BBA 3809

INFLUENCE OF RADIATION ON THE INCORPORATION OF RADIOACTIVITY IN DIFFERENT CELL CONSTITUENTS

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(Received May 4th, 1962)

SUMMARY

The effect of radiation on the incorporation of ^{32}P into cold trichloroacetic acid-soluble fraction, lipid and nucleic acid has been studied in *Escherichia coli*. The fraction of the total cells capable of giving rise to visible colonies after a dose of X-rays was determined from the agar plating. It was observed that the process of colony formation was about four times as sensitive as the nucleic acid synthesis in *E. coli*. Incorporation of radioactivity in lipid and acid-soluble phosphate was even less sensitive to radiation than nucleic acid synthesis. It has been suggested that radiation possibly breaks the DNA–enzyme complex, one such break resulting in the inhibition of cell division but in only a slight decrease in nucleic acid synthesis. This was considered to be the reason for the observed greater radiation sensitivity of colony formation than that of nucleic acid synthesis.

INTRODUCTION

The cause of the inhibition of cell division by radiation in microorganisms is still uncertain. But it has definitely been established that the process is very sensitive to ionizing radiation. The effects of radiation on various metabolic processes in cells are now being increasingly used as a means of understanding the radiation effects in microorganism[1–5]. Metabolic processes in cells can be studied from the uptake of various radioactive elements in the different fractions of the cells. An external agent which causes injury to cells, disturbs these processes and it is possible that a differential study of these disturbances will lead to the origin of the damage.

In the present work, the influence of radiation on the uptake of phosphorus in the different fractions of *E. coli* has been studied. For this purpose the experiments have been carried out in two ways: (a) by growing the cells in a growth medium containing radioactive phosphorus and noting the rates of incorporation of radioactivity in lipid, acid-soluble phosphates and nucleic acid, both in the control and the irradiated cells, and (b) by growing tagged cells in non-radioactive medium and measuring the loss in activity from the above cell constituents. The results have been compared with the radiation sensitivity of *E. coli* to give rise to a macro-colony on agar plates after exposure to ionizing radiations.

THEORETICAL CONSIDERATIONS

Equations for the study of biosynthetic activities in *E. coli* have been developed by ROBERTS *et al.*[6]. Fig. 1 is a simplified model of phosphorus kinetics for the study of the increase in activities in the lipid, acid-soluble and nucleic acid phosphorus of cells growing in a tagged medium. It is assumed that the cell mass per ml of the medium is increasing exponentially with time at the rate $Q = Q_0 e^{at}$. At time $t = 0$, the radioactive phosphorus (^{32}P) is added to the growth medium. In the model, X represents the total phosphorus atoms in the lipid pool, a product directly formed from the nutriment containing radioactive phosphorus. Y and Z represent the total phosphorus atoms in acid-soluble phosphorus and nucleic acid pools respectively.

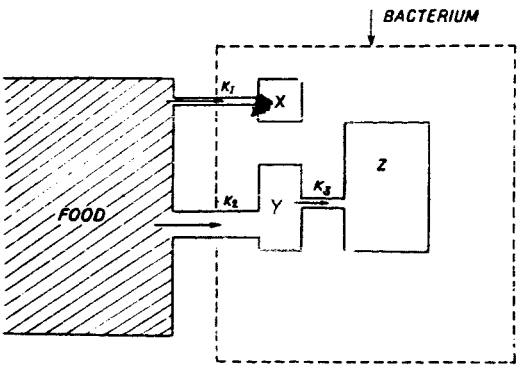

Fig. 1. Simplified model for the distribution of radioactivity in cells.

Acid-soluble phosphorus is assumed to be the precursor of the final product Z. X^*, Y^* and Z^* are the total radioactive phosphorus atoms in the lipid, acid-soluble phosphorus and nucleic acid respectively at any subsequent instant t. K_1 and K_2 are the rate constants per unit mass at which the lipid and the acid-soluble phosphorus draw the radioactive atoms from the medium, K_3 is the rate constant at which the radioactivity is drawn from the acid-soluble phosphorus pool by the nucleic acid pool. It is assumed that the loss of radioactivity from the acid-soluble phosphorus pool is equal to the gain by the nucleic acid pool.

