injected into the eyes of guinea pigs, the serum completely prevented the corneal reaction and the production of complement fixing antibodies. Pooled sera collected from normal mumps susceptible children, when mixed with mumps virus and injected into the eyes of guinea pigs irregularly prevented the corneal reaction and the production of complement fixing antibodies. (2) Subsequent to the injection of mumps virus into the eye of the guinea pig, complement fixing antibodies appear in the convalescent sera between the 4th and 8th day, persist at significant levels until the 20th to 24th days, and then decline. (3) Mumps virus persists or grows in guinea pig eyes for at least 8 days after inoculation. (4) Chick embryo adapted strains of mumps virus recently recovered from parotid tissue, saliva and testicular fluid when injected into guinea pig eyes produce the corneal reaction, and complement fixing antibodies were demonstrated in the convalescent sera.

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Influence of Temperature upon Radiation Sensitivity of Thermophilic and Mesophilic Bacteria. (19256)

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Thermobiosis has been the subject of considerable speculation, but of little experimentation. Gaughran(1) indicated that there was no fundamental difference in the effect of temperature on the respiratory systems of stenothermophilic and mesophilic bacteria. He postulated that the thermophilic organism may produce a substance to protect its proteins against heat. Allen(2) postulated that growth at thermophilic temperatures was possible because of an increased rate of synthesis of cellular proteins. She pointed out the relative ease with which thermophilic strains may be obtained from mesophilic cultures, and suggested that a very simple genetic change must be involved. Militzer *et al.*(3-5), and Georgi *et al.*(6) have recently shown that a number of thermophilic enzymes are much more heat stable than are those of mesophiles. The influences upon radiation sensitivity of temperature and of oxygen tension during irradiation have been examined by several workers. Hollaender *et al.*(7) reported an increased X-ray sensitivity when *Escherichia coli* was irradiated in an aerobic atmosphere. The same was also observed at low temperatures (2°C), but only when oxygen was present. Wyss(8) reported that hydrogenase-producing organisms can resist radiation much more effectively in an atmosphere of hydrogen than of nitrogen or methane, and that an oxygen atmosphere markedly increased their sensitivity. The demonstration of heat-stable enzymatic proteins in thermophiles suggests the presence of heat-stable nuclear proteins. Since thermophilic strains may be readily selected from mesophilic cultures, it seems doubtful that an independent genic alteration would be necessary for rendering each specific protein heat-stable. It appears more reasonable to believe that the modification resulting in an increased heat stability is established in the non-specific protein molecule before its final specificity is determined, *i.e.*, early in its synthetic sequence.

The present investigation was undertaken

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in an attempt to determine whether the intact genetic apparatus is heat-stable in thermophilic organisms when subjected to ultraviolet radiation at different temperatures should give data indicative of their relative nuclear heat-stability, since this radiation is heavily absorbed by such material. A heat-stable protein would not be expected to be affected by radiation as strongly at different temperatures as would be a heat-labile one. The latter would suffer concomitant heat and radiation denaturation.

