DEGRADATION OF BIOCHEMICAL ACTIVITY IN SOIL STERILIZED BY DRY HEAT AND GAMMA RADIATION

KATHERINE L. SHIH and KENNETH A. SOUZA

Extraterrestrial Biology Division, Ames Research Center, NASA, Moffett Field, Calif. 94035

Abstract. The activities of three enzymes present in soil, phosphatases, urease, and decarboxylase, were monitered as indicators of the loss of biochemical information occurring when soil was sterilized by dry heat (0.08% relative humidity), gamma radiation, or a combination of both. More enzymatic activity was retained in soil sterilized by a long exposure to dry heat at relatively low temperature (8 weeks at 100.5° C) than by a shorter exposure to a higher temperature (2 weeks at 124.5° C). No enzymatic activity was detectable in soil sterilized by an even higher temperature (4 days at 148.5° C). Soil sterilized with 7.5 Mrads of radiation retained much higher enzymatic activity than with heat sterilization. Combining sublethal doses of heat radiation effectively sterilized the soil and yielded enzymatic activities higher than those of soil sterilized by dry heat alone but lower than those of soil sterilized by radiation.

1. Introduction

On July 20, 1976 the first of two Viking spacecraft settled onto the surface of Mars and began to search for life. Regardless of whether the Viking mission detects or fails to detect life, it is only a beginning. Beyond Viking there will be other missions to Mars and it is likely that one of those will be a mission to return a soil sample of Mars to the Earth. Only within a terrestrial laboratory can the soil be examined with all the resources modern science and technology can muster.

When such a sample is returned to Earth it must be treated as a potentially hazardous material. Many scenarios can be proposed which describe the havoc wreaked upon us by unleashing foreign and potentially harmful organisms into our environment. The true risk, however, cannot be measured, and for this reason any returned sample should be thoroughly contained during Earth entry, recovery, and during the scientific investigations which follow. Should it be necessary to sterilize the sample within a terrestrial containment facility, one would want to do it efficiently, with the utmost safety, and yet in a way that retains as much scientific information as possible.

This study was undertaken to determine if two commonly used sterilants, dry heat and gamma radiation, could be combined to obtain a sterilizing regime which would maximize the destruction of microorganisms and minimize the loss of biochemical information in samples of soil.

2. Material and Methods

The soil used in this study was a sandy loam collected in Siskiyou County, near Macdoel, California in 1967. It is a gray-brown podzol of acid reaction which was sieved through

0.25-in. mesh, air dried, and mixed before being stored at room temperature in covered bottles [13].

Soil and sample preparation and sterilization assay. All soil samples consisted of 200 mg in glass tubes, 7.5 cm x 1.0 cm. Twenty samples were cotton plugged, placed in 400-ml beakers, and sterilized by heating to 170° C for 5 days. These samples were used as sterile controls for all experiments. Another 20 tubes (unsterile) were then placed in the same beaker, and the total of 40 tubes represent one experimental set. These sets were exposed to a test sterilization regime, and then each tube was filled with 2 ml of a sterile broth containing per 100 ml of Siskiyou soil extract: 1.0 g Trypticase soy broth (BBL), 0.1 g soluble starch (Fisher), and 0.2 g yeast extract (Difco). Soil extract was prepared by shaking 100 g of soil with 1 liter of water at room temperature for 1 h, autoclaving at 121° C and 15 psi for 90 min, and centrifuging at 12,000 x g for 15 min at 5° C. The supernatant fluid was filtered through Whatman No. 2 filter paper, and the filtrate constituted the final Siskiyou soil extract.

Incubation of all tubes was at 30° C, and turbidity was determined after 2 weeks. If a tube was scored turbid, the cause of the turbidity was determined microscopically, and in all such cases the turbidity was easily traced to numerous bacterial forms. A 0.1-ml sample from all tubes lacking turbidity was plated on the above described medium solidified with 1.5% (w/v) agar and incubated at 30° C for 2 weeks. In no-case were colonies observed. The 20 cotton-plugged tubes which were heat sterilized prior to experimentation served as a check on the level of contamination introduced by the assay procedure. Sterilization was considered to be achieved in the experimental tubes only when none of the 20 tubes became turbid.

In the experiments to test the synergistic effects of heat and irradiation on the inactivation of soil organisms, a different assay method was used. Instead of adding media to the experimental tubes and looking for the development of turbidity, each soil sample was mixed with 1.0 ml of water, and survivors were estimated on the solid medium described above. Colonies were counted after 2 weeks of incubation at 30° C.

