Second Australasian Conference on RADIATION BIOLOGY

MARTIN

RADIATION BIOLOGY

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FOREWORD

SIR F. MACFARLANE BURNET, O.M., F.R.S.

Opening remarks to the Conference, December 15, 1958

INTRODUCTION

THIS Conference is concerned with the action of ionizing radiation on living cells. Its objectives are purely scientific and, in one sense, it is only indirectly concerned with the human implications of radiobiology.

But in my position, I feel that it is necessary that I should underline those implications. Radiation of the sort we are interested in is perhaps the most two-edged of all weapons. We all know the medical and industrial benefits of the use of X-rays and natural and artificial radio-active substances. And equally we are aware of the dangers that, in one way or another, are associated with atomic radiation.

I shall say nothing about the value and the problems of radio-therapy for cancer and other diseases, though much of the programme will, of course, be relevant to this field.

But I should like to speak for a few minutes on the problem of assessing the dangers of radiation to human beings.

* * *

At the present time we are all only too conscious of the dangers of radiation—and if there is one practical task for radiobiology it is to provide a realistic understanding of the extent of the danger—and to express this in terms that can be clearly understood by anyone.

It is becoming a common practice in scientific publication to put a summary of conclusions at the beginning of the paper and I think that it will make my position clearer if I start with two simple statements which I believe do express the practical essence of current thought on radiobiological hazards.

1. That a major war fought with nuclear weapons would be an unimaginable calamity, resulting in the complete destruction of Western civilization and the death, directly or indirectly, of probably more than half the human population of the world and a tremendous increase in the burden of genetic damage carried by the survivors. The prevention of such a war is the overriding political and social necessity of our time.

2. That the danger associated with limited exposure to ionizing radiation, whether from cosmic rays and other natural sources, fall-out from test explosions, the medical uses of X-rays, or from work in laboratories and industrial establishments concerned with atomic power, is unimportant in comparison with the ordinary hazards of life.

I should like to elaborate the second of those statements because it is within its field that most of the work to be discussed at this conference will have its human relevance.

It is the duty of any medical officer of health to do his utmost to ensure that no unnecessary illnesses or deaths should occur within the community for which he is responsible. If he has the final responsibility of a whole nation of 10 million people, he will still regard it as very serious if even five individuals die from bubonic plague, or hydrophobia, from explosion of industrial boilers, or from massive exposure to a source of atomic radiation. These are all things which should *never* cause death in a country like Australia. If they do, something is wrong which must be righted immediately. But in that same nation approximately 120,000 persons covering every age will die each year from causes distributed over all ages and showing a fairly uniform pattern, so many from cancer, so many from accident, and so on. The vast majority of these are not in any realistic sense of the word preventable.

Death is inevitable and in any community likelihood of death has a characteristic pattern in relation to age. This can be simply expressed for any age as the chance of dying within the next 12 months. At birth, in Australia it is of the order of 1:40 but once the first year is past falls steadily till it reaches a minimum at the age of 12, when it is 1:2500. Thereafter it climbs slowly to about 1 per cent, *i.e.* 1:100 at the age of 50 and then progressively more steeply to the end of life.

People of my age know that there is a chance lying between 1 and 2 per cent that they will be dead a year from now. They also know that to drive a car 5000 miles in the year involves a risk of dying by road accident of the order of 1:1000 and that if one flies 20,000 miles there is a further risk of the order of 1:10,000 of death in an air crash. Neither adds significantly to the ordinary hazards of life and in neither case does the knowledge of the risk modify our behaviour. The product of the risk, multiplied by the number of people in the country, however, is quite large enough to justify plenty of thought and action to maintain and improve road and air safety.

In the radiation field, we have a reasonably accurate knowledge of the dose of radiation that will kill a man within a week or two and we know that to a fair approximation a very large dose of X-rays given for the treatment of ankylosing spondylitis may induce fatal leukaemia in about 1:1000 of the patients treated, the actual likelihood being directly related to the size of the dose. Virtually all the rest is inference. But at least we know that all the changes we talk about, medical diagnostic X-rays, fall-out from bomb tests, *etc.*, involve vastly smaller amounts of radiation than those I have mentioned.

