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RESULTS OF SOME EXPERIMENTS
IN BIOCHEMICAL ELECTRICITY

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SPACE SCIENCES LABORATORY

AEROPHYSICS SECTION

RESULTS OF SOME EXPERIMENTS IN
BIOCHEMICAL ELECTRICITY*

By

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GENERAL  ELECTRIC

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INTRODUCTION

Chemical reactions which give rise to electrical energy in living and non-living systems have been extensively studied for many years.

Potter (1911)¹ measured the production of electrical energy by growing yeast cultures in one half cell of a typical oxidation reduction cell. At that time, he reported that the "chemical action of their (the micro-organisms) vital processes was utilized to develop electrical energy in the manner parallel to the production of EMF by means of an ordinary galvanic battery".

Subsequent publications concerning the study of oxidation-reduction potentials derived from the metabolism of micro-organisms are too numerous to mention in this paper.

Rather, the scope of this paper will be limited to a discussion of bio-electrogenic systems, which may serve as a source of useable low-level electrical energy.

Recently, systems called "Biochemical Fuel Cells"* have been partially described by Sisler, 1961,³ 1962,⁴ and Welch,⁵ Rohrbach, 1961,² have reported that BFC's have been developed that produce enough energy to "operate radio beacons, signal lights, and other apparatus at sea".

* B. F. C.

The interest at the Space Sciences Laboratory, Missile and Space Division, in studying the production of electricity by means of biochemical reactions, stems from a previous association with the problems of waste management in closed ecological systems, and the study of living systems in space. As a result, therefore, algae and fecal bacteria were among the first biological organisms used in the early empirical phase of this work, which then led to the development of another system for the purpose of studying the biological mechanism by which the electricity was produced. Fleischmann's yeast which was grown in 5% glucose was the sole organism used in these experiments.

Concurrent with this research into the biochemical fuel cell, an empirical study was conducted, investigating the utilization of bioelectric potentials as a primary source of power for implanted electric devices.

EXPERIMENTAL

Two separate and distinct studies are described in this section, both of which relate to biochemical electrical production (Bio-Electrogenesis). These are:

1. Biochemical Fuel Cell
2. Bioelectric Potentials

I. Biochemical Fuel Cell

The first bio-electrogenic system tested in this laboratory consisted of one half cell containing algae and another half cell containing fecal bacteria. The two half cells were joined by a KCl-agar bridge and the emf was measured by inserting copper electrodes into each culture. The emf obtained was 0.3V at 1.4 - 2.02 ma/ft². (Fig. 1).

A more convenient cell assembly was designed so that the agar bridge could be replaced by various membranes thus lessening the internal resistance of the cell. (Figs. 2 and 3). Flattened quad rings were cemented onto pieces of plastic 4" x 4" x 1/4". The two half cells were formed by simply placing a membrane between the quad rings and by joining the two plastic plates with clamps. The test materials were allowed to flow into the cells via inlet tubes located at the bottom of the assembly. The outlet, located at the top of the cell, served as a gas vent.

Various membranes, such as cellophane, and anion and cation exchange materials were tested in this device. These membranes were evaluated by the shape of the polarization curves which were obtained by adding a series of resistances to the circuit. The change in voltage was plotted vs. the calculated current values.

The best membrane was composed of a cation exchange membrane which had been covered with platinum-black* (Fig. 4). Two pieces of platinum screen 12.5 cm^2 were used as electron collectors. (This type of membrane and electrode has been routinely used successfully by DECO in conventional $\text{H}_2 - \text{O}_2$ fuel cells).

All data reported in this paper were obtained by using the device described above. Although various mixtures of micro-organism were tested in this cell, it was necessary to simplify the system in order to study the biological mechanism. For this reason, it was decided that yeast would be used in one half cell and H_2O or air in the other half cell.

This particular organism was chosen because it will grow for a time in a non-electrolytic substrate, glucose. Therefore, the production of an emf could not be ascribed to a high concentration of electrolytes in the culture medium, since one half cell contained the sugar solution while the other contained tap water or air. A more important reason for choosing yeast lies in the fact that the metabolism of glucose by yeast has been studied extensively for many years.

The culture was prepared by adding one packet of Fleischmann's yeast to 500 ml of a 5% aqueous glucose solution. Fig. 5 shows that the open circuit voltage** varies as a function of the age of the culture. A maximum O. C. V. occurred at two hours; hence, two-hour cultures were used in all of the experiments conducted thus far.

At the end of a two-hour incubation period, about 20-30 ml of the yeast solution was admitted to one side of the membrane and air saturated water or air alone was allowed to flow into the other side of the cell.

Using this technique an O. C. V. of 0.65 to 0.75 volts was obtained.

*Obtained from G. E. DECO, Lynn, Mass.

** O. C. V.

The polarization curve (Fig. 6) shows that a maximum power output of $.66\text{w}/\text{ft}^2$ was obtained at a current of $2.2\text{ amp}/\text{ft}^2$.

The same emf was recorded when the whole culture was replaced by the cell-free supernatant fluid.

Fig. 7 shows a plot of O. C. V. vs. time without a load and with a load of 100 ohms. The drop of emf can be partially attributed to ageing of the culture. The utilization of a continuous culture technique should alter the slopes of these curves appreciably.

The effect of the organism upon the production of electricity was determined by measuring the O. C. V. under different experimental conditions. No appreciable O. C. V. was observed when both half cells were filled with H_2O or with the 5% glucose solution. No measurable O. C. V. was observed when one cell was filled with H_2O and the other with 5% glucose. An emf was generated only after the addition of yeast to the glucose solution. Various metabolites, such as ethanol and acetaldehyde may be involved in a catalytic reaction with the platinum-black to produce the observed effect.

A preliminary study of the biological mechanism was initiated by using various chemicals that are known to inhibit enzymatic reactions involved in the glycolytic metabolism of glucose.

A $3.3 \times 10^{-3}\text{M}$ solution of iodo acetetic acid, which blocks the action of glyceraldehyde-3 phosphate, was added to the culture medium. After two hours' incubation, the culture was admitted to one half cell and the O. C. V. was observed. In this case no appreciable O. C. V. was observed. (Fig. 8).

A new culture was grown in which the enolase reaction had been inhibited by the addition of a 10^{-2}M Na fluoride and 10^{-2}M PO_4 . The observed O. C. V. was 0.35 - 0.4V.

Finally, the action of alcohol dehydrogenase was blocked by the addition of 10^{-2}M SO_3^- . The O. C. V. was 0.6 - 0.65 as compared with an O. C. V. of 0.7 - 0.75V for the control culture.

Paper chromatography was used in order to further investigate the biological mechanism. The yeast cells were removed from the inhibited and non-inhibited cultures by centrifugation and about 0.1 ml was spotted on Whatman 3 MM filter paper. The solvent system used in developing the papers

was composed of ethyl acetate, acetic acid, water, 3:3:1. The developed papers were dried and sprayed with alkaline silver nitrate spray. Fig. 9 is a schematic representation of the separation of reducing metabolites.

Spot #1 is the 5% glucose solution without yeast added; two additional reducing metabolites were detected in the control culture supernatant (#2 and 3). The iodo acetate poisoned culture contained metabolite #2 but not #3; therefore, #3 probably is an active metabolite insofar as emf production is concerned.

The fluoride poisoned culture, as expected, contained #2, but a different metabolite #4 was observed. The SO_3^- poisoned sample was the same as the control except that the sodium sulfite itself contributed one low R_f spot.

To date no qualitative or quantitative analyses have been carried out on these metabolites.

At any rate, these data do indicate that an emf is observed only after the first electron transport reaction occurs, i. e. D-glyceraldehyde-3-phosphate to 1, 3, di phospho-D-glycerate. Also, the different metabolites which are associated with different emf's in poisoned cultures seem to indicate a catalytic interaction of reducing compounds and the platinum-black electrode. That is, the yeast produces ionizable metabolites which give up an electron(s) by reaction with the platinum-black electrode which is a conventional fuel cell reaction. In fuel cell terminology, this means that the yeast half cell is the anode, or the place where a loss of electrons occurs.

By definition, then the other half cell is the cathode (electrons gained) and will be treated as in a conventional hydrogen-oxygen fuel cell. This half cell reaction will be touched upon later in this report.

In order to further investigate the role of the platinum-black in the overall reaction, the following experiments were conducted:

The platinum-black was removed by an abrasive from one side of the cation exchange membrane. The yeast was added to this side of the membrane and H_2O added to the other side. No appreciable emf was observed (Fig. 10). When the yeast was added to the other side, reversed conditions; an emf of 0.35 - 0.4V was observed. When platinum-black was removed from both sides, no emf was observed.