Dry heat exposure. Dry heat was applied to soil samples with a vacuum oven (Precision Scientific with internal dimensions of 18 x 13 x 13 in.). Temperature was measured with a YSI model 42 SC telethermometer. Measurements at different locations within the oven indicated a variance of no more than 1° C between any two points within the oven at a temperature of 124.5° C. Three temperatures, 100.5° C, 124.5° C, and 184.5° C, were chosen for this study. Dry nitrogen gas and nitrogen gas carrying water vapor (dry nitrogen gas bubbled through temperature-controlled water) were mixed to give the combined gas a relative humidity of 0.08% at the exposed temperature, as measured with a hygrometer (model VK 36, Veekay). The gas passed through a copper coil within the oven to preheat the gas and flowed through the oven at a rate of 3 liters per minute. Beakers containing soil samples in glass tubes were covered loosely with aluminum foil during the dry heat exposure, and the covers were firmly secured following the completion of the treatment.

Gamma irradiation. Soil was irradiated with either of two ^{60}Co gamma cells with dose rates of 93 and 250 krads per hour. In combined treatment of dry heat and gamma irradiation, dry heat exposure was followed within 2 to 24 h by gamma irradiation.

Enzymatic activity assay. (i) Urease and decarboxylase. The evolution of $^{14}CO_2$ from [^{14}C]urea (2 mCi/mM, New England Nuclear) and [^{14}C]lactate (sodium $L - [1 - {}^{14}C]$lactate, 7.77 mCi/mM, New England Nuclear) was used to test urease and decarboxylase activity in soil. A test chamber to measure $^{14}CO_2$ evolution was prepared by using a short piece of rubber tubing to join two 10×20 mm tubes to form a cylindrical chamber (2). Soil (200 mg) and substrate (0.01 μCi, in 0.2 ml phosphate buffer, 0.05 M, pH 7, unless otherwise noted) were introduced into one tube of the chamber. A 12×14 mm strip of filter paper, moistened with 0.1 ml saturated $Ba(OH)_2$, was inserted in the opposite tube. Urea-containing samples were incubated 4 h at 23° C while those with lactate were incubated 7 h. These incubation periods were selected after determining that in unheated, unirradiated soil, the rate of label release from urea and lactate was constant for 4 h and 8 h, respectively. Beyond these intervals, the rate decreased markedly, suggesting the exhaustion of substrate. $^{14}CO_2$ collected by the filter paper was counted in a scintillation counter using a scintillation fluid described by DeVincenzi and Deal (2).

In order to determine if the assay methods described above were actually detecting enzymatic activity, inhibitors of urease (thiourea and *p*-hydroxymercuribenzoate) and decarboxylase (Pronase and $AgNO_3$) were used. Various concentrations of inhibitors were added to soil in 0.1-ml quantities and incubated with the soil prior to the addition of substrate (0.01 μCi of [^{14}C]urea or [^{14}C]lactate in 0.1 ml buffer or H_2O). In dry heat sterilization experiments, the differences in CO_2 evolution from samples treated or not treated with inhibitors were used to represent enzymatic activities.

(ii) Phosphatase. Phosphatase activity was determined by a flourimetric technique based on the use of β-naphthylphosphate (NP) as the flourogenic substrate (7). For each treatment, samples where divided into two groups. To each sample (200 mg) of the first group, 1.2 ml H_2O, 0.2 ml Modified Universal Buffer (7) (MUB, pH 7.35 unless otherwise mentioned), and 0.2 ml of 2×10^{-2} M NP in the same buffer were added. The second group served as a control and contained H_2O, MUB, but no NP. Each sample was shaken at room temperature (23° C) for 1 h, followed by the addition of 0.4 ml of 0.5 M NaOH to stop the reaction. NP was then added to the second group and all soil suspensions centrifuged at 27,000 x g for 15 min at 5° C. The flourescence of aliquots of the supernatant fluid was deterrmined in a Turner fluorometer containing a Corning 7--60 filter (peaking at 360 nm) in the activating beam, and with a 3--73 filter (peaking at 430 nm) in the emitted beam. The difference in readings between the samples with substrate added before incubation and those with substrate added after incubation represented the phosphatase activity.

As with urease and decarboxylase, various inhibitors [16] were tried to determine if the activity observed as in fact enzymatic. However, due to the complex mixture of

phosphatases in soil, no specific inhibitor of purified phosphatases was able to clarify this issue. Therefore, the effects of pH and a chelating agent (ethylenediamine-tetracetate [EDTA]), were investigated.

