Table 2. Data for the equilibrium of Eq. 5.

Parameter	Value	
	1400°C	1500°C
P_{N_2}(atm)	0.05	0.05
Alloy composition (X_{Si})	0.38	0.36
$\Delta G^\circ_{(Eq.\ 5)}$(kcal)	−32.6	−30.3
$\Delta G^\circ_{Si_2N_2O}$(kcal)	−104.4	−100.3

glass tube, sealed under vacuum, were two silica boats, one containing a chromium nitride (Cr_2N)-metallic chromium buffer, the other containing a mixture of silica and a silicon-nickel alloy of known composition, with a large excess of the alloy phase. The Cr_2N-Cr buffer maintains within the silica tube a constant nitrogen pressure determined by the equilibrium

$$2Cr_2N_{(s)} = 4Cr_{(s)} + N_{2(g)} \quad (3)$$

at any selected temperature. The silica-silicon–nickel mixture defines the oxygen pressure inside the silica tube, at any selected temperatures, according to the equilibrium

$$SiO_{2(s)} = (Si)_{alloy} + O_{2(g)} \quad (4)$$

The alloy composition is varied until a composition is found at which just a trace of Si_2N_2O is formed. When this condition exists, the free-energy change for the reaction

$$1/2\ SiO_{2(s)} + 3/2\ (Si)_{alloy} + N_{2(g)} = Si_2N_2O_{(s)} \quad (5)$$

is calculated as

$$\Delta G^\circ_{(Eq.\ 5)} = \Delta G^\circ_{Si_2N_2O} - 1/2\ \Delta G^\circ_{SiO_2} = -2.303\ RT \log_{10} \frac{1}{P_{N_2\,equil} \cdot a_{Si\,equil}^{3/2}} \quad (5a)$$

The Cr_2N-Cr equilibrium is known from the work of Seybolt and Oriani (*6*), and a_{Si} values of Si–Ni alloys are known from the work of Schwerdtfeger and Engell (*7*). Although the latter data were obtained at slightly higher temperatures than those in our work, the data can be applied with good accuracy to the present problem because the temperature coefficient of the activity coefficients is known, and is small.

In some of the initial experiments, difficulties were encountered as a result of diffusion of nitrogen and oxygen from the air through the wall of the silica glass tubes. This difficulty was eliminated in succeeding runs, either by using another silica tube outside the reaction tube, with a Cr getter between the two tubes, or by using thicker-walled silica glass tubes.

Runs were made at 1400° and 1500°C. In the former the equilibration time was about 72 hours, and, in the latter, about 12 hours (Table 2).

The free-energy values obtained by the two experimental methods are in reasonable agreement. The ratios of oxygen to nitrogen pressures of the gas phase in equilibrium with SiO_2 and Si_2N_2O at a total pressure of 1 atm calculated from the closed-tube runs are $10^{-16.0}$ and $10^{-15.2}$ at 1400° and 1500°C, respectively, as compared to the values of $10^{-15.5}$ and $10^{-14.5}$ obtained in the runs in the open system. These values provide a basis for speculations concerning the environmental conditions under which the enstatite chondrites were formed, or to which they have been exposed at some time during their existence.

Combination of the free-energy data for Si_2N_2O obtained in our work and the previously available free-energy data for SiO_2 and Si_3N_4 gives the free-energy change for the reaction

$$SiO_{2(s)} + Si_3N_{4(s)} = 2Si_2N_2O_{(s)} \quad (6)$$

as $\Delta G^\circ_{(Eq.\ 6)} \cong -10$ kcal at 1400° and 1500°C. This value should be considered to be only approximate, because of the possibility of some mutual solid solubility among the three phases involved. Attempts to react SiO_2 and Si_3N_4 by solid-state reaction in a closed silica tube in the temperature range of 1300° to 1650°C for periods up to 72 hours failed to produce any silicon oxynitride, presumably because of extreme sluggishness of the reaction. However, mixtures of SiO_2, Si_3N_4, and Si_2N_2O reacted slowly at 1500°C and higher temperatures to increase the proportion of Si_2N_2O, thus confirming the negative value of $\Delta G^\circ_{(Eq.\ 6)}$.