The same data were obtained when a cell-free supernatant of a two-hour culture was tested as above.

An inquiry into the electro-chemical reactions in the cathodic cell was limited to an observation of changes in emf as a function of oxygen concentration.

Yeast was used to fill the anode, water was used to fill the cathode half cell, and the O. C. V. was recorded. The cathode cell was emptied and pure oxygen was allowed to flow through the cell. The O. C. V. increased from 0.1 to 0.2 volts. The same effect was observed when a 10% solution of hydrogen peroxide was substituted for the oxygen. These reactions strongly indicate that the cathode reaction is essentially the same as that postulated for the conventional H₂ - O₂ fuel cell.

II. Bioelectric Potentials*

The following preliminary experiments were conducted in order to ascertain whether bioelectricity present in rats was of sufficient magnitude to power an electrical circuit.

The rats used in this study weighed approximately 150 grams and were anesthetized with Nembutal. An electrode of platinum/Pt-black was inserted into the coelom through a small hole in the abdominal wall. An alligator clip which was attached to the skin in the brachial region served as the second electrode.

The leads were connected to a variable resistance that led to a Sensitive Instrument Voltmeter. The current was calculated by varying the resistance and observing the change in voltage. A polarization curve was prepared by plotting the voltage vs. current. The power was also calculated and plotted (Fig. 11). A maximum of 64_μ watts was obtained at a resistance of 1000 ohms.

It should be noted that the polarization curves show a strange zig-zag effect in the lower resistance range. These curves indicate that at a given amperage a different voltage can be obtained with a different resistance. This phenomenon has not yet been explained but may be caused by the measuring devices used in these preliminary studies. On the other hand, this may be an indication of some adaptive biological process that comes into play when too much current is allowed to flow.

Next, the alligator clip was replaced by a platinum/Pt-black electrode implanted subcutaneously. The abdominal electrode was allowed to remain in situ. The appropriate electrical measurements were made as stated above. A maximum of 16_μ watts was obtained at a resistance of 1000 ohms (Fig. 12).

Finally, the subcutaneous Pt/Pt-black electrode was replaced by a stainless steel artery clamp, which was slipped under the skin.

A maximum of 300_μ amps at an emf of 0.3V and a resistance of 1000 ohms was observed. This results in a power output of 90_μ watts (Fig. 13).

A small 500 kc sine wave oscillator was designed (Fig. 14) and constructed which would operate on the B. E. P. output of a rat. Initially, the sine wave from this oscillator was observed on a cathode ray oscilloscope, using a

*B. E. P.

"C" battery to supply 0.5V through a potentiometer. Replacing the battery with the rat, containing a Pt/Pt-black electrode in the coelom, and a Pt electrode implanted subcutaneously, resulted in an equivalent amplitude sine wave on the oscilloscope. This simple experiment demonstrates the feasibility of utilizing the B. E. P. available from the rat to furnish power to a small high frequency oscillator, or extrapolating, other similar electronic devices. The electronic oscillator constructed for these experiments was designed for lower power requirements than are really available from the B. E. P. of the rat. Although this circuit was designed for low power performance, this is not to imply that circuits of much lower power requirements cannot be made.

A short duration experiment was then undertaken to demonstrate the constancy of this electric power output. For this study, the circuit containing the oscillator and oscilloscope was powered by the rat, having a Pt/Pt-black electrode in the coelom and a stainless steel electrode implanted subcutaneously, for a time period of four hours (Fig. 15). The output remained constant. The experiment was terminated by an intracardial injection of Nembutal. Voltage readings were measured at 15 minute intervals after death, and are shown below:

Before death	0.95V
15 minutes	0.8
30 "	0.6
45 "	0.35
60 "	0.2
75 "	0.0

DISCUSSION

The experiments presented in this paper indicate that a useful source of electrical energy may be produced by any one of several bio-electrogenic systems. The two primary systems studied here are:

- 1) Biochemical Fuel Cell
- 2) Bioelectric Potentials

and will be discussed separately.

I. Biochemical Fuel Cell

Our experiments have led us to conclude that the production of an emf in the yeast-water system is the result of a double catalytic reaction.

First, the biological or intra-cellular enzymatic reactions produce the

ionizable metabolites which diffuse through the cell wall.

Secondly, a catalytic reaction involving an interaction of the appropriate metabolites with the platinum-black results in the loss of electrons which then become available in the external circuit.

This particular reaction will be more completely understood when the active metabolites have been identified.

One very important aspect of Bioelectric Systems has not yet been investigated. Experiments must be conducted for the purpose of defining the Faradaic Efficiency of these biologically assisted devices.

Theoretical calculations of efficiency based on turnover rates and numbers of electrons involved in these enzymatic reactions cannot be used in this type of system. Actual experiments have to be conducted to measure the Faradaic Efficiency because at present it is not possible to know exactly how many of these electrons are actually available for the production of usable electricity.

II. Bioelectric Potentials

It is a well-known fact that electricity is generated in the living organism (Ref. 6). This can be demonstrated by observing the difference in potential when electrodes are placed at different sites on the body. It is also known that one part of the body may have a higher or lower potential than some other part.

These Bioelectric Potentials have been studied for many years but thus far have not been utilized as a primary source of electrical energy.

At present, B. E. P. are used primarily for diagnostic purposes. The most familiar and common instances are the electrocardiogram and the electroencephalogram devices which amplify and record these variations in potential. The diagnosis is dependent upon characteristic changes in the electropotentials in the particular organ being tested.

The recent development of miniature electronic circuits designed for low power operation strongly suggests that another utilization of the B. E. P. can be as a primary source of electrical power (Ref. 7). Factors that may possibly be limiting are current density and adverse physiological responses at the sites of the implanted electrodes.

Experiments have been conducted for the purpose of measuring and evaluating the power derived from anesthetized rats with implanted platinum/Pt-black and other electrodes. Polarization data have been collected and evaluated. These preliminary curves indicate that more than enough useful power is available. This B. E. P. has been used to power a 500 kc oscillator for a period of 4 to 8 hours, at which time the experiments were terminated by an intracardial

injection of Nembutal.

The successful utilization of B. E. P. may revolutionize many aspects of design and use of implanted electronic devices. There will be no need for batteries; the implant will be extremely small and lightweight; the reliability will be excellent and, finally, a number of different devices may be implanted in a single animal without undue traumatic effects.

SUMMARY

The production of electrical energy by biochemical methods was studied.

A Biochemical Fuel Cell system consisting of algae and fecal bacteria produced 0.3V at 1.4 to 2.0 ma/ft². The biological mechanism was studied by using a simplified system which contained only one organism, Fleischmann's yeast. Electrical properties were evaluated by analyzing polarization curves. These curves show a power density of 0.66 w/ft² and a current density of 2.2 amps/ft² at 0.3 volts. Finally, the catalytic action of the platinum-black was investigated.

Bioelectric Potential has been measured in rats by placing electrodes in different parts of the body. The electrical output has been evaluated as above and indicates a power output of 90 μ watts at a current of 300 μ amps. For this study the electrode geometric area was about 75 mm². A 500 kc sine wave oscillator was designed and constructed and has been operated from the energy obtained from Bioelectric Potential of a 200 gram rat with implanted electrode.

