cle stretch reflexes are presumably mediated by the 2*N* pathway in the woodchuck, they indicate the prominence of a central locus of action of the toxin.

Nociceptive reflexes were not quantitatively studied. Such reflexes could, however, be elicited at all degrees of paralysis. Furthermore, no evidence of impairment of polysynaptic reflex pathways was obtained from the dorsal root–ventral root experiments.

Murnaghan (*14*) and Emmons and McLennan (*8*) have recently presented data indicating that the toxin decreases conduction in peripheral nerves in addition to block of terminal branches of motor fibers (*7*). If impairment of reflexes were due only to the failure of neuromuscular transmission and to nerve block, reflex activity should be present in proportion to the degree of neuromuscular transmission. Stretch reflexes were, however, absent for all degrees of paralysis studied (see Table 1).

The present experiments do not indicate a mechanism by which such a selective block may occur. However, it is well known that the afferent fibers in the monosynaptic pathway branch extensively into fine terminals, as do the motor fibers at the neuromuscular junction. It may be conjectured that the toxin blocks fine terminal fibers at various sites in the central and peripheral nervous systems. If such is the case, its action may depend only upon the organization of the nervous pathway and not upon the type of chemical mediator released or the functions subserved.

Impairment of stretch reflexes is compatible with the symptoms of early paralysis (see *3*) and with the ascending nature of the paralysis; indeed, such symptoms can hardly be explained solely on the basis of peripheral motor block (see *6*). Incoordination, the earliest sign of impending paralysis, is not seen with curariform drugs but is observed with drugs and with surgical procedures which impair spinal reflex function. For example, interruption of afferent pathways from the hindlimbs by dorsal root section produces complete incoordination and full functional paralysis, although neuromuscular transmission is unchanged (see, for example, *12*). Thus the signs of early tick paralysis may be attributable almost entirely to the loss of stretch reflexes rather than to the slight degree of neuromuscular paralysis at this time.

DON W. ESPLIN
Department of Pharmacology, University of Utah College of Medicine, Salt Lake City
CORNELIUS B. PHILIP
LYNDAHL E. HUGHES
National Institute of Allergy and Infectious Diseases, Rocky Mountain Laboratory, Hamilton, Montana

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References and Notes

1. G. A. Mail and J. D. Gregson, *Can. Med. Assoc. J.* **39,** 532 (1938); W. L. Jellison and J. D. Gregson, *Can. Dept. Agr. Tech. Publ. No. 2599* (1947).
2. C. M. McCue, J. B. Stone, L. E. Sutton, Jr., *Pediatrics* **1,** 174 (1948).
3. K. H. Abbott, *Proc. Staff Meetings, Mayo Clinic* **18,** 39 (1943); J. B. Stanbury and J. H. Huyck, *Medicine* **24,** 219 (1945).
4. I. Rose and J. D. Gregson, *Nature* **178,** 95 (1956).
5. M. F. Murnaghan, *Nature* **181,** 131 (1958).
6. P. Emmons and H. McLennan, *Nature* **183,** 474 (1959).
7. M. F. Murnaghan, *Can. J. Biochem. and Physiol.* **38,** 287 (1960).
8. P. Emmons and H. McLennan, *J. Exptl. Biol.* **37,** 355 (1960).
9. This work was supported by senior research fellowship no. SF-145 and by grant no. B-872, National Institutes of Health, U.S. Public Health Service.
10. J. D. Gregson, *Can. Entomologist* **90,** 421 (1958).
11. L. E. Hughes and C. B. Philip, *Proc. Soc. Exptl. Biol. Med.* **99,** 316 (1958).
12. D. W. Esplin and R. J. Laffan, *Arch. intern. pharmacodynamie* **113,** 189 (1957).
13. D. W. Esplin, *J. Pharmacol. Exptl. Therap.* **120,** 301 (1957).
14. M. F. Murnaghan, *Science* **131,** 418 (1960); *Federation Proc.* **19,** 298 (1960).

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Reduction of Radiation Sensitivity of Dry Bacterial Spores with Hydrogen Sulfide

Abstract. Hydrogen sulfide reduces the lethal effect of x-rays in dry spores by about 50 percent when given after irradiation, and by approximately 75 percent when present during irradiation. The first effect is the result of removal of radicals that are toxic when combined with oxygen; the second, the removal of radicals that become toxic in the absence of oxygen. With these results we construct an explanation of the action of sulfhydryl compounds in protection against radiation damage.

With the use of dry spores of *Bacillus megaterium*, we have demonstrated the participation of free radicals and other chemical species in the lethal effect of x-rays, and the relationship of oxygen to this participation (*1–4*). Free radicals and oxygen have been discussed at length [see (*5*) for recent review] in connection with the mechanism of action of chemical compounds that protect biological organisms from the deleterious effects of high energy radiation. In this study we have tested a number of compounds related to those that are efficacious in higher organisms, and the results enable us to present a theory of the mode of action of certain protective chemicals.

It is well known that the most effective protective compounds are those containing -SH and -NH_2 groups. Those tested here are gases at ordinary temperatures since our system gives accurate results most conveniently with gases. In this communication we report results obtained with the simplest sulfhydryl compound, hydrogen sulfide (*6*).