The equations relating to the radioactive atoms in different compounds per ml of the growth medium are

$$X^* = \frac{K_1 Q_0}{a}(e^{at} - 1) \tag{1}$$

$$Y^* = \frac{K_2 Q_0}{a + K_3}(e^{at} - e^{-K_3 t}) \tag{2}$$

$$Z^* = K_3 \frac{K_2 Q_0}{a + K_3}\left(\frac{e^{at} - 1}{a} + \frac{e^{-K_3 t} - 1}{K_3}\right) \tag{3}$$

Eqn. 2 contains two terms, one increasing with time, the other decreasing. If t is sufficiently large, the contribution of $e^{-K_3 t}$ is negligible and Y^* increases approximately according to the relation

$$Y^* \approx \frac{K_2 Q_0}{a + K_3} e^{at} \tag{4}$$

Thus from the slope of the curve log. Y^* *vs.* t, when t is large one can determine a, both for the control and irradiated cells.

If the activities are expressed as fractions of the total activities in phospholipid, acid-soluble phosphorus, and nucleic acid pools (x, y and z respectively), Eqns. 1, 2 and 3 can be written as follows:

$$x = \frac{X^*}{X^* + Y^* + Z^*} = \frac{K_1}{K_1 + K_2} \tag{5}$$

$$y = \frac{Y^*}{X^* + Y^* + Z^*} = \frac{K_2}{K_1 + K_2} \cdot \frac{a}{a + K_3} \cdot \frac{e^{at} - e^{-K_3 t}}{e^{at} - 1} \tag{6}$$

$$z = \frac{Z^*}{X^* + Y^* + Z^*} = \frac{K_3 K_2}{K_1 + K_2} \cdot \frac{1}{a + K_3} \left(1 + \frac{a}{K_3} \cdot \frac{e^{-K_3 t} - 1}{e^{at} - 1}\right) \tag{7}$$

By adding Eqns. 6 and 7, it is found that $(y + z)$ is independent of time and is given by $y + z = K_2/(K_1 + K_2)$. Hence when t is large

$$y_\infty + z_\infty = \frac{K_2}{K_1 + K_2} \tag{8}$$

Also from Eqn. 6 it can be seen that when t is very large, y approaches the limiting value

$$y_\infty = \frac{K_2}{K_1 + K_2} \cdot \frac{a}{a + K_3} \tag{9}$$

By combining Eqns. 8 and 9, one gets

$$\frac{y_\infty}{y_\infty + z_\infty} = \frac{a}{a + K_3} \tag{10}$$

Since a has already been obtained from Eqn. 4, K_3 is determined.

The equations governing the flow of radioactive material from one compound to another in the prelabelled cells grown in non-radioactive medium have been developed by COWIE AND WALTON[7] and are given by

$$Y^* = Y_0^* \, e^{-K_3 \cdot \frac{K_2 - K_3}{a} \cdot t} \tag{11}$$

$$Z^* = Z_0^* + Y_0^* \left(1 - e^{-K_3 \cdot \frac{K_2 - K_3}{a} \cdot t}\right) \tag{12}$$

where Y_0^* and Z_0^* are the initial activities in acid-soluble phosphorus and nucleic acid, respectively. The constants

$$K_3 \cdot \frac{K_2 - K_3}{a}$$

can be determined with the help of Eqn. 11 from the decreasing slope of the curve log. Y^* *vs.* t. a and K_3 are already known from Eqns. 4 and 10 respectively, so that K_2, the rate at which the activity is drawn by acid-soluble phosphorus from the medium, is also determined.

MATERIALS AND METHODS

Specimen and growth medium

E. coli cells for the inoculation were obtained from 12-h-old Morton and Engely slants (tryptone 2 %, NaCl 0.5 %, glucose 0.5 %, KH_2PO_4 0.25 %, yeast extract 0.2 % and agar 2 %). Inoculum was either in non-radioactive or radioactive M[illegible] Engely medium (composition as given above, but excluding agar). Radioactive M and E medium contained 1 μC carrier-free ^{32}P, in the form of isotonic orthophosphate solution at pH 7.00, per ml of the medium.

Growth

Cells were grown for 1.5 h in non-radioactive medium to obtain non-tagged cells. To obtain tagged cells, cells were grown in radioactive medium for 3 h at 37°. Collected cells were washed with cold normal saline.

Source of irradiation and the method of exposure

The source of irradiation was a Picker Army Medical X-ray unit, run at 70 kV, 4 mA with an aluminum filter of 0.25 mm thickness. The dose rate at a distance of 4 in from the centre of the tube, measured with a Victoreen radiation meter, was 300 rads per min. Both tagged and non-tagged cells were exposed to X-rays, 4 in from the centre in 2-cm-wide glass tubes.