3. Results

Enzymatic activity inhibition study. To determine that the CO_2 evolution was due to the activity of the enzyme in question, experiments with various chemical inhibitors were performed. Autoclaved soil (90 min at 15 lb pressure and 121° C) completely lost the ability to evolve $^{14}CO_2$ from [^{14}C]urea (Table I). Thiourea, a competitor of urea for urease [3], was added at 1 M and 2 M concentrations and reduced $^{14}CO_2$ evolution to 15 and 6%, respectively. p-Hydroxymercuribenzoate (HMB), a chemical that reacts with SH groups which are the active sites of urease [12] to form S—Hg bonds, reduced the evolution of CO_2 in soil to 5% (1.9×10^{-3} M) and 0.5% (1.9×10^{-3} M), respectively. Furthermore, urease activity inhibited by HMB (1.9×10^{-3} M) could be restored to 70% of the initial activity after the addition of dithiothreitol (1.9×10^{-3} M), which restores the SH groups. Evolution of CO_2 from lactate (Table II) in soil was inhibited almost completely by either autoclaving the soil or by the addition of $AgNO_3$ (1 mg ml^{-1}), a respiratory poison. Pronase at 100 mg ml^{-1} reduced the total decarboxylase activity to less than 0.2% of the original activity. The inhibitor experiments described above indicated that the evolution of CO_2 from the substrates used was most likely due to enzymatic activity.

The effect of pH on the release of β-paphthol from β-naphthy-phosphate (NP) was similar to the effect of pH in the activities of a wide variety of enzymes (Table III). The

TABLE I
Inhibition of $^{14}CO_2$ evolution from [^{14}C]urea in soil

| Time of incubation (h) | ^{14}C activity (cpm)[a] | | | | | | Autoclaved[d] (90 min) | Without[d] soil |
| | Thiourea[b] | | | p-Hydroxymercuribenzoate[c] | | | | |
	Control	1 M	2 M	Control	1.9×10^{-3} M	1.9×10^{-2} M		
2	4163	640	290	2642	132	22	12	12
4	11252	1326	569	5360	325	39	15	14
6	14279	2088	890	7055	487	66	15	12

[a]Background: 14 cpm.
[b]Samples consisted of 200 mg of Siskyou soil; 0.1 ml of 1 or 2 M thiourea in phosphate buffer (0.05 M, pH 7.0) was added to samples. After 1 h incubation at room temperature, 0.1 ml of [^{14}C]urea (0.01 μCi activity) in the same buffer was added.
[c]A 0.1-ml aliquot of HMB in phosphate buffer (0.025 M, pH 8.5) or buffer only (control) was added to the samples. After 30 min incubation at room temperature, 0.1 ml of [^{14}C]urea (0.01 μCi) in buffer was added.
[d]A 0.2-ml aliquot of [^{14}C]urea (0.01 μCi) in 0.05 M phosphate buffer, pH 7.0, was added to each sample.

TABLE II

Inhibition of $[^{14}CO_2]$ lactate in soil

Time of incubation (h)	^{14}C activity (cpm)[a]							
	AgNO$_3$[b]			Pronase[c]			Autoclaved[d] (90 min)	Without[d] soil
	Control	0.1 mg ml^{-1}	1 mg ml^{-1}	Control	10 mg ml^{-1}	100 mg ml^{-1}		
2	782	55	14	1101	270	15	15	15
4	4399	214	14	4805	1365	22	12	16
8	7860	430	13	8896	2593	29	17	16

[a]Background: 15 cpm.

[b]Samples consisted of 200 mg of Siskyou soil; 0.1 ml of AgNO$_3$ in H$_2$O, or H$_2$O only, was added to samples. After 1 h incubation at room temperature, 0.1 ml of $[^{14}C]$ lactate (0.01 μCi) in H$_2$O was added to each sample.

[c]A 0.1-ml aliquot of Pronase (in 0.05 M phosphate buffer, pH 7) or buffer only (control) was added to the samples and incubated at 37° C for 90 min; 0.1-ml of $[^{14}C]$ lactate (0.01 μCi) was then added.

[d]A 0.2-ml aliquot of $[^{14}C]$ lactate (0.01 μCi) in 0.05 M phosphate buffer, pH 7.0, was added to each sample.

TABLE III

Inhibition of β-naphthol release from β-naphthylphosphate in soil

pH[a]	Arbitrary units of β-naphthol[b]
Control soil	
pH 4.55	57
pH 6.35	350
pH 7.35	582
pH 8.35	114
pH 4.55 + EDTA[c]	13
pH 7.35 + EDTA	320
Autoclaved soil	
pH 7.35	2

[a]Modified Universal Buffer was adjusted to indicated pH with 1 N NaOH before adding to soil samples.

[b]One unit represents 0.029 μg of β-naphthol.