The spores, mounted on Millipore filters, were exposed to 50-kv (peak) x-rays in containers that allowed control of temperature and gaseous environment as previously described (*7, 8*). Colony formation was the index of survival, with the slope of the survival line being the measure of sensitivity to radiation. Methods are described fully in other papers (*1, 2, 4, 7, 8*). In this system reproducibility from experiment to experiment is good and variances are low (*4*). The differences reported in this paper are highly significant. H_2S is not toxic to the dry spore.

In Fig. 1 the data are presented. H_2S, given to the spores after irradiation in N_2 but before exposure of the irradiated spores to O_2 (curve 3), results in protection to the same extent as that brought about by the radical scavenger nitric oxide (*3, 4*). The slope is 0.0141 kr^{-1} compared to 0.0380 kr^{-1} observed when the spores are irradiated in O_2 (*8*). When O_2 is introduced to the spores irradiated in N_2 before H_2S exposure (curve 1) this reversal is not observed, the slope being 0.0270 kr^{-1}. The interpretation is the same as for the NO results: radicals are formed that are long-lived and that can be scavenged by H_2S. These radicals become irreversibly toxic to the cell if they react with O_2.

In mixtures of H_2S and O_2, sensitivities intermediate between those observed in each alone are observed (curve 2). This preliminary result is interpreted as evidence for competition between these two molecules for the radicals in question. Detailed studies of

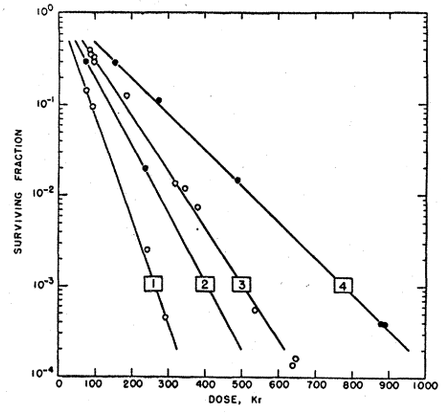

Fig. 1. Survival of dry spores of *Bacillus megaterium* when irradiated and treated as follows. Curve 1, spores irradiated in N_2, then exposed to 20 percent O_2 for 10 minutes, and then exposed to 20 percent H_2S for 15 minutes. The slope is 0.0270 kr^{-1}. Curve 2, spores irradiated in 20 percent H_2S and 80 percent air. The slope is 0.0173 kr^{-1}. Curve 3, spores irradiated in N_2, then exposed to 20 percent H_2S for 15 minutes. The slope is 0.0141 kr^{-1}. Curve 4, spores irradiated in 20 percent H_2S and 80 percent He. The slope is 0.0090 kr^{-1}.

this competition are being carried on.

When H_2S is present during irradiation (curve 4) even greater protection is observed, with the slope decreasing to 0.0090 kr^{-1}. This result indicates the presence of very short-lived (that is, lifetimes shorter than minutes, the time required for gas transfer after irradiation) radicals that can be scavenged by H_2S. These can ordinarily become toxic to the cell in the *absence* of oxygen. [It should be recalled here that nitric oxide, although it gives results equivalent to H_2S when given to the spores after irradiation, does not protect as well when present during irradiation (*3, 4*).]

In Fig. 2 we present a summary of the information at hand today concerning the radiation response in dry spores. We call this diagram the "radiation sensitivity profile." This particular profile is obtained when one uses photons of average energy about 20 kev, delivered at room temperature at about 20 kr/min to spores prepared according to our described methods. (We expect the profile to depend upon linear energy transfer, temperature, and perhaps dose rate.) This profile demonstrates that 62 percent of the total effect is dependent on O_2, 38 percent being independent of O_2. The former portion can be resolved into one associated with radicals having appreciable lifetimes, and one consisting of radiation-induced species that are toxic only if O_2 is present at the time of their formation. Possibilities concerning this class have been discussed (*1, 4*) and are under current investigation.

The new information in this note indicates that the portion which is independent of O_2, formerly called class I (*1*), is now divisible into two portions: one (class Ia) that is independent of H_2S, and therefore a measure of the "direct" effect, or else of a species that cannot be reversed by H_2S; and one (class Ib) that is reversible by H_2S only if present at the time of irradiation, and therefore one conceivably due to a very short-lived radical. Note that the radicals of class Ib are almost immediately toxic in the absence of oxygen, in contrast to those of class III that become rapidly toxic only when they react with oxygen.

