ADVANCES IN RADIOBIOLOGY

G. C. de HEVESY, A. G. FORSSBERG

AND

J. D. ABBATT

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PROCEEDINGS OF THE FIFTH INTERNATIONAL CONFERENCE ON RADIOBIOLOGY HELD IN STOCKHOLM ON 15th-19th AUGUST, 1956

EDITED BY

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This book is respectfully dedicated to

PROFESSOR H. J. MULLER

in appreciation of his pioneer
work in genetics

PREFACE

IN THE PREFACE to the Proceedings of the Fourth International Conference on Radiobiology, Professor Mitchell and his co-editors state that at that meeting more than ever before, the value of collaboration between investigators trained in a wide range of scientific disciplines was demonstrated as an obvious necessity for progress in the very difficult field of scientific inquiry represented by radiobiology. The truth of this statement was fully brought out in the course of the Fifth International Conference on Radiobiology held in Stockholm in August 1956. A great variety of subjects were discussed by scientists experienced in a wide range of scientific disciplines. The effect of irradiation on the hæmopoietic system being the first and that of the application of induced mutation in plant breeding the last items of the vast field covered. In the single year after the Cambridge meeting, marked progress has been achieved in the whole field covered by the Stockholm Conference. To mention one example only, our knowledge was markedly advanced both of the technique and the mechanism of replacement of damaged marrow cells by healthy ones. Though these and other investigations in the realm of radiobiology were carried out without regard to practical applications, these applications are in the future, but the immense difficulties which are involved in cellular replacement without damage to the organism will have to be overcome.

At the Stockholm meeting about 180 delegates attended from 16 different countries; these included, for the first time, members of the Chinese Medical Association representing China. The appreciation by this Association of the importance of radiobiology is reflected in the statement of their President, Dr. Fu Lien-Chang, in a letter to the Chairman of the Fifth International Conference on Radiobiology, when he said: "Radiobiology is closely connected with man's health; hence the holding of your conference at this juncture is of great significance and importance to human welfare."

It is planned that the 1958 meeting shall be held in the United States of America.

G. HEVESY
ARNE FORSSBERG
JOHN D. ABBATT

Stockholm and London

March 1957

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SECTION I

INITIAL OR PRIMARY CHEMICAL EFFECTS OF IRRADIATION

RADIATION SENSITIVITY OF MOLECULES IN INTACT CELLS

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Any reasonably satisfactory understanding of radiobiology will require a way of estimating quantitatively, even in a crude way, the effects of ionising radiation on the molecules of which the cell is composed. The direct effect may be calculated, under some conditions at least, by methods previously discussed by Pollard *et al.* (1955). The indirect effect in cells is caused by the migration of chemically active intermediates (such as OH, HO_2, H_2O_2) created by the ionising radiation. The magnitude of the indirect effect on a given molecule is specified by two parameters. One of these is the sensitivity of the molecule in question to the intermediates, and is frequently given in terms of the number Y of molecules inactivated per ionisation in a dilute water solution, under such conditions that all the intermediates formed react with the molecules. The value of Y is different for each kind of molecule. The other parameter is the mean distance P that the intermediates diffuse before they react with another molecule. The magnitude of P can be different for different parts of the cell.

Zirkle and Tobias (1953) developed a 'migration model' in which the dose and the biological effects were related through the 'diffusion distance' P. They applied it to the survival of yeast cells, but since so little is known about the targets involved, they had to calculate a value of P from other data and see if it was consistent.

In the present experiments yeast cells were irradiated both in a wet state and dry in vacuum, then assays carried out on the enzymes invertase and alcohol dehydrogenase (ADH) and on coenzyme A. The difference between the wet and the dry irradiations was assumed to measure the indirect effect only. By working with comparatively simple enzyme systems about which a good deal is known, it was then possible to interpret the results in terms of a numerical value of the parameter P.