[c]In EDTA treatment, samples were pre-incubated with 0.4 ml of 0.2 M EDTA for 30 min followed by the same procedure as described in Methods. The final concentration of EDTA was 0.05 M.
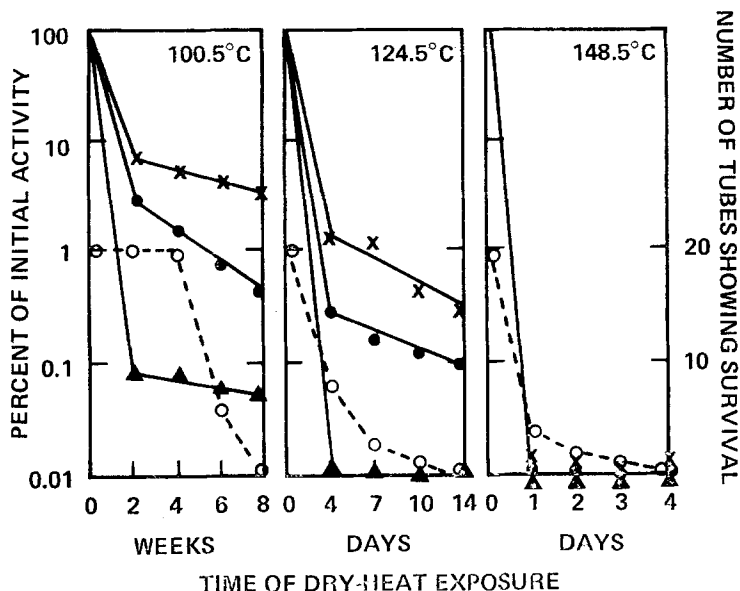
Fig. 1. Effects of dry heat sterilization on enzymatic activity in soil: urease activity (●) (the difference in $^{14}CO_2$ evolution after 4 h of incubation between samples treated or not treated with p-hydroxymercuribenzoate (1.9×10^{-2} M), as described in Table I); decarboxylase activity (▲) (the difference in $^{14}CO_2$ evolution between samples treated and not treated with Pronase (100 mg ml^{-1}), as described in Table II, after 7 h of incubation); phosphatase activity (x) (phosphatase activity was determined as described in Methods); number of tubes showing viability (○).

TABLE IV
Sterilization of soil by dry heat and gamma irradiation[a]

Gamma irradiation (Mrads)	Temperature								
		100.5° C				124.5° C			
	22° C[b]	3 days	5 days	1 week	2 weeks	8 h	16 h	24 h	48 h
0	20	20	20	20	20	20	20	20	20
0.75	20	7.5	5	1.5	0	14	10	8.5	0
1.50	20	5	5	0	—	6.5	2	3	—[c]
2.25	20	3.5	2	—	—	4	4	0	—
3.0	20	5	0	—	—	2	0	—	—
3.75	17.5	3	—	—	—	2	—	—	—
4.5	11	0	—	—	—	0.5	—	—	—
5.25	8	—	—	—	—	0	—	—	—
6.0	2	—	—	—	—	—	—	—	—
6.75	1	—	—	—	—	—	—	—	—
7.5	0	—	—	—	—	—	—	—	—

[a]Numbers are the average number of tubes showing viability from two experimental sets, each set with 20 tested tubes and each tube containing 200 mg soil.
[b]Room temperature.
[c]No measurements.

activities at pH 4.55 and 8.35 were 10% and 20%, respectively, of that at pH 7.35. Since metal ions are required for the activity of certain purified phosphatases and since the activity can be inhibited by chelating agents [16], the effect of EDTA on soil phosphatase activity was studied. This chelating agent (0.05 M) reduced the activity to 55% at pH 7.35 and to 23% at pH 4.55. Autoclaved soil possessed a very low activity. These results suggested that the release of β-naphthol from NP in soil was enzymatic.

Effects of dry heat on enzymatic activity and on sterilization. Three different temperatures, 100.5° C, 124.5° C, and 148.5° C were used to test the effects of dry heat on enzymatic activity (Figure 1). At all three temperatures, a rapid initial inactivation of urease, decarboxylase, and phosphatase was observed which was followed by a steady but slower exponential rate of decline. At 100.5° C, the activity level of all three enzymes dropped to less than 10% of the initial activity within the first 2 weeks of exposure, even though there was no drop in the number of tubes having organisms capable of growth. However, there undoubtedly was a drop in the number of viable organisms per tube after such an exposure, and this reduction could contribute to the reduced enzymatic activity.
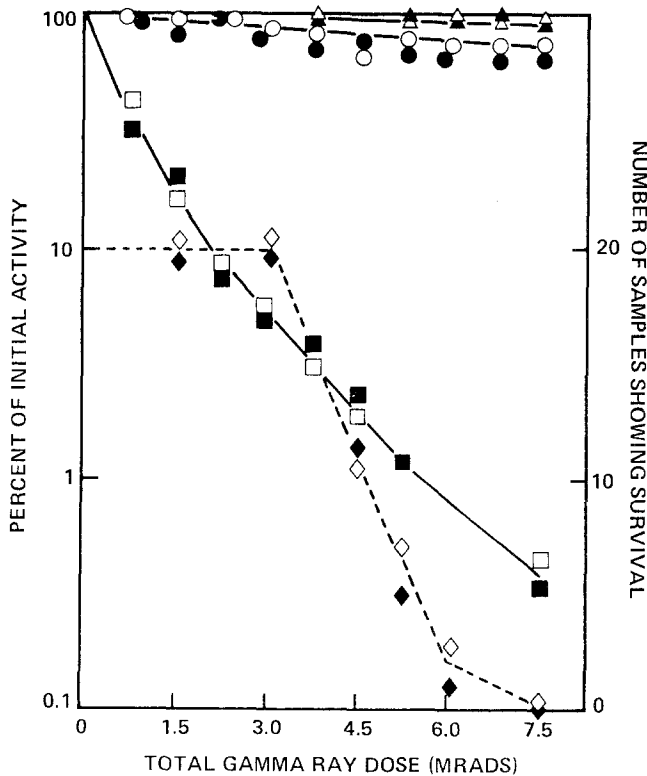

