uranium, a superconductor in which 5*f* electrons might play a role (*5*).

In the light of the present results on β-uranium, we would like to offer an entirely different explanation. Namely, that the reported superconducting behavior of the α-phase of uranium at and above 0.7°K may be due only to superconducting filaments of retained stabilized α'-, β-, and γ-phases, or perhaps even filaments of superconducting compounds. Either the γ-phase or the superconducting compounds could account for the transition region above 0.8°K. This transition region can easily be destroyed by mechanical means, such as filing, pulverizing, or rolling. By such means we succeeded in reducing the superconductivity of all uranium samples to the transition temperatures near 0.8°K. The reproducible coincidence between transition temperatures near 0.8°K found for the β-stabilized phases and those found for the α-uranium phases points to the existence of an additional network of what might be β-stabilized filaments of a much finer mesh. We believe that both networks of filaments, which might be retained β- and γ-phases, are situated in the grain boundaries. They are stabilized by impurities insoluble in the α-phase and, therefore, precipitated in just those grain boundaries. Preliminary results of electron microscopic investigations by Arrhenius and collaborators (*6*) show two different domain patterns which could well correspond to the two networks we postulate. The *fine* mesh network is on such a scale that it cannot be destroyed by mechanical means. It has been shown in detail for the LaRh system (*7*) how an extremely fine and well-distributed network of filaments can give rise to complete diamagnetic shielding even though the total concentration was only on the order of a few percent.

The similarity in superconducting behavior recently reported for α-uranium and protactinium (*8*) suggests that aside from the tetragonal form of protactinium, stable at room temperature, there may be other crystallographic modifications which, again, could be stabilized in the grain boundaries. Most elements in which the possibility of 5*f* electrons exists also show different crystallographic modifications. Why should protactinium be an exception since the 5*f* series starts with protactinium (*9*)?

The phase stable at temperatures above those of the β-phase is γ-uranium. Specific heat data on γ-uranium stabilized with 13.7 atomic percent of molybdenum have already been shown to have the expected anomaly with sharp transitions near 2°K (*10*). We have also found that U_6Fe, the uranium compound with the highest known transition temperature, 3.8°K, equally displays the expected caloric features of a normal superconductor. The failure to observe a superconducting heat anomaly (*4*) above 0.15°K for α-uranium, together with the broad transition can be simply explained by the absence of superconductivity of the α-uranium above 0.15°K as was postulated by Dempesy *et al.* for their sample, or by the presence of some state involving the coexistence of magnetic order and superconductivity (*11*). Further heat capacity measurements will be necessary to decide if and where α-uranium becomes superconducting.

Finally, we would like to point out that an anomalous superconducting behavior will frequently be caused by an anomalous metallurgical situation. While previous metallurgical investigations have not clearly shown evidence for the presence of other phases in α-uranium we believe that the present results give strong evidence to the contrary.

