onstrated—the initial crack must be large enough to supply heat for ten or more years in a practical power system. The LASL team believes that such a crack, extending 1.25 km from the borehole (compared with an estimated 40-m crack in their first experiment), is well within the realm of the hydrofracturing technique, although whether problems will be encountered in scaling up to this size remains to be seen.

The LASL experiment appears to have removed two of the principal concerns over the feasibility of tapping dry geothermal deposits in igneous rocks—it has shown that granite can be hydrofractured and that it is, at least in one region, impermeable enough to hold water tightly. (Even drilling in the granite, the most expensive operation in establishing such a geothermal well, turned out to be not as difficult as had been expected.)

Thinking about the optimum method of recovering heat from the earth is only beginning, however. One concept that could greatly increase the economic life of hot rock geothermal systems (and decrease their cost) is that proposed by B. Rayleigh of the Menlo Park, California, research center of the U.S. Geological Survey. Observing that in many regions the stresses in subsurface rock are reasonably constant over large areas, Rayleigh suggests that geothermal wells be drilled at an angle, in a direction perpendicular to the expected orientation of fractures. Then a series of parallel, vertical cracks could be fractured from a single well, possibly spaced as close as every 30 m. Slant drilling techniques are common in the oil industry, although not yet developed for crystalline rock, and Rayleigh's preliminary cost estimates indicate that such a system could be competitive with existing sources of electricity, even with cracks of relatively modest size. Still other ideas may emerge as more hydrofracturing experiments are performed and the design of geothermal systems moves from the feasibility stage to engineering design.

Assessing the extent of dry geothermal resources and drilling to prove out suspected deposits has also barely begun. Preparations for such assessments are going forward at a site in the vicinity of Marysville, Montana, however, at what may turn out to be the "mother lode" of geothermal deposits, at least in this country. The deposit is an apparent remnant of a geologically recent magma intrusion that very nearly became a volcano and that left what is believed to be billions of dollars worth of heat within 1 or 2 km of the surface. The project, directed by D. Stewart of the Battelle Memorial Institute laboratory in Richland, Washington, and funded by the National Science Foundation, will determine, among other things, whether groundwater is present or whether the deposit is dry.—ALLEN L. HAMMOND

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Singlet Oxygen: A Unique Microbicidal Agent in Cells

When the electrons of molecules in living cells are excited to higher energy levels, the process is invariably associated with light. Molecules that can be electronically excited by light serve as light receptors in two fundamental life processes, photosynthesis in plants and vision in animals. Electronically excited molecules generated by chemical reactions serve as light sources in the bioluminescence of certain insects and fish. Until last year, no one ever claimed to have demonstrated the existence in animals of functional excited molecules unrelated to light.

Then, in the spring of 1972, Robert C. Allen, Rune L. Stjernholm, and Richard H. Steele of Tulane University, New Orleans, Louisiana, presented evidence that electronically excited singlet oxygen acts as a microbicidal agent in phagocytosis. Their experimental approach and the evidence for their hypothesis were summarized by Allen at the recent American Chemical Society meeting in Chicago. If that hypothesis is substantiated, the system they have described represents the first demonstration of the capability of mammalian cells to use stored chemical energy for the generation of electronically excited molecules with a role in cellular function.

Phagocytosis is the process in which some types of white blood cells such as polymorphonuclear leukocytes engulf and destroy microorganisms. The mechanism of microbial digestion by these cells has been the subject of intermittent speculation for nearly a century. Intense interest in the system was revived in 1967, however, when Seymour J. Klebanoff of the University of Washington in Seattle showed that the digestion is mediated by a tripartite system consisting of an oxidizing enzyme called myeloperoxidase, hydrogen peroxide, and halide ions. Similar systems have subsequently been found in rat liver microsomes, cellular structures that, among other roles, participate in the detoxification of drugs and other foreign chemicals; in mouse spleen cells; in guinea pig polymorphonuclear leukocytes and bone marrow cells; and in rat alveolar macrophages, phagocytic cells from the lung.

Neither myeloperoxidase nor halide ions exhibit any intrinsic microbicidal activity, and the activity of hydrogen peroxide is much less than that of the complete system. Various mechanisms have thus been proposed to account for the observed microbicidal activity, including halogenation of the microorganism, production of reactive intermediates such as hypohalite anions (OI^-, OCl^-, and OBr^-) and chloramines, and formation of toxic aldehydes. Iodinated microbial substrates have, in fact, been isolated among the products of phagocytosis, but no one has been able to isolate comparable chlorinated or brominated products. Similarly, no one has been able to demonstrate the existence of hypohalites, chloramines, or any other reactive intermediates during the digestion. It is now considered likely that the system operates through more than one mechanism; one of these mechanisms, Allen argues, is the formation of singlet oxygen.

A singlet molecule is one in which the absorption of energy has shifted a valence electron from its normal bonding orbital to an antibonding orbital of higher energy, and in which the electron spins are paired. (Oxygen is an unusual diatomic molecule in that the spins of the two valence electrons of lowest energy are not paired in the most stable or ground state.) The resultant excited molecule is highly unstable and must release the excess energy by one or a combination of four methods.

The excited molecule can, for example, react with another molecule that is close by. If sufficient energy has been put into the system, the molecule can dissipate energy by cleavage of a bond to form reactive free radicals or, in the case of a diatomic molecule, dissociate into atoms. The excited molecule can relax directly to the ground state by the emission of light energy in a process known as fluorescence if the excitation was stimulated by light or as chemiluminescence if the excitation was stimulated chemically. Or, the excited electron can become unpaired to form the more stable triplet state; the triplet can, in turn, relax by emission of light of a longer wavelength in a process known as phosphorescence.