W. R. Ryall
Arnulf Muan

Department of Geochemistry and Mineralogy, Pennsylvania State University, University Park 16802

References and Notes

1. W. D. Forgeng and B. F. Decker, *Trans. AIME* **212**, 343 (1958).
2. M. E. Washburn and R. W. Love, *Amer. Ceram. Soc. Bull.* **41**, 447 (1962); M. E. Washburn, *Refractories J.* **39**, 412 (1963).
3. D. M. Brown, P. V. Gray, F. K. Heumann, H. R. Philipp, E. A. Taft, *J. Electrochem. Soc.* **115**, 311 (1968); C. M. Drum, *J. Appl. Phys.* **39**, 4458 (1968).
4. C. A. Anderson, K. Keil, B. Mason, *Science* **146**, 256 (1964).
5. B. Mason, *Geochim. Cosmochim. Acta* **30**, 23 (1966).
6. A. U. Seybolt and R. A. Oriani, *Trans. AIME* **206**, 556 (1956).
7. K. Schwerdtfeger and H.-J. Engell, *ibid.* **233**, 1327 (1965).
8. Contribution No. 68-55 from College of Earth and Mineral Sciences, Pennsylvania State University, University Park. Supported by the U.S. Atomic Energy Commission under contract No. AT(30-1)-2781, AEC publication No. (NYO-2781-37).

13 May 1969; revised 30 June 1969

Extraparticulate Chain Interaction between Different Electron Transport Particles

Abstract. *Interaction or cross-linking between the respiratory chains of the electron transport particles of bacterial origin occurs with a mixture of active and inactive particles. Interaction between bacterial particles and liver submitochondrial particles also occurs. Irradiation of the bacterial particles at 360 nanometers resulted in the destruction of quinone and consequent loss of ability of reduced nicotinamide adenine dinucleotides to reduce cytochromes b, c_1, c, and a plus a_3. A mixture of both irradiated and untreated particles in the presence of the reduced dinucleotide resulted in the reduction of cytochromes c and a plus a_3 in an amount equivalent to the total concentration of these cytochromes in both types of particles. In contrast, the amount of cytochrome b reduced was equivalent to half the particle concentration or to that observed with the active particles alone. The rate of reduction of cytochromes c and a plus a_3 with the mixture of particles was similar to that with the active particles alone. The interaction or cross-linking between the particulate respiratory chains of bacteria or of bacterial and mammalian systems occurs after cytochrome b and before or at cytochrome c.*

The possibility of intra- and extra-chain interaction of the respiratory assemblies as well as the possibility of cross-linking of respiratory components within the respiratory chain has been suggested from kinetic studies (*1*) by the use of selective respiratory inhibitors or dyes (*2*, *3*) and from studies

with a reconstituted mitochondrial system (*4*). Interaction of the respiratory carriers within the respiratory chains is thought in part to be restricted to those respiratory components in close proximity to one another. Evidence has been presented to support the conclusion that two or more cytochrome b components interact (*1, 2, 5*) and also that cytochrome (c_1 + c) and cytochrome a (*6, 7*) interact in mammalian mitochondria.

The respiratory components associated with the coupled electron transport chains occur in highly organized membrane systems and demand a strict spatial orientation of the carrier molecules. Although the respiratory carriers may be physically restricted in their interaction, "communication" within the respiratory chain or between adjacent chains may occur by (i) interaction between different forms of the respiratory carrier (for example, cytochrome b and b_1), (ii) cross-linking between different respiratory chains [cytochrome b_{NADH} (*8*) and $b_{succinate}$], and (iii) interaction between different respiratory carriers on the same chain, bypassing one or two respiratory carriers, or cross-linking between different respiratory carriers on different respiratory chains (flavin$_{NAD^+}$ or quinone with cytochrome b of the succinoxidase pathway). In addition, cross-linking or chain interaction may occur between the respiratory chains of subcellular structures such as mitochondria and other subcellular structures which possess a partial respiratory chain.

