THE MECHANISM OF CATHODIC DEPOLARIZATION EXHIBITED BY SULPHATE-REDUCING BACTERIA DURING METALLIC CORROSION PROCESSES

Thesis submitted to

The University of Cape Town

in fulfilment of the requirements for

the Degree of Doctor of Philosopphy

by

JOHN ANTHONY COSTELLO

B.Sc. (Cape Town)

M.Sc. (Tech.) (Manchester)

Council for Scientific & Industrial Research,
Scientia
PRETORIA

August 1975

A B S T R A C T

The mechanism of the corrosive action of sulphate reducing bacteria of the genus *Desulfovibrio* towards ferrous metals has been investigated. This corrosive action is anomalous when considered in the light of established knowledge of corrosion processes, since it occurs in the absence of oxygen, at more or less neutral pH values and at ordinary ambient temperatures.

The hypothesis of von Wolzogen Kühr and van der Vlugt, published in 1934, proposing that enzymic catalysis of sulphate reduction by cathodic charge constituted a "cathodic depolarizing" process, which appears to be widely accepted as the most likely mechanism for this corrosive effect, is evaluated critically here in the light of established electrochemical and biochemical knowledge. The theoretical arguments presented in this thesis show that this process, if it does occur, is unlikely to affect corrosion rates.

The experimental results presented here indicate that all cathodic depolarizing activity demonstrated in pure cultures of these organisms is attributable to the cathodic activity of dissolved hydrogen sulphide produced by the organisms. The conclusions of other workers, quoted in support of proposed enzymic catalysis of charge transfer from cathodes to redox dyes in laboratory systems, are considered, in view of experimental work performed during this investigation, to be a misinterpretation of experimental results.

Based on these observations a mechanism is proposed, accounting for corrosion rates observed in natural environments, and is presented in terms of formal electrochemical reasoning.

A C K N O W L E D G E M E N T S

I wish to acknowledge and express my sincere appreciation for the valuable assistance received from the following :

PROFESSOR E.C. LEISEGANG, Head, Department of Theoretical Chemistry, University of Cape Town, for his guidance and supervision during the fulfilment of this research project.

COMMANDER W.J. COPENHAGEN for his active interest in the progress of the research and for his encouragement generally.

DR. C.E. BIRD, Head of the Corrosion Research Division, National Chemical Research Laboratory, for his co-operation in the completion of this work and for editing the final draft of the manuscript.

MR. D. SANAN for his assistance with the experimental procedures.

THE COUNCIL FOR SCIENTIFIC AND INDUSTRIAL RESEARCH for the granting of financial assistance and for facilities made available.

JILL, my wife, for her inspiration and enthusiasm and for the typing of this manuscript.

C O N T E N T S

CHAPTER ONE

1 INTRODUCTION

1.1 The economic and ecological import of the problem

It is widely recognised by those concerned with the study and the prevention of metallic corrosion that sulphate-reducing bacteria considerably accelerate the deterioration of metals by corrosion in certain environments. Much experimental work has been devoted to the study of this problem during the past forty years, and the knowledge available on the subject has been prominently reviewed[1-4]. Accelerated corrosion of iron, steel and other metals, takes place when they are in contact with actively growing organisms of the genus *Desulfovibrio*. Species of this group of bacteria, characteristically anaerobic, are ubiquitous, and have caused damage to metals in soils, lakes, seas, rivers, harbours, oil-wells, industrial cooling systems, fuel tanks and sewerage systems.

The evaluation of financial losses resulting from corrosion processes is difficult because of the need to assess such primary losses as initial costs of materials, the costs of design, fabrication and maintenance, as well as secondary losses such as loss of production time, spoilage of products, and repair costs. It has been estimated however, that sulphate-reducing bacteria, in accelerating the corrosion of iron and steel pipe in underground environments, are responsible for losses of hundreds of millions of dollars annually in the U.S.A.[5,6], and tens of millions of pounds annually in Britain[7].

Since these organisms are responsible for accelerated corrosion in a far wider range of situations than the deterioration of buried iron and steel pipe, it must be concluded that they are responsible for much greater economic losses than these.

In the Republic of South Africa, awareness of this type of corrosion is increasing, and instances have been recorded in sea water-, fresh water-, and underground environments[8-10].

1.2 Metals affected, rates of corrosion and distribution of attack

Iron alloys (mild steel and cast iron) are the metals most commonly affected by accelerated corrosion caused by sulphate-reducing bacteria, these metals being widely used in environments suitable for active growth of these organisms (anaerobic soil environments). In South Africa, perforation of galvanized (zinc-coated) steel pipes by corrosive attack by sulphate-reducing bacteria has also frequently been observed[8], particularly in clay soils, where failures of this type after a two-year service life are common.

