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DOSE RESPONSE EXPERIMENTS USING THE MOUSE TAIL MODEL

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The radiation response of the mouse tail has been shown to be similar to that of normal connective tissue in man, one of the limiting tissues in clinical radiation therapy. However, this response depends on the temperature and oxygenation at the time of irradiation (HENDRY et coll. 1976, HENDRY 1978). The purpose of the present series of experiments was to investigate in detail the effect of these external parameters so that a set of conditions could be found under which the animal model most closely resembled the human situation—in other words to tune the system. Such tuning would greatly increase the usefulness of the experimental system and allow predictions of models such as NSD and CRE (ELLIS 1968, KIRK et coll. 1971, PERRY et coll. 1979) to be tested under conditions where clinical trials could not be justified but which might prove therapeutically beneficial in the future.

Materials and Methods

All experiments were carried out using BALB/c mice, either bought from Bantin and Kingman Ltd of Hull or bred locally from the same stock. Unanaesthetised mice, about 14 weeks old, were immobilised and irradiated in a jig developed from that reported by HENDRY et coll. (Fig. 1). Medial lengths of mouse tail (2 cm long) were irradiated at a dose rate of 0.87 Gy per min using a Pantak unit operated at 250 kV, 15 mA with a HVL of 2.5 mm of Cu. The probabilities of tail necrosis were estimated for 22 dose points at tail temperatures of 32.5, 35 or 37°C during irradiation with a constant flow of either air or oxygen maintained over the tails. Mice were generally irradiated in groups of 10; no dose point was assessed on less than 30 mice (excepting one of 28 when 2 mice had to be discarded) and the average number was 43.8. Tail reactions were observed for 7 weeks, at which time it was presumed that no further significant change in response would occur and that those tails which had not necrosed would eventually heal.

Results

The biologic effect of single doses of radiation on the tissues of the mouse tail was measured by the fraction of necrosed tails for any given set of experimental conditions. The fractional tail necroses obtained are shown in Fig. 2 as a function of absorbed dose for mice irradiated in air at temperatures of 32.5, 35 and 37°C, and in oxygen at 35°C. All dose points have fractional responses other than 0 or 1 in order to be analytically meaningful. In order to facilitate interpolation, the data were linearised using standard logit analysis as described by HEWLETT & PLACKETT (1979); in particular, using the minimum weighted logit chi-square procedure proposed by BERKSON (1953). The doses, ND_{50}, and slopes, dP/dD_{50}, for 50 per cent tail necrosis obtained for these dose response curves are listed in Table 1. It can be readily seen that, while the ND_{50} values for irradiation in air show no marked trend (except

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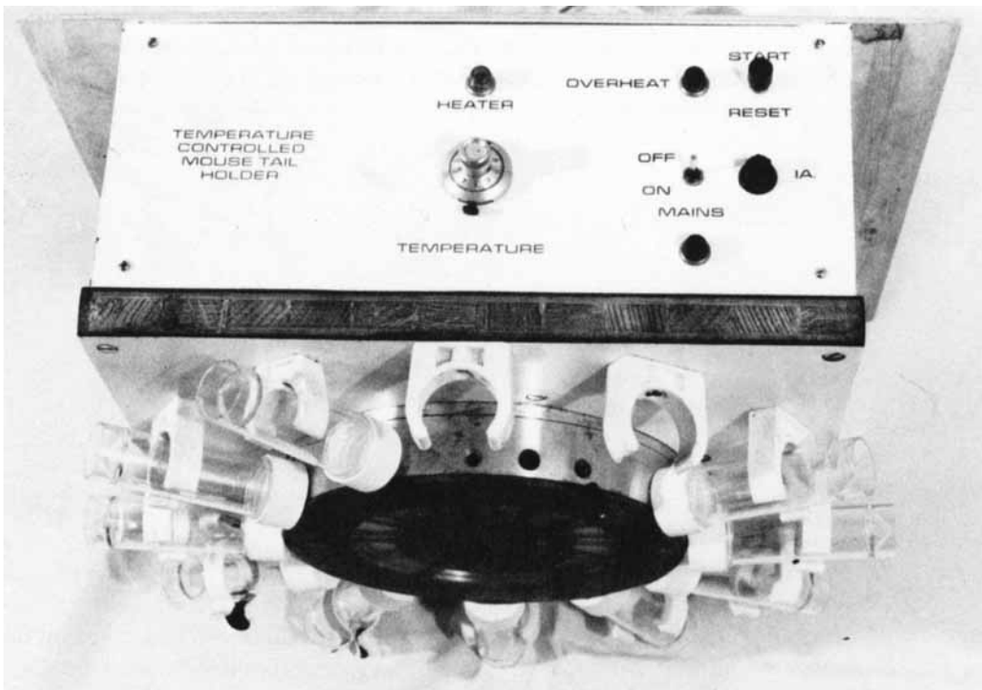