The interaction or cross-linking of the respiratory chains of different subcellular structures has been investigated with mixtures of electron transport particles containing active and inactivated electron transport chains. The electron transport particles from *Mycobacterium phlei*, which are capable of coupling phosphorylation to oxidation, were used to determine whether respiratory chain interaction can occur. *Mycobacterium phlei* (ATCC 354) was grown, and the cell-free homogenate and electron transport particles were prepared as described (*9*). Inactivation of the particles from *M. phlei* was accomplished by irradiation at 360 nanometers. Chemical, enzymatic, and kinetic studies with the light-treated particles have revealed that, with the exception of the quinone, the particles contain all of the respiratory carriers present in the untreated particles and that the respiratory carriers are functional (*10, 11*). In addition, it would appear that light treatment does not affect the physical orientation of the respiratory carriers because phosphorylation between cytochrome c and oxygen can be demonstrated with the quinone-depleted system (*12*). Since the quinone functions between flavoprotein and cytochrome b, it was possible to study the interaction of respiratory chains in the region between cytochrome b and oxygen.

The electron transport particles from *Mycobacterium phlei* are capable of oxidizing NADH. Complete enzymatic reduction of cytochromes b, c, and a occurred at transition (Fig. 1A). In contrast, after irradiation the particles failed to oxidize NADH, and the cytochromes remained in the oxidized state (Fig. 1B). However, incubation of an equal mixture of irradiated and untreated particles with NADH resulted in the reduction of cytochromes c and ($a + a_3$) in an amount equivalent to the concentration of the cytochromes in both particulate fractions (Fig. 1D). The level of cytochrome b reduction, in the experiment containing the mixture of particles, was not increased but was equivalent to that observed with the active particles alone. The total amount of enzymatically reducible cytochromes with a concentration of particles equal to the mixture of treated and untreated particles (12 mg of particulate protein) is shown in Fig. 1C. A comparison of the amount of enzymatically and chemically reducible cytochromes in the mixed particle population is given in Table 1.

The observations with the mixed particle population suggested that an interaction or cross-linking between particulate electron transport chains existed and that this cross-linking or transfer of electrons between particles occurred after cytochrome b and before or at cytochrome c. It appeared likely that the transfer of electrons between particles may have been carried out by a soluble enzyme or carrier which was liberated from the particles during preparation or light treatment. Thus, both types of particle preparations were subjected to two additional washings in an attempt to remove soluble materials released during preparation. After these washings, the rate of reduction and the total amounts of cytochromes c and ($a + a_3$) reduced with the mixture of irradiated and untreated particles was similar to that of the same particle mixture before additional washing.

To determine whether a molecule of low molecular weight was responsible for the transfer of electrons between

