monocytogenes and stresses the inadequacy of present isolation techniques in which a nonliving culture medium is used.

Studies identical with those described above have been carried out on silage from other areas where listeric infection developed shortly after the initiation of silage feeding, but the bacterium has not been isolated from any of these, to date (*8*).

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7. After the results of this study were made known to Dr. Palsson of the University of Iceland, he wrote that in 1945 in their laboratory Dr. Gislason and Mr. Vigfusson had isolated *L. monocytogenes* from guinea pigs inoculated with silage extracts and that the bacterium had been isolated directly from silage on one occasion since that time. Regretably, these important findings were never published.
8. The technical assistance of Mrs. Jean Martin is gratefully acknowledged. This work was supported in part by research grant No. E-2571 from the National Institute of Allergy and Infectious Diseases, U.S. Public Health Service. This report is a contribution (paper No. 515, journal series) from the Montana Veterinary Research Laboratory (Montana Experiment Station and Livestock Sanitary Board cooperating), Montana State College, Agricultural Experiment Station.

15 August 1960

Thermal Annealment and Nitric Oxide Effects on Free Radicals in X-irradiated Cells

Abstract. Four kinds of radicals are identified in dry spores (*Bacillus megaterium*) after x-irradiation: those associated with singlet, doublet, triplet, and oxygen-complex types of spectra. The singlet, present only at low temperatures, gives rise irreversibly to the doublet at 25°C. All hyperfine structure is depressed after annealment at 100°C and is lost when oxygen or nitric oxide is added. The physical results support the hypothesis that reactions of long-lived free radicals can account for the radiobiological phenomena of thermorestoration, nitric oxide protection, and latent oxygen effect.

Studies by Powers *et al.* (*1*) on the influence of physical and chemical factors on the response of nonmetabolizing dry spores to x-irradiation, as measured by colony-forming capacity, have revealed a systematic sensitivity to such factors as gas and temperature not only during but also after irradiation. These results suggest several classes of damage, one of which is thought to be due to long-lived free radicals. Upon reaction with oxygen these radicals may form lethal complexes; alternatively, they may be rendered harmless if scavenged by chemical recombination or annealed by thermal energy. This report presents physical evidence for the existence of such long-lived radicals with the postulated properties in irradiated dry spores.

The electron spin resonance spectrometer was a conventional microwave system (9.35 kMcy/sec) with a reflection cavity; a double modulation technique was used. The resonance patterns are approximately the second derivative of the absorption signal (*2*). The basic theory and practice of electron spin resonance spectroscopy have been treated by Ingram (*3*). Lyophilized bacterial spores were irradiated in 25-mg lots in a beryllium-window exposure chamber in an atmosphere of helium gas, at a temperature of −195°C. The x-ray source was equipped with a wolfram target and a beryllium window, operated at 50 kv and 30 ma (constant potential), at a dose rate of 50 kr/min. After irradiation the spores were quickly dumped into another section of the chamber, composed of a quartz tip (protected from radiation), shaped like the spectrometer cavity. The tip was then transferred into the cavity (also at —195°C). The spectra shown in this study were obtained after irradiation at doses of 4000 kr; essentially similar but quantitatively weaker signals were obtained at doses as low as 250 kr. No signals were found with unirradiated spores.

The initial spectrum (no warm-up) is shown at upper left in Fig. 1*A*. The spores were then warmed up to 25°C for specified intervals and returned to the cavity for measurement at −195°C. On warming over a period of 22 minutes, a singlet component of the initial spectrum appears to be converted into a doublet with 20-gauss spacing. A triplet present in the initial spectrum remains unchanged. Growth of the doublet at the expense of the singlet at 25°C is shown in Fig. 1*B*. the five-line spectrum at 22 minutes (Fig. 1*A*), consisting of a 1:2:1 triplet with 30-gauss spacings and the 1:1 doublet superimposed, is essentially the same as that seen when spores are irradiated at room temperature (25°C, Fig. 2*a*).