Fig. 2. Effects of gamma irradiation on enzymatic activity in soil. Solid and open symbols represent a dose rate of 250 krad h^{-1} and 93 krad h^{-1} respectively: phosphatase (● and ○); decarboxylase (■ and □); urease (▲ and △); number of tubes showing survival out of 20 tubes tested (♦ and ◊).
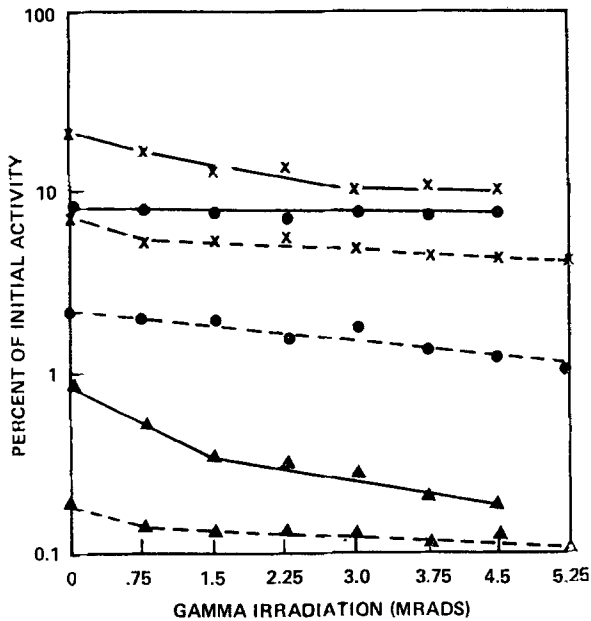
Fig. 3.   Enzymatic activity of soil exposed to a constant heat exposure coupled with various doses of radiation. Symbols: ———100.5° C for 3 days; - - - - -124.5° C for 8 h; x phosphatase; ● urease; ▲ decarboxylase.

At 124.5° C, a sharp drop in both viability and enzymatic activity was observed within the first 4 days of heating, and an even greater drop occurred within the first day at 148.5° C. After 8 weeks at 100.5° C, viability was not observed in any of the 20 tubes although enzymatic activities of 2.5, 0.5, and 0.06% of the original levels remained for phosphatase, urease, and decarboxylase, respectively. In contrast, sterility was achieved in 14 days at 124.5° C and 4 days at 148.5° C, but in both cases the activities of all three enzymes were below those in soil heated at 100.5° C for 8 weeks, and in some cases the activities of enzymes were not detectable by the present assay method.

Effects of gamma irradiation on sterilization and on enzymatic activity in soil. Soil samples were exposed to different doses of gamma rays with a dose rate of 250 krads/h. No viability was observed in soil samples exposed to a total dose of 7.5 Mrads (Fig. 2). However, enzymatic activity was only partially reduced at the same level of irradiation. Urease was insensitive to a 7.5-Mrad dose of gamma rays, phosphatase retained 70% of the original activity, while decarboxylase activity in the irradiated soil was reduced to 0.5% of the initial level.

A similar experiment was performed by administering a total dose of 7.5 Mrads to soil samples at a dose rate of 93 krads/h. Results of this experiment did not differ significantly from the preceding one either in the fraction of tubes showing viability or in the residual enzymatic activity.