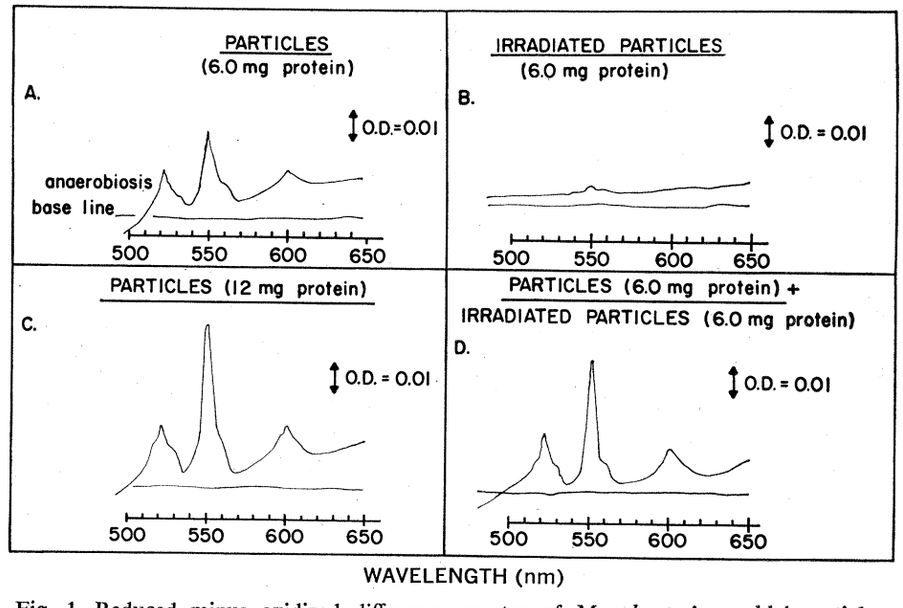
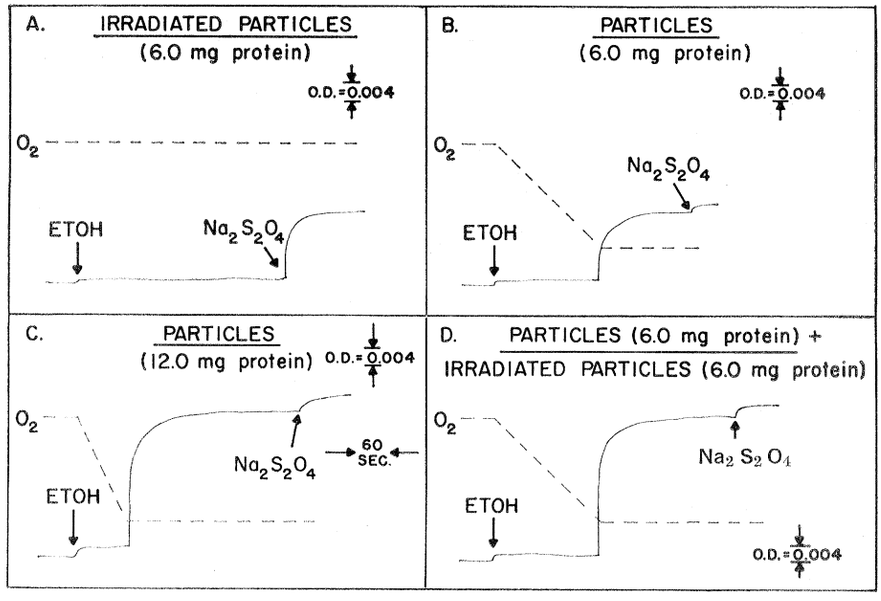


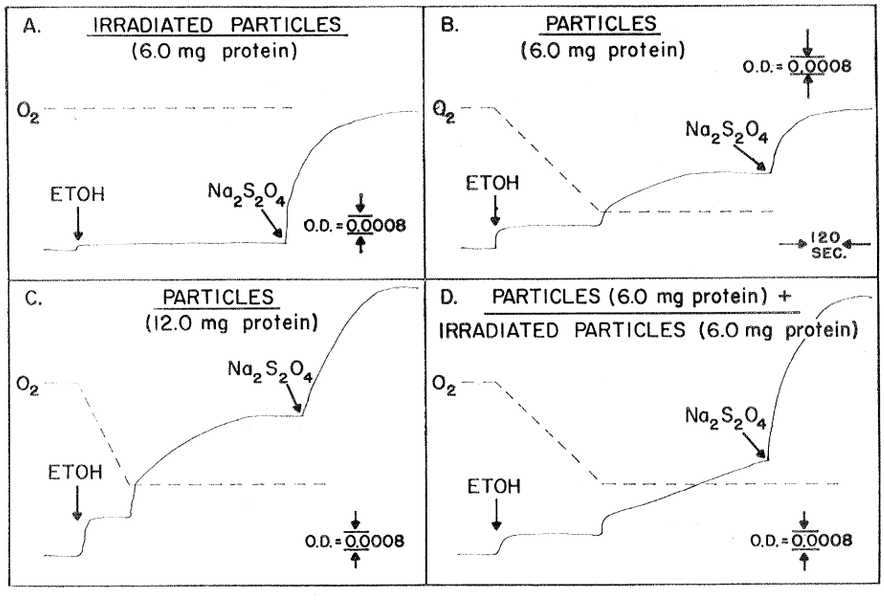
Fig. 1. Reduced minus oxidized difference spectra of *Mycobacterium phlei* particles. The reaction mixture contained particles (6.0 or 12.0 mg of protein as indicated), 100 μmole of HEPES-KOH buffer (*p*H 7.5), 9 μmole of $MgCl_2$, 50 μmole of hydrazine, 0.5 mg of crystalline alcohol dehydrogenase from yeast, 1 μmole of NAD^+, and water to a final volume of 1.0 ml. The reaction was started by the addition of 100 μmole of ethanol. Continuous scanning was performed until the anaerobic condition was obtained. The spectra were measured with a Cary 14 spectrophotometer with between 0.0 and 0.1 optical density (O.D.) units. The difference spectra were measured by the method of Chance (*17*).