The general scheme of experiments is given in Table I.

TABLE I

SCHEME OF THE EXPERIMENT

Treatment	*Nature of the sample*	*Procedure after treatment*	*Times of withdrawing the samples*	*No. of sets*
Non-tagged *E. coli*	Control	Grown in radioactive M and E at 37°	At intervals of 5 or 15 min	4
	Exposed to 1500, 3000, 4500 and 6000 rads	Grown in radioactive M and E at 37° immediately after irradiation	At intervals of 5 or 15 min	Each 3 sets
	Control	Grown in non-radioactive M and E medium at 37°	At intervals of 5 or 20 min	4
Tagged *E. coli*	Exposed to 1500, 3000, 4500 and 6000 rads	Grown in non-radioactive M and E medium at 37° immediately after irradiation	At intervals of 5 or 20 min	Each 3 sets

Collection of samples and chemical fractions

Control non-tagged cells were mixed with radioactive M and E medium and allowed to grow at 37°. Cells were collected at definite intervals as given in Table I and washed free of radioactivity with cold normal saline. Radiated non-tagged cells were mixed with radioactive medium immediately after the radiation. Collection of cells was similar to that of control cells.

Control tagged cells were mixed with non-radioactive M and E medium and

allowed to grow at 37°. Cells were collected at definite intervals as given in Table I. Radiated tagged cells were similarly mixed with non-radioactive medium immediately after radiation. Cells were collected as before.

Chemical fractionation of the collected cells was done by the method of KATCHMAN AND FETTY[8]. The cold trichloroacetic acid-soluble fraction was regarded as the acid-soluble phosphate, the ether–alcohol extract as the lipid, and the rest as the nucleic acid. Protein was not separated from nucleic acid, as it was known that protein contained very little ^{32}P.

Measurement of radioactivity

Radioactivity measurements were done with a TGC-1 end-window Geiger–Müller counter (Tracerlab). For expressing the activity, as activity at the time per ml of the growth medium, these measurements were multiplied by the volume of the sample and divided by the volume of the growth medium taken.

Determination of the number of viable cells

The number of viable cells was determined from the visible colonies developed on agar plates after suitable dilutions of the sample[9].

RESULTS

The rise in activity with time in the acid-soluble phosphorus of all bacteria in 1 ml of growth medium *i.e.* Y^* *vs.* t is shown in Fig. 2. The various curves in this figure are for control cells and for cells exposed to different doses of radiation from 1500 to 6000 rads; since it was the aim of these experiments to determine the growth constant a

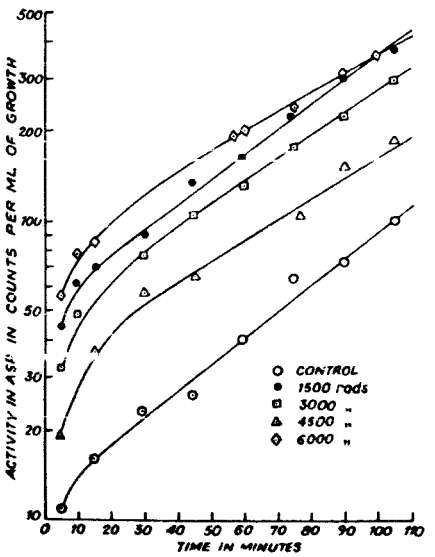

Fig. 2. Growth of activity with time in the acid-soluble fraction of the non-irradiated and irradiated non-tagged bacteria.

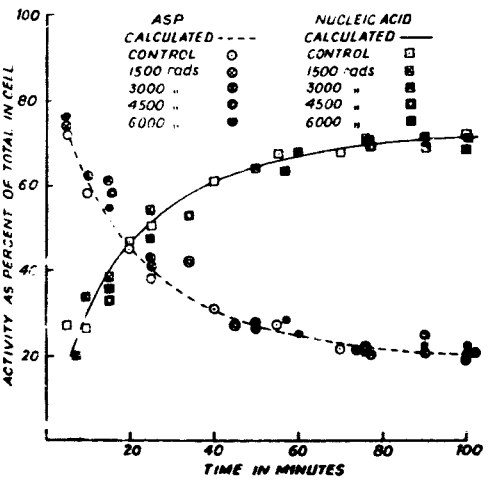

Fig. 3. Activity in acid-soluble and nucleic acid fraction of the non-irradiated and irradiated bacteria, expressed as per cent of the total activity in cells.

from the slopes of these curves, the activities (in counts per ml of growth medium) found in experiments on different dates were not normalized at any particular time. The concentration of bacteria per ml of growth medium varied somewhat from day to day thus causing the relative shifts of these growth curves with respect to each other. After a short period of growth the rise in activity in all cases was found to be exponential. The slope of the exponential region gave the growth constant of the control and the irradiated cells according to Eqn. 4. It was found that a decreased from 0.022/min to 0.014/min when the dose of exposure of the cells varied from 0 to 6 krads (Table II, column 2). The value of $a = 0.022$/min for the control cells corresponded to a time of 31 min required for doubling the cell mass.