Experimental methods. Spore suspensions of *Bacillus stearothermophilus,* strain No. 1503 (obtained from the National Canners Association), and *Bacillus globigii* were prepared from Difco nutrient agar flats (containing 0.2% soluble starch) which had been incubated for 5 days at 55 and 37°C, respectively, (at which time most of the vegetative cells had lysed). The spores were then washed with M/200 phosphate buffer, resuspended in the buffer, and filtered through cotton to remove the clumps. These suspensions were irradiated in the buffer at pH 7.0, either in large petri dishes containing 50 ml of spore suspension, or in special quartz cuvettes (17 ml) designed for irradiation through the side of the upright vessel. The latter suspensions were agitated during irradiation with air or hydrogen gas, which was brought to the proper temperature prior to its being bubbled into the suspension by passing it through a coil immersed in a water bath. Suspension temperatures during irradiation were 4, 24, 37, 55, 75, 90, and 100°C, and suitable viability controls were effected for each series. Temperatures above 24°C were held constant by the use of a small thermostatically controlled hotplate. The buffer was heated to the proper temperature prior to the addition of spores in order to limit possible germination during a slow heating, and the suspension was then immediately irradiated. Predetermined amounts of heated buffer were added at intervals where needed to compensate for loss by evaporation. Water vapor over the open plates was removed by passing a stream of sterile air directly over the surface of the spore suspension. Aliquots were withdrawn at intervals and plated for survivors and zero-point mutants and for inoculation into Difco nutrient broth tubes for the later determination of end-point mutants. Cultures were plated as soon as turbidity appeared in an effort to minimize selection factors, since growth rates of the streptomycin resistant and sensitive strains were found to be the same during the initial stages of growth, whereas the final turbidity attained by the resistant strains was lower. A level of streptomycin (2.0 μg/ml) was selected which yielded 1.1 and 0.5 spontaneous mutants/million respectively, for *B. globigii* and *B. stearothermophilus*. The colonies appearing upon this level of antibiotic were tested further for confirmation of their resistance. The activity of the level of streptomycin employed remained constant over the 3 or 4 days involved in incubation at 55°C. Mutation incidence was also determined in control experiments using the triphenyltetrazolium method of Lederberg(9) for the detection of fermentation mutants, and in all cases the results were found to be comparable with those obtained using streptomycin-resistance as the criterion of mutability. The incidence of thermophilic biochemical mutants(12) was too low to enable comparisons to be made. Determination of zero-point mutation incidence in spore experiments was complicated by germination factors, and such determinations yielded erratic results. Therefore, only end-point mutation incidences were reported. In early experiments the spores were uniformly heat-shocked for 10 minutes at 95°C(10) but such treatment applied during experiments designed to determine an effect of temperature itself is not desirable. Mefferd and Campbell(11) have shown that the same order of germination of thermophilic spores can be secured when a plating medium containing 10^{-6} parts furfural is substituted for a heat shock. Direct plating upon the starch-nutrient agar medium containing furfural was, therefore, employed here. No detectable photoreactivation was noted in the irradiated spores of any strain. Because of the many variables present, very carefully and empirically standardized conditions were employed throughout all operations. Even so, results of individual experiments varied to a small degree, but trends were constant.

Experimental results. Survivor and endpoint mutation incidence curves for *B. globigii* spores irradiated in air at different temperatures demonstrated a marked increase in ultraviolet sensitivity at 4 and 55°C as compared with 24 and 37°C. Thermophilic spores, on the other hand, were only slightly more sensitive at 4 and 75°C than at 24, 37, or 55°C. Representative experiments are reported in Fig. 1 and 2, respectively. Changes in radiation sensitivity at different temperatures are more apparent if the data are reported as in Fig. 3 and 4. To obtain these, a constant ultraviolet dose, *i.e.*, seconds of irradiation, was selected and the number of survivors and mutants were plotted against the temperature

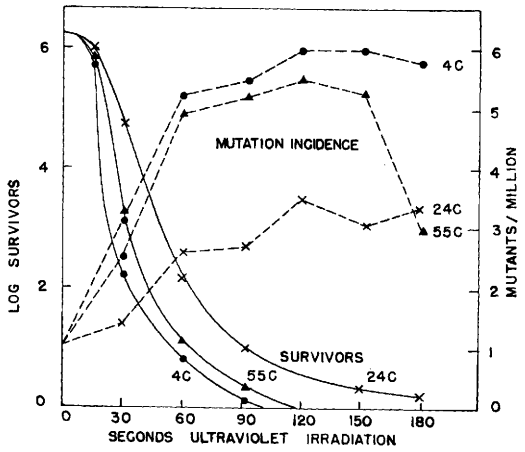

FIG. 1. Survivor and mutation incidence curves for *Bacillus globigii* at different temperatures.

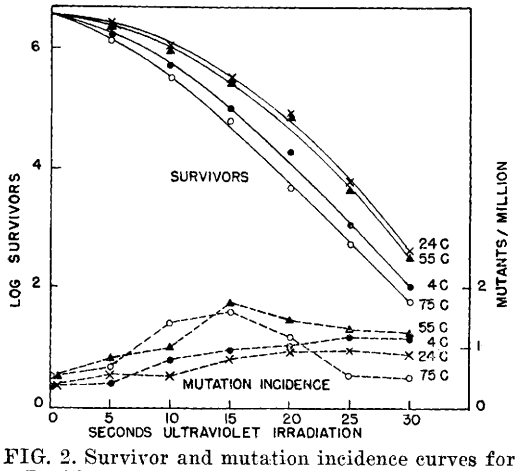

FIG. 2. Survivor and mutation incidence curves for *Bacillus stearothermophilus* spores at different temperatures.