Cytochrome c (551–540 nm)

Fig. 2. Rate of oxygen uptake and rate of cytochrome reduction of *Mycobacterium phlei* particles. The reaction mixture consisted of particles, 100 μmole of HEPES-KOH buffer (*p*H 7.5), 15 μmole of $MgCl_2$, 50 μmole of hydrazine, 0.5 mg of crystalline alcohol dehydrogenase from yeast, 1.0 μmole of NAD^+, and water to make a total volume of 2.5 ml. The spectra were measured with a dual-beam spectrophotometer equipped with a vibrating platinum-oxygen electrode (551–540 nm).

active and inactive particles, the experiment was conducted under anaerobic conditions, with active and inactive particles being separated by a semipermeable membrane. The active particles were incubated with substrate (generated NADH) in a dialysis chamber (Diaflo) containing a filter which would exclude molecules having a molecular weight higher than 25,000. The chamber was flushed with nitrogen, and the enzymatic reaction was allowed to proceed until all of the cytochromes reached complete reduction. After complete reduction the nitrogen pressure was increased to 0.68 atm, and 2.5 ml of fluid that contained material having a moleclular weight below 25,000 was permitted to flow into a sealed cuvette, previously flushed with nitrogen and containing inactive particles, cofactors, and substrate in a volume of 0.5 ml. Although it was possible to show that the active particles had undergone complete reduction, the diffusible material from the active particles (molecular weight less than 25,000) failed to reduce cytochromes c or $(a+a_3)$ of the inactive particle preparation. Thus it would appear that the transfer of electrons from active to inactive particles was not mediated by a compound of low molecular weight.



Cytochrome b (562–574 nm)

Fig. 3. The rate of oxygen uptake and the rate of cytochrome b reduction in *Mycobacterium phlei* particles (562–574 nm). The conditions were similar to those described in Fig. 2.

The rates of oxidation and reduction of the individual cytochromes were measured with the system containing mixed populations of active and inactive (light-treated) particles. The rates of reduction of cytochromes c (Fig. 2) and $(a+a_3)$ were rapid and greater than the overall rate of oxygen uptake observed with generated NADH. Of particular interest was the finding (i) that the kinetics of reduction in the mixed particle population were similar to the kinetics observed with the active particles alone and (ii) that the transfer of electrons from the active to the inactive particles occurred at a rate equivalent to the rate of reduction of cytochromes c or $(a+a_3)$ in the untreated preparation. The combination of active and inactive particles failed to increase the rate of oxygen uptake (Fig. 2D). The fact that there was no further increase in the rate of oxidation may indicate that the rate-limiting step in the electron transport chain exists at a point before that of particle interaction or before cytochrome c (*13*). As indicated by the results obtained from the difference spectra of the cytochromes in a mixed population, the amount of reduction of cytochromes c and $(a+a_3)$ was equivalent to the concentration of these cytochromes in both types of particles, whereas the reduction of cytochrome b (Fig. 3D) was equivalent to that of the active particles alone.

