ACTA RADIOLOGICA

OFFICIAL ORGAN OF THE RADIOLOGICAL SOCIETIES OF
DENMARK, FINLAND, NORWAY AND SWEDEN

Vol. 4 | THERAPY PHYSICS BIOLOGY | 1966
Fasc. 3 | | June

ELECTRON BEAM IRRADIATION OF BRAIN OF RAT AND DOSE-MODIFYING EFFECT OF ANOXIA

by

N. A. SHARPLES and A. G. JACOBS

The susceptibility of cells to high LET radiation damage is profoundly influenced by the concentration of oxygen present in the cell at the instant after the radiation energy arrives, and, according to GRAY (1953), both specific lesions and whole body effects can be modified according to the prevailing level of oxygenation. From their work on E. coli B, ALPER & HOWARD-FLANDERS (1956) showed that radiosensitivity was maximally affected by environmental oxygen tensions of the range 0 to 10 mm Hg partial pressure, wherein a two- to threefold alteration in radiosensitivity could be obtained. This work has been applied to mammalian tissues by WRIGHT & HOWARD-FLANDERS (1956) using mouse tails; by VAN DEN BRENK et coll. (1962) using mouse limbs; and by GRAY et coll. (1952) using whole body irradiation.

The malignant cell whose oxygen tension is low is able to survive doses of irradiation that would damage or destroy fully oxygenated cells. The anoxic radioresistant cell has long troubled radiotherapists because the radiocurability of a tumour depends on being able to prevent all the malignant cells repro-

From the Department of Pathology, St. Mary's Hospital Medical School, London, England. This research was supported in part by a grant from the Medical Research Council and the British Empire Cancer Campaign. Submitted for publication 22 March 1965.

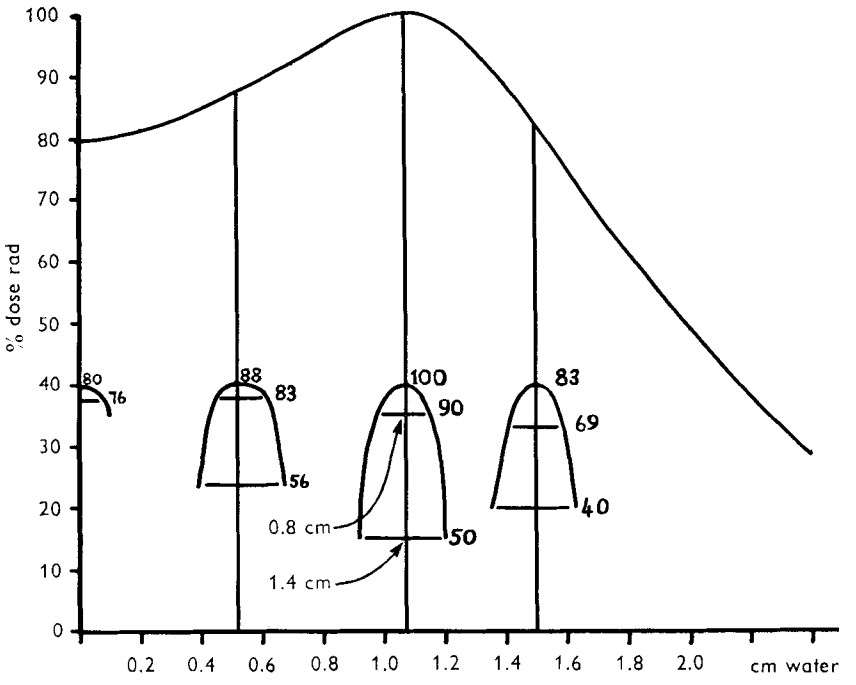

Fig. 1. Depth dose data of the electron beam showing rapid fall-off at depth.

ducing, including any anoxic cells, even though these latter may only amount to 1 % of the total malignant mass. Some workers, CHURCHILL-DAVIDSON (1957), EMERY (1960), have tried to solve the problem by subjecting the whole patient to increased partial pressures of oxygen to try and raise the oxygen content of the tumour, prior to, and during irradiation therapy. Apart from the technical problems involved in this form of pre-treatment therapy, its effectiveness is directly dependent on the efficiency with which the capillary network of the tumour transports the extra available oxygen to each and every cell in the mass. The vascular network on which this mode of therapy relies is often poorly developed. Further, whilst the rise in oxygen content of the malignant cells is possibly subject to variation, the normal tissue in the area has its radiosensitivity raised to its maximum value, which may result in more damage than can be accepted being suffered by normal structures in the region under treatment. An alternative approach is to try and take advantage of the poor vascularity of the tumour, which is the limiting factor to alterations in tissue gas tensions. Instead of attempting to raise the oxygen level of tumour and adjacent tissue, it is suggested that the normal tissue has its oxygen tension lowered by de-

