though the subunits on the "bottom" surface are usually less distinct, they may add to any estimation based solely on total counts of *visible* capsomeres in the negative.

Measurements on close-packed arrays of intervirus/intersubunit ratios (*6*), and their comparison with values obtained from models, have little meaning in the light of the parallax involved in measuring the spacings between long hollow capsomeres, and the assumption that all morphological subunits are identical in size and shape needs further consideration. While this may be nearly so for the papova viruses, there is strong evidence that the subunits of turnip yellows mosaic virus and the entero-viruses are much smaller than those of the papova group and the larger animal viruses (*13*).

It is common knowledge that in any biological species not all the members are perfect specimens and it can be expected that virus particles are no exception. Observation of negatively stained virus preparations in the electron microscope reveals that many virus particles are assembled more "symmetrically" than others. We must bear in mind that in any system of classification based on fine structure, the basic symmetry and capsomere number for a given virus species relates to the most morphologically favorable members in the preparation. It is not unlikely that during the course of construction of a virus capsid from an intracellular source of capsomeres, errors may occur and particles with variable numbers of subunits could be formed. Although we have seen many disordered particles in our papova virus preparations, none suggest a possible count of $N = 92$.

The evidence continues to mount that the papova viruses ($N = 42$, $n = 3$) are approximately 45 to 50 mμ in diameter and that their capsids consist of 30 hexagonally faced and 12 pentagonally faced hollow capsomeres. Next in the icosahedral series ($N = 92$, $n = 4$) come the reoviruses and wound-tumor viruses, approximately 60 to 70 mμ in diameter and possessing a capsid again with 12 pentagonally faced, but with 80 hexagonally faced columnar capsomeres. Proper interpretations of patterns of cubic symmetry are proving to be powerful tools in building new systems of virus classification (*14*).

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Preliminary Experiments on a Microbial Fuel Cell

Abstract. Experiments were initiated to determine whether microbes using a hydrocarbon as food could generate electrical energy. Experiments with ethane were unsuccessful, but when microbes or glucose oxidase were added to a solution of glucose, electrical output was observed, confirming in part previous observations. As far as we know, no one had previously tested hydrocarbons in this manner, although the several effects of microbial activity on redox potential are well known. Failure of the microbes employed to dehydrogenate ethane in the absence of molecular oxygen is discussed in the light of recently published experimental evidence.

Since biological dehydrogenations take place in the absence of an immediate direct participation of oxygen, it is conceivable that a wire could couple oxygen with the microbial dehydrogenation and hydrogen ionization reactions. The electrons transferred would react at the oxygen electrode to produce hydroxyl ions which could migrate through a semipermeable membrane to react with hydrogen ions, completing the cyclic reaction. It is obvious that the crucial link in the series of reactions is the substitution of a wire for the ordinary electron transport mechanism, at least so far as the final reaction with oxygen is concerned.

A practical experimental system required as a prime consideration the selection of a semipermeable membrane which would separate the "biological electrode" from the oxygen electrode. Such a membrane, according to theory, would allow passage of hydroxyl ions but not free oxygen. The system used was divided into three compartments by common dialysis membranes. The two outer compartments contained the electrodes, while the middle compartment served as a "buffer zone." The dialysis membrane, plus continuous bubbling of O_2-free nitrogen through this zone, effectively prevented any oxygen from reaching the biological electrode compartment.

The volume of each of the three compartments was approximately 400 ml. Platinum sheets (10 by 3 in.) were used in the two outer half-cells as electrodes. Nitrogen and oxygen were bubbled continuously into the biological and oxygen half-cells, respectively. Electrical measurements were performed with the usual laboratory devices.

Nocardia were used in the experimental systems because of their established ability to oxidize hydrocarbons. *Escherichia coli,* a facultatively anaerobic bacterium, and glucose oxidase, an oxygen-requiring enzyme, were selected on the basis of their oxygen requirements. The basal solution employed in all compartments consisted of 1 percent sodium chloride in 0.05*M* phosphate buffer, *p*H 7. When glucose was used as substrate it also was added to all three compartments.

In the first test of the experimental cell the glucose-glucose oxidase system was used. This enzyme catalyzes the aerobic oxidation of glucose to gluconic acid. However, in our system, no reaction occurred when the electrode wire was substituted for oxygen. This could mean either that molecular oxygen is absolutely required for the reaction or that a substitute hydrogen acceptor is required to initiate the reaction in the absence of oxygen. Findings when methylene blue (0.25 mg) was added proved the latter premise to be correct. Open-circuit voltage (electromotive force) increased 80 to 180 mv, and 50 to 100 mv was maintained under a load of 1000 ohms.

Since the glucose-glucose oxidase system failed to react in the absence of oxygen but did react with methylene blue, it was reasoned that a facultative anaerobe, which requires neither of the two in its metabolism, might produce measurable current. Such was the case. When *Escherichia coli* was added to the biological half-cell with glucose as substrate, the open circuit voltage increased from 150 to 625 mv and, under a load of 1000 ohms, 500 mv was maintained for over 1 hour, at which time

the experiment was terminated. The addition of methylene blue had no effect, except that the methylene blue was rapidly decolorized.