In viability studies, exposure of the spores to higher temperatures after anoxic irradiation results in thermorestoration of a considerable fraction (*4*). The physical results are similar in that a reduction of the number of radicals (electron spin resonance) by as much as 50 percent is effected by annealment at 100°C for 10 minutes (Fig. 2*c*). Furthermore, in the viability studies, if the anaerobically irradiated spores are exposed to oxygen before annealment, no part of the potential damage is reversible. In these studies, if the anaerobically irradiated sample is first exposed to oxygen at 25°C, the spectra shown in Fig. 2*b* result (dotted line, 1.5 minutes; solid line, after 3 minutes). This represents a new oxygen-complex radical resulting from the reaction of radicals *R2* and *R3* (Fig. 1*A*) with oxygen. The residual radicals in the annealed system (Fig. 2*c*) also react with oxygen to form a typical oxyradical spectrum (Fig. 2*d*) but of reduced strength. This is in general agreement with the biological results.

Again, the discovery from the viability studies that nitric oxide enhanced the survival of anaerobically irradiated spores (*5*) led to the hypothesis that the protective action of nitric oxide could be explained by its scavenging action upon long-lived oxygen-reacting radicals. The physical results are in accord with this hypothesis in showing nearly total obliteration of the hyperfine spectra by nitric oxide (50 percent

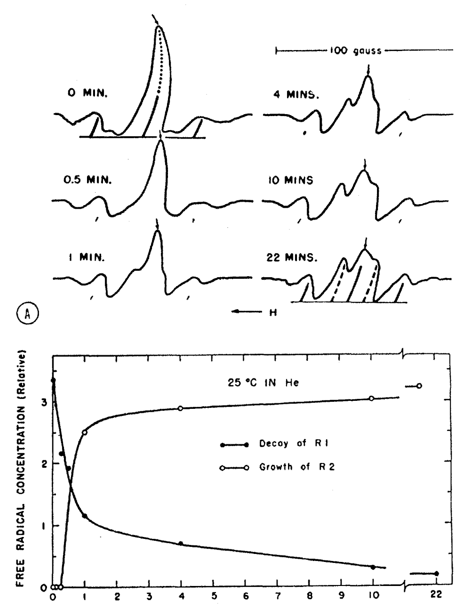

Fig. 1. (*A*) Paramagnetic resonance measured at −195°C in dry bacterial spores previously irradiated anaerobically (4×10^6 r in He at −195°C; warmed in He at 25°C). A hyperfine doublet (broken lines) appears between 1 and 22 minutes, lateral to the center line of an initial triplet (solid lines), while the superimposed initial singlet (dotted line) disappears. Arrow represents position of *g* (= 2.003). (*B*) Kinetics of destruction of the singlet component (*R1*) of the center line and growth of the doublet (*R2*) at 25°C anaerobically. [The amplitude of meter deflection is plotted directly for *R2*; the asymptotic values of the triplet (*R3*) center line were subtracted in computing *R1*.]

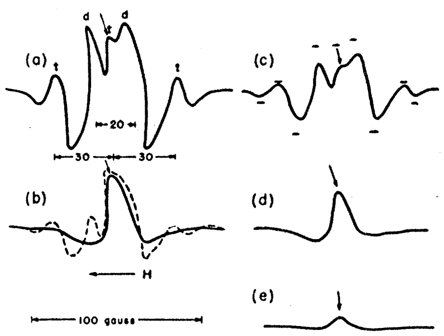

Fig. 2. Paramagnetic resonance measured at $-195°C$ in dry bacterial spores previously irradiated anaerobically at 25°C. (*a*) Hyperfine patterns composed of doublet *d* and triplet *t* spectra. (*b*) Spectra produced by reaction of these radicals with O_2 at 25°C (see text). (*c*) Anaerobic annealment of radicals in (*a*) at 100°C for 10 minutes; signal heights for this sample before annealment are given by the horizontal lines. (*d*) Reaction with oxygen of radicals remaining after annealment. (*e*) Reaction of radicals in (*a*) with nitric oxide.

in helium) applied after irradiation (Fig. 2*e*). Similar biological and physical results with nitric oxide have been reported independently by Sparrman *et al.* (*6*) in another system.

While the close correlation shown between the biological and physical measures of radiation damage does not require any causal relationship of events, the results do support with physical evidence derived from the same system the proposed mechanism (*1*) of the latent oxygen effect (*7*).