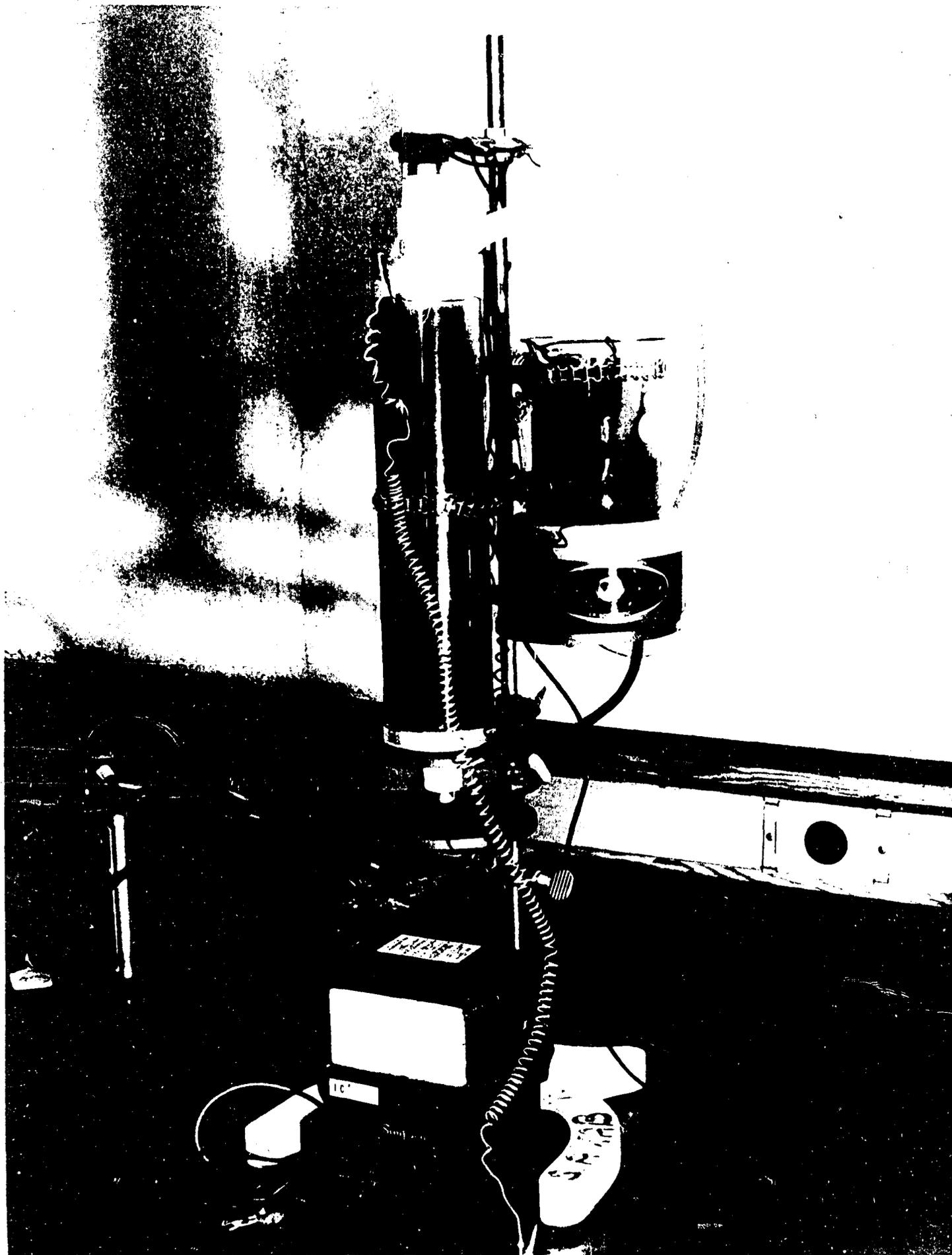
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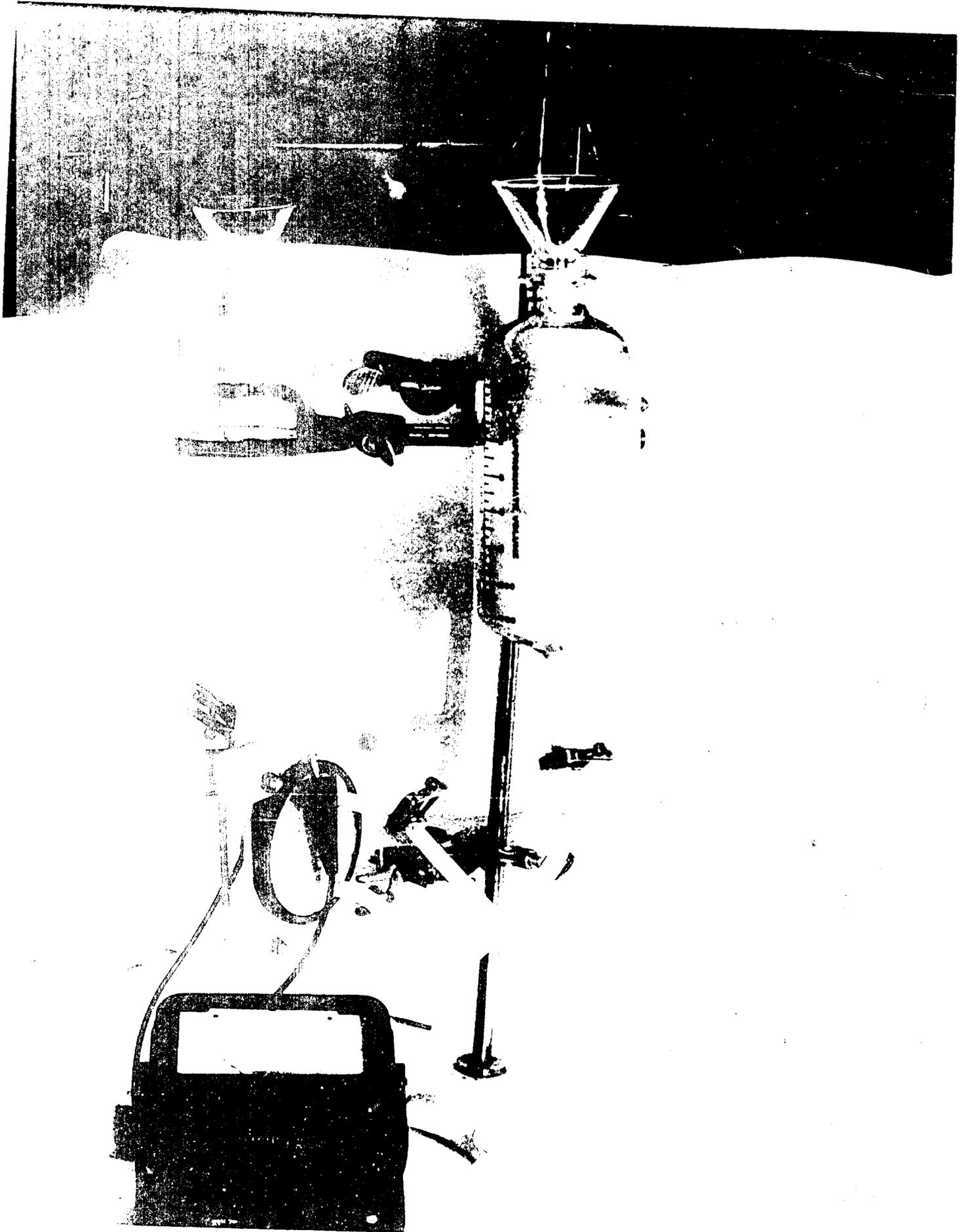