In recent years much evidence has been presented that corrosion of aluminium and its alloys is often due to the action of sulphate-reducing bacteria[11-17]. This is of particular importance to the aircraft industry, as cases of perforation of aluminium alloy aircraft fuel tanks have been attributed to the action of these organisms. Where tanks are an integral part of an aircraft wing structure, the danger of this type of corrosion may be extreme.

It is a common observation that the corrosion rate of iron in neutral de-aerated waters is negligible, and that the corrosion rate of steel in water increases linearly as the oxygen content is raised[18]. In most aqueous environments, highest corrosion rates are observed where oxygen has ready access to the metal-environment interface. In anaerobic environments where sulphate-reducing bacteria are active however, oxygen is absent, acids are absent and yet corrosion rates are many times higher than in well-aerated, neutral aqueous environments. Table I contains some rates of penetration of mild steel recorded in the presence of sulphate-reducing bacteria. Some rates of penetration of iron and steel in other corrosive environments are included for the purpose of comparison. It is apparent that the rates of penetration recorded in the presence of sulphate-reducing bacteria (cases 4,5,6,7,8,9,11 and 12) are considerably higher than any rates recorded in more or less neutral aqueous environments in the absence of these organisms (cases 1,2 and 3). The highest rate of penetration recorded in the presence of sulphate-reducing bacteria (case 12) is greater than the rate of penetration of iron in 1-M hydrochloric acid (case 10).

Values of i_{corr} given in the table are calculated values of corrosion current, assuming an electrochemical corrosion mechanism. These will be used later in mechanism discussions.

Attack on metals in the presence of sulphate-reducing bacteria is usually localised at points on the metal surface and takes the form of pitting to perforation rather than a general thinning. Pits are generally steep-sided and contain a black, loosely-adherent deposit of sulphides of iron, which on dislodgement reveals bright corroded metal. Figures 1 and 2 illustrate two typical cases of attack by sulphate-reducing bacteria in sea water environments. Figure 1 shows the perforation of the mild steel sea water intake pipe of a condenser system in which harbour water was used as a coolant. At the point of perforation shown, no corrosion was visible until probing of the apparently intact coal-tar epoxy paint coating revealed perforation of the metal beneath, indicating that the organisms had penetrated a discontinuity (pin-hole) in the paint coating and caused accelerated attack of the underlying steel. Figure 2 shows a perforated condenser tube from the same system. In this case, anaerobic silt rich in sulphate-reducing organisms had settled on the lower surface of the horizontal tube. The perforations illustrated in Figures 1 and 2 took place in periods of ten and seven months respectively, and the corresponding corrosion rates are entered as cases 11 and 12 of Table I.

1.3 The position of the problem among the sciences

Corrosion is a common phenomenon in nature and as such it has been subject to observation and definition by individuals whose methods of description are based in a number of scientific disciplines. Broadly, corrosion is the transformation of metal or alloy, used as a material of construction, from the metallic to the non-metallic or combined state by interaction with the environment. Generally, the metallic structure is brought into being by the combined agencies of chemists, metallurgists and engineers. The transformation to the non-metallic or combined state is most simply describable as a chemical reaction. It is axiomatic thus, that corrosion reactions are thermodynamically predictable, and may be studied by the methods of chemical thermodynamics and kinetics. Ultimately, some model of the reacting system at the atomic or molecular level may be constructed. This information may then be put to use in predicting methods of control of corrosion reactions, and prolonging the useful lives of metallic structures. The fruits of such an approach to the study of corrosion are evident in the successful development of corrosion-resistant alloys, cathodic protection techniques, the use of chemical corrosion inhibitors, corrosion resistant surface treatments for metals, and several other useful preventative and protective procedures.

Corrosion of steels in the presence of sulphate-reducing bacteria has been the subject of research, during the past forty years, of workers trained in various scientific disciplines. In this case, aggressive bacteria form part of the corrosive environment, and thus in order to describe and study the environment thoroughly, the methods of microbiology and biochemistry must be invoked. The combination of these disciplines with physical chemistry and metallurgy in studying this problem has inevitably led to bias, in one or the other direction, in the design of experiments and their interpretation. Among the unfortunate consequences of such an approach has been the use of faulty biological experimental technique by physical chemists, such as the use of mixed bacterial cultures in electrochemical experiments. Biologists, in studying corrosion problems have shown a lack of appreciation of the methods of physical chemistry: for example, mechanisms of corrosion reactions have been proposed at a molecular level by workers not in possession of the thermodynamic, kinetic and molecular structural data required as a basis for such proposals.

