

Fig. 4. Changes in the reaction time following two series of T.A.B. injections (250×10^6 organisms) (separated by a 7-week interval). Upper, first series of injections; lower, second series of injections

ation, but the bulk of the evidence so far strongly implicates the involvement of the intracellular electron transfer systems and hence supports the views of Tahmisan⁷ and many others that these systems are of paramount importance in the reaction of a tissue to radiation.

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Binding of Strontium in Blood

WE have reported¹ that, following the ingestion of radioactive strontium by normal subjects, the specific activity in urine was appreciably greater than that in contemporary plasma. Consequently, we postulated that strontium existed in blood in two different chemical forms. We were unable, however, to find any difference in the specific activity of strontium in plasma ultrafiltrate compared with that of the non-dialysable fraction. This result prompted further experiments which have now revealed an artefact in the original measurements. Heparin, which is known to be a source of adventitious stable strontium², was used in the collection of the blood. An incorrect allowance for this gave us falsely low values for the specific activity of plasma. A complete re-estimation of the relative specific activities of strontium in urine and plasma has therefore been made. The results of these measurements are given in Table 1. The prob-

Table 1. SPECIFIC ACTIVITY OF STRONTIUM IN URINE AND PLASMA

Subject	Specific activity of strontium (counts/min/ μ g)		Ratio specific activity urine/plasma
	Urine	Plasma	
R. H. M.	176	170	1.03
G. E. H.	208	145	1.43
A. J. P.	135	158	0.85
T. E. F. C.	240	192	1.25
P. W. E.	222	218	1.02
		Mean	1.1 \pm 0.1

ability of the ratio being greater than unity is 32 per cent, the 95 per cent confidence limits 0.84 and 1.40.

In some experiments already reported¹, and in others unreported, heparin (batch No. 64373) was added to the blood samples. This addition was 40 mg (equivalent to 4,800 units) to all blood samples of 100 ml. or more. As the strontium content of this amount of heparin is known, it was possible to correct these earlier determinations. The observed and corrected values for the specific activity of strontium in urine relative to that in plasma are given in Table 2. The mean corrected value of the ratio 0.98 ± 0.05 confirms the more recent values in Table 1.

Table 2. EFFECT OF HEPARIN ON THE OBSERVED RATIO OF SPECIFIC ACTIVITY OF STRONTIUM IN URINE RELATIVE TO PLASMA

Subject	Specific activity of strontium in urine relative to plasma	
	Observed	Corrected
A. J. P.	2.68	1.38
P. W. E.	1.70	0.67
"	1.18	0.98
R. H. M.	1.70	0.81
"	1.69	0.85
"	1.80	1.10
"	1.17	1.02
"	1.01	0.80
"	1.45	1.14
"	1.49	1.02
F. S. W.	2.20	1.23
	Mean	0.98 \pm 0.05

We conclude that the specific activities of strontium in plasma and urine are probably equal. It should be added that the same result has also been obtained for calcium by others^{3,4}. There is, therefore, no experimental evidence to support the view that there are two different forms of strontium (or calcium) in blood which the kidney is able to differentiate.

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A Multicomponent X-ray Survival Curve for Mouse Lymphosarcoma Cells irradiated *in vivo*

NEARLY all investigations hitherto reported of the survival of animal cell populations exposed to single doses of ionizing radiations have yielded data which have been fitted by curves of the same general shape: a shoulder of varying size (which may be absent) followed by a region of exponential decrease. Only a single exponential component has been found even in those investigations in which survival was studied to fairly low levels (less than 10^{-3}), irrespective of whether survival was measured by colony formation *in vitro*¹⁻⁵, or *in vivo* by tumour induction^{6,7}. These results have been obtained despite the fact: (a) that a given cell strain may be heterogeneous with respect to innate sensitivity⁸; (b) that cells may undergo marked fluctuations in radiation sensitivity during the division cycle⁹; (c) that inhomogeneities in degree of oxygenation might exist, particularly in a tumour cell population *in situ*¹⁰. It may be presumed, therefore, that one or more of the following has been pertinent in each study: (1) A particular population may be homogeneous with respect to inherent sensitivity, degree of oxygenation, and presence of protective agents or other modifiers of radiation response. (2) The overall sensitivity of cell populations heterogeneous with respect to radiation