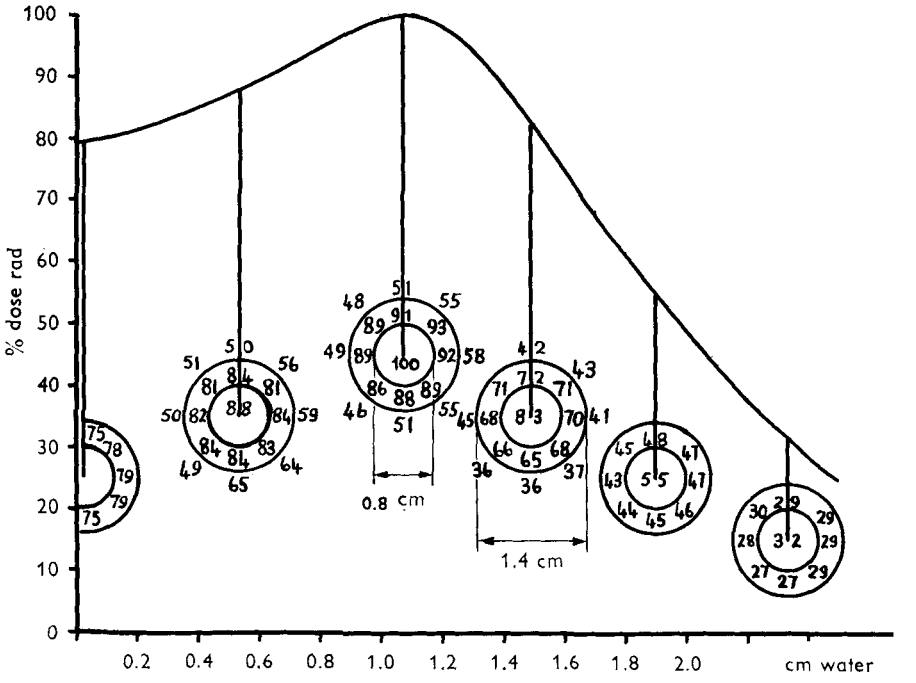

Fig. 2. Depth dose data of the electron beam showing fall-off from central axis.

priving it of its normal supply of oxygen. By virtue of its better blood supply the normal tissue can be brought into equilibrium with blood of very low oxygen content faster than the abnormal tissue can, so the tissue oxygen concentration of the normal tissue should fall to below that of the tumour tissue, provided that the normal tissue does not contain any reserves of oxygen. Thus by reversing the usual oxygen content status, the tumour is made relatively more radiosensitive than its adjacent normal tissue (WRIGHT 1960).

We have been investigating the possibility of applying such a technique to the only tissue that can quickly be made anoxic according to current methods of tissue oxygen determination, i. e. the brain. Using the brain of the rat we have attempted to determine the radiosensitivity of the cerebral tissue and the dose-modifying effect of nitrogen breathing before and during irradiation of the brain.

Materials and Methods. Two-hundred-and-thirty Wistar albino rats of both sexes, aged between 11 and 19 weeks, were subjected to electron beam irradiation from an 8 MeV linear accelerator. Anaesthesia was induced with an intraperitoneal injection of 40 mg/kilo bodyweight of methohexitone sodium,

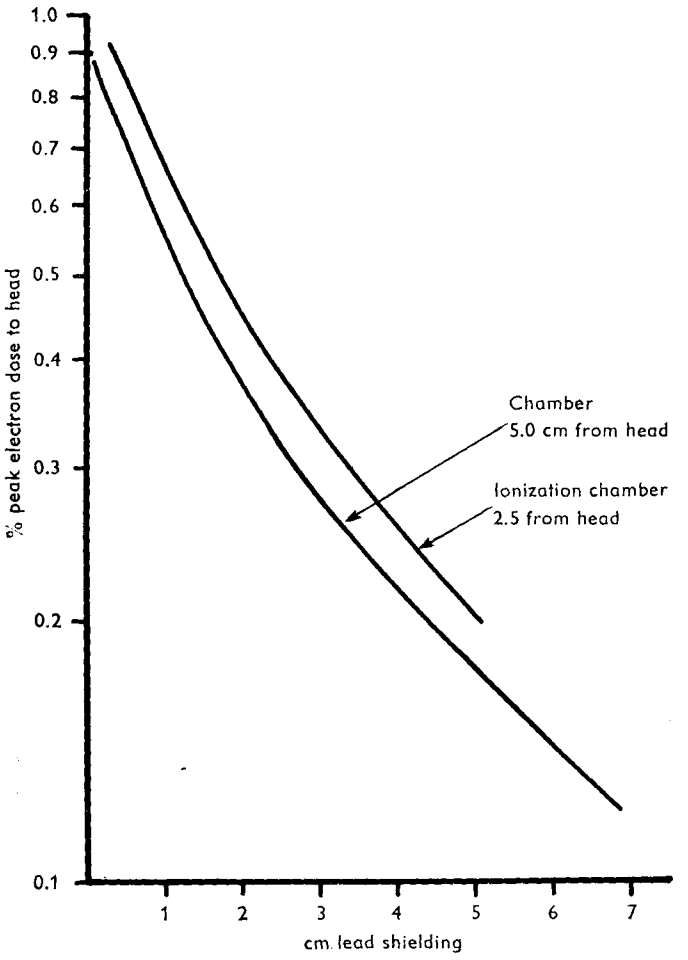

Fig. 3. Effect of lead shielding on whole body dose to rat when treating a local field on the head.

chosen for its rapidity of onset and clearance, so that respiratory depression due to the barbiturate was kept to a minimum. Anaesthesia was maintained on an air plus ether mixture delivered from a purpose-built anaesthetic machine. The machine allowed close control of the volumes of gases given to the animals by using rotameter tubes calibrated from 100 to 1 000 ml, and rapid changes could be made from one gas to another by the incorporation of solenoid controlled valves in the gas lines. The final gas mixture was administered to the rat via a close fitting face mask.

'Tissue oxygen' studies were carried out using polarographic needle elec-

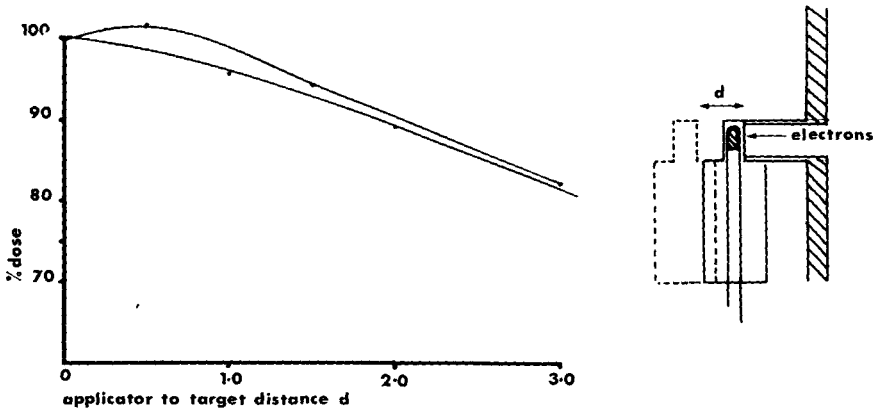

Fig. 4. Technique and dose measurements to assess final dose on increase of focal skin distance.