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References and Notes

1. A. N. Holden, *Physical Metallurgy of Uranium* (Addison-Wesley, Reading, Mass., 1958); J. H. Gittus, *Uranium* (Butterworth, Washington, 1963).
2. J. Bardeen, L. N. Cooper, J. R. Schrieffer, *Phys. Rev.* **108**, 1175 (1957).
3. D. Shoenberg, *Nature* **159**, 309 (1947); N. Alekseyevsky and L. Migunov, *J. Phys. (U.S.S.R.)* **11**, 95 (1947); R. A. Hein, W. E. Henry, N. M. Wolcott, *Phys. Rev.* **107**, 1517 (1957).
4. C. W. Dempesy, J. E. Gordon, R. H. Romer, *Phys. Rev. Letters* **11**, 547 (1963).
5. C. G. Kuper, M. A. Jensen, D. C. Hamilton, *Phys. Rev.* **134**, A15 (1964); T. F. Smith and W. E. Gardner, *ibid.* **140**, 1622 (1965); K. Mendelssohn, *Rev. Mod. Phys.* **36**, 144 (1964).
6. G. O. S. Arrhenius *et al.*, private communication.
7. G. Arrhenius, R. Fitzgerald, D. C. Hamilton, B. A. Holm, B. T. Matthias, E. Corenzwit, T. H. Geballe, G. W. Hull, Jr., *J. Appl. Phys.* **35**, 3487 (1964).
8. R. D. Fowler, B. T. Matthias, L. B. Asprey, H. H. Hill, J. D. G. Lindsay, C. E. Olsen, R. W. White, *Phys. Rev. Letters* **15**, 860 (1965).
9. We want to thank W. H. Zachariasen for enlightenment on this point.
10. B. B. Goodman, J. Hillairet, J. J. Veyssié, L. Weil, *Proc. 8th Int. Conf. Low Temp. Phys.* (Butterworth, Washington, 1963), p. 350.
11. See S. Barrett, M. H. Mueller, R. L. Hitterman, *Phys. Rev.* **129**, 625 (1963).
12. We acknowledge the assistance of J. P. Maita and L. D. Longinotti and thank Mrs. V. B. Compton for the x-ray diffraction analysis and many discussions and A. M. Clogston for one crucial discussion. Work at La Jolla supported in part by the U.S. Air Force Office of Scientific Research.
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Direct Evidence for the Cathodic Depolarization Theory of Bacterial Corrosion

Abstract. *Cathodic depolarization of mild steel by* Desulfovibrio desulfuricans *was demonstrated with benzyl viologen used as an electron acceptor. Direct measurement of the cathodic depolarization current indicated a maximum current density of 1 microampere per square centimeter. Aluminum alloys were also cathodically depolarized by the organism.*

In 1934, von Wolzogen Kühr and van der Vlugt proposed a theory for the anaerobic corrosion of iron by bacteria (*1*). In brief, the theory states that bacteria, primarily those of the genus *Desulfovibrio*, remove hydrogen that accumulates on the surface of iron as a result of their hydrogenase activity and reduce sulfate, yielding hydrogen sulfide. The electrons removed as a result of hydrogen utilization permit more iron to be dissolved or corroded at the anode. The hydrogen sulfide and the hydroxyl ions combine with the ferrous ions to form secondary reaction products at the anode.

Over the years evidence for and against this theory has accumulated. Much of this earlier evidence has been reviewed by Starkey (*2*). More recently, Raifsnider (*3*) and Scott (*4*) have presented observations that are not in agreement with this classical theory. Booth and his associates (*5*), using polarization techniques and weight loss measurements versus hydrogenase activity, have presented evidence for the theory. These methods, although involving standard techniques, are nevertheless indirect approaches and may be subject to various interpretations. The key step in the theory is the removal of hydrogen or electrons at the cathode and the subsequent dissolution of iron at the anode. If the *Desulfovibrio* cells are

able to remove hydrogen or electrons from the surface of iron via an electron acceptor (sulfate), a deficiency of electrons should be created at places where there are no cells (anodes). As a result, the equilibrium $Fe \rightarrow Fe^{++} + 2e$ is upset and the reaction should be displaced to the right, causing more Fe^{++} ions to go into solution (corrode).

Direct evidence for this step was obtained by substituting the dye, benzyl viologen, colorless when oxidized and violet when reduced (*6*), for sulfate as the electron acceptor to avoid the complicating factor of H_2S reaction with the iron and to make the reduction process visible.

Through use of the medium, trypticase soy broth (Baltimore Biological Laboratory) plus 2 percent agar, it has been possible to culture *Desulfovibrio* readily and in pure culture on the surface of agar plates under a hydrogen atmosphere. Cells of *Desulfovibrio desulfuricans* (Mid-Continent Strain A), possessing hydrogenase activity, were grown on the surface of this medium, removed with a bacteriological loop, and placed on a small area on the surface of solidified washed (Noble) agar (2 percent) containing 0.01*M* tris buffer [tris (hydroxy methyl) aminomethane] and benzyl viologen (0.01 percent). This medium was adjusted to *p*H 7.0 ± 1 with HCl and autoclaved at 15 lb (6.8 kg) for 15 minutes. Polished (emery cloth), degreased (acetone), and sterilized (alcohol plus flame) coupons of 1010 mild steel (8 by 15 mm) were placed on the surface of the agar plates (Fig. 1) with one end on the area with the cells. The plates with the coupons were placed in a jar suitable for evacuation (Brewer's jar, Baltimore Biological Laboratories), and the atmosphere was replaced by nitrogen.