Several studies have previously shown that artificially generated singlet oxygen reacts with regions of high electron density in microbial substrates to form substituted dioxetanes, molecules which contain a four-membered ring consisting of two adjacent oxygen atoms and two carbon atoms. Dioxetanes are generally very labile and dissociate to form electronically excited carbonyl groups that relax by chemiluminescence. It is this chemiluminescence that Allen, Stjernholm, and Steele believe they have observed in the digestion of engulfed microorganisms by polymorphonuclear leukocytes—although the possibility exists that they are observing spurious relaxation of singlet oxygen.

Using special photomultipliers to observe the very small amount of light emitted in the reaction, they found that the intensity of chemiluminescence correlates well with all variables (except iodide concentration) that alter microbicidal activity. The intensity of emitted light is thus directly proportional to the concentrations of chloride ion, bromide ion, hydrogen peroxide, and myeloperoxidase. The intensity is also proportional to the leukocyte's glycolytic activity in the hexose monophosphate pathway for conversion of glucose to carbon dioxide; glycolysis in the hexose monophosphate shunt generates reducing potential as nicotinamide adenine dinucleotide phosphate (NADPH), and is probably the most important source of the energy required for phagocytosis. The pH optimum for chemiluminescence, 4.5, is also the same as that for microbicidal activity.

No chemiluminescence is observed, however, when oxygen is excluded from the system, when only the leukocytes are present, or when only the microbial substrate is present. Chemiluminescence is also not observed with polymorphonuclear leukocytes from children with chronic granulomatous disease in which the leukocytes are unable to kill the ingested microorganism.

The first step in any proposed mechanism for microbicidal activity in polymorphonuclear leukocytes is the reaction of NADPH and molecular oxygen to produce hydrogen peroxide and oxidized nicotinamide adenine dinucleotide phosphate, $NADP^+$. (Formation of $NADP^+$ stimulates glycolysis via the hexose monophosphate shunt to produce more NADPH.) The reaction may proceed directly, but there is some evidence, Allen says, that the first product of NADPH oxidation is the superoxide anion radical, $\cdot O_2^-$. Other investigators have reported that the disproportionation of two such radical anions at low pH results in the formation of hydrogen peroxide and singlet oxygen.

A more likely explanation for the formation of singlet oxygen is as a product of the myeloperoxidase-catalyzed oxidation of the halide by hydrogen peroxide. The hypohalite anion thus formed can then either react directly with the microbial substrate, as in the case of iodination, or react with a second molecule of hydrogen peroxide. The reaction of a hypohalite anion with hydrogen peroxide is the classical chemical reaction for the production of singlet oxygen. Singlet oxygen has previously been shown by other investigators to have microbicidal activity of the magnitude observed.

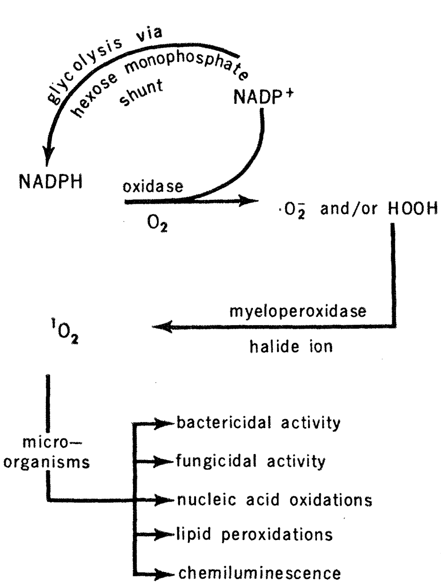

Fig. 1. A possible mechanism for the generation of singlet oxygen ($^1O_2$) in polymorphonuclear leukocytes. [Source: Richard H. Steele, Tulane University]

This scheme has met with a certain amount of skepticism from other investigators. Some, such as Howard Zelliger of Johns Hopkins University in Baltimore, Maryland, argue that singlet oxygen may not even be able to exist in solution. The hydrogen bonds responsible for solvation of the oxygen molecule in water may be able to dissipate energy so fast, he suggests, that the singlet species is never formed. John Lee of the University of Georgia in Athens, for example, has been trying for some time to produce singlet oxygen in solution with a laser—so far without success. Steele points out, however, that the singlet oxygen in the myeloperoxidase system could be bound in a hydrophobic lipid or protein region where it is not solvated.

Others, such as Erwin Fridovitch of Duke University, Durham, North Carolina, argue that a wide variety of chemical reactions, particularly those involving free radicals, can produce chemiluminescence. It is thus possible that the superoxide anion radical is the microbicidal species and that the chemiluminescence results from the decay of its reaction products. This mechanism would also be unique, however, since the anion radical is a highly reactive, destructive molecule that could just as easily react with the leukocyte itself. There are some cellular enzymes, such as superoxide dismutase, whose function is believed to be protection of the cell by destruction of anion radicals generated by external stimuli, but there is no conclusive evidence that such radicals have a functional role in cellular metabolism.

One approach that might yield more information about the mechanism, Fridovitch suggests, would be determination of the wavelength of the emitted light, since every molecule has a characteristic wavelength for fluorescence. Such a determination is very difficult because of the extremely small amount of light produced, but identification of the chemiluminescent species might make it possible to eliminate some of the postulated mechanisms and determine whether the system is actually as unique as it appears.—THOMAS H. MAUGH II

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