trodes after the type described by CATER et coll. (1957). We used 200 μ 'Diamel'-coated platinum wire mounted in the shaft of a No. 20 hypodermic needle with 'ARAL AT100/HY100' to give extra insulation. The face of the wire was ground off parallel to the bevel of the needle and polished. These recording electrodes were inserted singly into the fore brains of rats through fine burr holes made in the parietal area of the skull; a silver/silver chloride reference electrode was placed subcutaneously in one flank. A polarising voltage of 0.6 volt was used. The current developed by the system was recorded on a recording micrograph (Kipp & Zonen, type BD2 or BD3) set at 0.1 microamp sensitivity.

The source of radiation was the electron beam of the 8 MeV linear accelerator at the Radiotherapy Research Unit of the Medical Research Council, Hammersmith Hospital. This unit was chosen for two reasons. The first was that the machine could maintain a sustained high dose rate per minute with minimum 'run-up' time before peak output. Secondly, the type of radiation was in current use for therapy and research work, so that the machine was fully calibrated. The dose rate utilised was 35 kilorad per minute, using a short tunnel between the scattering foil window and the head of the animal undergoing irradiation, giving an effective focal distance of 91 cm. The exit port was an acrylic tube applicator of 1.5 cm internal diameter. This diameter was the optimum size available in relation to the rat's skull, since a smaller internal dimension would have caused too great a 'fall off' in dose on moving laterally off the central beam axis (Figs 1 and 2). These figures also show the rapid fall off at depth of the beam, which minimised radiation reactions on the

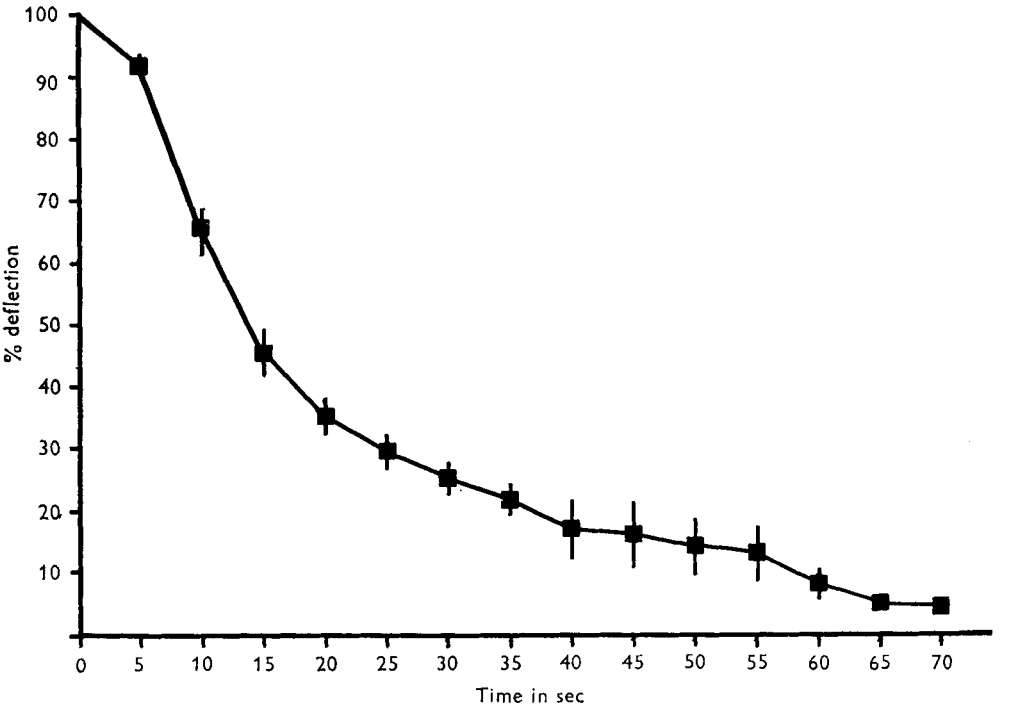

Fig. 5. Decline in current recorded from brain during nitrogen breathing.

structures in the pharyngeal region. It was anticipated that the body of the rat would receive a quantity of roentgen radiation derived from scatter during exposure of the brain to the electron beam. To assess this factor, dose measurements were made with an acrylic rat phantom containing a thimble ionisation chamber and bolus to occupy the residual air spaces. The dose on the midline of the 'rat' was recorded with the chamber at the head, 2.5 cm into the trunk from the head, and at 5.0 cm from the head. The results of this procedure, and the reduction in body dose effected by increasing the thickness of lead shielding between the wall of the applicator and the body of the phantom are shown in Fig. 3. In animal experiments involving the use of a 'tissue oxygen' electrode in the brain at the time of irradiation, it was necessary to increase the focal skin distance to allow the electrode to clear the end of the applicator.