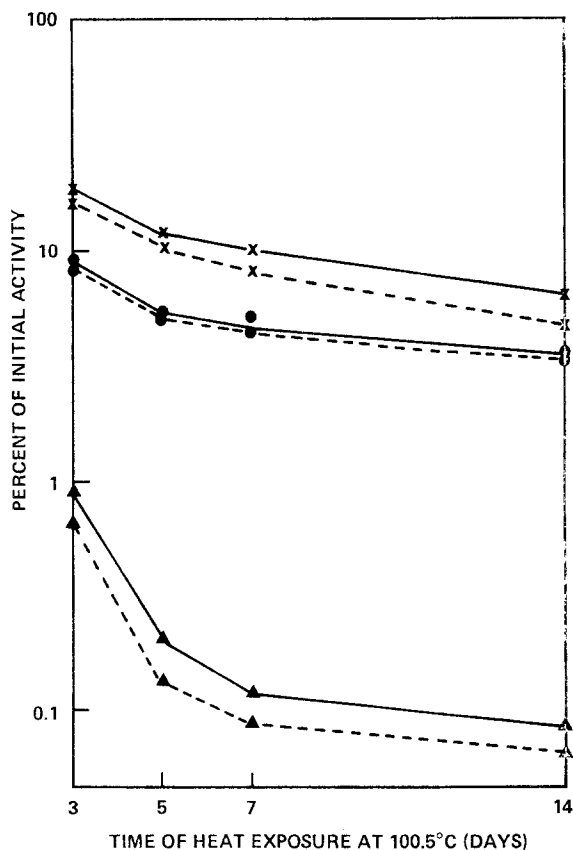*Sterilization of soil by heat and radiation.* Viability was not observed in any of 40

Fig. 4.   Enzymatic activity of soil exposed to dry heat (100.5° C) for different durations followed by 0.75 Mrads of gamma irradiation.. Symbols: ———— heat alone; - - -heat +0.75 Mrads of gamma radiation; x phosphatase; ● urease; ▲ decarboxylase.

samples heated for 2 weeks at 100.5° C and followed by a nonsterilizing dose (0.75 Mrads) of gamma irradiation (Table IV). Soil heated for 48 h at 124.5° C and followed by 0.75 Mrads of gamma irradiation yielded similar sterilization results. By relating the combination treatment to the levels of heat and radiation which, when applied separately, yield the same results (Figures 1 and 2), one sees that only 1/10 of the radiation dose and 1/4 and 1/7 the exposure of 100.5° C and 124.5° C, respectively, were required to sterilize soil.

Effects of heat and radiation exposure on enzymatic activity in soil. (i) Enzymatic activity of soil exposed to a constant heat coupled with various doses of radiation. Soil samples heated at 100.5° C for 3 days were subjected to doses of gamma irradiation ranging from 0.75 to 4.5 Mrads. As seen in Table IV, a combination of 0.75 Mrads with the aforementioned heat exposure was insufficient to render the soil sterile. The same heat exposure coupled with 4.5 Mrads was adequate to reduce the number of tubes
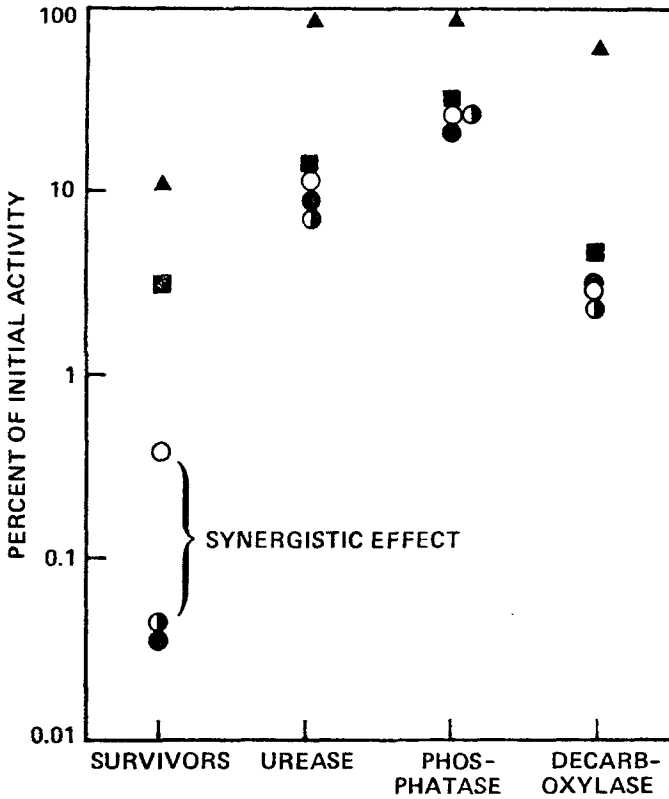
Fig. 5.   Synergistic effects of dry heat and gamma irradiation on inactivation of soil organisms and soil enzymes; gamma irradiation (▲) (250 krads); dry heat (■) (1.5 h at 124.5° C); combined treatment (●) (gamma irradiation was performed 24 h after dry heat treatment); combined treatment (◐) (gamma irradiation was performed 2 h after dry heat treatment); theoretical survival for combined treatment (○).

showing viability to zero. Enzymatic activity was not totally destroyed by this latter treatment as shown in Figure 3. Soil heated for 3 days at 100.5° C and exposed to 4.5 Mrads of gamma rays retained 10% of the phosphatase activity originality present, as well as 8.2 and 0.02% of the original urease and decarboxylase, respectively. Soil heated for 8 h at 124.5° C and irradiated with 5.25 Mrads of gamma rays, retained 4.0, 1.1, and 0.1% of the initial activities of phosphatase, urease, and decarboxylase, respectively.