It is a curious situation that one of the major political controversies of the twentieth century, the danger to mankind of radio-active fall-out from bomb tests, cannot be resolved in scientifically acceptable terms—and that in an attempt to clarify the fall-out situation people have been made seriously afraid of undergoing medically necessary X-ray examinations—again without any adequate scientific basis for their fear.

There are many things to be found out about the interactions of ionizing radiation and living cells and I hope that contributions made at this conference will provide much of interest and value. But I am certain that most of us will listen particularly for anything that is relevant to the half-dozen major questions which must be answered if we are ever to resolve our current practical perplexities. At the risk of restating the obvious, I shall put these questions in abbreviated form.

1. Does the genetic effect of radiation show a linear dose–effect relationship down to the levels of natural background and below?

2. If not, how does the genetic effect of relatively small amounts of ionizing radiation on a standard human being vary with the total dosage, its physical quality and the time course of its administration?

3. What is the relative susceptibility to genetic damage of the foetus, the child and the adult?

4. Are the carcinogenic and leukaemogenic effects of radiation due essentially to the same mutagenic processes as are responsible for genetic damage? If so, are secondary 'promoting' factors also concerned in allowing the emergence of overt disease? Or, as Kaplan has suggested, is the whole effect unrelated to mutagenic action of radiation?

5. Whatever the process of carcinogenesis, knowledge on the influence of dose, quality and rate of administration, and of the relative susceptibility at different ages will be needed.

Until these questions can be answered, I believe we are justified in accepting some empirical rules for action:

1. All ionizing radiation is potentially harmful and the degree of exposure should be reduced to the lowest possible level that is consistent with the fulfilment of acceptable medical, industrial or military needs.

2. Exposure to artificial radiation not greater than the dosage always being received from natural sources is of no significance in comparison with normal hazards of life.

3. The potential benefit of any intelligent medical use of X-rays will far outweigh any risk of radiation damage.

4. The accepted international levels of permitted exposure in nuclear laboratories, *etc.*, form a reasonable basis for action.

It is the task of conferences such as this to provide the background of quantitative knowledge that will allow us eventually to replace these empirical rules with scientifically valid recommendations. Only then are we likely to escape from the emotionally charged atmosphere of suspicion, controversy and misunderstanding that seems to rise in a stifling cloud whenever radiation hazards become a topic of political discussion.

I look forward very much to hearing the papers and discussions of this Conference. I am confident that it will help to stimulate research in Australia and aid understanding of the impact of radiobiology on human affairs.

I have much pleasure in declaring the Conference open.

CONTENTS

1

RADIATION DOSE—MACROSCOPIC, MICROSCOPIC AND SUB-MICROSCOPIC ASPECTS

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FOR the purpose of evaluating the physical, chemical, and biological effects of the interaction of radiation with matter, it is necessary to have some measure of the radiation, or its interaction, in physical terms.

The effects of radiation on a cell, or a tissue, are, of course, due to the radiation which is absorbed by the cell and independent of the radiation which happens to pass through the cell. It follows that for biological purposes the most suitable physical quantity can be defined in terms of energy imparted by ionizing particles to matter at the place of interest. It is in terms of this quantity that absorbed dose has been defined internationally[1].

> 'Report of the International Commission on Radiological Units and Measurements (I.C.R.U.) 1956. Handbook 62.
> 1.1. Absorbed dose of any ionizing radiation is the energy imparted to matter by ionizing particles per unit mass of irradiated material at the place of interest.
> 1.2. The unit of absorbed dose is the rad. 1 rad is 100 ergs/g.'

There is another physical quantity which is useful for the description of radiological phenomena, namely the photon or neutron energy which would, through interaction with matter, become transformed into the energy of ionizing particles originating in a given quantity of matter, divided by its mass. This quantity has not hitherto been explicitly defined or named by the International Commission. There might be certain advantages in introducing such a definition to describe the interaction between radiation and matter quite generally, in terms of energy and mass, in some such manner as that suggested above. As this is at present under consideration by the Commission, it would be inappropriate to discuss it further here. It will readily be seen that in the restricted case of the interaction of photon radiation with air, this quantity is essentially that which has been defined by the Commission as 'exposure dose'. It is the quantity of which the roentgen is a unit. The relation between absorbed dose and exposure dose, as at present defined, has been discussed elsewhere[2].