In order to determine whether different types of electron transport particles can interact, the inactive (light-treated) particles from *Mycobacterium phlei* were tested with submitochondrial

Table 1. Enzymatically (at transition) and chemically reducible cytochromes of *Mycobacterium phlei* particles and rat liver mitochondria. The system consisted of particles, 100 μmole of HEPES-KOH buffer (*p*H 7.5), 15 μmole of $MgCl_2$, 50 μmole of hydrazine, 1 μmole of NAD^+, 0.5 mg of yeast alcohol dehydrogenase, and water to a final volume of 2.5 ml. The reaction was started by the addition of 100 μmole of ethanol. β-Hydroxybutyrate (100 μmole) was used as the electron donor in the system containing the mitochondrial preparation. The spectrophotometric studies were performed with a split-beam spectrophotometer (American Instrument Company).

Addition of electron transport particles	Total cytochrome reduction (nmole)					
	Cytochrome a (601–623 nm)		Cytochrome b (562–574 nm)		Cytochrome c (551–540 nm)	
	Enzymatic	Chemical	Enzymatic	Chemical	Enzymatic	Chemical
M. phlei (6.0 mg)	0.31	0.33	0.28	0.67	1.16	1.19
M. phlei irradiated (6.0 mg)	.0	.32	.0	.66	0.0	1.12
M. phlei (6.0 mg) + *M. phlei* irradiated (6.0 mg)	.60	.61	.29	1.36	2.24	2.32
Mitochondria (2.0 mg)	.41	.45	.35	0.44	0.59	0.62
Mitochondria (2.0 mg) + irradiated *M. phlei* particles (6.0 mg)	.71	.80	.31	1.14	1.69	1.76

particles. Rat liver mitochondria were prepared according to the method described by Hogeboom (*14*). The submitochondrial particles were obtained by treating the mitochondria with high-frequency sound for 2 minutes (*15*). Since rat liver mitochondria can oxidize β-hydroxybutyrate whereas the particles of *M. phlei* lack this activity unless supplemented with the bacterial supernatant fraction, β-hydroxybutyrate was utilized as the electron donor to reduce the mammalian electron transport chain. In addition, the particles of *M. phlei* were irradiated to further insure that the electron transport from substrate proceeded by way of the mammalian electron transport chain. The oxidation of β-hydroxybutyrate occurred with the submitochondrial particle preparation (Table 1) but failed to occur with the irradiated *M. phlei* particles. The combination of rat liver submitochondrial particles and inactive *M. phlei* particles resulted in the reduction of cytochromes c and $(a + a_3)$ equivalent to the sum of these cytochromes in both types of particle preparations. The reduction of cytochrome b was equivalent to that in the submitochondrial preparation with little or no reduction of the *M. phlei* cytochrome b. The combination of submitochondrial particles and inactive bacterial particles failed to increase the rate of oxidation over that observed with the submitochondrial particles alone. Attempts were also made to determine whether the combination of mammalian and bacterial particles would result in increased phosphorylation. However, no additional phosphorylation was achieved by the mixture of particles even in the presence of bacterial coupling factors (*16*).

Interaction between extraparticulate chains does not occur between bacterial particles and mammalian microsomes. Rat liver microsomes were obtained by the method of Hogeboom (*14*). The cytochrome b_5 of rat liver microsomes was reduced by NADH, but the transfer of electrons did not occur between the microsomes and irradiated bacterial particles. Similarly, with succinate as the electron donor the cytochromes of active (untreated) bacterial particles were reduced. However, with a mixture of active bacterial particles and microsomes with succinate as substrate, reduction of microsomal cytochrome b_5 did not occur. The lack of chain interaction between microsomes and the bacterial electron transport particles may be related to the difference in potential between cytochrome b_5 of the microsomes and cytochromes b and c of the bacterial particle or to the inaccessibility of the bacterial cytochrome b or c.

