

Correspondence

THE EDITOR—SIR,
 HUMAN LYMPHOCYTE TRANSFORMATION BY
 PHYTOHAEMAGGLUTININ AFTER "IN VITRO" AND "IN VIVO"
 IRRADIATION

Your correspondents, Song and Levitt (1975), queried our results on lymphocyte transformation by PHA after *in vitro* irradiation. They suggested that our results conflicted with other published data and they referred specifically to the work of Conrad (1969) which, in their opinion, supported their own findings that PHA transformation of lymphocytes from rats was not significantly depressed by 1,000 rad of X-irradiation. Conrad (1969) constructed a response curve for maximum effect, *i.e.* after three days incubation, a time used in our own studies, and showed that for an X-ray dose of 750 rad inhibition of lymphocyte transformation occurred and the response was 38 per cent of that observed at zero dose, while at higher doses a levelling off in response was seen (Fig. 1). Our data after three days' incubation showed that the response was 25 per cent of that at zero dose for 750 rad of γ rays with a similar levelling off at higher doses. Ilbery *et al.*, (1971) carried out similar experiments with

human lymphocytes after *in vitro* γ irradiation and showed that the response was 35 per cent of that at zero dose for 750 rad with a similar tendency to level off at high doses (Fig. 1).

Work by Nias (1975) not yet published showed that 1,000 rad reduced PHA transformation to approximately 35 per cent of that at zero dose. These results with human lymphocytes are really very similar considering the different techniques used to measure lymphocyte response to PHA. In our experiments (Braeman and Moore, 1974) a microscopic morphological technique was used; Conrad (1969) used electronic counting and sizing of nuclei and Ilbery *et al.* (1971) and Nias (1975) used incorporation of tritiated thymidine. The results of all these investigations are plotted in Fig. 1. Since it is the radiosensitivity of lymphocyte response to PHA transformation that is being measured, the data from all three investigations were plotted using the conventional semi-logarithmic plot used in radiation studies. From this graph a dose of 400-600 rad reduced the response to 40 per cent of that observed when no radiation was given, and in each case a levelling off in effect is seen at higher doses.

Song and Levitt (1975) quoted the work of Neff and Cassen (1968) who demonstrated that damaged lymphocytes were rapidly removed from circulation following *in vivo* irradiation. This work was carried out with rabbits and caution must always be exercised when extrapolating animal results to humans. However, Neff and Cassen (1968) also measured the effect of *in vitro* irradiation on lymphocyte transformation by PHA and noted for 300 rad of X-irradiation the response was 80 per cent of that observed for zero dose after three days incubation, a loss of response comparable to that reported by these other workers for human lymphocytes. Care must also be taken when comparing data obtained after three days incubation with that seen after six days incubation.

It was possible to obtain blood samples, before and immediately after irradiation, from some patients being treated at Velindre Hospital. The samples taken before irradiation were then irradiated *in vitro* with the same dose as that given to the patient. Obviously the lymphocytes would receive a smaller dose from the *in vivo* treatment and a smaller loss in PHA transformation would be expected. The results are shown in Table I and demonstrate that both *in vivo* and *in vitro* irradiation depress response of lymphocytes to PHA. Patient E is additionally interesting since the tumour was a basal cell carcinoma on the nose which was irradiated with superficial X rays so no significant dose would be given to the lymphocytes; this is clearly seen in the unchanged response of the lymphocytes after *in vivo* irradiation.

