The Effect of Radiation on Microbiologic Characteristics of *M* Tuberculosis*

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The effect of irradiation on mutation (expressing itself as drug resistance) and on viability of *Mycobacterium tuberculosis* was studied in vitro. Forty-two identical cultures of *H37-Rv* (*M* tuberculosis) were exposed to different levels of cobalt radiation (10, 100, 1,000, 2,500, 5,000, 10,000, and 20,000 rads) with six samples used for each of the seven radiation levels. Equivalent samples exposed to zero rads and samples handled and stored identically formed the controls. Coded cultures were read in a double-blind fashion to determine the number of surviving organisms and sensitivities to nine different antituberculosis drugs. Organism viability began to decrease at radiation levels of 1,000 rads and decreased linearly with higher levels of radiation. Three of the 42 radiated cultures developed drug-resistant organisms (one to INH, one to PAS, a third to SM). This drug resistance occurred at levels of clinical significance (>1 percent control) as well as in amounts exceeding probability values for chance resistance mutation. High radiation levels such as occur in radiotherapeutic doses decrease the viability of *M* tuberculosis. Radiation may also induce genetic mutation expressed as primary drug resistance.

The coexistence of tuberculosis and broncho-epithelial carcinoma has prompted speculation, much of it convincing, that each disease may aid the other: carcinoma originating in granulomatous areas and latent foci of tuberculosis being reactivated by contiguous neoplasia. An interesting aspect of this interrelationship concerns therapeutic management of patients with both diseases, especially with reference to radiotherapy for concurrent lung cancer and tuberculosis.

Advanced active TB has been proposed as a contraindication to radiotherapy, either for reasons of patient intolerance or, more pervasively, for the old notion that radiotherapy would reactivate TB.

The data are clinical and retrospective. To the best of our knowledge no controlled prospective evaluation of radiation effects on *M tuberculosis* has been made. Accordingly, in vitro experiments were performed to determine (1) if radiation affects organism viability; and (2) if radiation would induce mutations in tubercle bacilli which would then be expressed as drug resistance.

**MATERIALS AND METHODS**

*M tuberculosis* (*H37-Rv*) kept at -70°C in a concentration of 5 × 10⁷ viable units/ml of Middlebrook 7H-9 served as the quality control strain for drug susceptibility testing and identification of mycobacteria. Five milliliters of frozen *H37-Rv* were defrosted and diluted into 45 ml of 7H-9 broth, resulting in a bacterial concentration of 5 × 10⁴ viable units/ml (Fig 1). This medium was then dispensed in 1 ml aliquots into 48 5-ml screwcapped plastic tubes numbered 0-47. The tubes were number coded from a table of random numbers and assigned to one of eight radiation levels. The radiation was delivered from a cobalt-60 source at a constant distance from the culture tubes. The overall size of the radiation field was 16 × 16 cm and easily allowed inclusion of 12 tubes at a time. Dose rate delivered was calculated to be 66.4 rads/minute. Six tubes were exposed for 0.15 minute = 10 rads, 6 tubes for 1.51 minutes = 100 rads, 6 for 15.07 minutes = 1,000 rads, 6 for 37.66 minutes = 2,500 rads, 6 for 75.32 minutes = 5,000 rads, 6 for 150.84 minutes = 10,000 rads, and 6 for 301.4 minutes = 20,000 rads. Six tubes were placed on the field with the cobalt source off, forming the "radiation control." The tubes were then frozen at -70°C.

During the eight weeks after radiation, six tubes were taken each week at random from the freezer and tested bacteriologically with the *H37-Rv* quality control strain within the framework of the routine mycobacteria susceptibility and identification tests. The weekly testing of a separate *H37-Rv* served as a "storage control" to monitor the effect of -70°C on the viability of the organisms under study, since it was stored and handled identically to the six radiated specimens each week. The microbiologist did not know the number code, and since the specimens had been taken at random from the freezer the bacteriologic testing was double-blind.

Standard plate counts on 7H-10 agar using four tenfold dilutions were performed using four-section 100-mm Petri dishes for all radiated and unirradiated specimens. In this manner survival rates were determined as well as drug susceptibility patterns to the following antituberculosis drug concentrations:

- Isoniazid (INH) 0.2, 1.0 and 5.0 μg/ml
- Streptomycin sulfate (SM) 2.0 and 10.0 μg/ml
- PAS 2.0 μg/ml
- Ethambutol (EMB) 5.0 μg/ml
- Ethionamide (ETA) 5.0 μg/ml

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I radied specimens exposed to a 8 wks.
Routine drug susceptibility, resistance levels:
- 1000 rads: 6 tubes
- 5000 rads: 6 tubes
- 10,000 rads: 6 tubes
- 20,000 rads: 6 tubes

Freeze (-70°C), defrost 6 tubes/wk x 8 wks.
Routine drug susceptibility, N/N, organism viability.

**FIGURE 1.** Experimental model showing (left) “storage control;” (center) radiated specimens; and (right) “radiation control.”