Figure 5

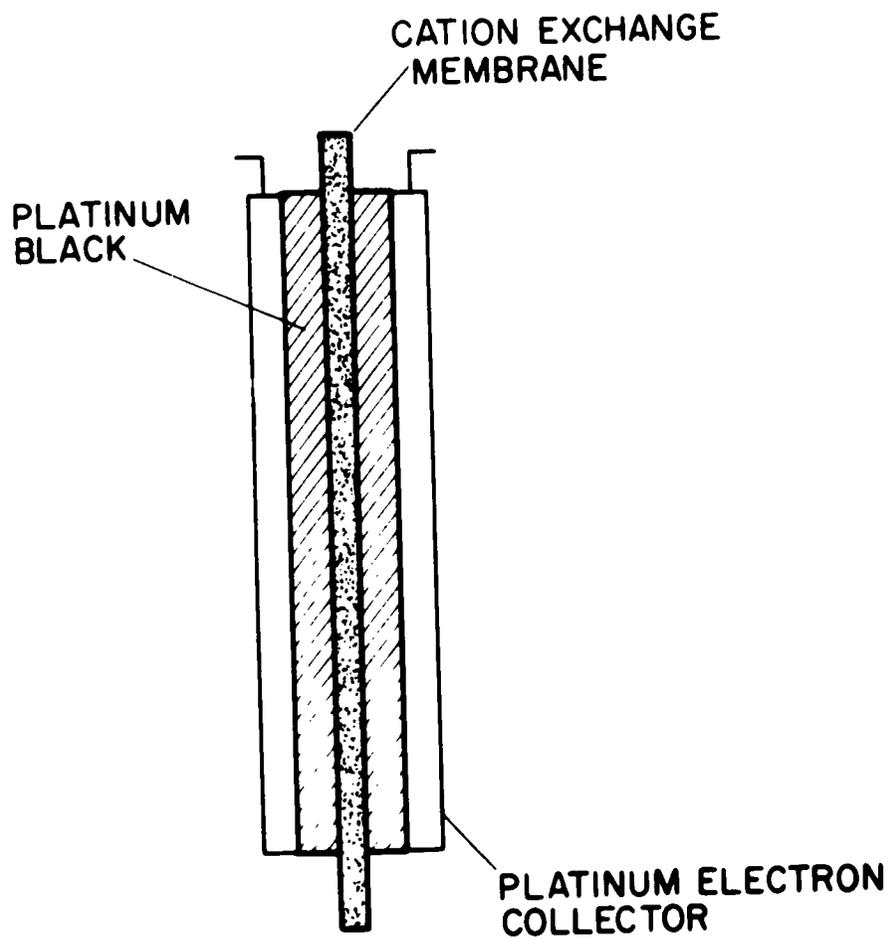


Figure 4. Cell Membrane

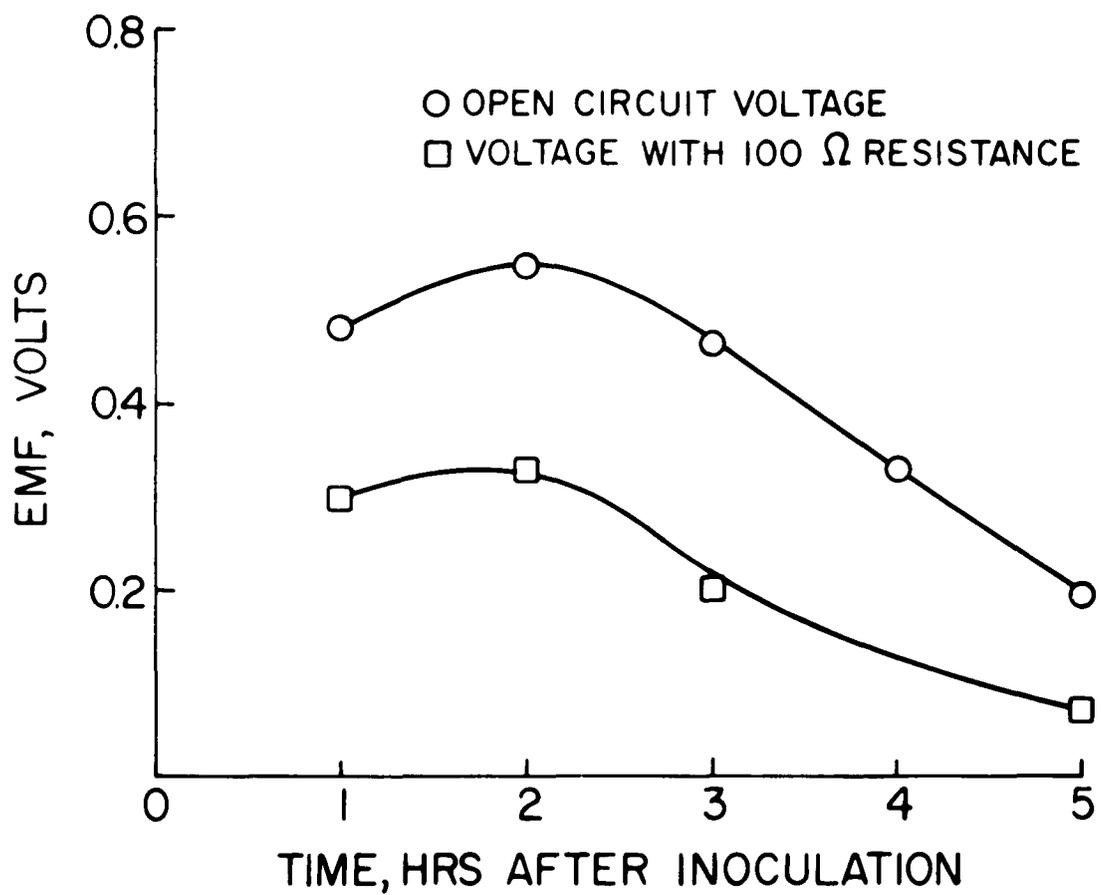


Figure 5. Activity of Yeast Culture

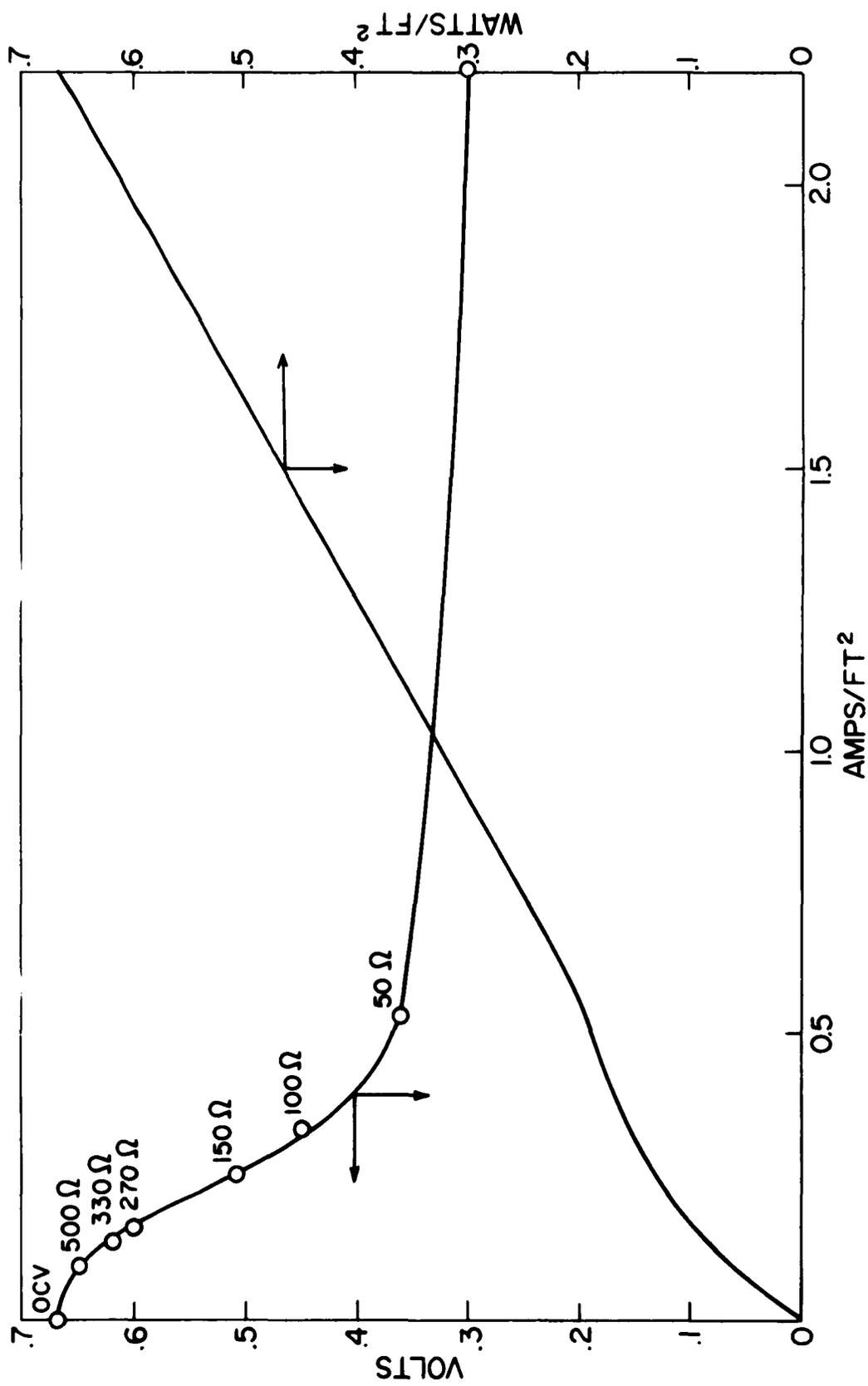


Figure 6. Typical Polarization Curve