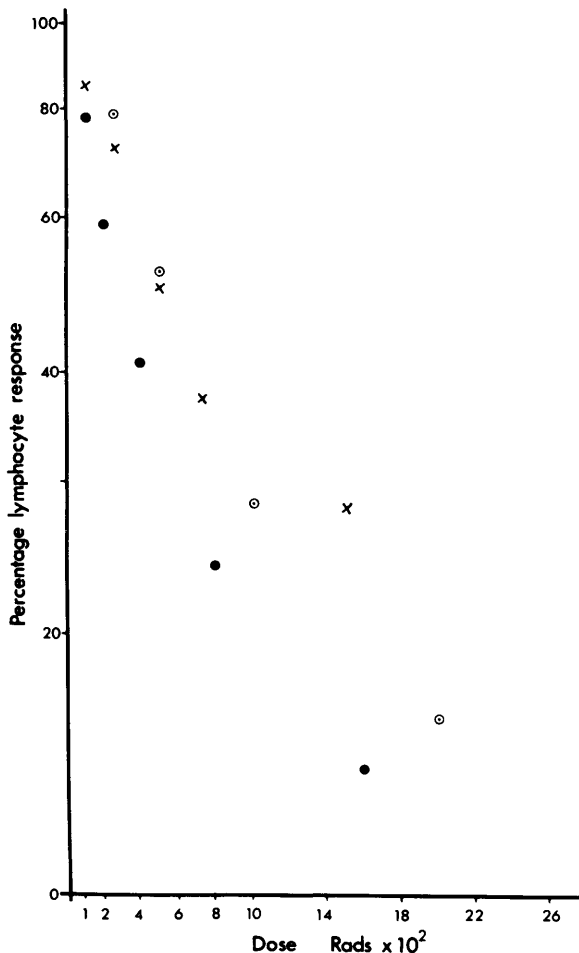


FIG. 1

Response of human lymphocytes to PHA after *in vitro* irradiation; ●, Braeman and Moore 1974; X, Conrad 1969; ○, Ilbery *et al.*, 1971.

TABLE I

LYMPHOCYTE TRANSFORMATION BY PHA AFTER "IN VIVO" AND "IN VITRO" IRRADIATION AS A PERCENTAGE OF THAT SEEN AT ZERO DOSE.

Patient	Dose (rad)	Tumour site	% PHA transformation	
			<i>in vivo</i> irradiation	<i>in vitro</i> irradiation
A	223	Larynx	91	89
B	500	Parotid	78	65
C	268	Breast	91	67
D	400	Breast	93	55
E	2,000	BCC	100	40

Patients A, B, C, D treated by ⁶⁰Co γ rays and Patient E with 110 kVp X rays.

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Where samples of blood have been taken before and immediately after irradiation, *i.e.* approximately 15 minutes after completion of treatment, total white cell counts and differential white cell counts were not significantly different.

We feel that in spite of Song and Levitt's (1975) accusation that our results are atypical, it does seem that a closer examination of other people's data shows that we agree with most *in vitro* studies that have been carried out on lymphocyte transformation after irradiation and the suggestions in the original letter are still valid.

Yours, etc,
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THE EDITOR—SIR,

HYPOXIC RADIOSENSITIZERS AND CELLULAR RESPIRATION

In a recent communication, Rauth and Kaufman (1975) presented data demonstrating radiosensitization of hypoxic tumour cells with specific drugs and injection techniques. They stressed that radiosensitization *in vivo* is dependent on (1) drug transport to the tumour, (2) diffusion of the drug to the hypoxic cells, and (3) drug effectiveness at high cell density. Inherent in these points, yet worthy of further emphasis, is the requirement that such drugs should have little or no metabolic effect on either the normal or malignant tissues. Changes in cellular metabolism, including respiration, may drastically alter the radiosensitivity of multicellular structures including both tumours (Biaglow *et al.*, 1970) and the *in vitro* spheroid system (Durand and Biaglow, 1974).

Rauth and Kaufman observed that the nitro-imidazoles were the only drugs which resulted in tumour sensitization after intraperitoneal injection. However, relatively good sensitization occurred after localized (intra-tumour) injection of both NDPP and metronidazole (Flagyl). These observations may be partially explained on the basis of the drug concentrations necessary in the tumour for radiosensitization. Another factor which should be considered is the influence of these drugs on cellular oxygen utilization.