Fig. 3 shows a plot of

$$\frac{Y^*}{X^* + Y^* + Z^*} \text{ and } \frac{Z^*}{X^* + Y^* + Z^*}$$

as a function of time, *i.e.* the variation in the activity in acid-soluble phosphorus and nucleic acid for irradiated and non-irradiated cells, expressed as per cent of the total activity in bacteria. It was found that the points for the irradiated and control cells fell almost on the same curve, so that $y_\infty + z_\infty = 0.90$ and $y_\infty = 0.19$, both for control and irradiated cells. With $y_\infty/y(y_\infty + z_\infty) = 0.21$, K_3 for varying doses of exposure was calculated with the help of Eqn. 10 from the previously obtained values of a. The values of K_3 so calculated are given in column 5 of Table II.

$$\left(\frac{X^*}{X^* + Y^* + Z^*}\right) = x$$

was at all times found to be about 0.1 for both control and irradiated cells, and hence is not shown in the figure. From Eqns. 5, 6 and 7, $x/(y + z) = K_1/K_2 = 0.1/0.9$ *i.e.* $K_2 = 9K_1$ for the control as well as the irradiated cells.

TABLE II

DETERMINATION OF THE CONSTANTS a, K_3, K_2 FOR CONTROL AND IRRADIATED CELLS

Dose of irradiation (rads)	Cell growth constant		$y_\infty + z_\infty$	y_∞	Uptake constant for nucleic acid		Uptake constant for acid-soluble phosphate	
	a/min	Per cent of the control			K_1/min	Per cent of the control	K_2/min	Per cent of the control
0	0.022	100	0.90	0.19	0.081	100	0.31	100
1500	0.019	86	0.90	0.19	0.071	88	0.30	97
3000	0.0175	79	0.90	0.19	0.065	80	0.294	95
4500	0.0155	70	0.90	0.19	0.0578	71	0.28	90
6000	0.014	63	0.90	0.19	0.052	64	0.27	87

Results with previously labelled cells when placed in non-radioactive medium have been presented in Fig. 4, where the loss of activity of the acid-soluble phosphate of the prelabelled bacteria in counts per ml of the growth medium, *i.e.* Y^* of Eqn. 11, has been plotted semilogarithmically against the time of growth. The various curves in the figure are for control cells and cells exposed to different doses of radiation from

1500 to 6000 rads. Here also no normalization was done thus accounting for the relative shift in the different figures representing results of different experiments. All curves were straight lines on a semilogarithmic plot. The rate constants determined from the decreasing slope of these log. Y^* vs. t curves gave the values of $K_3(K_2 - K_3)/a$. The values of the constant K_2 under the different conditions were then obtained from a

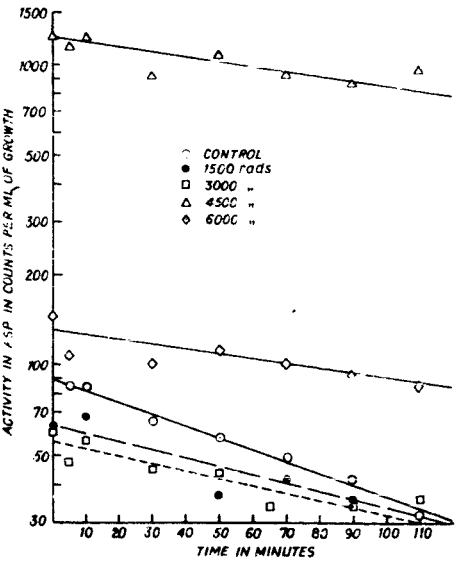

Fig. 4. Loss of radioactivity with time from the acid-soluble fraction of the irradiated and non-irradiated tagged cells.

and K_3 determined previously for those conditions of the cells. The values of the constant K_2, thus determined for the various values of exposure doses, are shown in column 8 of Table II and are found to vary from 0.31/min to 0.27/min for exposure doses from 0 to 6 krads. Thus K_1 ($= K_2/9$) varied from 0.034/min to 0.03/min for the same range of doses (not shown in Table II).

