COMPARISON OF EFFECTS OF ATOM DECAY AND BETA-RAY RADIATIONS ON THE INACTIVATION AND MUTATION OF A MYCOBACTERIUM

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INACTIVATION of bacteria by incorporated radioactive phosphorus (P^{32}) was reported by FUERST and STENT (1956) and KAUDEWITZ, VIELMETTER and FRIEDRICH-FREKSA (1958). It is not always easy to differentiate the effect of P^{32} decay from the effect of intracellular beta-ray irradiation, although differentiation from the effect of extracellular radiations could have been made by these authors. The present author (TSUKAMURA 1960a) previously made a comparative study on the mutagenic effects of P^{32} and S^{35} (radioactive sulfur) incorporated into cells of a *Mycobacterium*. Since, under the conditions tested, intracellular radiations with S^{35} could cause neither inactivation nor mutation in cells, it was suggested that decay of P^{32} atoms plays an important role in causing inactivation and mutation. Thereafter, some additional experiments were made (TSUKAMURA 1961). The organism was labeled with P^{32} -orthophosphate under different conditions, and the relationship between the intracellular distribution of P^{32} and inactivation and mutations in these cells was followed. The results revealed that increase of P^{32} incorporation into the deoxyribonucleic acid (DNA) fraction caused an increase of inactivation and mutation, and, in addition, an inactivation frequency that fitted a curve of a single-hit-type was found in cells containing a higher amount of P^{32} in their DNA fraction. It was considered thus that decay of P^{32} in the DNA mainly causes inactivation and mutation. However, inactivation and mutation were also caused but to a less extent in cells containing a large amount of P^{32} outside the DNA fraction and a slight amount of it in the DNA fraction. In the latter case, the possibility could not be excluded that intracellular beta-ray radiations may have taken a part in causing inactivation and mutation, although it was probable that, as well, accumulation of decays in cytoplasm is associated with such effects considering the occurrence of inactivation curves of a multihit type.

The present study was designed to determine what role is played by the beta-ray radiations that always accompany the decay phenomenon. For this purpose, the effect of radioactive strontium (Sr^{90}) having a very long half decay period (19.9 years) and a strong beta-ray radiation capacity ($E_{\max}$ 0.65 Mev, 2.35 Mev) was compared with the effect of P^{32} (half decay period 14.3 days, $E_{\max}$ 1.71 Mev).

MATERIALS AND METHODS

An avirulent strain Jucho of *Mycobacterium avium* was used. The organism was subcultured in 50 ml of Sauton medium (in 200 ml Erlenmeyer flasks) to

which were added P^{32} and Sr^{90} . Inoculation was made with 3 mg (wet weight) of cells per flask. The media inoculated were incubated at 37°C for six days and the resulting cultures were used for determining viable counts and mutation frequencies to streptomycin resistance and to isoniazid resistance. P^{32} -orthophosphate (specific activity CF, Radiochemical Center, Amersham, England) and Sr^{90} -nitrate (specific activity CF, Oak Ridge National Laboratory, Oak Ridge, Tennessee, U.S.A.) were used for the experiments.

Methods for determinations of the number of viable cells per mg (wet weight) and of the mutation frequencies were described previously (TSUKAMURA 1960a). Counts were made after five days of incubation and dilutions giving rise to 20 to 200 colonies per plate were used for the basis of calculation.

Supernatants of the cultures were obtained by centrifugation and, after drying, their radioactivities were counted by a Geiger-Mueller counter (count efficiency for P^{32} 5%). Cells of the cultures were washed five times in distilled water, measured for their wet weight and counted for their radioactivities. Radioactivity was expressed as counts per minute (cpm). The appropriate corrections for background and natural decay were applied.

RESULTS AND DISCUSSION

Growth: The results are shown in Table 1. In media containing different concentrations of isotopes and containing no radioisotope, almost similar amounts of growth were observed, although a culture growing in a medium containing $20\text{ }\mu\text{C}$ Sr^{90} /ml exhibited somewhat delayed growth during the first few days. Amounts of growth as measured after six days of incubation did not differ significantly (Table 1). It appears, therefore, that the growth phases of these cultures do not differ markedly (this organism enters the stationary growth phase after three days (TSUKAMURA 1960b)).

Viability of cells: The results are shown in Table 2. In number of viable cells per mg only the culture grown in medium containing $1\text{ }\mu\text{C}$ P^{32} /ml showed a marked decrease, to about one sixth of that for the control culture grown in medium containing no radioisotope. However, no decrease occurred in the culture grown in medium containing $20\text{ }\mu\text{C}$ Sr^{90} /ml, which was exposed to much more intracellular and extracellular beta-ray radiations than was true for the culture grown in medium containing $1\text{ }\mu\text{C}$ P^{32} /ml (Table 3).