In the next series of experiments an attempt was made to use a hydrocarbon as fuel. An active ethane-oxidizing culture of *Nocardia* was added to the biological half-cell, and ethane was bubbled into the solution. Apparently, no reaction occurred. On the addition of methylene blue, a slight increase in open circuit voltage was measured. However, this was later shown to be due not to the *Nocardia*-ethane system but, rather, to endogenous respiration. When glucose was substituted for ethane a definite increase in metabolic activity occurred. Additions of methylene blue to this system caused current flow eventually to reach a maximum of 2 ma (see Table 1).

Finally, a test was made of the effect of a strong oxidizing agent (potassium ferricyanide) on the system, with glucose as fuel. When 1 mg of $K_3Fe(CN)_6$ was added to the O_2 half-cell in the absence of microbial cells at the opposite electrode, only a slight increase in current was measured. However, when *Escherichia coli* cells were added to the biological half-cell the current increased markedly (from 0.1 to 1.6 ma in 30 minutes). The addition of large amounts of $K_3Fe(CN)_6$ at the O_2 electrode and of methylene blue at the biological electrode resulted in very little current flow unless metabolizing cells were also present at the biological electrode.

The experimentation described was exploratory, and no attempt was made to increase current measurements by using unit cells in series, as had been done previously by Cohen (*1*). A principal question at the outset was: Can microbes which ordinarily oxidize hydrocarbons release measurable quantities of electrical energy? The experiments performed revealed that while nocardia release measurable electrical energy in their metabolism, confirming somewhat similar experiments performed with bacteria and yeast 50 years ago by Potter (*2*), hydrocarbons were not successfully metabolized under the conditions employed.

The data clearly show that when a substitute hydrogen-acceptor for oxygen —namely, methylene blue—is successfully employed as an oxidant in endogenous respiration or glucose metabolism, by either living microbes or the enzyme glucose oxidase, a current is measurable. In fact, *Escherichia coli,* a facultative anaerobe capable of metabolizing glucose in the absence of either oxygen or methylene blue, yielded current in the experimental system in the absence of methylene blue.

Methylene blue did not serve as a successful hydrogen acceptor in the metabolism of ethane by ethane oxidizers. The simplest explanation for this is that the initial reaction of biological hydrocarbon oxidation involves a physical incorporation of molecular oxygen into the hydrocarbon molecule. This would involve an "oxygenase" enzyme, recently discussed in detail by Mason (*3*). Kallio and his associates give support to this idea in their recent work on the microbial oxidation of hydrocarbons (*4*). Since the biological half-cell must rigidly exclude molecular oxygen, no hydrocarbon oxidations will occur unless intermediate hydrogen acceptors are found. Current measurements can serve as a tool in searching for intermediate oxidants which will accept hydrogen from hydrocarbons.

Table 1. Biological electrode reactants. *E*, electromotive force; *I*, current. The load was 1000 ohms.

Condition	Glucose oxidase		*E. coli*		*Nocardia*	
	E (mv)	*E* under load (mv)	*E* (mv)	*E* under load (mv)	*E* (mv)	*I* (ma)
At equilibrium*	141	38	148	42	50	0
Added enzyme	198	50				
Added microbial cells			625	521	115	0
Ethane in *Nocardia* system					120	0
Methylene blue (0.25 mg)	280	75	625	521	192	0
Methylene blue (0.25 mg)	287†	90	625	521	245†	0.05
Glucose replacing ethane in *Nocardia* system					245	.05
Methylene blue (0.5 mg)					265	
Methylene blue (1.5 mg)					295	.45
Methylene blue (4.0 mg)					305	.60
Methylene blue (10.0 mg)					300	1.70
Methylene blue (35.0 mg)					300‡	2.00

* Glucose present in glucose-oxidase and *E. coli* systems only. † Methylene blue did not decolorize. ‡ Methylene blue was decolorizing but not completely decolorized when the experiment terminated. It was completely decolorized in all other cases.

Preliminary data of this nature can lead to only tentative conclusions. That microbial metabolism is a source of measurable electrical energy is established, but as far as we know, no one has made a serious attempt to employ this energy.

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Conditioning of Induced Electroencephalographic Sleep Patterns in the Cat

Abstract. Stimulation of the basal forebrain synchronizing area of the cat induces the behavioral and electroencephalographic manifestations of sleep. By pairing such stimulation with a neutral auditory stimulus we were able to evoke electroencephalographic synchronization and sleep preparatory behavior to the presentation of a tone, and to temporal factors associated with the conditioning procedure.

In his classical investigations on diencephalic functions, Hess (*1*) distinguished two regions in the hypothalamus to which he ascribed, in a general fashion, antagonistic actions. Stimulation in caudal hypothalamic areas (ergotropic zone) resulted in a generalized behavioral activation and sympathetic discharge, whereas similar stimulation in more anterior loci (trophotropic zone) produced generalized somatomotor suppression and parasympathetic discharge. Additionally, low frequency stimulation of the massa intermedia of the thalamus resulted in the onset of behavioral sleep. It has been shown more recently that electrical stimulation of a specific basal forebrain area induces a diffuse and sustained electroencephalographic (EEG) synchronization in immobilized short-term preparations as well as in behaving cats with permanently implanted electrodes (*2*). This area has been termed the basal forebrain synchronizing area. The EEG synchronization observed in unrestrained cats is accompanied by a parallel transition from alert, waking behavior to apparent sleep, all of which typically occurs within 1 or 2 minutes