(ii) Enzymatic activity of soil exposed to different durations of heat exposure coupled with a nonsterilizing dose of gamma irradiation. Figure 4 shows that enzymatic activity of soil exposed to heat and gamma irradiation mainly reduced by heat exposure, and that 0.75 Mrads of gamma irradiation further reduced the activity only slightly. Soil sterilized by the combination treatment of heat (2 weeks at 100.5° C) and irradiation (0.75 Mrads) retained 4.5, 3.2, and 0.07% of initial phosphatase, urease, and decarboxylase activities, respectively. Again, these activities were higher than those of the soil samples sterilized only by exposure to 100.5° C for 8 weeks (Figure 1).

Thus, the increase in biochemical information in soil gained by reducing thermal destruction was not offset by radiation damage. However, for all combinations of heat and radiation used to sterilize soil, biochemical activity retained was lower than that of soil sterilized by radiation alone.

Synergistic effects of heat and radiation on the inactivation of soil organisms and enzymatic activity. Figure 5 illustrates a typical experiment in which soil was subjected to 124.5° C for 1.5 h and a gamma ray dose of 250 krads (250 krads^{-1} h). The inactivation of soil organisms by the combined treatment was approximately 10 times greater than that expected by the product of individual inactivation of dry heat and gamma irradiation. Dry heat treatment alone and gamma irradiation alone reduced the soil organisms to 2.8 and 1.1% of the initial population, respectively. Theoretically, the combined treatment should have reduced the population to 2.8% x 1.1% = 0.31%. The observed surviving population of the combined treatment, nevertheless, was only 0.038%. The synergistic inactivation of soil organisms was similar regardless of whether the time between heat and radiation treatments was 2 or 24 h. The synergistic effects, however, were not so displayed in the reduction of enzymatic activity in soil.

A second study used a heat exposure of 100.5° C for 48 h and a radiation dose of 250 krads at a rate of 93.5 krads^{-1} h. This combination of heat and irradiation inactivated the initial microbial population to levels similar to those obtained with the heat and radiation exposure mentioned above, and a similar synergistic response was obtained.

4. Discussion

Enzymatic activity in soil may result from enzymes contained within soil organisms (viable or nonviable) and from microbially released enzymes which may persist in an active and stable state for prolonged periods in association with soil colloids [12]. Sensitivity to dry heat or gamma rays may depend on the environment of the enzyme (i.e., whether it is within a cell or bound to soil particles) and also the number of active and sensitive sites the molecule contains [6]. Of the three enzymes tested, decarboxylase was the most sensitive to both heat and irradiation. Phosphatase retained higher activity than urease after dry heat treatment, but urease retained the higher activity after gamma irradiation. All the enzymes were more sensitive to a sterilizing dry heat exposure, regardless of the temperature applied, than to a sterilizing dose of gamma rays. However, more activity was retained when the soil was sterilized by a long exposure at relatively low temperature than by a much shorter exposure to high temperature. The present study failed to detect any effect of dose rate on the destruction of viable organisms or enzymatic activity in soil. It is plausible to assume that the limited range of dose rates studied was not able to demonstrate the effect under the present assay system.

Many reports have shown that the combined effect of heat and irradiation on bacterial spores not only may be additive but also may result in synergism [5, 8, 14]. In this study a significant synergistic effect (about 10 times higher than the theoretically calculated level) on the inactivation of soil organisms was demonstrated. One possible explanation

for this result is that heat and gamma radiation affect two different systems. For example, heat may affect system A, i.e., protein [1, 11], and irradiation may affect system B, i.e., DNA [4]. When heat alone is applied, cells receiving a sublethal dose may require system B to repair damage, and conversely with radiation. Those cells that receive damage which is repairable if either heat or radiation alone is applied are not able to repair the damage when treatment is applied in concert and consequently fail to grow and reproduce. Another possible explanation is that dry heat may lead to metabolic injury of the organisms which sensitizes them to gamma rays [9, 10, 15]. That soil could be rendered sterile by a nonsterilizing dry heat exposure followed by a nonsterilizing dose of gamma radiation demonstrated the higher efficiency of the combined treatment.