Dose measurements were made as in Fig. 4, the final dose being increased to compensate for the extra distance. A closed circuit television unit (Pye Ltd) was used for viewing the animal during irradiation and a transducer

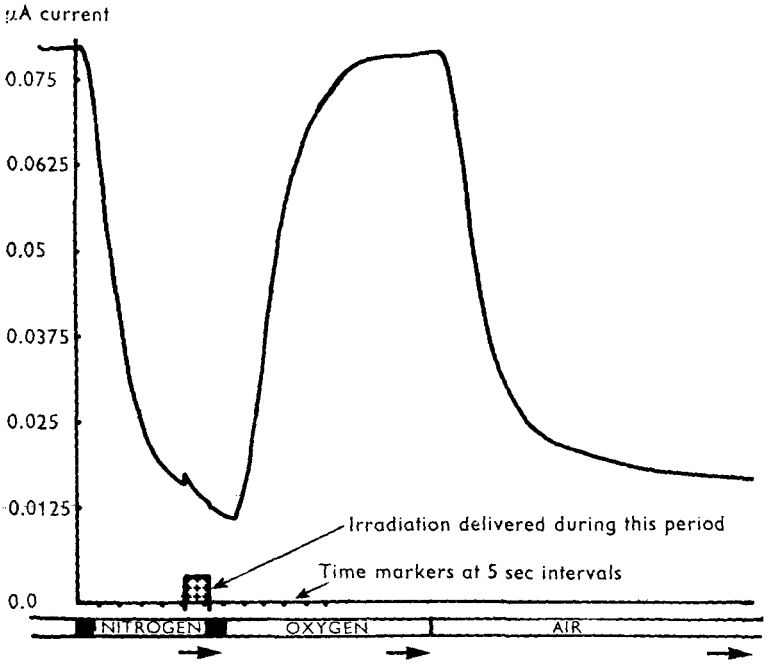

Fig. 6. Rat brain irradiation whilst breathing 100% nitrogen

(Boulton Paul Ltd, type F 16) was used to display respiratory movements on Pendeford-multimeter, C21 mark II (Boulton Paul Ltd).

Results

Preliminary investigations with the 'tissue oxygen' electrode in the brains of anaesthetized rats given various concentrations of oxygen and nitrogen to breathe, had shown that a rat could be maintained on 100 % nitrogen for between 50 and 60 seconds with a good chance of survival without any obvious respiratory or neurological sequelae.

The decline in current recorded from the brain, when animals were switched from 100 % oxygen to 100 % nitrogen as the inspired gas, are shown in Fig. 5, giving the mean values for 31 rats including plus or minus one standard error. The values are expressed as a percentage of the maximum deflection recorded with the rat breathing 100 % oxygen.

This mode of expression was chosen because the calibration of this form of electrode in terms of absolute partial pressures of oxygen is open to question. In tissue, the current recorded at the tip of the electrode is proportional to the

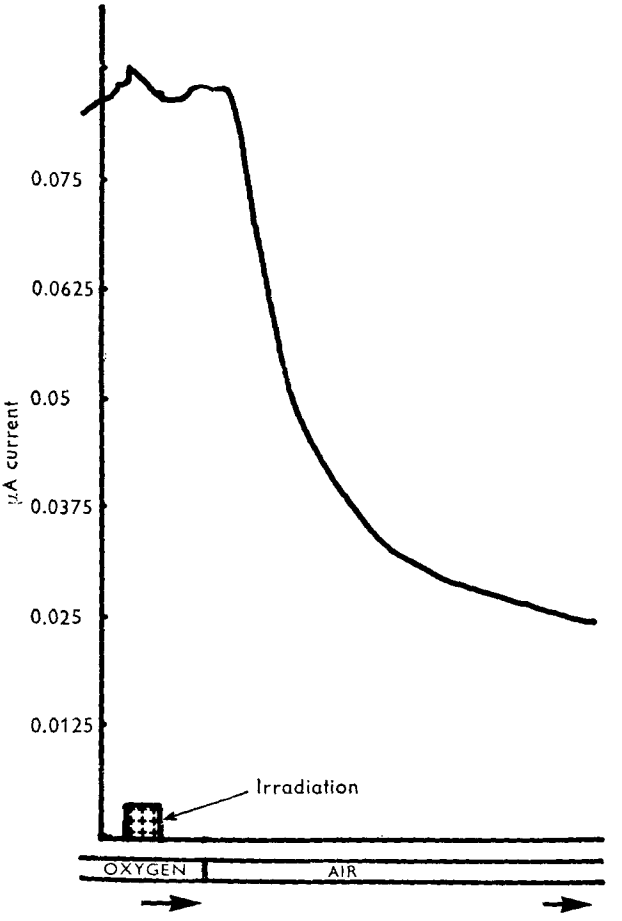

Fig. 7. Rat brain irradiation whilst breathing 100 % oxygen.

remainder of the sum: oxygen arriving at the tissue in the region of the electrode minus oxygen consumed by the tissue, and hence such types of electrode should be known as 'surplus oxygen' electrodes. Also no calibrating 'cell' has yet been devised that both supplies and consumes oxygen, so it did not seem correct to carry out direct calibration experiments in vitro and use the results for in vivo readings. We preferred to internally calibrate the electrode in the animal since our electrodes were large and gave only relative values of the 'surplus oxygen' in a large area of cerebral tissue.

Actual recordings made whilst the rat's brain was being irradiated are given in Figs 6 and 7, in Fig. 6 the course when the animal breathed nitrogen,