The mechanism by which chain interaction between particles occurred is unknown; however, the interaction occurred after cytochrome b and before or at cytochrome c. Direct contact between the particles may be required for interaction to occur. If this is the case, it is possible that cytochrome c is located on the periphery of the particulate membrane, so that electrons can flow from one particle to another by way of the interaction of cytochrome c molecules, while cytochrome b may be located at an inaccessible site within the membrane and thus be incapable of chain interaction.

The nature of the interaction between different types of subcellular electron transport particles remains unknown; however, particle interaction between mitochondria or between bacterial electron transport particles appears to occur upon collision *in situ*. Unlike the mitochondrion which can move about in the cytoplasm of the cell and contact other mitochondria, the electron transport particles in bacteria appear to be bound to the membrane. Thus, interaction of the respiratory chains in bacteria could only occur by close proximity of the various respiratory apparatus on the membrane or by dissociation of a segment of the membrane containing the respiratory apparatus and its reassociation with other membrane structures.

EITAN BOGIN
TANEAKI HIGASHI
ARNOLD F. BRODIE
Departments of Microbiology and Biochemistry, University of Southern California School of Medicine, Los Angeles

References and Notes

1. C. P. Lee, R. E. Estabrook, B. Chance, *Biochim. Biophys. Acta* **99**, 32 (1965); S. K. Lanyi, *Arch. Biochem. Biophys.* **128**, 716 (1968); E. R. Redfearn, P. A. Whittaker, S. Burgos, in *Oxidases and Related Redox Systems*, T. E. King, H. S. Mason, M. Morrison, Eds. (Wiley, New York, 1965), vol. 2, p. 943.
2. M. Jeng and F. L. Crane, *Biochem. Biophys. Res. Commun.* **30**, 465 (1968).
3. Y. Hatefi, A. G. Haavik, L. R. Fowler, D. E. Griffiths, *J. Biol. Chem.* **237**, 2661 (1962).
4. T. E. King, *Advan. Enzymol.* **28**, 155 (1966).
5. E. C. Slater, *Biochem. J.* **46**, 484 (1950).
6. E. Yakushiji and K. Okunuki, *Proc. Imp. Acad. Jap.* **16**, 299 (1940).
7. T. Yonetani, *J. Biol. Chem.* **235**, 3138 (1960).
8. Abbreviations: NADH and NAD^+, reduced and oxidized nicotinamide adenine dinucleotide; HEPES, *N*-2-hydroxyethylpiperazine-*N'*-2-ethane sulfonic acid; ATCC, American Type Culture Collection.
9. A. F. Brodie and C. T. Gray, *J. Biol. Chem.* **219**, 853 (1956).
10. A. F. Brodie and J. Ballantine, *ibid.* **235**, 226 (1960).
11. A. Asano and A. F. Brodie, *ibid.* **239**, 4280 (1964).
12. C. K. R. Kurup and A. F. Brodie, *ibid.* **241**, 4016 (1966).
13. P. B. Scholes and L. Smith, *Biochim. Biophys. Acta* **153**, 363 (1968).
14. G. H. Hogeboom, *Methods Enzymol.* **1**, 17 (1955).
15. W. W. Kielley and J. R. Bronk, *J. Biol. Chem.* **230**, 521 (1958).
16. E. Bogin, T. Higashi, A. F. Brodie, in preparation.
17. B. Chance, *Science* **120**, 767 (1954).
18. Supported by NIH grant AI 05637, NSF grant GB 6257X, AEC contract AT (11-1)-113, and the Hastings Foundation of the University of Southern California School of Medicine. This is the 45th paper in a series dealing with oxidative phosphorylation in fractionated bacterial systems.

8 July 1969