After about 17 to 24 hours in a temperature of 27° ± 1°C, the plates and coupons were removed. A dark violet area of reduced benzyl viologen was observed in the agar underneath the part of the coupon over the area previously covered with cells (Fig. 2*a*). Lighter areas of reduced benzyl viologen were observed in the agar underneath both ends of the coupon not in contact with the cells. These lightly reduced areas, probably due to the direct reduction of the dye by the metal, disappeared (oxidized) and left only the heavily reduced area produced by cellular reduction (Fig. 2*b*). A yellow-brown area can be observed on the surface of the agar surrounding the areas that were in contact with the coupon. This material appears to be an insoluble iron compound, probably Fe_2O_3 or $Fe(OH)_3$, which was formed as a result of iron oxidation by small traces of oxygen present in the nitrogen, the agar, or the metal surface.

The iron in the agar was made visible (developed) by adding either potassium thiocyanate (for Fe^{+++}) or potassium ferricyanide (for Fe^{++} or Fe^{+++}) with or without acid (HCl). In Fig. 2*c* equal portions of an aqueous 1-percent solution of potassium thiocyanate and HCl (10 percent concentrated, vol/vol) were added to the agar surface. Heavy concentrations of iron were indicated within several minutes where the insoluble iron "edge" was located. Since this iron was located at the surface, the iron thiocyanate complex was washed away by adding more of the reagents. The area of reduced benzyl viologen was still visible. After about 20 minutes, only the pink iron thiocyanate complex in the agar that had slowly developed (Fe^{++} slowly oxidized to Fe^{+++}) remained visible (Fig. 2*d*). The reduced benzyl viologen had by this time been oxidized (colorless). Within 30 to 45 minutes, the pink iron thiocyanate complex began fading and eventually completely disappeared.

The use of nonacidic potassium ferricyanide (10-percent aqueous solution) is much more satisfactory because the color complex does not fade, but it must be added immediately to the agar surface after removing the plate from the nitrogen atmosphere to detect the Fe^{++} ions. A plate developed with ferricyanide is shown (Fig. 2*e*) that indicates a heavy concentration of Fe^{++} ions under the coupon not in contact with the cells (anode) and relatively few Fe^{++} ions at the cathode (coupon in contact with the cells).

The results obtained seem to indicate that the steel coupon on the agar surface has corroded as a result of three reactions, which take up electrons or act as "electron sinks": (i) the combination of electrons with oxygen and water to form $(OH)^-$ ions, which react with the Fe^{++} ions and more oxygen to form the "edge" effect; (ii) the direct uptake of electrons by oxidized benzyl viologen to form the violet reduced benzyl viologen; and (iii) the reduction of H^+ ions at the iron surface to form molecular or atomic hydrogen, which is then removed. The electrons are utilized to reduce the benzyl viologen.

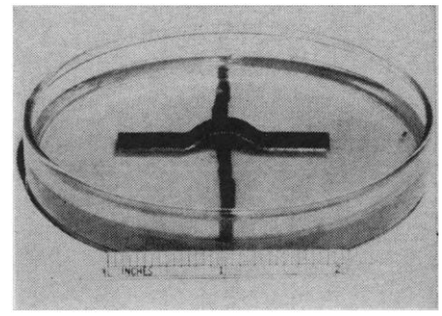

Fig. 1. Petri plate with 1010 steel coupon resting on agar surface.