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27 June 1960

Simultaneous Generalization Gradients for Appetitive and Aversive Behavior

Abstract. In the presence of a bright light five monkeys were trained to press a lever to avoid shock and to pull a chain for food reward. When tested with a series of lights dimmer than the conditioning stimulus, the monkeys showed a sharp gradient of effect for the rewarded response, in contrast to a very flat gradient for the avoidance response.

When an organism is trained to make a response in the presence of a particular stimulus, this response will also normally occur in the presence of other stimuli that are physically similar to the original conditioning stimulus. The result of this phenomenon is often a "gradient of stimulus generalization"—an orderly decline in the probability of response, which takes place as the physical difference between the original stimulus and various test stimuli is increased. Stimulus generalization is a major explanatory concept in such areas as learning theory, psychotherapy, and abnormal psychology (*1*).

The study reported here was designed to determine whether there is any difference between generalization gradients for reward-controlled and punishment-controlled behavior. The technique used to investigate this problem permitted a comparison of the two gradients for individual subjects and may be applicable to other problems in experimental psychology and psychopharmacology.

Five young male rhesus monkeys were the subjects. The experimental test chamber was a commercially produced model (Foringer) which provided an automatic mechanism for reward delivery, an electrifiable grid, implementation for two possible responses (pulling a chain which hung in the center of the chamber and pressing a lever mounted on one wall), and a 110 v a-c, 60-watt house light mounted above a circular screen of milk glass in the top of the chamber. During generalization testing the intensity of this house light was varied in discrete (though unequal) steps by means of a group of fixed resistors in series with the house light. The 11 possible test-light intensities were calibrated on several occasions with a General Electric foot-candle light intensity meter placed approximately 1 foot below the glass screen on which the house light was projected.

All the subjects were first trained to press the lever, which postponed shock for 10 seconds; by responding at least once every 10 seconds the subjects could avoid shock entirely (*2*). During this training period, and all subsequent training periods prior to the generalization test, the house light was on continuously at its maximum intensity (28.1 ft-ca).

After the animals became proficient at avoiding shocks, the chain was introduced into the apparatus, and each pull of the chain was rewarded with a pellet of food. The avoidance schedule was still in effect, so that lever-pressing continued even during the learning of the chain-pulling response. Eventually all the subjects learned to press the lever to avoid punishment and to pull the chain to produce food reward on a 2-minute variable-interval schedule (*3*).

After ten additional sessions of exposure to the concurrent schedules of reward and avoidance in the presence of the brightest light intensity (*4*), a generalization test session was programmed. During this test, light of 11 different intensities was presented, 12 times at each intensity, in a mixed order. Each stimulus presentation lasted 30 seconds. No rewards or punishments were obtainable during this test. The test procedure was quite similar to that of Guttman and Kalish (*5*).

For several experimental sessions after this generalization test the subjects were put on concurrent reward-avoidance schedules as before; they were then given a second generalization test (*6*).

Chain-pulls and lever-presses in response to each of the 11 light intensities were recorded. Generalization gradients, relating response strength (the ratio of total number of responses to each intensity to total number of responses to all intensities) to log intensity of the test light are shown in Fig. 1. These data are group means for the two generalization tests combined. Considered separately, the data for individual subjects are very similar to the group results.

Figure 1 displays a clear difference

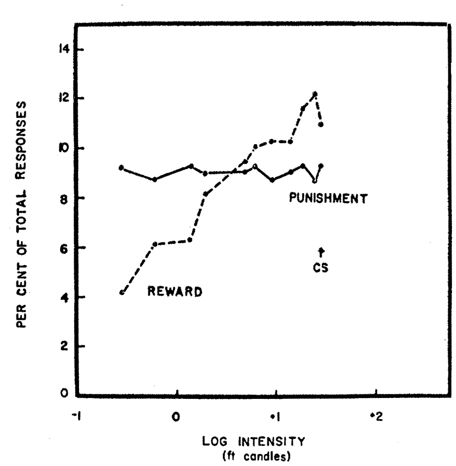

Fig. 1. Generalization gradients for reward-controlled and punishment-controlled behavior.