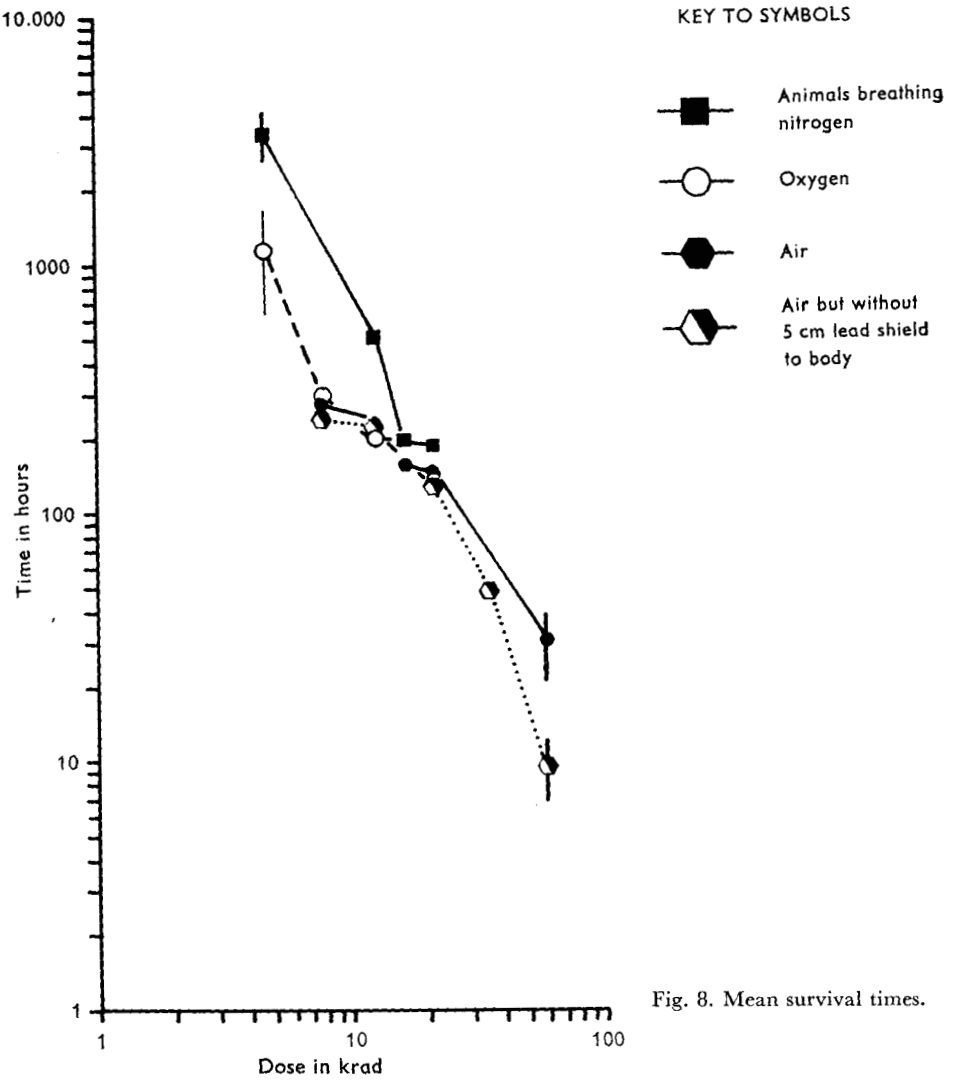

Fig. 8. Mean survival times.

and in Fig. 7 the course whilst the rat breathed 100 % oxygen during the procedure. With the dose rate available, we aimed to commence radiation after 35 seconds of 100 % nitrogen-breathing for animals irradiated with doses from 4 700 rad to 21 380 rad. These high doses were used to try and demonstrate the existence of the nitrogen dose modification on a rapid all-or-none basis. Rats were also given 35 500 rad and 58 700 rad, but only whilst breathing air.

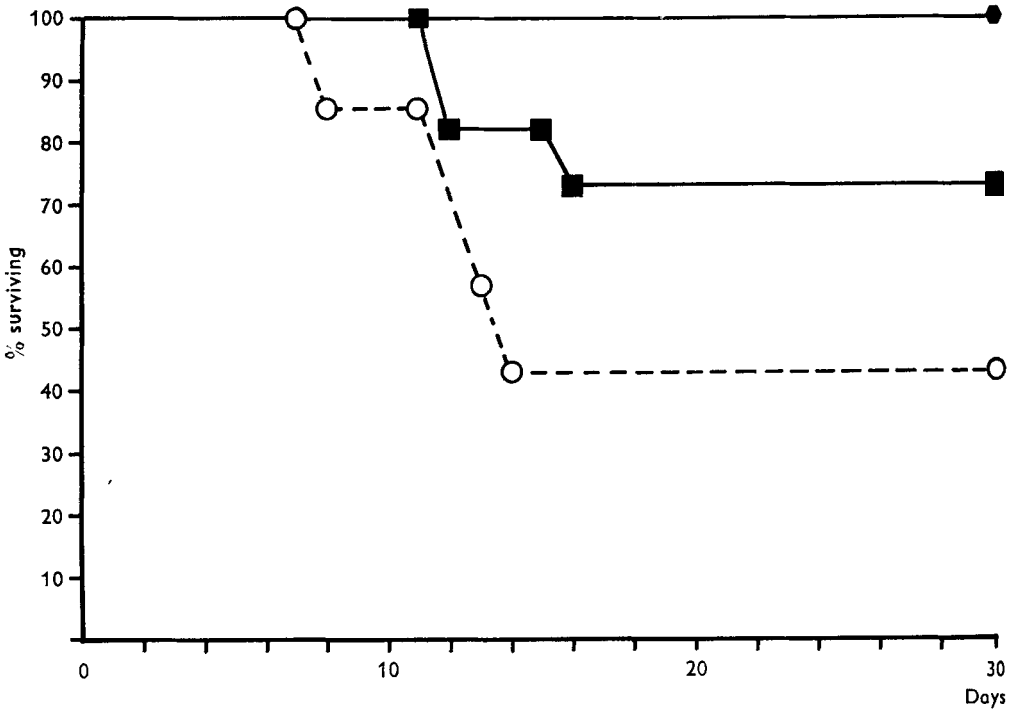

Fig. 9. 30-day survival pattern; dose to brain 4 700 rad. For key to symbols, see fig. 8.

The general shape of the mean survival time curve in Fig. 8 conforms to the pattern found by RAJEWSKY (1954) though the plateau where the survival time remained constant in spite of increased dosage of irradiation extended in our series from 8 500 rad to 20 000 rad, whereas Rajewsky's plateau ranged from only 1 200 rad to 15 000 rad. The marked shortening of the survival time after a dose of 58 700 rad with the rat breathing air, demonstrates that one has passed down the shoulder of the curve. We noted that at 35 500 rad and at 58 700 rad the presence of the extra 2 inches of lead shielding to the body of the irradiated animal exerted an effect on the survival times (Figs 3 and 8). In the dose range 16 600 rad to 21 380 rad we could not demonstrate any significant modification of the survival pattern by giving the animals nitrogen instead of air or oxygen to breathe, though the nitrogen breathers did have slightly longer mean survival times.