The iron that forms the "edge" can be distinguished from reactions ii and iii by omitting acid in the "development" process. The iron that forms in reaction ii (both anode and cathode) as distinguished from reaction iii may be detected by using control coupons (no cells in contact with either end of the coupon). By direct observation of the color intensity, it can be noted that the total quantity of Fe^{++} ions evolved from both ends of the control coupon in reaction ii is much less than the total amount of iron evolved at the anode in reaction iii.

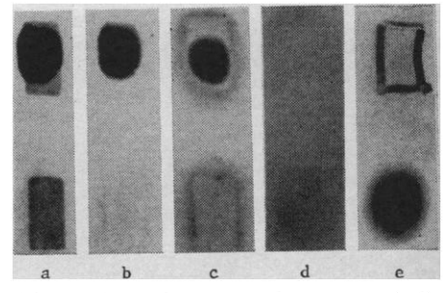

Fig. 2. Areas in agar under coupon indicating location of reduced benzyl viologen and ferrous ions. (*a*) Agar surface immediately after removal of steel coupon. Dark area due to reduction of benzyl viologen by *Desulfovibrio* cells. (*b*) Same plate 10 minutes later, after the benzyl viologen that was reduced directly by the steel has been oxidized (decolorized) by exposure to the air. (*c*) A similar plate to which HCl (10-percent concentration in H_2O, vol/vol) and potassium thiocyanate (10 percent wt/vol) have been added. (*d*) Same plate as 2*c*, 20 minutes after addition of HCl and potassium thiocyanate. (*e*) Portion of plate that was developed with aqueous potassium ferricyanide (10 percent wt/vol) showing a heavy Fe^{++} concentration at anode (no cells) and none at the cathode (area surrounded by black border of masking ink where a heavy concentration of cells was placed).

Since there was obviously a flow of electrons from the anode to the cathode, direct measurement of this cathodic depolarization current seemed possible. Two electrodes (each with a surface area of 1.1 cm^2), made from one of the coupons, were encased in lucite and secured by means of a holder that fit over a standard plastic petri dish bottom. After the two electrodes were dropped on the agar surface and secured, the petri dish was placed in a Brewer's jar and the air was replaced by nitrogen. The two electrodes were connected to a very sensitive vacuum tube voltmeter (Hewlett-Packard, model 412A) equipped with a recorder (Esterline-Angus, model AW).

With a very large number of cells (entire surface growth of 3-day-old trypticase soy broth + agar plate) under one electrode (cathode) and none under the other electrode (anode), a sustained current density of about 1 $\mu a/cm^2$ was obtained for a period of about 9 hours. This corresponds to a corrosion rate of about 2.5 mdd (milligrams/dm^2 day) or about 0.00046 ipy (inches per year) with the formula mdd = ipy × 696 × density (*7*) and taking the density of 1010 steel as 7.85. No appreciable current was obtained in the absence of cells under the electrode. The electrode in contact with the cells (cathode) always showed a positive polarity and the anode a negative polarity, a standard dry cell being used as reference.

By use of this technique, it has also been demonstrated that aluminum and aluminum alloys can be cathodically depolarized. Metals more noble than iron and aluminum in the electromotive series, such as tin, zinc, and lead, appeared to be resistant to this type of attack. This may be due to the toxic effects of these metals and their ions on the hydrogen or other electron transport systems in the cells.

It thus appears that the mechanism proposed by the theory does indeed operate, if it can be assumed that sulfate acts in a similar fashion to benzyl viologen at the iron electrode as an electron acceptor. If such an assumption is made, the corrosion rate appears too small to account for the extensive corrosion (attributed to these organisms in nature) entirely by the Wolzogen Kühr theory.