The energy defined by the absorbed dose is imparted to matter in the form of energy transfers between the moving charged particle and orbital electrons. These transfers result, in the first instance, in molecular excitation and ionization, and secondarily, in chemical change and heat.

The absorbed energy is usually about equally divided between ionization and excitation, but we have as yet no precise information concerning the relative magnitudes of these two forms of energy transfer in condensed systems. In certain gases, rather accurate information is available, and it is the fact that the average energy, W, expended by ionizing particles per ion-pair formed in gases is nearly independent of the speed of the particles, within very wide limits, which has made ionization a useful measure of absorbed energy.

By the application of the cavity theory of ionization, and the use of appropriate physical constants[1], the absorbed dose in a solid or liquid may be inferred from a measurement of gas ionization in a small cavity with an uncertainty which only slightly exceeds the uncertainty in our knowledge of W. The value of W for air which is recommended by the International Commission, is 34 eV for X- and γ-ray dosimetry, and 35 eV for neutron dosimetry. *Table 1*, compiled by Boag[3], shows the considerable measure of agreement between the recent determinations of W.

Table 1. The average energy expended by electrons per ion-pair formed in air

Author	*Radiation*	*W air* eV	*Standard error* eV
Emery[a]		33·5	Not given
Gross *et al.*[b]	^{35}S β	33·6	0·3
Bay *et al.*[c]	^{35}S β	33·7	0·3
Barber[d]	1 to 35 MeV electrons	33·8	1·2
Jesse and Sadauskise	^{3}H β	33·9	Not given
,, ,, ,,	^{63}Ni β	34·0	Not given
Weiss and Bernsteinf	2 MeV X-rays	33·9	0·8
Bernier *et al.*[g]	^{60}Co γ-rays	33·0	0·3
Skarsgard *et al.*[h]	22 MeV X-rays	32·8	0·6

a. Emery, E. W. *Brit. J. Radiol. N.S.* 29 (1956) 370
b. Gross, W., Wingate, C. and Failla, G. *Radiation Res.* 7 (1957) 570
c. Bay, Z., Mann, W. B. Seliger, H. H. and Wyckoff, H. O. *Radiation Res.* 7 (1957) 558
d. Barber, W. C. *Phys. Rev.* 97 (1955) 1071
e. Jesse, W. P. and Sadauskis, J. *Phys. Rev.* 97 (1955) 1668
f. Weiss, J. and Bernstein, W. *Phys. Rev.* 103 (1956) 1253
g. Bernier, J. P., Skarsgard, L. D., Cormack, D. V. and Johns, H. E. *Radiation Res.* 5 (1956) 613
h. Skarsgard, L. D., Bernier, J P., Cormack, D. V. and Johns, H. E. *Radiation Res.* 7 (1957) 217

Since the difference between the mean of these experimental values and 34 eV is still doubtfully significant, and the precise evaluation of W for particles of different speed is still under investigation in a number of laboratories, the International Commission recommends that the value for W of 34 eV should still be used in the interests of uniformity.

When we attempt to analyse dose response relationships in biological materials account has to be taken of the fact that energy is not delivered uniformly to matter, but discontinuously along the tracks of individual ionizing particles. Nearly 50 years ago C. T. R. Wilson[4], who celebrates his 90th birthday this year, made his first cloud chamber expansion in the presence of X-rays, and wrote, 'with little expectation of success, and in making an expansion of the proper magnitude for condensation on the ions while the

air was exposed to the rays, I was delighted to see the cloud chamber filled with little wisps and threads of clouds—the tracks of electrons, ejected by the action of the rays'. Photographs of these tracks, scaled down in the ratio of the electron stopping powers of gas and tissue, still provide us with the best available information as to the approximate distribution of ionizing events in matter exposed to all types of ionizing radiation. We are still not too sure of the precise value of the scaling factor because we do not know the exact value of the average energy expended per ion-pair produced in condensed systems. Moreover, these photographs tell us nothing about the location of the excited molecules which occur along the tracks with greater frequency than the ions. Nevertheless, photographs of the type reproduced in *Figures 1 and 2*