Fig. 1. The mouse tail irradiation jig. Air or oxygen is passed through the upper part of the equipment where it is heated to a preset temperature. The gas then emerges along the 12 channels into which are inserted the tails for irradiation. These channels, together with the animal holders and the annular gap in the lead shielding are seen in the lower part of the photograph.

perhaps a decrease with temperature), the slopes of the dose response curves increase monotonically with temperature.

In order to assess the effect of gas environment on the response of irradiated tail, the ratio of the ND_{50} value in air to that in oxygen was determined at 35°C and found to be

$$\frac{ND_{50}(\text{air})}{ND_{50}(\text{oxygen})} = 1.02 \pm 0.05$$

There is clearly no significant difference in the observed dose responses. This result is at odds with that reported by HENDRY who quotes unchanging ratios of 1.12±0.05 and 1.12±0.04 at 32 and 37°C, respectively. The dose response curves in air and oxygen can be further compared by considering the ratio of the dP/dD_{50} values at 35°C which are found to be

$$\frac{dP/dD_{50}(\text{air})}{dP/dD_{50}(\text{oxygen})} = 0.93 \pm 0.22$$

This result also demonstrates that there is no significant difference in biologic effect as assessed by fractional tail necrosis between irradiations in air and oxygen.

Table 1

Values of ND_{50} and dP/dD_{50} for 50 per cent tail necrosis for various experimental conditions

Temperature	ND_{50}	dP/dD_{50}
32.5°C air	32.1±1.0	0.052±0.013
35°C air	29.7±1.0	0.071±0.011
37°C air	30.3±1.0	0.110±0.019
35°C oxygen	29.1±1.0	0.076±0.013

Discussion

The salient feature of the results (Table 1) is the marked increase in the slope of the dose response curves with temperature for irradiation in air. No corresponding changes in slope have been identified by HENDRY et coll. The observed monotonic change in slope could conceivably arise from the changing hypoxic status of tissues of the tail with temperature, probably mediated through resultant changes in blood flow. Evidence for the existence of a proportion of anoxic but still viable cells in rodent skin under normal conditions has been reviewed by HENDRY et coll. and HENDRY, and need not be

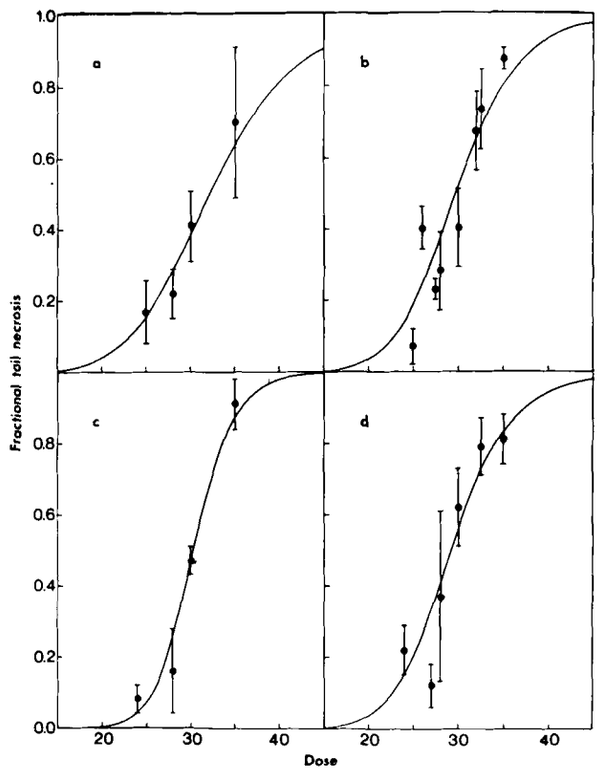

Fig. 2. Response curves of mouse tail to single doses (in Gy). Fractional tail necrosis is plotted against absorbed dose for various experimental conditions: a) 32.5°C in air, b) 35°C in air, c) 37°C in air, d) 35°C in oxygen.