Kanamycin (KM) 5.0 μg/ml
Rifampin 1.0 μg/ml
Cycloserine (CS) 50.0 μg/ml
Pyrazinamide (PZA) 20.0 and 50.0 μg/ml

These media were incubated for 21 days at 35 to 37°C in an atmosphere of 10 percent CO₂. Drug susceptibility was determined according to the proportion method, which expresses the emergence of drug-resistant mutants as a percentage of the whole population present on the control plate. Colony morphology on 7H-10 agar was expressed as rough-regular, with a central spot (RgRs.).

Approximately 0.2 ml of the 1:10 dilution of each radiated and unradiated bacterial suspension was inoculated onto Lowenstein-Jensen slants for the performance of the niacin and nitrate reductase tests four weeks after incubation at 35-37°C without CO₂.

The double-blind code was not broken until all the cultures were tested for drug susceptibility, survival rate, and biochemical properties.

**RESULTS**

All 42 radiated specimens, all six “radiation control,” and all eight “storage control” specimens showed typical morphology (RgRs) on 7H-10 agar and yielded positive niacin and nitrate reactions on Lowenstein-Jensen medium.

**Organism Viability**

Those cultures frozen and stored longer than four weeks exhibited decreasing numbers of viable organisms (Fig 2). It was clear that organism viability would have to be corrected for the apparent effect of storage-freezing on viability.

The radiated cultures frozen and stored for more than four weeks showed a greater loss of viable organisms than the nonradiated storage controls frozen and handled identically (p<0.02).

To determine organism viability, growth for the four dilutions of the radiated specimen was summed to give a total organism count for each radiated specimen. To correct this value for loss due to storage alone, a similar calculation was performed for the “storage control” for the week that specimen was evaluated. The radiated specimen’s total organism count divided by the “storage control’s” total organism count gave a percentage of corrected growth. Since there were six specimens at each radiation level, this procedure was repeated for the five other cultures receiving the same radiation dose, yielding six percentage values. The mean value of these percentages was then derived representing mean orga-

**FIGURE 2.** Effect of storage-freezing on organism viability. After four weeks decrement in viability occurs, much greater for radiated than for nonradiated cultures (p<0.02).

**FIGURE 3.** Effect of radiation (corrected for storage and freezing) on organism viability plotted semilogarithmically. At radiation dosages of 1000 rads or greater there is exponential increase in number of organisms killed.
nism viability (now corrected for storage) per given radiation dosage level. This was done for all seven radiation dosage levels (10 rads to 20,000 rads). Organism viability could then be plotted as a function of radiation dosage, or, subtracting organism viability percentage from 100, the mean percentage of organisms killed plotted as a function of radiation dosage (Fig 3). At radiation levels of 1,000 rads or greater there is a progressive increase in the number of organisms killed.

In comparing the number of viable organisms in the groups receiving 0, 10, and 100 rads with the number of organisms in their storage controls, there was no significant difference. However, in comparing the number of viable organisms in the groups receiving 1,000 rads or greater (1,000, 2,500, 5,000, 10,000 and 20,000 rads) with their storage controls there were significantly fewer organisms in these radiated groups than in their storage controls (p<0.01). Thus, the organisms radiated with 1,000 rads or more did demonstrate decreased viability, whereas those receiving less radiation did not.

**Drug Susceptibility**

Three radiated specimens showed the presence of drug-resistant organisms. A specimen receiving 1,000 rads had two colonies of *M tuberculosis* growing in a 2.0 µg/ml concentration of streptomycin sulfate. To determine the proportion of mutants this represented, the ratio of the number of mutants (drug-resistant colonies) to the total number of cells in the population was calculated. For streptomycin this was $1.42 \times 10^4$. The highest proportion of mutants to be expected on the basis of chance genetic mutation alone for identical cultures of *M tuberculosis* with streptomycin is $2.0 \times 10^4$. The incidence of drug resistance to streptomycin after radiation was greater by a magnitude of two logs than could be accounted for on the basis of chance genetic mutation alone.

A specimen receiving 10 rads had three colonies of *M tuberculosis* growing on 0.2 µg INH/ml, and two colonies on 1.0 µg INH/ml. The calculated proportion of mutants for INH thus was $1.42 \times 10^4$. The highest proportion of mutants to be expected on the basis of chance genetic mutation alone for identical cultures with INH (at 1.0 µg/ml) is $3.1 \times 10^4$. The incidence of INH resistance after radiation was greater by a margin of two logs that could be accounted for on the basis of chance genetic mutation alone.

A third specimen receiving 2,500 rads had 250 colonies of *M tuberculosis* on a PAS concentration of 2 µg/ml. The calculated proportion of mutants with resistance to PAS is $1.78 \times 10^2$ in the present study. The highest proportion of mutants for PAS due to chance genetic mutation alone is $1 \times 10^4$. The incidence of PAS resistance in this experiment was greater by a margin of four logs than could be accounted for on the basis of chance genetic mutation alone.

Quality control evaluations of identical cultures of nonradiated H37Rv for drug susceptibility performed weekly in the same laboratory for over two years revealed a level of drug resistance considerably below that demonstrated after radiating identical cultures.