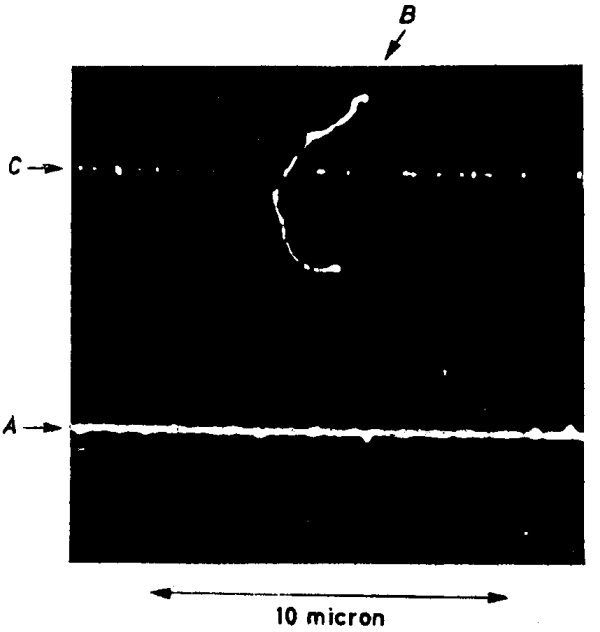

Figure 1. A, proton projected by a neutron; B, slow electron (20 kV) X-ray; C, fast electron (200 kV) γ-ray

suffice as a basis for the discussion of the physical factors to which differences between biological response to equal doses delivered by fast and by slow ionizing particles must be related. They provide us with a picture of the dose distribution within the living cell at the level of resolution of the electron-microscope.

The most obvious features of such pictures are the large amounts of empty space and the comparatively small number of particles which contribute to the energy deposited within a cell exposed to moderate doses of radiation. It is also important to note that the slower the particle, the higher the rate of loss of energy along the track, and the smaller the total number of tracks which contribute to a given dose. This has important biological consequences.

Consider, for example, the nucleus of a cell about 10 microns in diameter exposed to 25 rad of soft X radiation(Ag_K). The ionizing particles in this case will be photo-electrons having an energy of 21 kV and range slightly

smaller than the diameter of the nucleus. The dissipation of 21 KeV ($3{\cdot}4\ 10^{-8}$ ergs) in a sphere 10 microns in diameter represents an energy dissipation of 67 ergs per g, or 0·67 rad. It therefore requires $\frac{25}{0{\cdot}67}$, or approximately

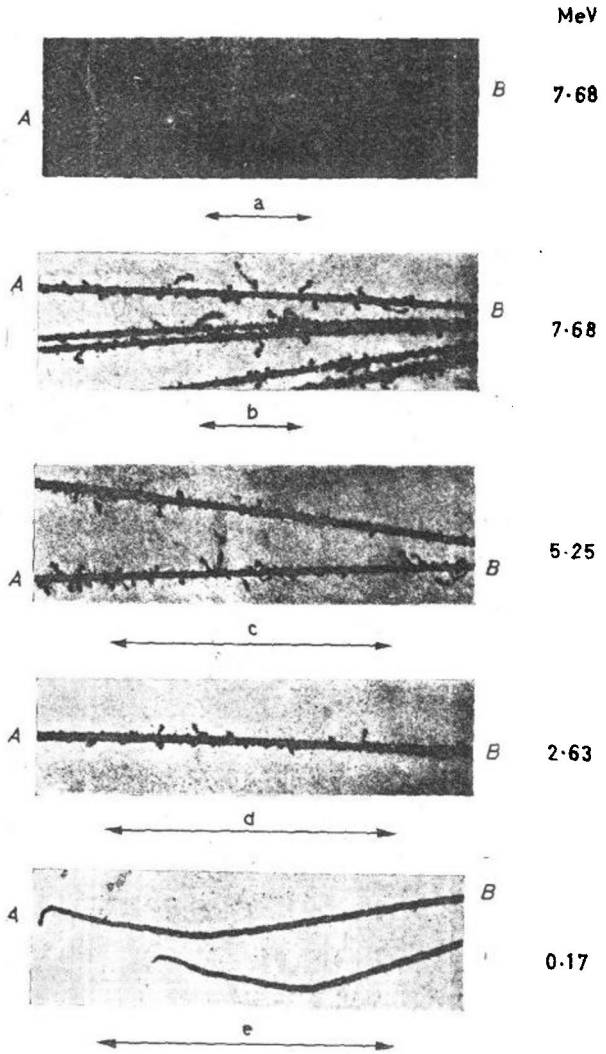

Figure 2. Wilson cloud chamber photographs of α particles showing δ-rays

(Reproduced by kind permission of T. Alper from Z. Phys. 76 (1932) 172)