Table 2

Fraction of anoxic cells in mouse tail at different temperatures in air

Temperature	Anoxic fraction
32.5°C	0.00374
35°C	0.00186
37°C	0.000092

repeated here. The possibility that the temperature dependent change of response is associated with changing hypoxic status can be investigated qualitatively with the aid of a simple model.

Consider an over-simplified model based on the following assumptions: (1) The dose response is governed by Poisson statistics so that the probability of necrosis depends on the number of surviving clonogenic cells. (2) The response curves are only concerned with large single doses of radiation, so that cell survival fractions can be described by the high dose approximation to the multi-target formula. (3) The critical normal tissue in the mouse tail is composed of either aerobic or anoxic cells—the hypoxic status of the tissue is dependent on the fraction, x, of anoxic cells. (4) Oxygen acts as a dose modifying agent so that the extrapolation members of aerobic and anoxic cells are the same, but the mean lethal doses differ. (5) The fundamental parameter values adopted are: oxygen enhancement ratio = 3, mean lethal dose (aerobic) = 3 Gy, so that mean lethal dose (anoxic) = 9 Gy.

The choice of parameters throughout the model is in no way unique and is guided only by the need for values typical of mammalian cell types and compatible with available data.

On the basis of this simple mathematical model for the number of surviving cells, the fractions of anoxic cells present at each temperature can be evaluated from the data presented in Table 1 for irradiation in air, and are shown in Table 2. The anoxic fraction is seen to exhibit a very marked and monotonic decrease with temperature.

The wider implications of the postulate that changes in dose response with temperature are mediated through consequent alterations in hypoxic status can best be appreciated and its validity qualitatively confirmed by considering the way in which the ND_{50} and dP/dD_{50} values depend on temperature as predicted by the simple model. To that end, some relationship must be presumed between the anoxic fraction and tail temperature. As the qualitative features of the model are not critically dependent, even for modest extrapolations beyond the range of experimental observations, on the choice of function, a smooth curve drawn through the data points of Table 2 was considered adequate. However, to evaluate the ND_{50} and dP/dD_{50} values it is necessary to assume a value for the product, nN_0, of the extrapolation number, n, and the original number of clonogenic cells, N_0. The value of nN_0 can be derived from the data presetned in Table I and is found to be about 7000, although estimates are rather variable. It is interesting to note that if n is taken to be 2, which is typical of many mammalian cell lines, N_0 is found to be 3500. This number presumably represents the original number of basic clonogenic cells of the most critical tissue in the mouse tail. If a 2 cm length of tail has a diameter of 3 mm, the density of these basic cells is about 2.5×10^4 per cm^3.

The functional dependences of ND_{50} and dP/dD_{50} on temperature can then be calculated from the model (Fig. 3). The dP/dD_{50} line is a sigmoid and

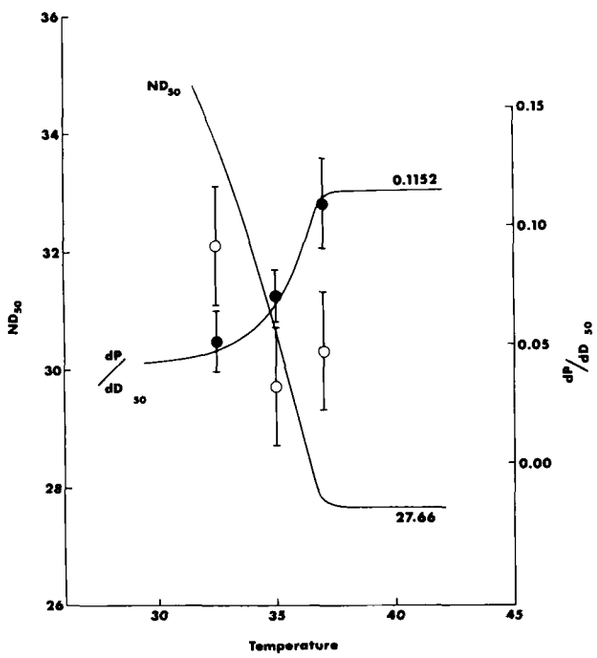

Fig. 3. Graphical presentation of the ND_{50} (in Gy, ○) and dP/dD_{50} (in Gy^{-1}, ●) values recorded in Table 1 for irradiations in air at various temperatures. The lines through the experimental points represent the functional dependences on temperature of ND_{50} and dP/dD_{50} as calculated from the model. D_0=33 Gy. nN_0=7 000.