·The synergistic effect on inactivation of soil organisms by the combination treatment of heat and irradiation was not observed with soil enzymatic activity. Soil sterilized by heat and irradiation maintained a higher level of residual enzymatic activity than soil sterilized by a longer exposure to the same heat alone. In fact, heat was the deciding factor in the inactivation of enzymatic activity in the combined treatment, since irradiation only slightly increased inactivation of enzymes following heat exposure. Therefore, sterilization by a combination of heat and irradiation decreases the biochemical information in soil less than does sterilization by heat alone.

This study suggests that to retain the maximum amount of biochemical activity in soil and yet sterilize it, gamma irradiation rather than dry heat should be used. Dry heat, however, because of its penetrating power is frequently the method of choice when sterilization alone is considered. A combination of heat and irradiation has the desired qualitites of each, yet has the advantage of producing a synergistic inactivation of soil microbes and thus may prove of value in reconciling problems of the science and safety of an extraterrestrial soil sample return mission or whenever sterilization with a minimum of biochemical destruction is desired.

References

[1] Bhaumik, G. and Bhattacharjee, S. B.: 1975, 'Repair of Heat Injury in Thymine Auxotrophs of *Escherichia coli*,' *Can. J. Microbiol.* **21**, 1274–1277.

[2] DeVincenzi, D. L. and Deal, P. H.: 1976, 'Studies Related to the Development of the Viking 1975 Labeled Release Experiment,' NASA TR-460. U.S. Government Printing Office, Washington, D.C.

[3] Kistiakowsky, G. B. and Shaw, W. H. R.: 1952, 'On the Mechanisms of the Inhibition of Urease,' *J. Am. Chem. Soc.* **75**, 866–871.

[4] Okada, S.: 1970, 'DNA as a Target Molecule Responsible for Cell Killing,' in *Radiation Biochemistry*, Vol. I, Academic Press, New York, pp. 101–147.

[5] Pallas, J. E. III and Hamdy, M. K.: 1976, 'Effects of Thermoradiation on Bacteria,' *Appl. Environ. Microbiol.* **32**, 250–256.

[6] Pettit, N. M., Smith, A. R. J., Freedman, R. B., and Burns, R. G.: 1976, 'Soil Urease: Activity, Stability, and Kinetics Properties,' *Soil Biol. Biochem.* **8**, 479–484.

[7] Ramirez-Martinez, J. R. and McLaren, A. D.: 1966, 'Determination of Soil Phosphatase Activity by Fluorimeter Technique,' *Enzymologia* **30**, 243–253.

[8] Reynolds, M. C., Lindell, K. F., David, T. J., Favero, M. S., and Bond, W. W.: 1974, 'Thermoradiation Inactivation of Naturally Occurring Bacterial Spores in Soil,' *Appl. Microbiol.* **28**, 406–410.

[9] Richmond, R. C., and Powers, E. L.: 1974, 'Modification of Radiation Sensitivity of Bacterial Spores by Silver Salts,' *Radiat. Res.* **58**, 470–480.

[10] Richmond, R. C., Simic, M., and Powers, E. L.: 1975, 'Radiation Sensitivity of *Bacillus megaterium* Spores in the Presence of Co(III) Complexes,' *Radiat. Res.* **63**, 140–148.

[11] Rosenthal, L. J. and Iandolo, J. J.: 1970, 'Thermally Induced Intracellular Alteration of Ribosomal Ribonucleic Acid,' *J. Bacteriol.* **103**, 833–835.

[12] Rowell, M.: 1974, 'Production and Characterization of Enzymes Stabilized and Humic Acid Analogues,' University Microfilms International, Ann Arbor, Michigan.

[13] Silverman, M. P., and Munoz, E.: 1974, 'Microbiol Metabolism and Dynamic Changes in the Electrical Conductivity of Soil Solutions: A Method for Detecting Extraterrestrial Life,' *Appl. Microbiol.* **28**, 960–967.

[14] Sivinski, H. D., Garst, D. M., Reynolds, M. C., Trauth, C. A. Jr., Trujillo, R. E., and Whitfield, W. J.: 1972, 'The Synergistic Inactivation of Biological Systems by Thermoradiation,' in G. B. Phillips and W. S. Miller (eds.), *Industrial Sterilization*, Duke University Press, Durham, North Carolina, pp. 305–335.

[15] Tallentire, A. and Jones, A. B.: 1973, 'Radiosensitization of Bacterial Spores by Potassium Permanganate,' *Int. J. Radiat. Biol.* **24**, 345–354.

[16] *Worthington Enzyme Manual*, 1972, Worthington Biochemical Corporation, Freehold, New Jersey.