36 particles, to produce a dose of 25 rad. If we consider a large number of nuclei exposed to this dose, the number of photo-electrons crossing individual nuclei will be distributed about the mean, in accordance with the Poisson formula, and the standard deviation in the number of particles per nucleus

will be $\sqrt{36}$—(6). There will thus be appreciable and possibly important differences between the quantities of energy dissipated in each nucleus, but the chance that any given nucleus altogether escapes ionization is e^{-36}, which is quite negligible. Suppose, on the other hand, that the total dose were that corresponding to the maximum permissible weekly exposure, namely 0·3 rad, the mean number of particles will then be $\frac{0{\cdot}3}{0{\cdot}67} = 0{\cdot}45$ and no energy at all will be deposited in a fraction $e^{-0{\cdot}45}$, or 64 per cent of the nuclei. Again, if the total dose were 25 rad but the radiation were α radiation instead of X radiation, the nuclei would be traversed by an average of one particle and e^{-1}, or 37 per cent of the nuclei would be unaffected. Thus, whether or not the statistical aspects of dose are important in any given case would be dependent on:

(*1*) the size of the element of the tissue under consideration,
(*2*) the magnitude of the dose, and
(*3*) the type of radiation.

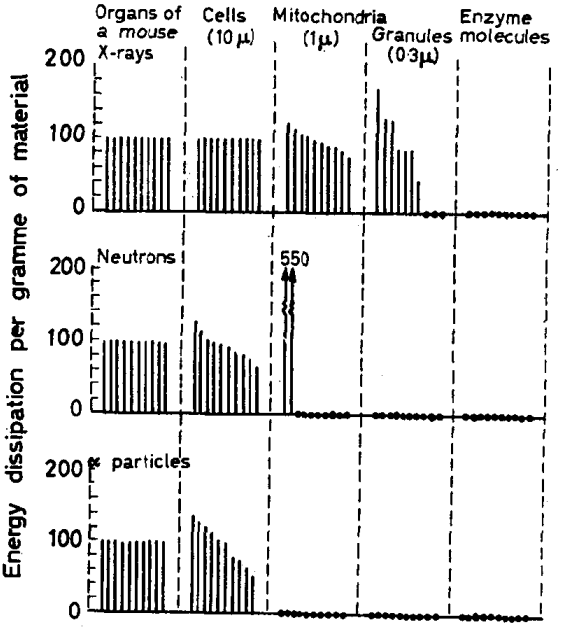

Figure 3

Figure 3 shows the actual estimated amount of energy deposited in each of 10 elements of tissue of the same size when exposed to 100 rad of X-rays, neutrons, or α-particles respectively. It is evident that in general 10 mitochondria or smaller particles could be selected at random from a group exposed to 100 rad of α-particles, and none would have been ionized. In the case of objects exposed to neutrons, a similar situation obtains for particles of 0·3 μ in diameter and less. In the case of X-rays, the critical size in relation to the dose of 100 rad occurs at the level of macromolecular dimensions.

The above statements all refer to the direct deposition of energy by ionizing particles within the objects under consideration. Some of the energy

transfers between the ionizing particle and orbital electrons give rise to the production of free radicals and to new stable molecular configurations. A proportion of these may be able to diffuse away from the track to distances determined by their lifetime and chemical reactivity. They can, in principle, react selectively with certain types of biological molecule, thus affecting a much larger proportion of these particular kinds of molecule than can be directly ionized by the moving particle. It is known that such indirect inactivations take place in dilute aqueous solutions of enzymes, desoxyribose nucleic acid (DNA) and other molecules of biological importance. We have only meagre evidence as to the extent in which such indirect inactivations take place in the living cell. Hutchinson and his colleagues[5,6] have concluded that in the living yeast cell the effective diffusion range of products formed by ionizing particles is of the order of 30 Å. Thus, a dehydrogenase molecule which has a radius of 36 Å is inactivated by energy deposited within a region about twice its own volume, and Co-enzyme A, of radius 6 Å, by energy deposited within 60 times its own volume. Such allowances for indirect inactivation do not significantly alter the statistical aspects of dose for any structure larger than an enzyme molecule.