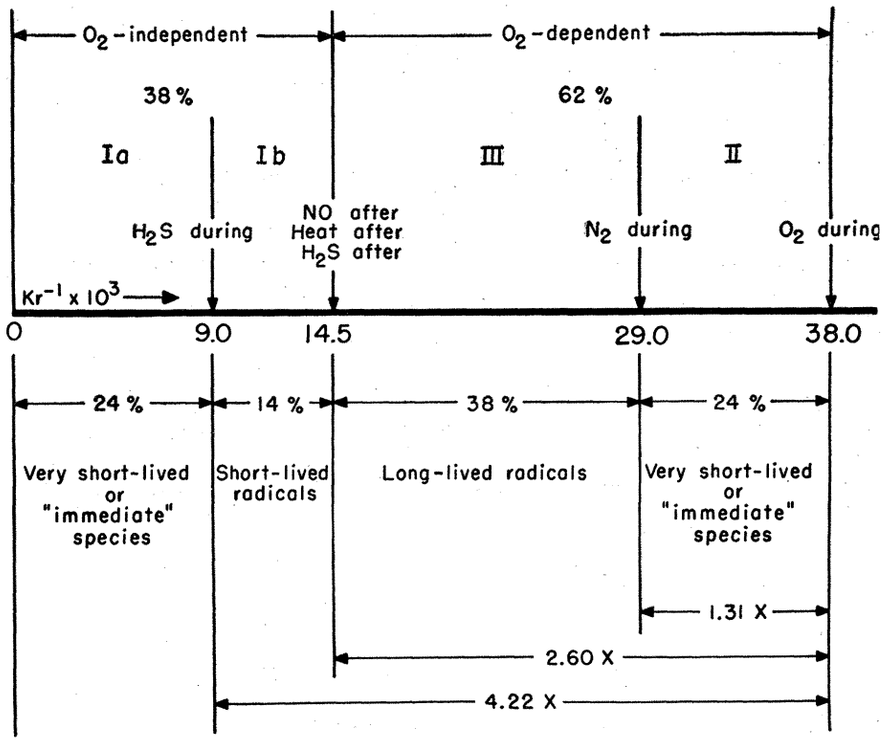

Fig. 2. The "radiation sensitivity profile" of dry spores irradiated at room temperature with 50-kv (peak) x-rays at approximately 20,000 r/min. The heavy, central, horizontal line represents slopes of survival curves. The top vertical arrows are at the values of the slopes observed under the various conditions noted. The Roman numerals refer to former terminology for describing the kinds of damage (*1*), with the subdivision of class I damage into Ia and Ib being an innovation in this report. The numbers at the bottom are the ratios of slopes observed under the indicated conditions relative to the maximal sensitivity observed.

The ratios of effectiveness observed under different conditions are noted at the bottom of Fig. 2. Sensitivity seen when the spores are irradiated in O_2 is 131 percent of that when irradiated in N_2. This is a small O_2 effect. But when the comparison is made with the sensitivity observed after postirradiation treatment with radical scavengers, the effect is seen to be 262 percent, a value of the order of the ordinarily observed O_2 effect in radiation biology.

On the basis of these results in dry spores we can suggest a general explanation of the action of sulfhydryl compounds in this and other biological systems. Sulfhydryl compounds can affect both portions of the oxygen effect by reducing O_2 tensions. When O_2 concentrations are low, the species immediately dependent upon O_2 (class II) do not become toxic, and the radicals of class III (which in the spore are very long-lived) cannot form toxic oxyradical complexes. Thus, they can be scavenged by the chemical compound before O_2 is readmitted. But the total effect of the sulfhydryl is in excess of the O_2 effect (in the case of the spore 422 percent versus 262 percent). The action of the sulfhydryl in this portion of the general effect is to scavenge very reactive, O_2-independent radicals (class Ib).

While the exact relationships between the dry and wet systems are not yet recognized, this model explains the protective action of sulfhydryl compounds under anoxic conditions demonstrated in bacteria (*9*) and in T2 bacteriophage (*10*). It also accounts for the protective action of H_2S in dry seeds (*11*).

E. L. POWERS
B. F. KALETA

Division of Biological and Medical Research, Argonne National Laboratory, Argonne, Illinois

References and Notes

1. E. L. Powers, R. B. Webb, C. F. Ehret, *Radiation Research* Suppl. 2, 94 (1960).
2. R. B. Webb, E. L. Powers, C. F. Ehret, *Radiation Research* **12**, 682 (1960).
3. E. L. Powers, B. F. Kaleta, R. B. Webb, *ibid.* **11**, 461 (1959).
4. E. L. Powers, R. B. Webb, B. F. Kaleta, *Proc. Natl. Acad. Sci. U.S.* **46**, 984 (1960).
5. D. R. Kalkwarf, *Nucleonics* **18**, 76 (1960).
6. This work was performed under the auspices of the U.S. Atomic Energy Commission.
7. R. B. Webb, C. F. Ehret, E. L. Powers, *Experientia* **14**, 324 (1958).
8. E. L. Powers, R. B. Webb, C. F. Ehret, *Exptl. Cell Research* **17**, 550 (1959).
9. A. Hollaender, W. K. Baker, E. H. Anderson, *Cold Spring Harbor Symposia Quant. Biol.* **16**, 315 (1951); H. I. Kohn and S. E. Gunter, *Radiation Research* **12**, 449 (1960); **11**, 732 (1959).
10. P. Howard-Flanders, *ibid.* **12**, 443 (1960).
11. N. Nybom, A. Gustaffson, L. E. Ehrenberg, *Botan. Notiser* **4**, 344 (1952).

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