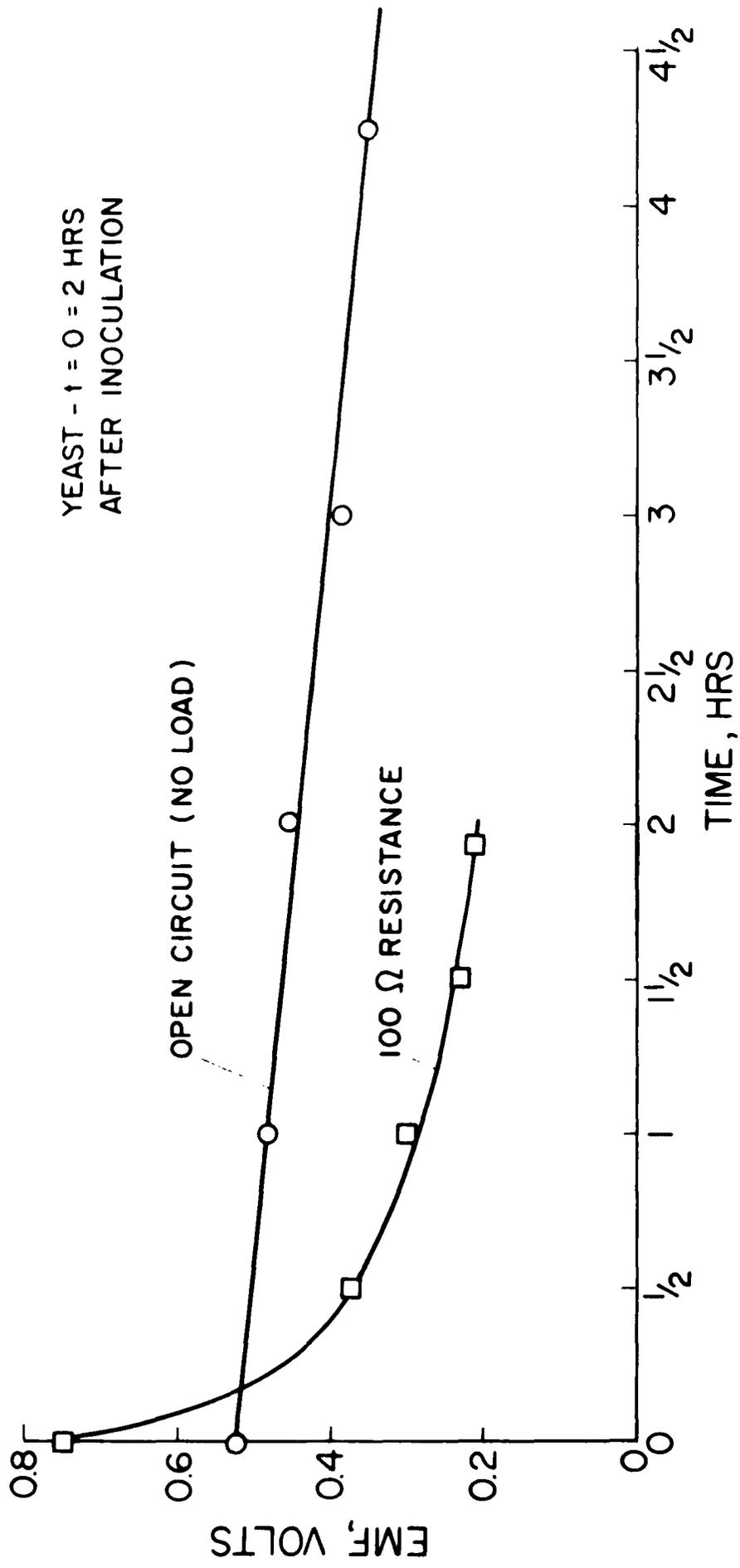


Figure 7. Voltage Decline vs. Culture Age

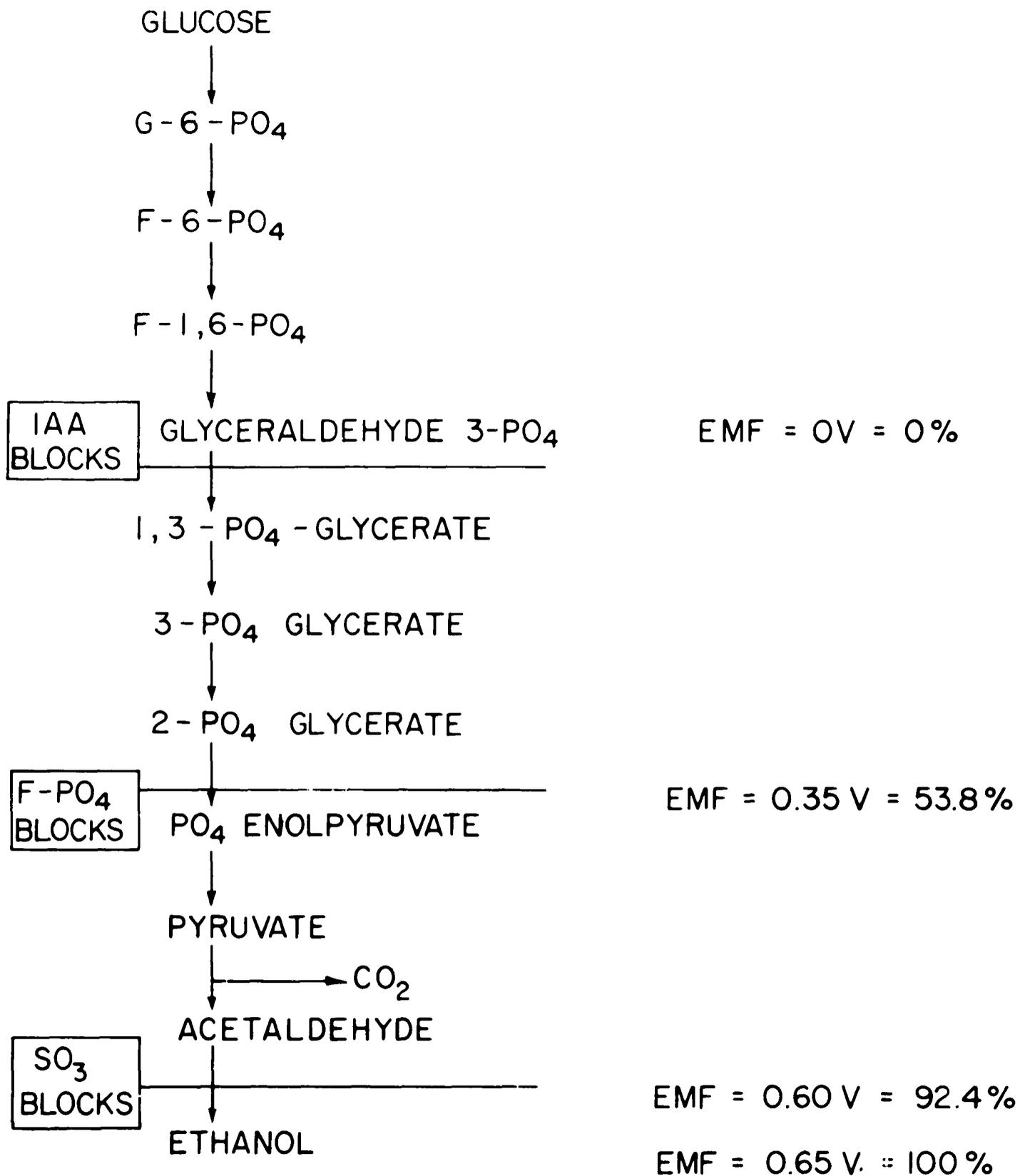


Figure 8. Glycolytic Metabolism

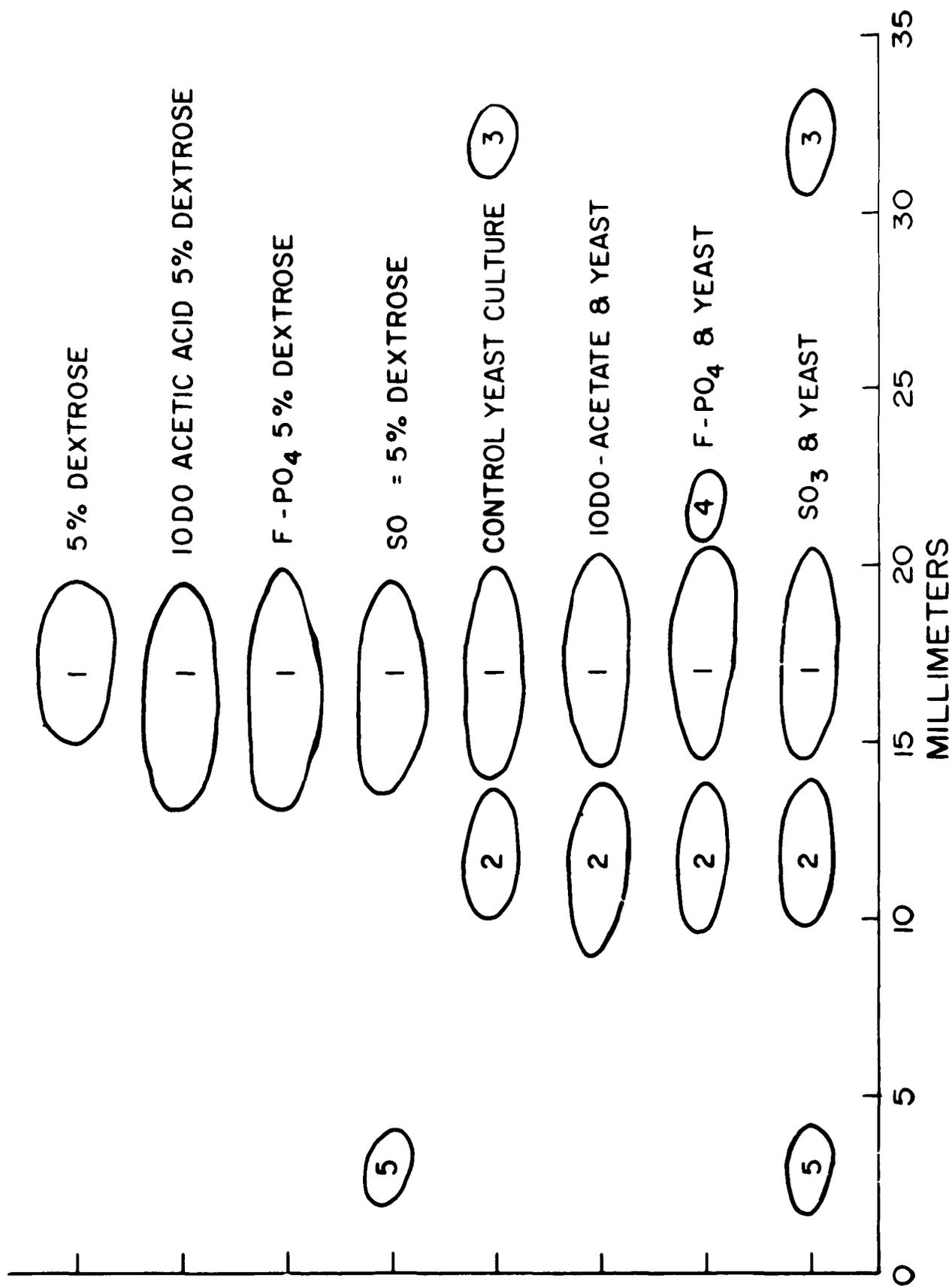