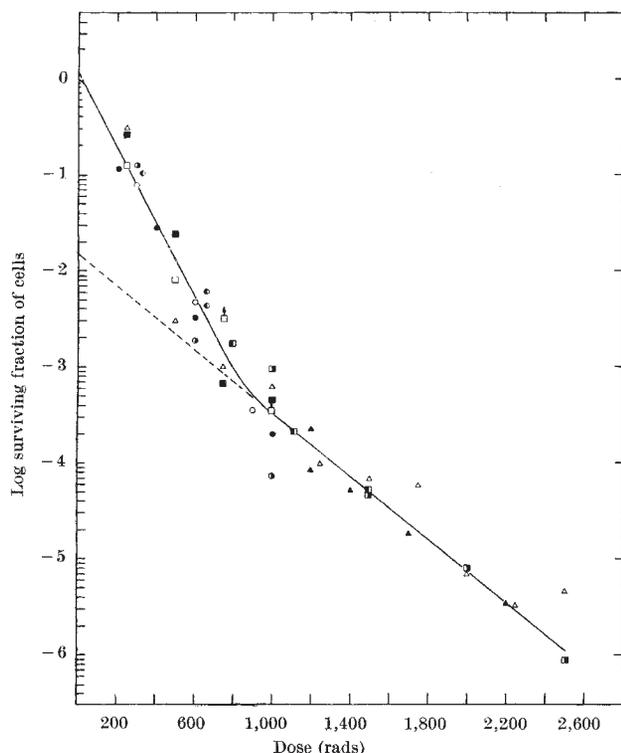


Fig. 1. Survival of 6C3HED mouse lymphosarcoma cells irradiated *in vivo*. The curve has been fitted by eye. Each symbol refers to a separate experiment. Each point involved determination of the number of cells required to produce tumours in 50 per cent of 30 mice, using the end-point dilution method of Hewitt (ref. 14). This number for unirradiated cells was generally in the range 2-5 cells. The arrows indicate minimum values

response may, under certain conditions, exhibit relatively simple survival kinetics^{9,11,12}. (3) A less-sensitive cell type may be present in amounts too small to be detected unless many decades of inactivation are effected¹³. (4) The precision of cell survival measurements, particularly those carried out *in vivo*, is limited.

We have observed a very different radiation response in the cell populations which constitute solid, subcutaneous 6C3HED mouse lymphosarcomas, 1-2 cm in diameter, in C3H mice. When the viability of such cells is measured by Hewitt's method¹⁴ immediately after total body exposures to graded doses of X-rays (220 kV C.P.; 0.5 mm copper + 1.0 mm aluminium added filtration; half-value layer 1.9 mm copper; 70-90 rads per min), survival is found to follow a multicomponent exponential curve, preceded by a small shoulder (Fig. 1). The results suggest that the survival kinetics are determined mainly by cells of only two sensitivities. The relative proportions of these remained essentially constant in four experiments. The initial exponential slope corresponds to a mean lethal dose (D_0) of 110 rads; its extrapolation number is about 1.2. The terminal slope indicates a D_0 of 260 rads, and extrapolates to about 0.01 on the ordinate. Neither the precision of the data nor the size of the resistant fraction warrants resolution of the measured survival curve into components for the sensitive and resistant fractions at doses less than 1,000 rads.

When tumours containing 10^8 - 10^9 cells (about 1 g) are irradiated with 3,000 rads, the surviving cells, which should be of the resistant type exclusively, give rise, on injection into new mice, to populations which exhibit a survival curve identical to that shown in Fig. 1. Hence, the resistant cells do not appear to constitute a sub-group the low sensitivity of which is inheritable.

The ratio of the exponential slopes, 2.3, is typical for oxygen enhancement of radiation sensitivity as observed in many studies¹⁵, including those concerned with cytological

damage¹⁶ and survival^{7,17,18} in tumour cells. It is proposed, therefore, that about 1 per cent of the cells of these tumours are anoxic and that the terminal slope characterizes their radiation sensitivity. The following preliminary results tend to support this hypothesis: (1) Irradiation in air of cells suspended in chilled fetal calf serum yields a survival curve which has only one exponential slope; the value of D_0 is close to that for the majority population irradiated *in vivo*. (2) Irradiation of cells *in situ* in mice killed by either cervical fracture¹⁸ or asphyxiation in nitrogen, all of the tissues of which are presumably anoxic, yields a survival curve from which it is estimated that the fraction of radiation-resistant cells is about 10 times larger than in live mice. Further tests, both *in vitro* and *in vivo*, of the effects of oxygen and of anoxia on the radiation sensitivity of these tumour cell populations are under way.

The possibility that a given tumour cell population may be heterogeneous with respect to oxygenation has been considered previously^{15,16}, and the implications of the foregoing type of cell population response for the radiation therapy of tumours has been discussed theoretically by several authors^{10,19,20}. Such discussions would now appear to rest on a much firmer foundation.

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Carbohydrate Metabolism in the Sapwood of *Pinus radiata*

THE use of carbon-14 produced in nuclear bomb testing in biological investigation has been discussed in an earlier communication¹. Since 1954 the specific activity of the carbon dioxide in the atmosphere over New Zealand has been increasing, as is shown in Fig. 1². We have used this effect to examine the carbohydrate metabolism of *Pinus radiata*.

Annual rings of a *Pinus radiata* tree felled in January 1960 in the Kaingaroa Forest were ground up and acetone extracted in a Soxhlet extractor. The ground rings were treated with steam in an autoclave and then exhaustively extracted with water. Cellulose was separated from the residue. The water-extracted material and the cellulose were converted to carbon dioxide and counted in the Institute of Nuclear Science low-level carbon-14 counter. The ¹⁴C/¹³C ratio of each sample was measured so that the results could be corrected for any isotope effect in