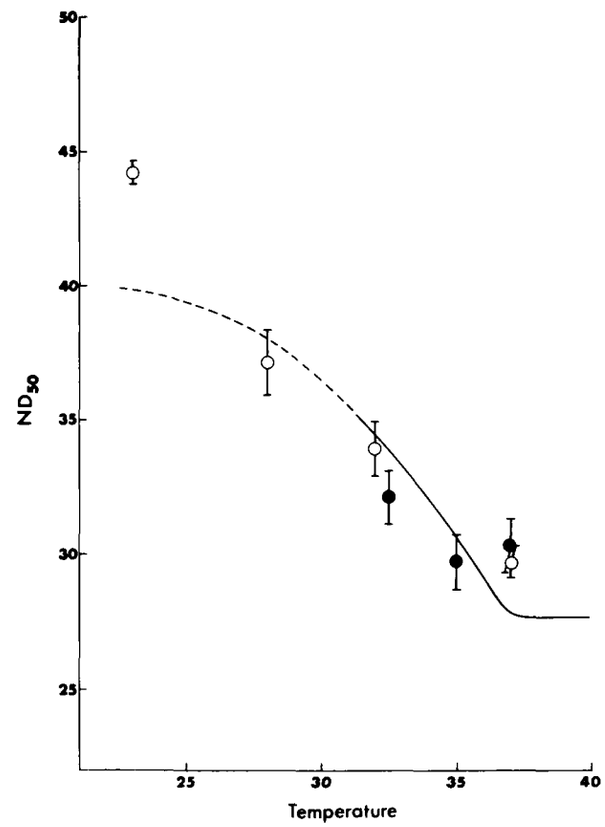

Fig. 4. Relationship of ND_{50} (in Gy) to temperature from ambient to 40°C. The experimental points shown are those of HENDRY (1978; ○) and the present authors (●). The solid line is a fit of the model to the present work; the dashed line is an extrapolation calculated from the model.

demonstrates a comparatively rapid change in slope of the dose response curves by a factor of almost 3 over a range of temperature from about 30 to 38°C, corresponding to the OER value of 3. The dP/dD_{50} curve becomes asymptotic: (1) for higher temperatures, when all the cells of the tissue have become aerobic, and (2) for lower temperatures, when the increasing anoxic fraction dominates the response of the population. The ND_{50} line falls steadily, demonstrating a distinct increase in radiation sensitivity, as temperature increases, before becoming asymptotic as all the cells become aerobic. For decreasing temperatures, the ND_{50} line rises with decreasing rapidity as the tissue approaches a constant hypoxic level. This rapid increase in ND_{50} for lower temperatures was observed by HENDRY, whose data are reproduced with those of the present work in Fig. 4. Although of different strain, these data have been combined as the qualitative features of response should not depend on any specific mouse strain. The predicitions of the model for the dependences of ND_{50} and dP/dD_{50} on temperature are in good general qualitative agreement with experimental observation (Figs 3, 4).

It would therefore appear that the postulate that changes in dose response with temperature are mediated through consequent changes in hypoxic status is completely consistent with the experimentally observed data from mouse tail irradiations.

In the treatment of patients with malignant tumours by the combined modalities of radiation and hyperthermia, whole or partial body temperature may on occasion be raised to about 40°C to prevent excessive heat loss from local volumes elevated to potentially curative temperatures. The substantial increase in sensitivity of radiation response with increasing temperature up to about 40°C (Fig. 4) has important implications for patients treated by heat

and radiation at the same time. If a patient with elevated body temperature is irradiated, the radiation response of normal tissue may be substantially greater than at ambient temperatures. While given doses of radiation will presumbably be less than those for radiation treatment alone, they may well be limited by normal tissue response with consequent reduction in the therapeutic ratio.

SUMMARY

Radiation induced tail necrosis in BALB/c mice was used to investigate the effects of tissue temperature and local oxygenation on the radiation response. Single doses of 250 kV roentgen-rays were given over the temperature range 32.5 to 37°C and dose response curves obtained. The temperature dependence of the response was shown to be consistent with changing hypoxic status by the derivation of a simple model; this has practical implications for the treatment of patients using simultaneously combined radiation therapy and hyperthermia.

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