy h^{-1}. Experiments are in progress to determine whether the use of the anaesthetic Ethrane in the low-dose-rate treatments, in contrast to the Nembutal used in the acute irradiation treatments, might be responsible for the differences observed.

Model studies with hypoxic sensitizers

1606A

L. M. van Putten and T. Smink
Radiobiological Institute TNO, Rijswijk, The Netherlands

TNO
20743

Many model tumour studies have indicated that the application of hypoxic sensitizers is not effective when combined with tumour irradiation in conventional small fractions. It appears, as in the case of hyperbaric oxygen treatment, that this is due to re-oxygenation of the tumour. For this reason, studies were performed with Ro-07-0582 in combination with small dose fractions of radiation on a poorly re-oxygenating tumour—mouse osteosarcoma C22LR. In initial studies it seemed not to respond to application of the sensitizer, but it appeared that this was due to the fact that re-growth delay was used as the treatment end-point and that, owing to a rapid repopulation rate, re-growth was not dependent on the hypoxic cells. When this was modified by pretreatment of the tumour with a large single dose of radiation, the subsequent application of small fractions of radiation were significantly affected by the hypoxic sensitizers. Since toxicity data for the nitroimidazole compounds seem to limit their application to a small number of doses, we then investigated whether,

in therapy with higher dose fractions (ten daily doses of 600 rad in 2 weeks), the sensitizer was more effective when given early or later in the course of treatment or early or later in each treatment week. In a first experiment the results show only small differences, but this result needs confirmation.

Misonidazole (Roche-07-0582)-a cytotoxic agent specific for hypoxic cells

I. J. Stratford

Gray Laboratory of the Cancer Research Campaign,
Mount Vernon Hospital, Northwood, Middlesex, England

Some nitroheterocyclic compounds that act as radiosensitizers can also function as cytotoxic agents. There is a large differential between toxicity for hypoxic cells and for aerated cells. The 2-nitro-imidazole, Misonidazole, falls into this class of compound, and this paper describes some studies into the mechanism of this differential toxic effect. In particular, experiments were done on the influence of intracellular pH, since there have been suggestions that tumour pH is low, this being brought about in hypoxic regions by anaerobic glycolsis.

Asynchronous, log-phase Chinese hamster cells in MEM plus 7·5 per cent fcs were held at 37°C under 95 per cent N_2/5 per cent CO_2 and extracellular pH was adjusted with bicarbonate. In the presence of Misonidazole, plots of cell survival versus contact time with drug show curves which, after an initial shoulder region, are exponential. After 5 hours contact with 2 mM Misonidazole at pH values from 7·4 to 6·8 surviving fraction was reduced to 10^{-2}. Lowering pH to 6·35 increased the slopes of the survival curves, so that after 5 hours at pH 6·35 survival was reduced to 10^{-5}.

This increase in the cytoxic effect of Misonidazole with lowering pH is a bonus if drugs of this type are to be used as chemotherapeutic agents. This result also has implications for the mechanism of anaerobic toxicity, which were discussed.

Combination of fractionated irradiation with 5-fluorouracil or actinomycin D

D. Gonzalez and K. Breur

Department of Radiotherapy, Wilhelmina Gasthuis,
University of Amsterdam, Amsterdam, The Netherlands

The effect of fractionated X-ray irradiation with the concomitant administration either of 5-fluorouracil or actinomycin D has been studied on an experimental mammary carcinoma in mice. Two different time-sequences in the administration of the drugs with relation to every fraction of radiation were investigated: the effect of actinomycin D at a dose of 0·1 micrograms/mouse 15 min before every fraction of 480 rad X-rays was compared with the same dose administered 18 hours after every fraction; the effect of 5-fluorouracil at a dose of 10 milligrams/kg 15 min after every fraction of 480 rad X-rays was compared with the same dose administered 18 hours after every fraction. The treatment by X-rays alone consisted of nine fractions of 480 rad (three fractions a week during three weeks). The fractions were given on alternate days. It was expected that administration of the drug shortly before or after every X-ray fraction could interfere with the repair of radiation induced sublethal damage and thus would be more effective than if administered when most of repair is already completed.

The combined treatments using either actinomycin D or 5-fluorouracil achieved more tumour growth delay than could be expected from the addition of the delay produced by irradiation alone and drug alone. This effect was found to be independent of the interval between every fraction of radiation and the administration of the drug. Some possibilities explaining the results obtained are discussed.