Methods

Because of the very high doses needed (1–1,000 million rads) most of the irradiations were carried out on the Yale cyclotron, using 4 MeV deuterons and 8 MeV alpha particles, with a few points using the 40 MeV alpha particle beam of the Brookhaven cyclotron. The dry irradiations were

carried out in vacuum by techniques previously described (Pollard *et al.*, 1955). Briefly, 1–3 mg. (dry weight) of yeast cells in 0·5 ml. water were pipetted on a ½-inch round microscope glass cover slip and dried by slow pumping in a vacuum desiccator. After irradiation, the samples were re-suspended in 1 ml. of water.

For the wet irradiations, 1–3 mg. (dry weight) of yeast cells suspended in 0·5 ml. of 0·01 M phosphate buffer (*p*H of 7) were pipetted on to a ½-inch diameter disk of Millipore 'molecular sieve' type filter which was placed on an absorbent pad saturated with buffer.

Invertase and ADH activities were measured on a haploid strain SC–7 obtained from Zirkle's laboratory. The Co–A activity of these cells was quite low, so a commercial brand (Fleischman's) of dried yeast was used.

Results

For invertase and ADH, if the logarithm of the activity surviving a given irradiation were plotted against the dose expressed in incident particles per square centimeter, the resultant curves were all straight lines. For Co–A, the dry curves were also straight lines. The wet curves indicate logarithmic inactivation down to 30% survival, with this percentage activity surviving doses about ten times the 50% dose. Thus the survival curves were of the form

$$f = e^{-SB}$$

where f is the fraction surviving a dose of B particles per square centimeter, and the parameter S, having the dimensions of an area, is usually referred to as a cross section. A large cross section denotes a high sensitivity to radiation.

The experimental values are collected in Table I. It is seen that the invertase in wet cells is about twice as sensitive to radiation as in dry cells, or the direct and indirect effects are about of the same order of magnitude. ADH, which is a sulphhydryl enzyme, is about twenty times as sensitive wet. Co–A is of the order of one hundred times as sensitive wet. Its dry cross section is in reasonable agreement with that expected on the basis of it low molecular weight of about 750. The direct effect on invertase is in excellent agreement with earlier measurements by Pollard, Powell and Reaume (1952) on purified invertase and by Powell and Pollard (1955) on invertase in dried cells.

Discussion

To obtain quantitative information on the movement in the cell of the intermediates which are active in the case of indirect action, use will be made of Zirkle and Tobias' migration model (1953). Under the assumption that the probability of an intermediate reacting with the surface of a molecule

is large compared with the ratio of the mean free path of the intermediate in water (the order of an Ångstrom or so) to the radius r_0 of the molecule, Wijsman (1952) has shown that the probability that an intermediate formed a distance r from the molecule will react with that molecule is given by

$$\frac{r_0}{r} e^{-\frac{r-r_0}{P}} \quad \text{.....(1)}$$

where P is a convenient measure of the distance that an intermediate can travel, and is equal to $\sqrt{Dt}$, where D is the diffusion constant of the intermediate, and t is the time it takes a given concentration of intermediates to drop to $1/e$ of its initial value by collision with cell constituents. By integrating this expression, it was shown by Zirkle and Tobias that the fraction f of activity which survives a dose of beta particles per square centimeter is

$$f = e^{-(S'+S'')B} \quad \text{.....(2)}$$

where S′ is the cross section for direct action, S″ the cross section for the indirect effect

$$S'' = 4\pi Y i(P^2 r_0 + P r_0^2) \quad \text{.....(3)}$$

where i is the number of ion pairs per unit path length along the particle track, and Y is the ionic yield.

From the data in Table I, Table II can be constructed, adding values of Y determined from previous work. The values of the radii of the target molecules used are those calculated from the molecular weight under the assumption of a spherical molecule.

TABLE I

Particle	Rate of energy loss ev/100 Å in protein	Cross section A²	
		Wet	Dry
Invertase			
4 MeV deuterons	230	4,600	2,300
8 MeV alphas	1,000	23,000	11,000
ADH			
4 MeV deuterons	230	25,000	1,000
8 MeV alphas	1,000	23,000	—
Co-A			
4 MeV deuterons ...	230	10,000	150
40 MeV alphas	250	> 3,000 (one run)	180 (one run)