Biological response may be influenced by dose rate as well as by dose. On a macroscopic scale, a cell irradiated at constant dose rate is being continuously exposed to injurious agents and a level of damage is reached which is a balance between the rate of injury and the rate of recovery. There is, however, a statistical aspect of dose rate as well as dose. In terms of particles, a volume element of the cell is discontinuously affected by events which are randomly distributed in time as well as in space. A dose-rate dependence may thus arise from interactions between successive particles which pass through the same volume element. These interactions may be at the chemical level, in which case the time constant which describes the dose-rate dependence will be related to the lifetime of intermediate species, as discussed by Lea[7], and exemplified by the experiments of Chapiro[8], Ghormley[9], Sutton and Rotblat[10], and others.

Alternatively, the time constant may be related to cell metabolism, as is thought to be the case when aberrant chromosome configurations are produced by the union of two or more tracks produced by different ionizing particles[11,12,13]. Clearly, in the case of any form of biological damage which arises in a uniform population of cells from the action of a single ionizing particle, biological response cannot be dose-rate dependent. Dose-rate dependence may, however, be observed even when injuries are induced by single particles if the population itself is heterogenous and changing with time in such a way that the distribution in sensitivity among the individual cells depends on the duration of exposure.

One example of the influence of dose rate on biological response is given in *Figure 4 (a)* which reproduces the experimentally observed growth inhibition in *Vicia* roots exposed to γ radiation at different dose rates. Each member of the family of curves corresponds to a constant exposure time. It is seen that a given dose was most effective when delivered in 8 min. Longer exposures were less effective, but prolongation of the duration of exposure from 12 to 24 hours resulted in no further decrease in biological effectiveness. It will also be seen that there is an interdependence between dose and dose

rate in that the duration of exposure has no influence on the degree of biological damage sustained by roots exposed to less than about 50 rad. The dose required to produce three different levels of biological damage is shown as a function of duration of exposure in curves *A*, *B*, and *C*, of *Figure 4* (*b*). A logarithmic plot of minimum growth rate relative to that of control roots (*Figure 5*) shows that the dose-response relationship is exponential in the case of 12- and 24-hour exposures, but sigmoidal for shorter exposures. The shapes of the sigmoidal curves for both X and γ radiation are dose-rate dependent, but the exponential curve is dose-rate independent, as would be expected for an injury induced by a single particle.