	Oxygen	Air	Nitrogen	
21 380 rad	136	147	186	hours
16 600 rad	192	158	197	hours

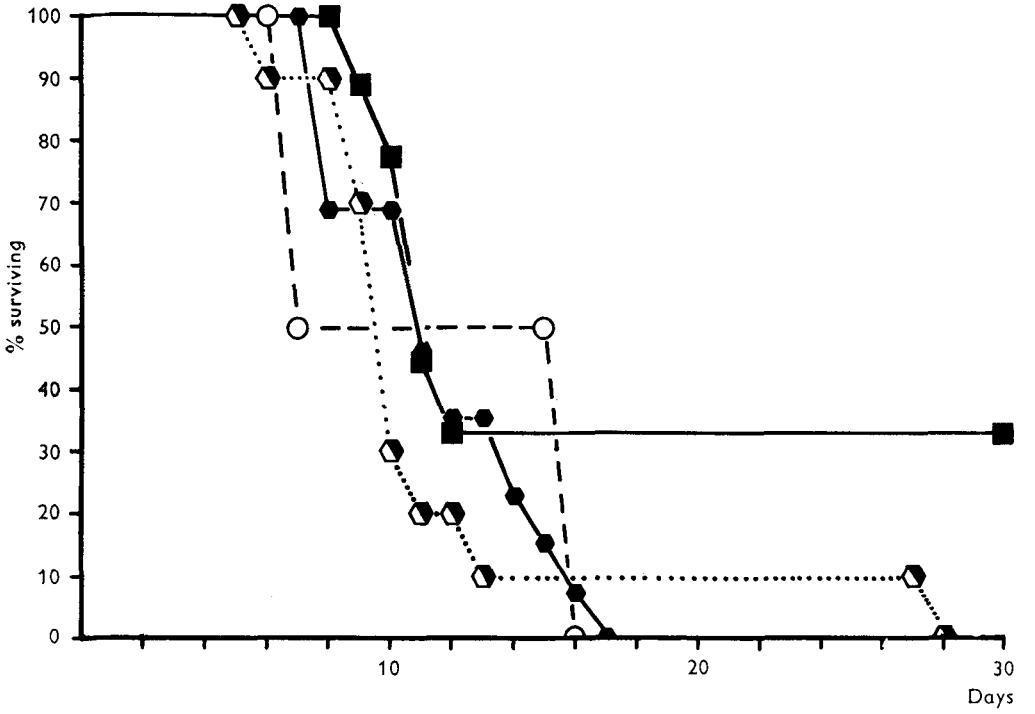

Fig. 10. 30-day survival pattern; dose to brain 7 800 rad. For key to symbols, see fig. 8.

Between 12 800 rad and 4 700 rad the oxygen and nitrogen survival lines diverge but do not run parallel to each other. There is an apparent time of survival equivalence at 6 000 rad under oxygen to 11 500 rad under nitrogen, giving a dose modification factor of 1.9.

The 30-day survival patterns, as shown in Figs 9 to 12, disclose that 43 % of the animals survived 30 days after receiving a dose of 4 700 rad whilst breathing oxygen at the time of irradiation, whereas 41 % of the animals who breathed nitrogen at the time of irradiation survived 30 days after a dose of 12 800 rad, a dose modification factor in this case of 2.7.

Discussion

There is no doubt that a sufficient lowering of the oxygen content of cells results in a modification of the effects of irradiation, allowing the cell to survive incident doses of irradiation that would kill a fully oxygenated cell. This effect

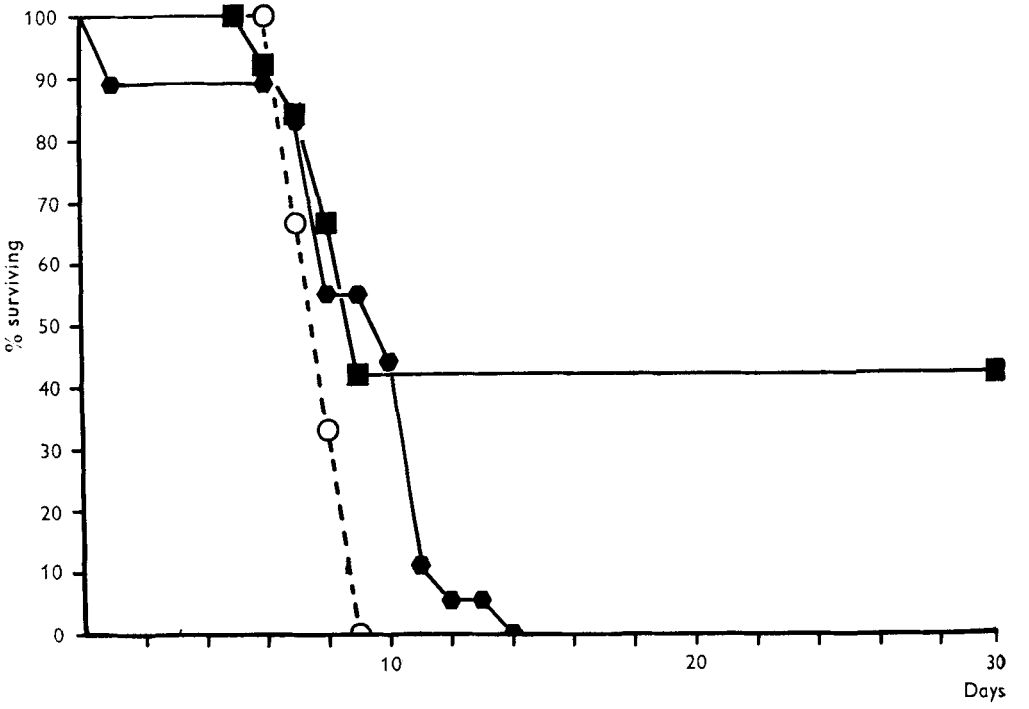

Fig. 11. 30-day survival pattern; dose to brain 12 300 rad. For key to symbols, see fig. 8.