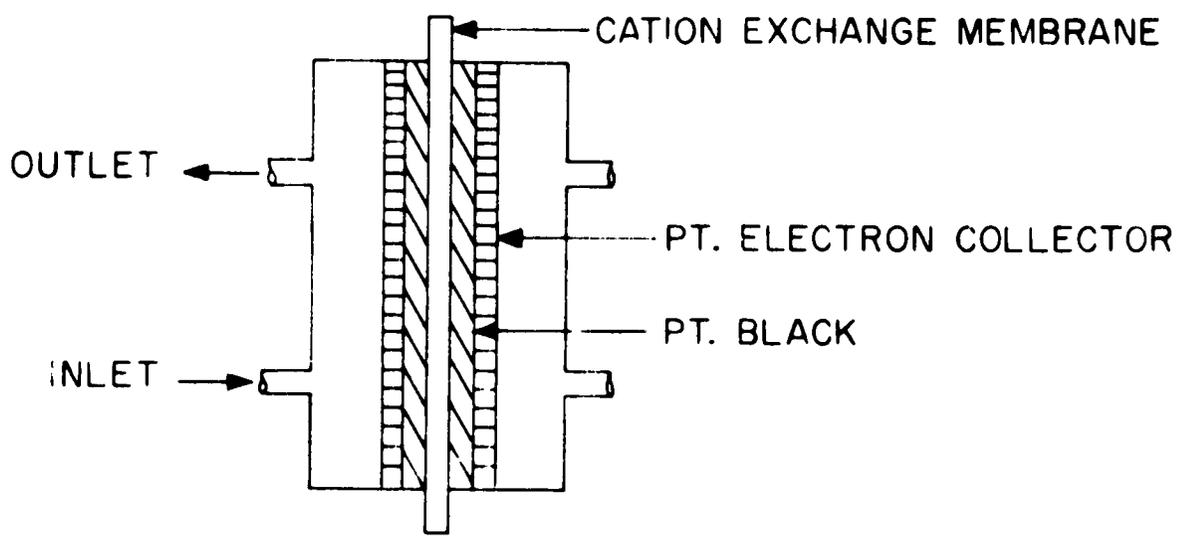
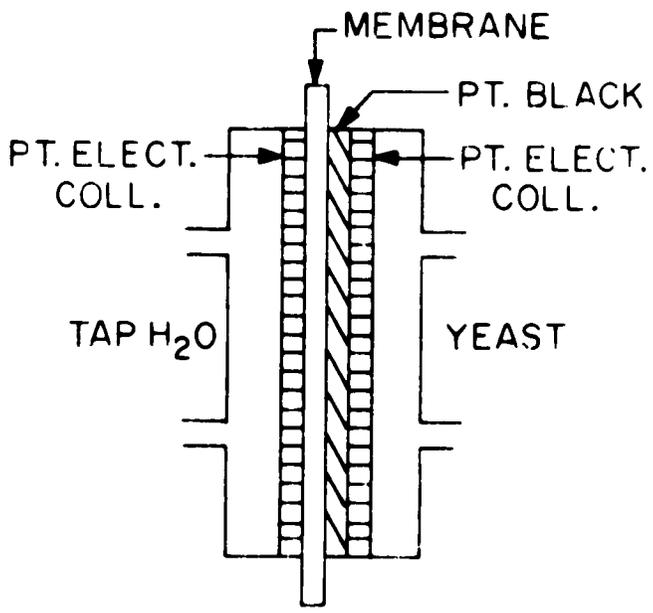


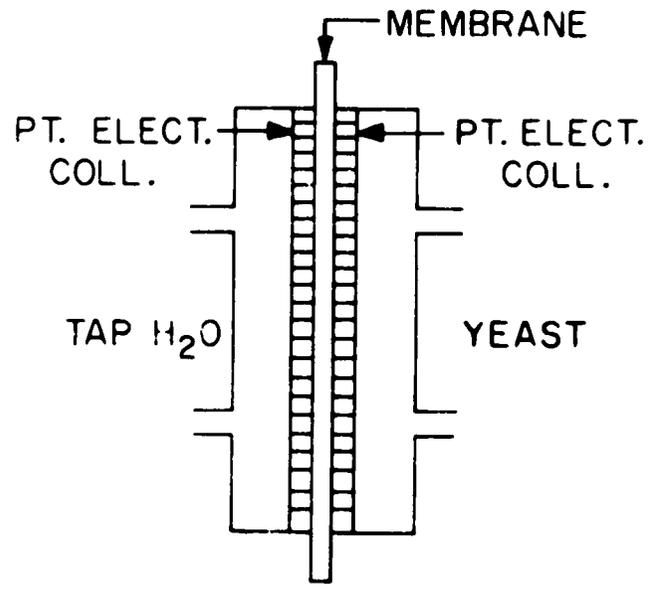
Figure 9. Paper Chromatographic Separation of Reducing Compounds



OCV = 0.65 TO 0.75 VOLTS
(COMPLETE SYSTEM)