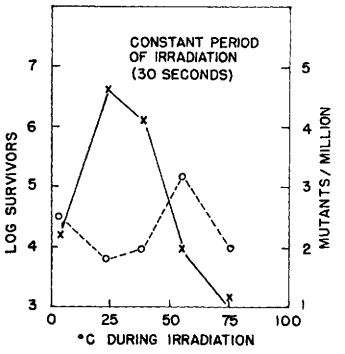

FIG. 3. Influence of temperature upon the effects resulting from aerobic ultraviolet irradiation of *Bacillus globigii* spores. Suspensions irradiated contained 301 million spores/ml. Spontaneous mutation incidence was 1.1 mutants/million (2 μg/ml streptomycin). × = survivors; O = mutation incidence.

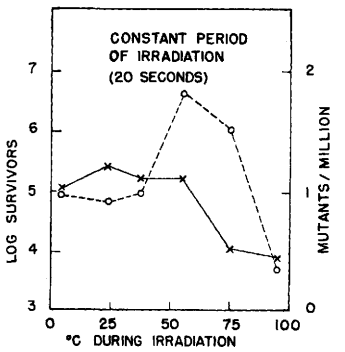

FIG. 4. Influence of temperature upon the effects resulting from aerobic ultraviolet irradiation of *Bacillus stearothermophilus*, Strain 1503, spores. Suspensions irradiated contained 7 million spores/ml. Spontaneous mutation incidence was .5 mutants/million (2 μg/ml streptomycin). × = survivors; O = mutation incidence.

range for which killing and mutability curves had been determined. These points were arbitrarily taken as 30 and 20 seconds, respectively, for *B. globigii* and *B. stearothermophilus*, since the latter is the more sensitive to the lethal action of the radiation. Other time intervals might just as well have been selected, but these lay approximately halfway along the logarithmic portion of the survivor curves. Mutability data may alternatively be reported by plotting the mutation incidence resulting from that ultraviolet radiation dose which yields 99.9% killing. This method gives information which is more suitable for comparison purposes, and such data are reported in Table I.

TABLE I. Influence of Temperature upon Incidence of Streptomycin Resistant Mutants Following an Aerobic Ultraviolet Irradiation Sufficient to Yield a 99.9% Kill of *Bacillus globigii* and *Bacillus stearothermophilus* spores.

Temp. of irradiation	4	24	37	55	75	90
*B. globigii**	2.5†	3	3.1	3.2	2	—
*B. stearothermophilus**	1	1.1	1.4	1.6	1.3	.4

* Incidence of spontaneous mutants: 1.1 and .5 mutants/million, respectively.

† Figures are incidence of end-point mutants in mutants/million.

Comparable information was obtained over a temperature range from 4 to 55°C for log phase vegetative cells of each organism; the temperature effects were much more marked than were noted with spores. Even thermophilic cells became markedly sensitive at 55°C. Comparison at the higher temperatures were not reliable, however, because many of the cells were killed by the action of heat alone.

The high solubility of oxygen in the buffer at low temperatures undoubtedly explains in part the enhanced radiation sensitivity noted at 4°C. The maximum resistance to radiation of the spores of each organism occurred in the vicinity of the optimum temperature for its growth. Temperature controls in each experiment indicated that many of the spores of *B. globigii* were destroyed by the heat alone at temperatures above 75°C, but at lower temperatures viable counts remained constant. The viability of the spores of *B. stearothermophilus* was practically unaffected by heat at any temperature used.