TABLE 1
Amount of growth occurring after six days of incubation

Medium	Amount of growth per flask
Control (no radioisotope)	1.8 gm (wet weight)
Sr^{90} -nitrate $2\text{ }\mu\text{C}/\text{ml}$	1.9
Sr^{90} -nitrate $20\text{ }\mu\text{C}/\text{ml}$	1.7
P^{32} -orthophosphate $1\text{ }\mu\text{C}/\text{ml}$	1.6

Each medium was inoculated with 3 mg (wet weight) of cells.

TABLE 2

Viability and mutation frequencies in cultures grown in media containing no radioisotope and in media containing radioisotopes in concentrations of 2 μ C Sr⁹⁰/ml, 20 μ C Sr⁹⁰/ml and/or 1 μ C P³²/ml

Medium	No. of viable cells per mg (wet weight)	Ratio of INH-resistant mutants* per 10 ⁵ viable cells‡	Ratio of SM-resistant mutants† per 10 ⁵ viable cells‡
Control	$(2.89 \pm 1.43) \times 10^6/\text{mg}$	19.8 ± 12.4	2.91 ± 0.550
Sr ⁹⁰ 2 μ C/ml	$(1.91 \pm 1.14) \times 10^6/\text{mg}$	11.9 ± 4.04	4.55 ± 2.33
Sr ⁹⁰ 20 μ C/ml	$(3.25 \pm 1.73) \times 10^6/\text{mg}$	67.7 ± 44.3	10.8 ± 5.38
P ³² 1 μ C/ml	$(4.40 \pm 2.95) \times 10^5/\text{mg}$	$174. \pm 45.0$	89.2 ± 41.0

* Mutants resistant to 10 μ g isoniazid/ml.

† Mutants resistant to 1 μ g streptomycin/ml.

‡ Mean and standard deviation obtained by three independent determinations on the same culture. In each determination, the number of viable cells was calculated as an average of five plates. The relatively high deviations in the data seem to have resulted from doubling of the errors involved in estimations of the number of viable cells and the amount of cells (mg) or the errors involved in calculations of the number of total viable cells and the number of mutants.

TABLE 3

Radioactivities of culture filtrates and of bacterial cells

Medium	Radioactivity of culture filtrate	Radioactivity of bacterial cells
Control	0. cpm/0.2 ml	0. cpm/mg (wet weight)
Sr ⁹⁰ 2 μ C/ml	38,265.	1,397.
Sr ⁹⁰ 20 μ C/ml	380,200.	11,425.
P ³² 1 μ C/ml	16,332.*	1,781.*

cpm = counts per minute.

* Corrected into cpm at the beginning of incubation.

In view of the results, it is concluded that inactivation of cells occurring in the culture to which 1 μ C P³²/ml was added is not due to intracellular beta-ray radiations but due to decay of P³² atoms.

Mutation frequencies: The highest mutation frequencies were found in the culture to which 1 μ C P³²/ml was added, and a slight increase in mutation frequency was seen in the culture given 20 μ C Sr⁹⁰/ml. No significant increase was seen in the culture receiving 2 μ C Sr⁹⁰/ml, which would have been irradiated by about the same amount of intracellular beta-ray radiations and by a much greater amount of extracellular radiations than was true for the culture getting 1 μ C P³²/ml.

In view of these results, it is conceivable that increase of mutation frequencies occurring in medium containing 1 μ C P³²/ml is mainly the result of decay of P³² and is not due to the beta-ray radiations whether they were intracellular or extracellular. It also seems probable that the increase in mutation frequency, which was observed previously (TSUKAMURA 1961) in cells whose DNA fraction was labeled with only a little amount of P³², was due to decay of P³² atoms in the cytoplasm. On the other hand, a large amount of Sr⁹⁰ (i.e., beta-ray radiations) could cause mutations, since the effect of decay is negligible in this iso-

tope. It appears remarkable thus that in contrast to the effect of P^{32} , Sr^{90} is mutagenic in a large amount without inactivation.

SUMMARY

Effects of P^{32} and Sr^{90} added to culture media were compared in a *Mycobacterium* to determine whether decay of atoms or intracellular beta-ray radiations takes an important role in causing inactivation and mutation. Both inactivation and mutation occurred significantly in a culture grown in medium containing $1 \mu C P^{32}/ml$, and only a moderate increase of mutations occurred without inactivation of cells in a culture grown in medium containing $20 \mu C Sr^{90}/ml$. No significant increase of mutation was seen in a culture grown in medium containing $2 \mu C Sr^{90}/ml$.

Since large amounts of intracellular and extracellular beta-ray radiations from Sr^{90} were found to be ineffective in causing inactivation of cells and much less effective in causing mutation than is true for a small amount of P^{32} , it is conceivable that inactivation and mutations occurring in cells grown in medium containing such a small amount of P^{32} (i.e., $1 \mu C P^{32}/ml$) are due neither to intracellular nor extracellular beta-ray radiations but mostly to decay of P^{32} atoms incorporated into cells.

LITERATURE CITED

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