Each value in table 1 represents the average of 3 to 8 independent observations. From these experiments it appears that the lag period is appreciably lengthened by as little as 500 ergs per mm^2 of ultraviolet, but that doubling this dose results in only a slight further increase for cells grown on solid medium and no increase for cells grown in nutrient broth. The most striking feature of these observations is the marked reduction in the lag period as the result of exposure of irradiated cells to reactivating light. The reactivation treatment appears to have no effect on cultures not previously exposed to ultraviolet light. None of the treatments used affected the generation time during the logarithmic growth period. In all cases this remained at 19 to 20 minutes.

VARIATION OF IRRADIATION EFFECTS ON MICROORGANISMS IN RELATION TO PHYSICAL CHANGES OF THEIR ENVIRONMENT

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It has been shown that slow cathode ray irradiation of bacteria *in vacuo* requires an unusually high dose compared to measurements made with soft X rays of comparable energies under normal air pressure (Moos, 1946, unpublished data; Moos, Nucleonics, **8**, 50, 1951; Hardung, Helv. Phys. Acta, **18**, 45, 1944; Haskins, J. Applied Phys., **9**, 553, 1938). Experiments by C. G. Dunn *et al.* (J. Applied Phys., **19**, 605, 1948) produced similar results with *Saccharomyces cerevisiae*. Hardung and Moos (Experientia, **5**, 155, 1949) pointed out that dehydration might be a cause of increased resistance of organisms irradiated *in vacuo* as compared with organisms irradiated in a liquid environment.

This hypothesis suggested that a more quantitative investigation might disclose some interesting information about the interaction of radiation with biological matter in different physical environments.

Pseudomonas aeruginosa and *Escherichia coli* were grown in horse meat infusion broth for 24 hours and 16 hours, respectively, at 36 C. For the experiments, 0.1 ml of these cultures was introduced into 2 ml "lusteroid" containers, then placed into pyrex test tubes and connected with an ordinary lyophilizing unit. After 2.5 hours of evacuation about 97.5 per cent of volatile material was removed from the broth leaving a residue of the bacteria, proteins, metabolic products, and salts; further drying did not change this result.

A 186 Kv X-ray unit served as radiation source. The beam was filtered by 0.25 mm copper and 0.55 mm aluminum. The samples were irradiated at a dose of approximately 250 rpm.

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Three samples prepared under identical conditions were employed in a typical experiment in the following manner. One sample, after drying, was resuspended with 0.1 ml distilled water and irradiated simultaneously with the second, but dry, specimen. The third dried specimen was kept as a control. All samples including the controls were kept at 2 C during irradiation to inhibit additional growth. After irradiation the two specimens were suspended in 0.1 ml water. Subsequent diluting and plating in agar media were done in accordance with standard procedures.

In a second series of experiments distilled water was employed in preparing the bacterial suspensions. The original broth cultures were centrifuged and washed several times with distilled water which was boiled shortly before its application to remove as much free oxygen as possible. At the end of this procedure the remaining bacteria were resuspended in distilled water, dried, and treated in the same manner as the organisms in broth.

TABLE 1

Effect of the state of the solvent on the survival of Pseudomonas aeruginosa when subjected to X-irradiation

SERIES	STATE SOLVENT	PERCENTAGE SURVIVAL AFTER IRRADIATION				
		2,325 r	3,000 r	3,550 r	4,800 r	6,000 r
a.	Liquid broth	12.9 ± 1.6*	12.9 ± 2.1	—	1.9 ± 0.2	1.8 ± 0.2
	Dried broth	88 ± 3	—	—	51 ± 5.4	—
b.	Liquid dist. H_2O	6.3 ± 1.7	0.97 ± 0.14	1.5 ± 0.8	0.65 ± 0.1	0.15 ± 0.1
	Dried water	10.5 ± 4.4	—	5 ± 2.7	1.48 ± 0.3	—
c.	Frozen broth	—	—	—	30.1 ± 4.6	—

* Standard error.

In another experiment the bacterial broth solution was frozen and not dried. The first of the three samples was defrosted at room temperature just before irradiation and the remaining samples shortly after irradiation.

A 24 hour incubation time at 36 C followed the plating. The colonies that appeared were counted by a procedure described in an earlier paper (Moos, *in press*).

The data obtained from experiments with *P. aeruginosa* irradiated under different environmental conditions are tabulated in table 1.

Drying of the bacteria suspended in broth or in distilled water had considerable protective effect against radiation. In both cases the difference between the samples irradiated in a liquid state and those irradiated in a dry state is statistically (95 per cent) significant. Dried organisms, originally suspended in broth, seem to be better protected against effects of radiation than dried bacteria suspended in distilled water. This suggests some additive protective actions of the two factors, components of the broth and drying.

(A film of the residue left by the broth after the drying probably covers the bacteria.)

The effect of radiation on a frozen bacterial-broth suspension was of the same order of magnitude as the effect in experiments with the bacteria in dry state. Here, as expected, the indirect action[2] of radiation must have been inhibited to a great extent.

Irradiation of *E. coli* in broth solution and in the dried state showed that the results observed with *P. aeruginosa* can be obtained also with another organism. For example, at 3,000 r the number of survivors in the irradiated broth suspension is approximately one-fourth the number of bacteria surviving after an aliquot of the same broth suspension was dried and irradiated.

The experiments show that bacteria in a dry state are much less affected by irradiation than bacteria in solution. This observation cannot be explained on the purely physical basis of X-ray absorption. The indirect action of radiation on dried organisms must be greatly reduced, but it has not been shown to be the sole reason for the high protective effect of dehydration.

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[2] The definition of indirect action of radiation in this case covers only lethal events due to absorption of radiation outside the bacteria.

FURTHER USE OF DIPHENYLAMINE FOR THE STUDY OF CAROTENOID BIOSYNTHESIS IN MYCOBACTERIUM PHLEI

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It has been demonstrated that carotenoid production in *Mycobacterium phlei* is strongly inhibited by diphenylamine at concentrations which do not affect growth (Turian, G., Helv. Chim. Acta, **33,** 1988, 1950). There is good reason for thinking that diphenylamine acts as an antioxidant, directing metabolism toward the formation of the less oxidized representatives of the C_{40}-polyene series. In *M. phlei* this inhibition was found to be most marked at the terminal synthetic steps, i.e., apparent conversion of neutral hydrocarbons into acidic compounds (keto-enol polyenes such as chrysoflein). More recently it has been reported by T. W. Goodwin (Biochem. J., **49,** xxiii, 1951) that in *Phycomyces* grown in the presence of diphenylamine the observed decrease in colored polyenes is accompanied by an increase in hydrogenated C_{40}-polyenes such as phytofluene and phytoene. Similar results have been obtained by the authors in *Neurospora* inhibited by diphenylamine or grown in the absence of light (unpublished observations).

From the nature of its inhibitory action it was felt that diphenylamine might

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