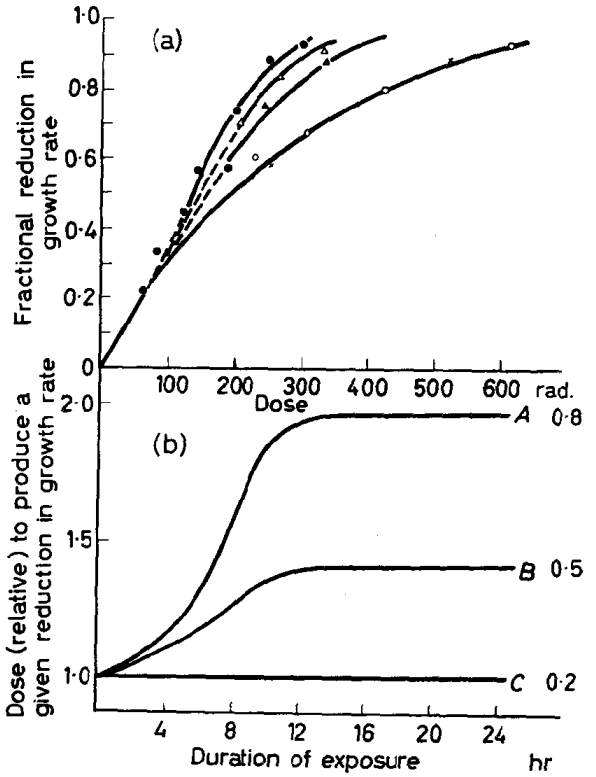

Figure 4. Influence of duration of exposure on γ-ray damage to roots

The range in duration of exposure studied in biological experiments has generally not been large enough to reveal the transition from dose-rate dependence to dose-rate independence illustrated in *Figure 4* (*b*). In view of the particle nature of ionizing radiation, dose-rate independence is, however, to be expected on physical grounds for the reasons given earlier whenever cells are exposed to sufficiently small doses at low dose rates. The doses and dose rates to which living organisms are exposed on the count of background radiation (0·1 rad in a year) would be likely to fall within the dose-rate independent range.

When two different qualities of radiation, given at the same dose rate, are compared, it is frequently found that the doses required to bring about the

same biological response are unequal. If the dose-response curves are of the same shape, *i.e.* if they may be superimposed by a simple change in the dose scale for one of the radiations, the inverse ratio of the doses which produce identical effects is defined as the relative biological efficiency (R.B.E.) of the two radiations (I.C.R.U. 1956, p. 7). Some seventy-five determinations of R.B.E. of supervoltage X radiation relative to ordinary deep-therapy X radiation have recently been reviewed by Kohn[14]. Plotted as a histogram, these observations show a Gaussian distribution with a mean at 0·85 and standard deviation of about 0·15. The difference from unity is small, and it is unfortunately the case that no inconsiderable fraction of the 300 scientific man-years of labour, represented by this histogram, is wasted through inadequate dosimetric precision. After doubtful results have been discarded, there nevertheless remains a substantial body of data indicating an R.B.E. of megavoltage relative to 200 kV X radiation of about 0·8. This is indicated

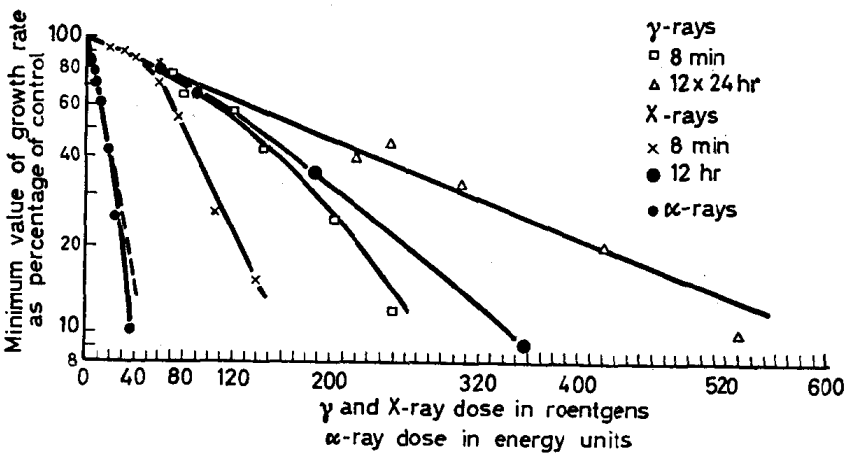

Figure 5. Inhibition of growth produced by δ, X and α radiation

by the very careful work of Kohn himself, and his colleagues in San Francisco[15,16], and of a group of investigators at the Christie Hospital, Manchester[17].

Large differences in R.B.E. are only found when radiations of very different quality are compared, *e.g.* when X and γ radiation on the one hand is compared with neutron or α radiation on the other. *Figure 5* shows a family of experimental curves for growth inhibition by α-rays, X-rays, and γ-rays[18]. The α-ray curve is dose-rate independent over the range investigated. The X- and γ-ray curves have already been discussed in connection with dose-rate dependence. It is evident that in this case R.B.E. of any two radiations cannot be represented by a single parameter. A horizontal line, representing the growth rate ratio of irradiated to control roots of 0·8, intersects the curves in a series of doses which have quite different ratios from the intersections with a horizontal line at the 0·15 level. R.B.E. is thus seen to be a function of dose and dose rate. It is also a function of the physiological condition of the cells and the oxygen tension in their environment at the time of irradiation. Since the effects of densely ionizing particles are much less influenced by oxygen tension than those produced by X or γ radiation,