was first demonstrated by HOLTHUSEN (1921) who showed that Ascaris eggs in the anaerobic state required three times as much roentgen radiation to prevent their hatching as eggs irradiated under aerobic conditions. Similar results have been obtained with isolated cell cultures, single organs such as thymus or tail, and for whole body irradiation. VAN DEN BRENK (1963) in his work on tourniquet anoxia accepts a figure of 2.7. From our results with rats' brains we have been unable to demonstrate a consistent degree of modification of the effect of irradiation by anoxia. The 25-day survival equivalence of 6 000 rad in oxygen to 11 500 rad in nitrogen represents only a modification of 1.9, and the alteration in 30-day survival only held for 4 700 rad in oxygen to 12 800 rad in nitrogen. This improvement in survival would hardly be considered worthwile from a tumour therapy standpoint, even though our doses are rather beyond the accepted therapeutic range.

As to the inconsistency in our results, a simple explanation could be that we didn't render the brains of our rats sufficiently anoxic before commencing irradiation. As 83 % of the animals that were irradiated whilst breathing

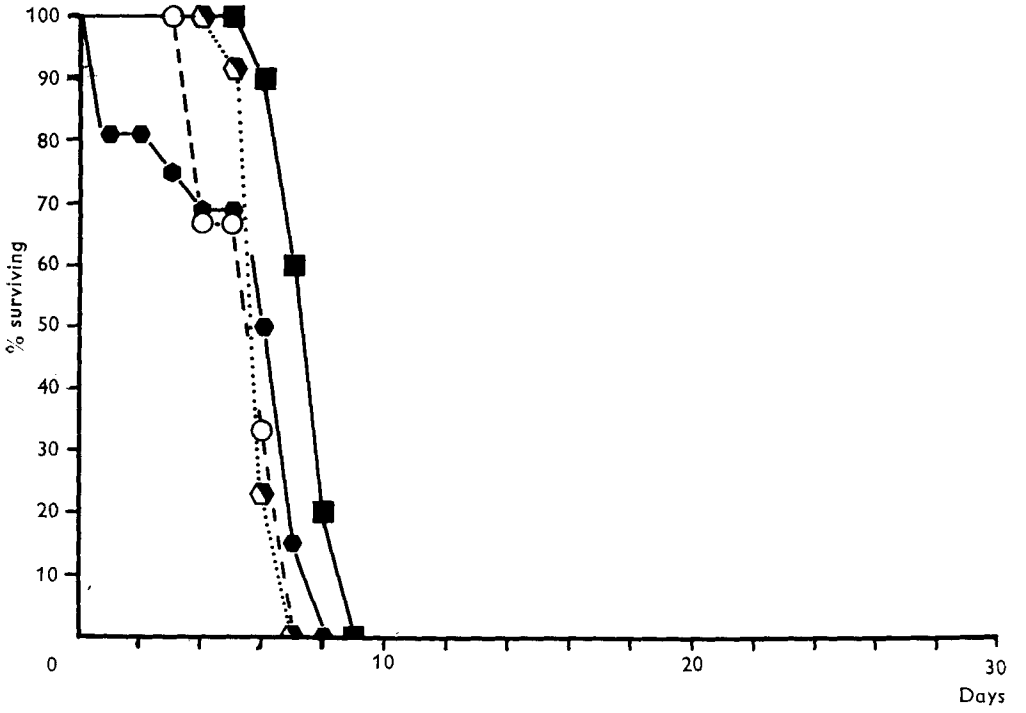

Fig. 12. 30-day survival pattern; dose to brain 21 380 rad. For key to symbols, see fig. 8.

nitrogen stopped breathing before the end of irradiation, and required revival by artificial respiration, we think that our rats reached critically low levels of oxygen tension, for some groups incurred a 10 % mortality because the animals didn't restart respiration. In our hands, 60 seconds of breathing 100 % nitrogen was the tolerable limit that would give the rat a good chance of survival, for to try and increase this time was to risk killing the brain tissue by hypoxia alone, which would have confused the situation. Readings from our platinum electrodes show that the brain quickly becomes anoxic when the animal is given nitrogen to breathe. DAVIES & BRONK (1957), using more refined methods, claim that cerebral tissue is always on the brink of oxygen lack. CROSS & SILVER (1962) have shown that there is some variation in the current recordable, and hence in 'tissue oxygen concentration' even in closely related parts of the brain. Our experience confirms this. We had hoped to use the polarographic system as a monitor to indicate when irradiation could commence, but in irradiated rats the prolongation of anaesthesia necessary to

effect suitable placement of the electrodes caused further hazard to the animals. Precise information of the actual levels of oxygen concentration in cerebral cells is not available, and one must avoid the circular argument that brain tissue can only be truly anoxic when dose modification can be demonstrated. The question awaits the introduction of a method of measuring intracellular oxygen tensions that do not interfere with the cell's metabolism, and can be accurately calibrated in terms of partial pressures of oxygen with values directly referable to cells in tissue.