OCV = 0.35 TO 0.4 VOLTS



OCV = 0 TO 0.1 VOLTS

Figure 10. Catalysis Studies

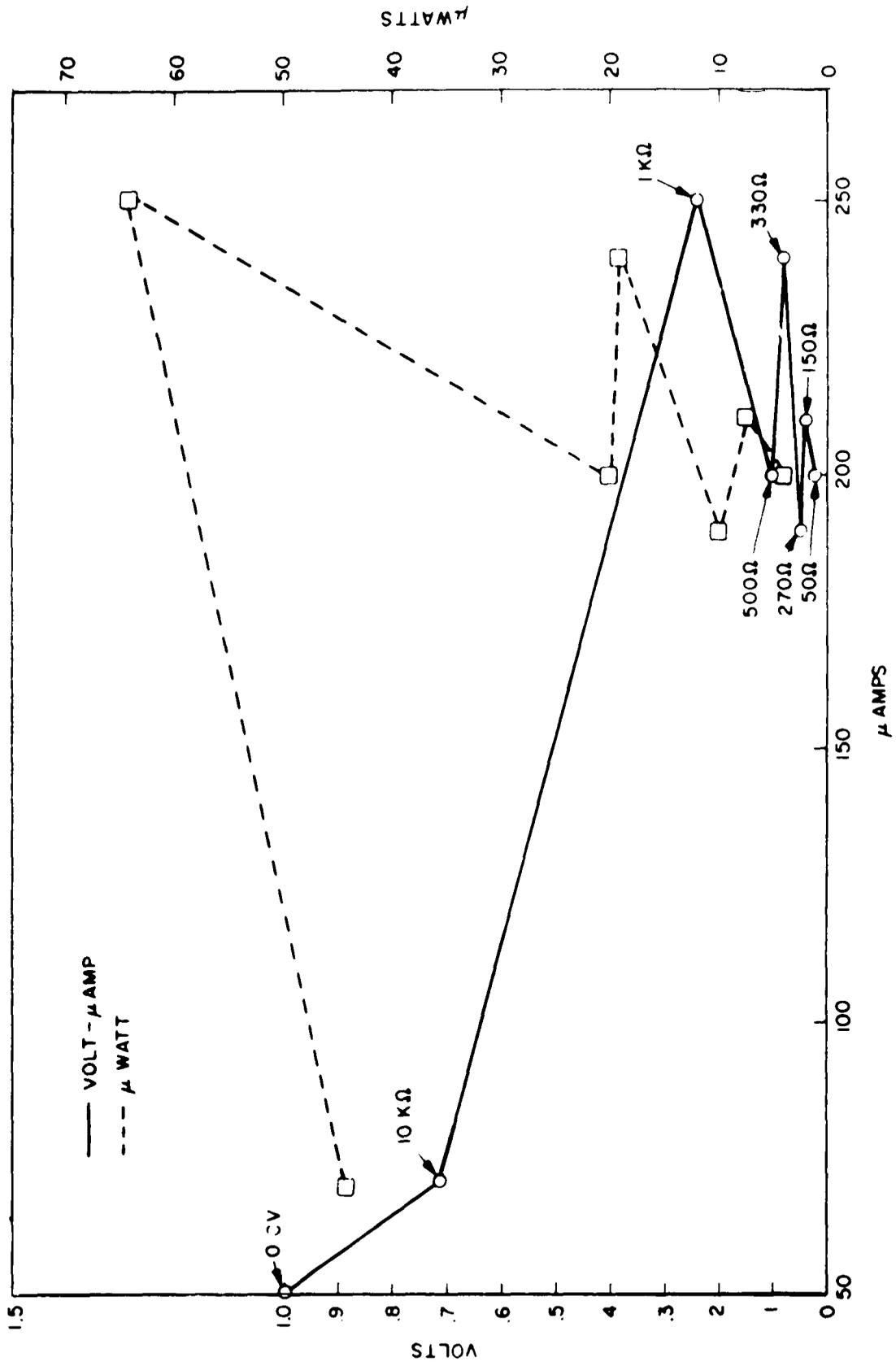


Figure 11. Rat #1 - Polarization Curve
 Pt - Pt - Blk - Sub Q (-)
 Allig - Clip - Skin (+)

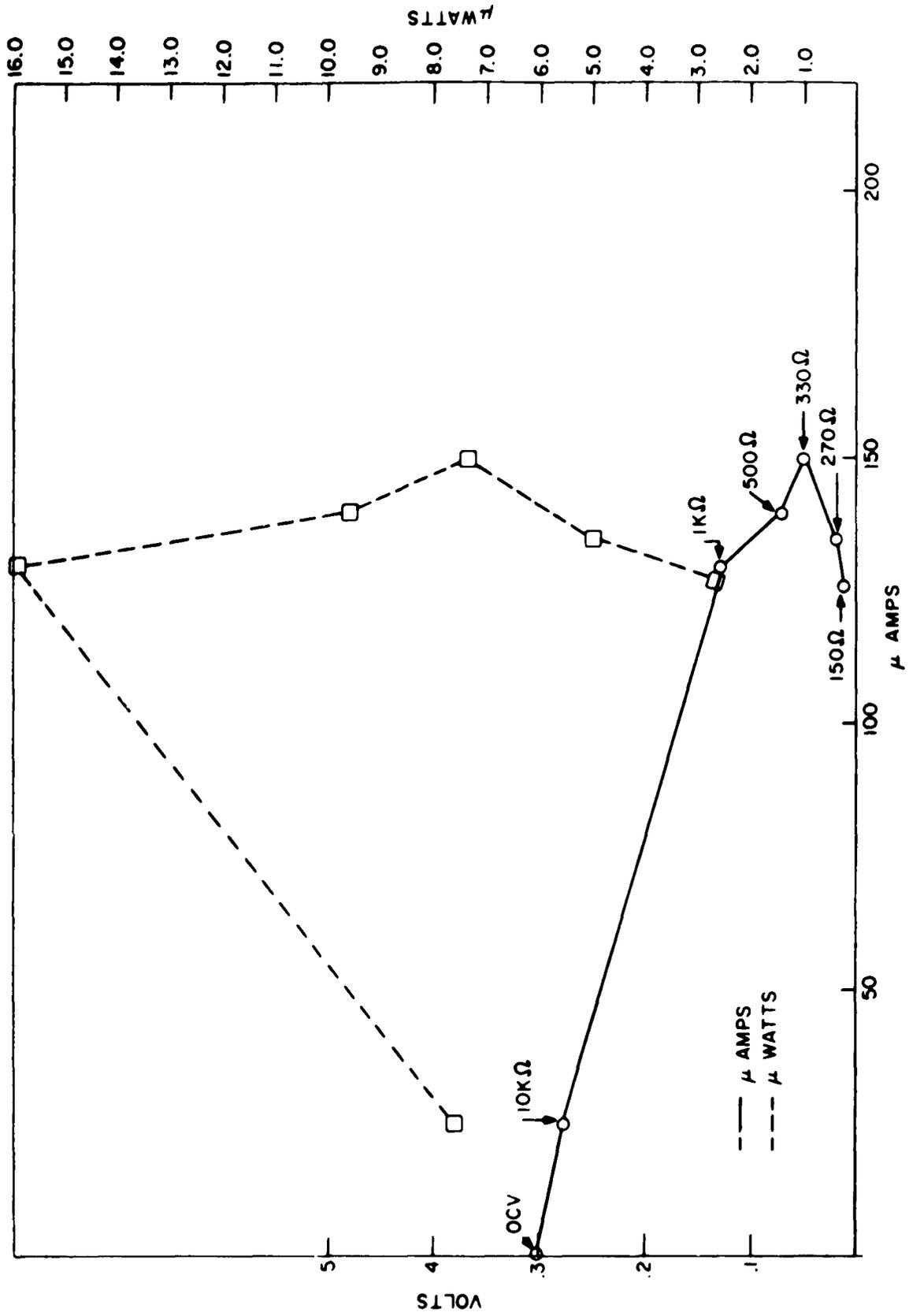


Figure 12. Rat #2 - Polarization Curve
 Pt - Pt - Blk - Coelom (+)
 Pt - Pt - Blk - Sub Q (-)