The influence of oxygen upon radiation sensitivity is demonstrated in Fig. 5, which reports data resulting from experiments in which *B. stearothermophilus* and *B. globigii* spores were irradiated in a hydrogen atmosphere at different temperatures. Survivor curves are not reported, since only high temperatures had any effect upon *B. globigii*, whereas, the thermophile was unaffected at the temperatures tested. The data are, therefore, reported in the same way as was done in Fig. 3 and 4. However, it will be noted that with both organisms there was a steady increase in sensitivity to radiation with increasing temperature, but that *B. globigii* became much more sensitive than did *B. stearothermophilus*. There was no increased sensitivity at 4°C, confirming the observation of Hollaender *et al.*(7) that oxygen is required for such an effect. The end-point mutation incidence of the thermophile and mesophile dropped below the spontaneous level when irradiated at 100 and 75°C, respectively, possibly due to some selective factor.

Conclusions and summary. (1) Temperature and atmosphere exert an important influence upon the effects produced by ultraviolet irradiation of the mesophile and thermophile studied. These effects are noted even in the relatively inert spore stage of each. Although the thermophile was more sensitive than the mesophile to the lethal action of a given dose of ultraviolet radiation, it was affected to a lesser degree by changes in temperature during irradiation in the air. Temperature changes exerted almost no effect upon the thermophile when oxygen was excluded from the suspension during irradiation, while the mesophile was markedly affected. This would be the expected result if the thermophile possessed heat-stable nuclear proteins, and damage due to the radiation were not additive with that resulting from heat-denaturation. Mutation incidence at 99.9%

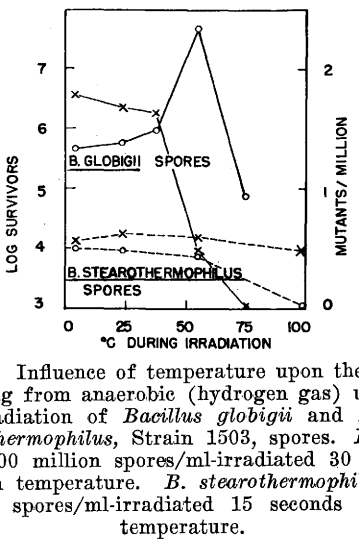

FIG. 5. Influence of temperature upon the effects resulting from anaerobic (hydrogen gas) ultraviolet irradiation of *Bacillus globigii* and *Bacillus stearothermophilus*, Strain 1503, spores. *B. globigii*: 200 million spores/ml-irradiated 30 seconds at each temperature. *B. stearothermophilus*: 8.2 million spores/ml-irradiated 15 seconds at each temperature.

killing fell to below normal levels at 75 and 100°C for the mesophile and thermophile, respectively, possibly due to selection. (2) The relative mutability of the two was also confirmed by the determination of fermentation (mannose) mutants, and by isolation of biochemical mutants using the Davis(12) technic. Such mutants were readily isolated from *B. globigii* cultures, but only a limited number of vitaminless and amino acidless, and no purineless or pyrimidineless mutants could be isolated from the thermophilic cultures. Generally, the thermophile was much less mutabile than the mesophile as compared by streptomycin resistant, fermentation and biochemical mutants.

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Depression Red Cell Iron Turnover by Transfusion. (19257)

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One of the commonly employed methods for study of the regulation mechanisms of a steady state is to observe the events which follow an increase or decrease in the regulated quantity.

This paper is concerned with the change in erythrocyte production rate, subsequent to an increment in the red cell volume (RCV) of rats, as measured by plasma and red cell iron turnover using Fe^{59}.* The method for increasing the red cell volume was by intraperitoneal transfusions of blood obtained from the same highly inbred strain.

Hess(1) discovered that rabbits which had been rendered polycythemic by repeated transfusions developed marrow aplasia and fibrosis. Boycott and Douglas(2) contended that only excessive red cell destruction, *not* inhibition of production, explained the disappearance of cells from artificially plethoric rabbits. Although some of their own data would now be considered clearly indicative of lessened production, this concept was not accepted until Robertson's(3) work. Later Krumbhaar(4), Boycott and Oakley(5), and others used the reticulocyte percent as an index of marrow erythrocyte production, and they noted a decline in reticulocytes during the early weeks of transfusions. Although most of the early workers used blood which was initially compatible, several noted development of agglutinins. Possible causes for the inconsistent results in early work will be discussed.

Methods. The iron turnover in plasma and red cells was determined using tracer amounts of Fe^{59}. Plasma iron turnover is the quantity

* No attempt is made here to elaborate on the mechanism through which a change is induced in rate of production by the increased RCV.