We have observed pronounced effects on the oxygen consumption of V-79 cells by Flagyl, NF-167 and NDPP, three of the drugs used by Rauth and Kaufman (Fig. 1). Flagyl inhibited and NF-167 stimulated oxygen utilization at all concentrations. A more complex response was observed with NDPP, as oxygen utilization was stimulated at low drug concentrations and inhibited at higher drug levels. Concentrations of NDPP in excess of 0.5 mM were found to be cytotoxic, and the decreasing respiration at these concentrations (Fig. 1) probably reflects, in part, this toxicity.

Spheroids irradiated in the presence of 0.5 mM NDPP (Fig. 2) showed an initial increase of radio-resistance. This

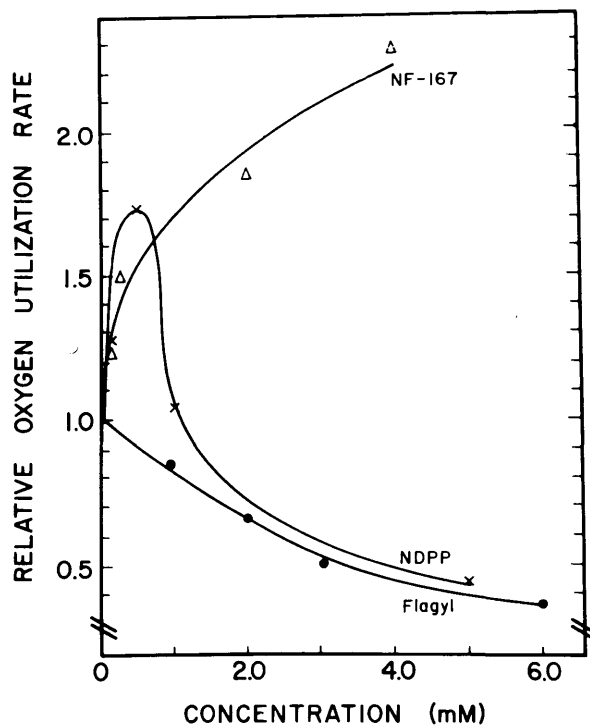


FIG. 1.

Immediate effects of increasing concentrations of freshly prepared NDPP, NF-167 and Flagyl on respiration rate of V79 Chinese hamster cells in suspension. For experimental details, see Biaglow and Durand, 1976.

concentration produced a 70 per cent increase in the rate of oxygen utilization (Fig. 1) and apparently increased the hypoxic fraction of the spheroid sufficiently to offset the "direct" sensitizing effect of NDPP. Slight sensitization occurred initially at the lower NDPP concentration of 0.125 mM where the hypoxic fraction was not increased as markedly. Sensitization with this low drug concentration reflects both adequate transport of the sensitizer to the hypoxic cells of the spheroids, and virtually "unlimited" drug replacement from the surrounding medium. A similar situation would be extremely difficult to achieve *in vivo* unless the drug were not metabolized and were uniformly distributed. The decline in effect with time, both *in vivo* and *in vitro* (Fig. 2), is probably linked to drug metabolism. This would, of course, develop more slowly in the spheroid system due to the smaller number of cells.

In the tumour system used by Rauth and Kaufman, as in the *in vitro* spheroid, the net radiosensitivity is in part a function of cellular oxygen utilization and hence the size of the hypoxic cell population. Thus, we would predict that sensitizers which markedly increase oxygen utilization of the well-vascularized areas of a tumour (*e.g.* NF-167, Fig. 1) would always lead to an increase in the hypoxic fraction that could only be counterbalanced by a potent radiosensitizing potential. Conversely, sensitizing concentrations of drugs that inhibit cell respiration (*e.g.* Flagyl, Fig. 1) would always be expected to increase radiosensitivity through both oxygen-like radiosensitization and partial reoxygenation. Both predictions are consistent with Rauth and Kaufman's observations with their *in vivo* system and