DISCUSSIONS

Fig. 5 shows the variation of the constants a, K_1, K_2 and K_3 with increasing doses of radiation. The constants are plotted as per cent of the control value *i.e.* the a/a^0, K_1/K_1^0, K_2/K_2^0 and K_3/K_3^0 where a and a^0, K_1 and K_1^0, K_2 and K_2^0, K_3 and K_3^0 are the values of the constants at doses D and 0 rads respectively. These curves showed that at a dose of 6 krads K_1/K_1^0 and K_2/K_2^0 were 0.87 whereas both K_3/K_3^0 and a/a^0 were about 0.63. The ability of the cells to give rise to a macro-colony on an agar plate was reduced to 18%. These results indicated that K_2 was more sensitive to radiation than K_1 and K_3 or, in other words, radiation retarded the process of phosphorus incorporation into nucleic acid (*i.e.* nucleic acid synthesis) to a greater degree than the other biochemical processes studied, *viz.* phosphorus incorporation into phospholipid or acid-soluble compounds. The colony-forming ability of the cells was the most

sensitive amongst all the processes including nucleic acid synthesis, *i.e.* about four times as sensitive as the latter. Similar observations have also been reported by POLLARD AND KENNEDY[1].

If one considers that the radiation-induced damage to DNA was in some way responsible for the inhibition of cell division[10,11] the sensitive volume of cells, determined from the survival of colony-forming ability, was about 0.02 of [illegible] DNA volume in the cell[9,12]. Thus a control part of DNA, roughly 0.02 of the total DNA chain, is effective as the control region for the colony-forming ability of the cell. The

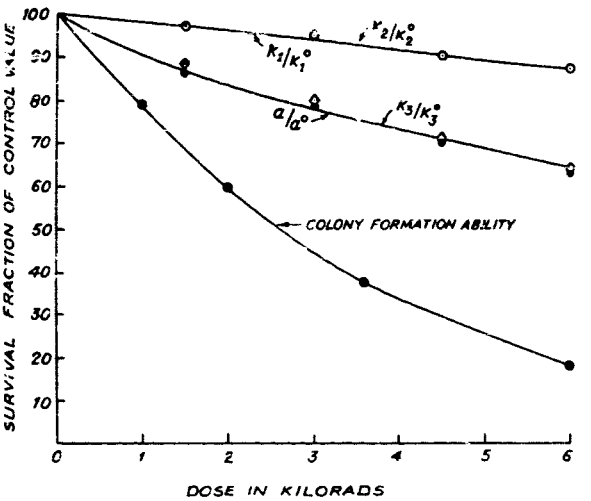

Fig. 5. Variation of the survival fractions for different processes with increasing doses of irradiation to the cell.

concept of this control region in DNA was also supported by studies, both *in vitro* and *in vivo*, of the inhibition of induced enzyme synthesis by irradiation of DNA or by its inactivation due to the decay of ^{32}P incorporated in DNA (see ref. 13).

As bacterial RNA is supposed to be synthesized through DNA (see ref. 14) any damage to DNA would soon be reflected by the loss of enzyme-forming capacity of the cell. This idea has been presented in another way by ORD AND STOCKEN[15] and LAJTHA *et al.*[16] who postulated that inhibition of DNA synthesis was due to (a) a block in certain enzymes responsible for DNA synthesis, and (b) damage to DNA molecules preventing them from serving as templates for replication. The radiation-induced inhibition of mitotic activity of the cell is thus a secondary effect of the damage to DNA.

If the contention is correct that DNA coils, in contact with an enzyme, carry on their synthetic activities[17], the control region of DNA may be that portion of the chain which is in contact with the enzyme. Radiation then may be supposed to act on any of these contact regions of DNA in such a way as to disrupt the DNA–enzyme complex. Whereas a change in any of the contact regions might lead to the inhibition of cell division, such damage might not equally inhibit nucleic acid synthesis. That is possibly why in the present work radiation has been found to inhibit colony formation to a greater degree than nucleic acid synthesis.

ACKNOWLEDGEMENTS

This work was carried out under the financial sponsorship of the Council of Scientific and Industrial Research, India. One of the authors (M.S.) is indebted to the Ministry of Scientific research and Cultural affairs, Govt. of India for a Research Training Scholarship.

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