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References

1. C. A. H. von Wolzogen Kühr and L. S. van der Vlugt, *Water* **18**, 147 (1934).
2. R. L. Starkey, *Producers Monthly* **22**, 12 (1958).
3. F. J. Raifsnider, 19th Annual Conference, National Association of Corrosion Engineers (1963).
4. W. R. Scott, *Mater. Protect.* **4**, 57 (1965).
5. G. H. Booth and A. K. Tiller, *Trans. Faraday Soc.* **56**, 1689 (1960); G. H. Booth and F. Wormwell, *1st International Congress on Metallic Corrosion* (Butterworths, London, 1961), p. 83; G. H. Booth and A. K. Tiller, *Trans. Faraday Soc.* **58**, 2510 (1962).
6. L. Michaelis and E. S. Hill, *J. Gen. Physiol.* **16**, 859 (1933).
7. H. H. Uhlig, *Corrosion and Corrosion Control* (Wiley, New York, 1963), pp. 39, 361.

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Acrylamide-Gel Electrophorograms by Mechanical Fractionation: Radioactive Adenovirus Proteins

Abstract. *A mechanical fractionator was developed to produce electrophorograms by extrusion of polyacrylamide gels through a narrow orifice in a continuous, sequential stream. The system permits separation of uniform fractions free of zone distortion. An electrophorogram of radioactive type-2 adenovirus proteins so fractionated gave a pattern in excellent agreement with the pattern obtained by laborious manual sectioning and in agreement with the pattern obtained on a replicate gel stained with Coomassie brilliant blue R250. The adenovirus particle yielded about ten resolvable protein components in unequal amounts. Like picornaviruses, these icosahedral animal viruses have multiple protein components in the viral coat.*

Electrophoresis in polyacrylamide gels (*1*) is a technique for resolving mixtures of macromolecules. It has revealed previously undetected complexity in many types of samples and is especially adaptable to analysis of protein mixtures. The separated components, if not colored, are usually detected by stains or chromogenic reactions. While such methods may be qualitatively adequate, they are quantitatively reliable only when pure reference substances are available for standardization. Isotopic quantitation of the fractions is usually less variable and more direct, and double isotopic labeling, or combined isotopic labeling and staining, permits quite accurate identifications of mixed components. Autoradiographic methods developed for acrylamide gels (*2*) give high resolution but do not permit easy identification of components by double isotopic labeling.

A particularly suitable configuration for gel electrophoresis is a round column through which the samples migrate vertically (*1*). Methods in which gels are sliced have been previously used (*1*) and I now describe a simple device for sectioning gels. I also describe a mechanical fractionator for round gel columns that produces samples suitable for determination of radioactivity by direct counting in planchet and liquid-scintillation counters and for other forms of analysis. Uniform fractions permitting high resolution are produced by continuous extrusion of the gel through a narrow orifice. When this fractionator was applied to production of electrophorograms of radioactive adenovirus proteins, eight to ten components were electrophoretically separable, indicating a degree of complexity not previously reported for the adenovirion (adenovirus particle).

The extrusion system is shown schematically in Fig. 1*A*, and the fractionator and its drive mechanism are shown in Fig. 1, *B* and *C*. Gels to be fractionated were removed from the glass tubes in which electrophoresis was performed, either by rimming with a 20-gauge 8.9-cm needle and syringe filled with a viscous lubricant (noncross-linked polyacrylamide) or by pushing with a tight-fitting plunger. The gels were completely inserted into the stainless steel sleeve (*2* in Fig. 1) and the plunger (*3* in Fig. 1) was inserted partway. With the gel inside, the sleeve-plunger assembly was screwed into the plexiglass block (*1* in Fig. 1) and the plunger was pushed by hand until the end of the gel was seen to be at the tip of the needle valve (*6* in Fig. 1). The assembled fractionator was then placed in the drive mechanism, and 0.05 percent sodium dodecyl sulfate to carry the crushed gel was pumped into the carrier-fluid inlet (*4* in Fig. 1) at the rate of 4 ml/min with a peristalic pump (*3*). The needle valve was adjusted to an opening of about 0.125 mm and the fractionator motor was started. The gel was crushed as it was pressed through the orifice and the small fragments were carried through a flexible tube, attached to the carrier-fluid out-