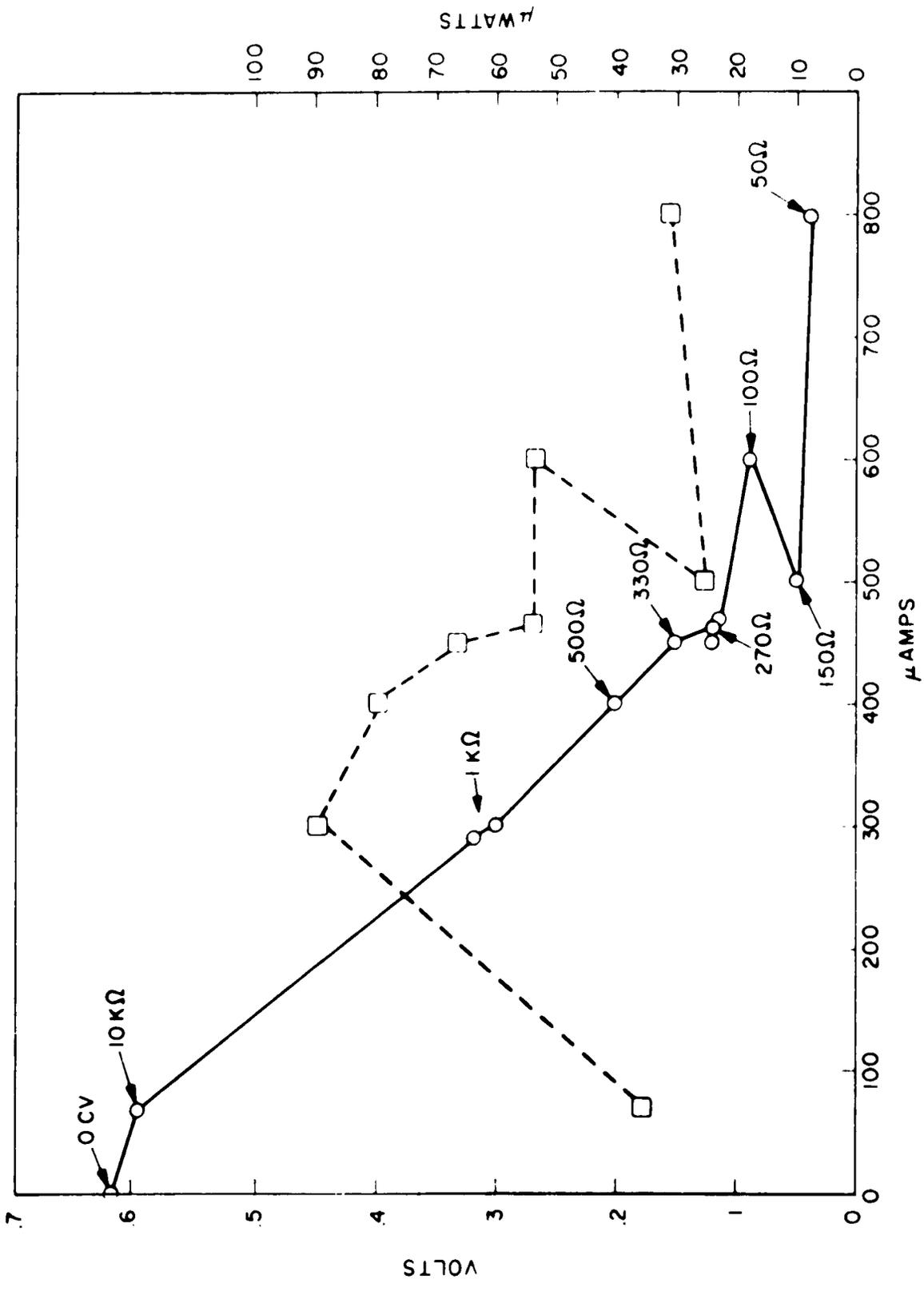


Figure 13. Rat #3 - Polarization Curve
 Pt - Pt - Blk - Coelom (+)
 Stainless Steel Clip - Sub Q (-)

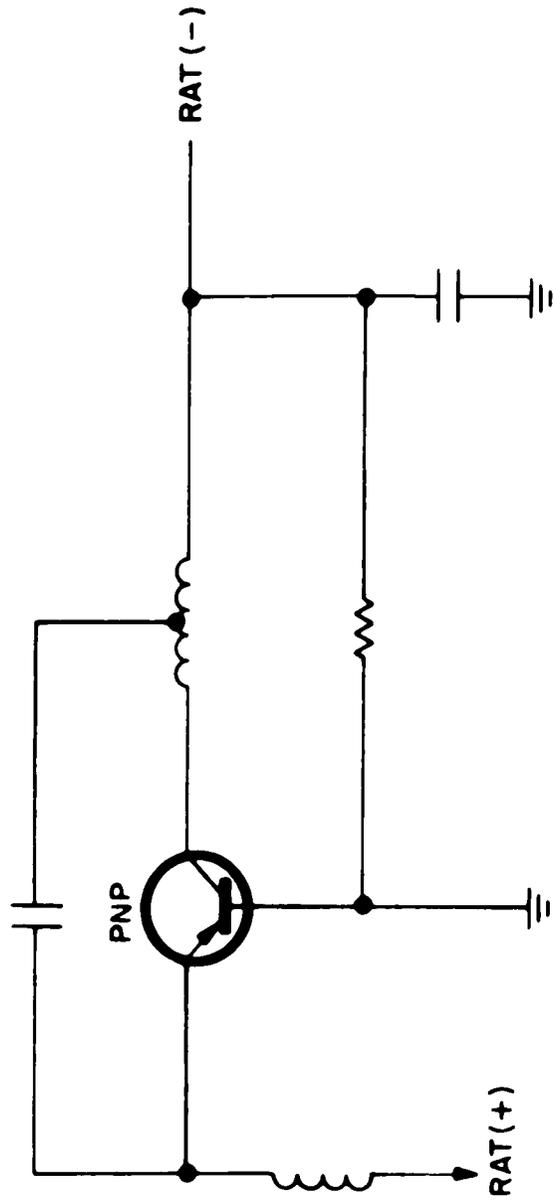
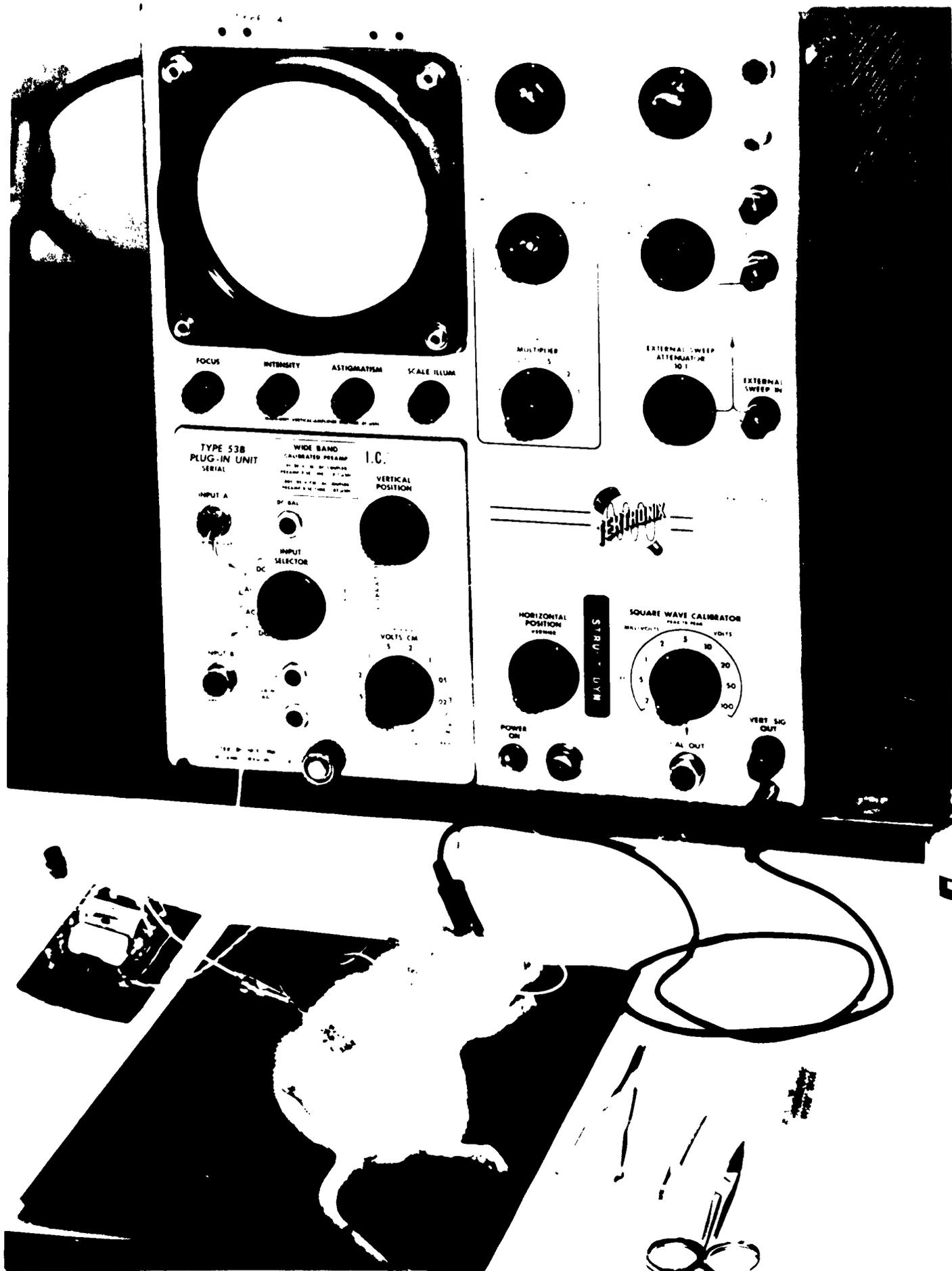


Figure 14. Schematic of Electronic Circuit



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SUMMARY <p> A Biochemical Fuel Cell system consisting of algae and fecal bacteria produced 0.3V at 1.4 to 2.0 ma/ft². The biological mechanism was studied by using a simplified system which contained only one organism, Fleischmann's yeast. Electrical properties were evaluated by analyzing polarization curves. These curves show a power density of 0.66 w/ft² and a current density of 2.2 amps/ft² at 0.3 volts. Finally, the catalytic action of the platinum-black was investigated. </p> <p> Bioelectric Potential has been measured in rats by placing electrodes in different parts of the body. The electrical output has been evaluated as above and indicates a power output of 90 μwatts ^{μV} at a current of 300 μamps ^{μA}. For this study the electrode geometric area was about 75 mm². A 500 kc sine wave oscillator was designed and constructed and has been operated from the energy obtained from Bioelectric Potential of a 200 gram rat with implanted electrode. </p>		

xx ag ft
 xx-microwatt
 xxxx microampere
 xxxx ag mm

By cutting out this rectangle and folding on the center line, the above information can be fitted into a standard card file.

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