The mechanism whereby irradiation causes death is another factor that must be considered. Death of the brain following irradiation can be grossly divided into that caused by destruction of brain cells or permanent impairment of their metabolism, and that caused by alterations to the blood supply. These factors cannot readily be separated though one series of changes may appear to dominate. Death from the high doses of irradiation with the animal surviving only a few hours or days is thought to be primarily due to death of brain cells although this is uncertain. In the symposium on the 'Response of the nervous system to ionizing radiation' (1962), no one seemed to be able to give a definite opinion supported by experimental evidence. The brains of our rats who died after high doses of irradiation showed marked oedema and small areas of haemorrhage. When death occurred months after a lower dose of irradiation the blood vessels showed more extensive changes with thrombus formation. The brain substance was markedly necrotic in both grades of irradiation. Therefore anoxia, to be truly effective, must protect not only the cells comprising the brain tissue, but also the blood vessels from the effects of irradiation. The brain is at a disadvantage compared to other tissues following trauma of any form, since it cannot replace dead neurons with new cells of the same function. It may be that nitrogen does protect a percentage of cells in any tissue, which, if these can grow and divide and replace the dead cells, can restore full function to the tissue, but that in the brain the percentage of cells surviving is not high enough to ensure continued function of the brain as an integrated unit.

Since this paper was prepared, the dose range has been extended to include groups irradiated in air, oxygen, or nitrogen at 1 010 rad and 2 030 rad. All groups show a 100 per cent 30-day survival.

Acknowledgements

We wish to thank Dr E. A. Wright for his guidance and Mr P. D. Laming for his technical assistance. We also thank Mrs A. Silvester (Hammersmith Hospital) for her preparation of the physical dose measurements and Mr G. Harding for his help with the linear accelerator.

SUMMARY

The brains of rats have been irradiated with 8 MeV electrons whilst the animals breathed 100 % nitrogen before and during irradiation. No consistent degree of dose modification effect by nitrogen in the dose range 4 700 rad to 21 380 rad was shown. The level of hypoxia necessary to achieve dose modification is thought to be too close to that which causes brain cell death by itself. The inability of the brain to regenerate functional neurons is probably another factor.

ZUSAMMENFASSUNG

Die Gehirne von Ratten wurden mit 8 MeV Elektronen bestrahlt, wobei die Tiere vor und während der Bestrahlung 100 % Stickstoff einatmeten. Bei Stickstoffatmung ergab sich im Bereich von 4 700 bis 21 380 rad kein bestehender Unterschied. Es wird vermutet, dass der Grad von Hypoxie, der notwendig wäre um Dosismodifikationen wirksam zu machen, zu nahe dem Zustand ist, der den Zelltod selbst zur Folge hat. Die Unfähigkeit des Gehirnes funktionelle Neurone zu regenerieren, ist wahrscheinlich ein weiterer Faktor.

RÉSUMÉ

Le cerveau de rats a été irradié par des électrons de 8 MeV pendant que ces animaux respiraient de l'azote pur avant et pendant l'irradiation. On n'a pas observé de modification constante de la dose sous l'effet de l'azote entre 4 700 et 21 380 rad. Les auteurs pensent que le degré d'hypoxie nécessaire pour entraîner des changements de dose est trop proche de celui qui, par lui-même, cause la mort des cellules cérébrales. L'incapacité du cerveau à régénérer des neurones fonctionnels est probablement un autre facteur.

REFERENCES

ALPER T. and HOWARD-FLANDERS P.: Role of oxygen in modifying the radiosensitivity of E. Coli B. Nature 178 (1956), 978.

CATER D. B., PHILLIPS A. F. and SILVER I. A.: Apparatus and techniques for the measurements of oxidation-reduction potentials, pH and oxygen tension in vivo. Proc. roy. Soc. B. 146 (1957), 289.

CHURCHILL-DAVIDSON I., SANGER C. and THOMLINSON R. H.: Oxygenation in radiotherapy. II. Brit. J. Radiol. 30 (1957), 406.

CROSS B. A. and SILVER I. A.: Some factors affecting oxygen tension in the brain and other organs. Proc. roy. Soc. B. 156 (1962), 483.

DAVIES P. W. and BRONK D. W.: Oxygen tension in mammalian brain. Fed. Proc. 16 (1957), 689.

EMERY E. W., LUCAS B. G. B. and WILLIAMS K. G.: Technique of irradiation of conscious patients under increased oxygen pressure. Lancet 1 (1960), 248.

GRAY J. L., TEW J. T. and JENSEN H.: Protective effect of Serotonin and of para-aminopropiophenone against lethal doses of radiation. Proc. Soc. exp. Biol. (N. Y.) 80 (1952), 604.

GRAY L. H., CONGER A. D., EBERT et coll.: The concentration of oxygen dissolved in tissues at the time of irradiation as a factor in radiotherapy. Brit. J. Radiol. 26 (1953), 638.

HALEY T. J. and SNIDER R. S. (editors): Response of the Central nervous system to ionizing radiation. Academic Press, New York and London 1962.

HOLTHUSEN H.: Beiträge zur Biologie der Strahlenwirkung: Untersuchungen an Askarideneiern. Pflügers Arch. ges. Physiol. 187 (1921), 1.

RAJEWSKY B.: Radiation death in mammals. Radiobiology Symposium, p. 81. Butterworth's Scientific Publ., London 1954.

VAN DEN BRENK H. A. S., MADIGAN J. P., KERR R. C. et coll.: The treatment of malignant disease of the extremities by megavoltage irradiation under tourniquet anoxia. J. roy. Coll. Radiol. Aust. 7 (1963), 142.

— ELLIOTT K. and HUTCHINGS H.: Effect of single and fractionated doses of X-rays on radiocurability of solid Ehrlich tumour and tissue reactions in vivo for different oxygen tensions. Brit. J. Cancer 16 (1962), 518.

WRIGHT E. A.: Experimental radiotherapy of anoxic mouse tumours. Brit. J. Radiol. 33 (1960), 657.

— and HOWARD-FLANDERS P.: The use of growing mouse tail as a test object in radiobiology, p. 449. Progr. in Radiobiol. (Editors: Mitchell & Holmes & Smith). Oliver & Boyd, Liverpool 1956.