**DISCUSSION**

Two major findings emerged from these experiments. First, radiation dosages greater than 100-1,000 rads cause a progressive decrease in the viability of *M tuberculosis*. Second, the emergence of drug-resistant organisms at levels greater than could be accounted for on the basis of chance genetic mutation raises the question of mutagenicity of radiation for drug resistance.

This effect of irradiation on other bacterial species shows a dose-response curve not dissimilar in shape but different in magnitude to the dose-response curve for *M tuberculosis*. The D37 (the dose level required to decrease the number of remaining viable organisms to 37 percent) for oxygenated *Escherichia coli*, is 3,000 rads. The D37 level for *M tuberculosis* was not reached in our studies. At 3,000 rads the number of surviving viable organisms would be about 68 percent (extrapolated from Fig 3) or almost twice as many as in *E coli*. This comparative radioresistance of *M tuberculosis* (as compared with *E coli*) has been demonstrated also for UV radiation in which the tubercle bacillus is two to three times more resistant than *E coli*.

Since the mycobactericidal dosage range (100-1,000 rads or greater) is in the usual clinical radiotherapeutic range, certain clinical implications may arise concerning radiotherapy concurrently with pulmonary TB. The coexistence of pulmonary TB and pulmonary neoplasia resulted in some instances in tuberculous patients receiving radiotherapy for the neoplasm. It has been axiomatic for years that irradiation of the chest with a history of TB would produce rapid reactivation and dissemination of the mycobacterial disease, and older radiotherapy tests stress this point. Fulkerson described a patient who with negative sputa for TB received 6,500 rads for carcinoma of the lung. One month after radiotherapy he developed positive sputum and had disseminated TB at autopsy. It was concluded that irradiation to the lung with possible tuberculous background might cause reactivation of tuberculosis.

Other investigators, however, have found irradiation actually helpful in tuberculosis. In 1940,
Lahey\(^9\) reported satisfactory results in 80 to 90 percent of cases of TB lymphadenitis treated with irradiation, and others\(^{10-12}\) have demonstrated equally successful results. The critical factor for optimal response was the simultaneous use of antituberculosis chemotherapy, which seemed to obviate the hazards implicit in irradiation to tuberculous areas.\(^{12}\) Fulkerson and one of us\(^{14}\) studying 15 patients with coexistent pulmonary TB and carcinoma over a 15-year period found that radiation to the lung for neoplasia in the presence of TB is not contraindicated provided that the patient is receiving adequate antituberculosis therapy. None of these irradiated patients developed activation or spread of their TB following tumoricidal irradiation.

It seems safe to administer radiotherapy to a tuberculous patient with carcinoma, provided he is on concurrent antituberculous therapy. If one is willing to extrapolate in \textit{vivo} data to an in \textit{vivo} setting, moderate to high-dose radiation may in fact have a salutary effect on pulmonary tuberculosis, at least by decreasing, as our study indicates, the number of viable organisms.

The second major finding of the present study was the appearance of drug-resistant organisms following irradiation. This resistance occurred for INH and SM at orders of magnitude two logs greater than could be accounted for on the basis of chance genetic mutation alone, and for PAS at four logs greater than could be accounted for on the basis of chance genetic mutation alone. Assuming mutation to be responsible for the drug resistance, the three resistant cultures might represent mutants induced by the mutagenic effects of irradiation, rather than by spontaneous mutation alone.

The interaction of ionizing radiation with bacterial systems results in a spectrum of effects including: reparable genetic deletions;\(^3\) changes in subsequent radioresistance;\(^{18}\) and cell death.\(^{14}\) To a certain extent these effects are dose-dependent, but environmental factors such as oxygen concentration, the presence and amount of chemical modifiers, and the stage of DNA synthesis all contribute to the degree of damage caused by ionizing radiation.\(^{16}\) The development of resistant strains of bacteria results from the selection of spontaneous mutants.\(^{17}\) Ionizing radiation which is known to increase the rate of bacterial mutation\(^{15}\) might increase the incidence of antibiotic resistance, as demonstrated by the present study.

One patient observed by Bobrowitz\(^{18}\) and two patients by one of us\(^{18}\) had the emergence of primary drug-resistant TB after receiving tumoricidal doses of radiation. In light of the present results, it is intriguing to consider whether their drug-resistant TB was in fact initiated by the mutagenic effects of the radiation.

Two factors limit the ability to extrapolate these experimental data to a clinical setting. First, the data are in \textit{vivo} and are at best an approximation of in \textit{vivo} biologic phenomena. Second, dose-fractionation was not performed in the experiment. Radiation dosages of these magnitudes would surely be fractionated in a clinical setting. However, in view of a possible risk of inducing drug resistance in \textit{M. tuberculosis} in \textit{vivo} with moderate to high-dose irradiation, it would seem prudent that judicious followup with drug susceptibility testing be undertaken in patients with inactive TB receiving radiotherapy.

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REFERENCES

7 Moss EWT: Therapeutic Radiology. St. Louis, CV Mosby Co, 1959, p 327
8 Fulkerson LL: Personal communication
9 Lahey FH, Hare HF, Hau AD: Treatment of tuberculous adenitis. Lahey Clin Found Bull 1:2, 1940