1.4 The aims of this study

The aim of the present study is to describe the phenomenon of corrosion caused by sulphate-reducing bacteria in the language of physical chemistry. It is proposed to draw the information required to describe the system by thermodynamic and kinetic methods, from established information and from the results of the author's experimental work. Ultimately, the corrosion mechanism will be described.

CHAPTER TWO

2 ELECTROCHEMICAL CONSIDERATIONS

It is necessary to describe the phenomenon of corrosion in formal physical chemical terms, to develop a body of fundamental theory upon which to base discussions of bacterial corrosion mechanisms. It shall be accepted as a first premise that all aqueous corrosion reactions proceed *via* electrochemical mechanisms. This fact, established by the work of Evans[25], Hoar[26], and Agar[27] at Cambridge in the period 1920-1940, is integral to all theories of corrosion in aqueous environments. This early work was the foundation of corrosion kinetic studies, and demonstrated the faradaic equivalence between corrosion rates and electrical currents flowing in metals immersed in corrosive media. An indication of the relationship between polarization phenomena (overpotentials of electrode processes) and corrosion rates also emerged from this work, and subsequent work of the Cambridge school.

Having accepted the electrochemical mechanism of aqueous corrosion, it is possible to develop a fundamental body of corrosion theory in which the absolute reaction rate theory of Glasstone, Laidler and Eyring for a single electrode process[28] may be applied, *via* the mixed potential theory of Wagner and Traud[29], to the case of two or more interacting electrode processes, of which corrosion is an example.

This kinetic approach, coupled with technological developments in the field of electronics, has made possible the electrochemical determination of corrosion rates and the study of corrosion mechanisms. It is the kinetics of corrosion reactions which are of importance in the structural and other engineering and technological uses of metals. Thermodynamic predictions of corrosion tendencies developed, notably by Pourbaix[30], from Nernstian equilibrium electrochemical reasoning, are of very limited practical value: some thermodynamically predictable corrosion reactions proceed at such slow rates as to make the metals involved useful, in the engineering and technological sense, for considerable service lives.

It is the opinion of the author that existing theories of bacterial corrosion mechanisms ascribe too great a role in the corrosion process to some vital biological influence on electrode processes, at present inaccessible to the quantitative methods of chemistry. This gap in the extension of the main body of corrosion theory, expressed in physical chemical language, to the case of bacterial corrosion is caused more by the lack of theoretical knowledge of workers in this field, than by a deficiency in the main body of theory itself.

2.1 Single electrode processes

We shall consider a single electrode process describable as

$$OX + ne \rightarrow RED \qquad 2.1$$

OX represents an ion in solution and RED a steady state of discharged OX at a metal electrode surface. If we consider 2.1 as a charge-transfer reaction which takes place at an electrode-electrolyte interface under zero field conditions ("zero field conditions" means that no electric field exists across the charge-transfer interface), absolute reaction rate theory predicts that the rate may be written

$$\vec{k} = \frac{\kappa T}{h} e^{-\Delta\vec{G}^{o\ddagger} / RT} \qquad 2.2$$

where $\vec{k}$ is the specific reaction rate for the heterogeneous reaction (it has dimensions cm s^{-1}), κ is the Boltzmann constant, h is Planck's constant, and $\Delta\vec{G}_c^{o\ddagger}$ the standard free energy of activation for the process of reduction of OX.

The specific reaction rate multiplied by the activity, or, for the purpose of readily measurable approximation, the concentration C_{ox} of OX ions gives the actual reaction rate $\vec{\nu}_c$ under zero field conditions (subscript c indicates a chemical, or zero-field rate).

$$\vec{\nu}_c = \frac{\kappa T}{h} C_{ox} e^{-\Delta\vec{G}_c^{o\ddagger} / RT} \qquad 2.3$$

The reasoning thus far is the same as might be applied to any chemical reaction. Electrochemical reactions however, have the unique property that they are heterogeneous redox reactions and charge transfer is effected *across an interface*. For this charge transfer to take place continuously, a charge gradient must exist across the interface. Such a charge gradient has the nature of a potential field. If, as would be expected from the laws of physics, the rate of charge transfer (or electrical current) is influenced by the magnitude of the charge gradient, the rate of the chemical charge transfer reaction would also be expected to be affected. If a potential gradient $\Delta\phi$ exists so as to favour the left-to-right direction of reaction 2.1, the rate of the reaction is controlled not only by the standard free energy of activation for the charge-free state, $\Delta\vec{G}_c^{\,o\ddagger}$, but also by an additional electrically-controlled activation energy term.

The electrochemical free-energy consequences of potential field $\Delta\phi$ would be expected, from faradaic considerations, to be a function of $F\Delta\phi$. This gives the value of this voltage, $\Delta\phi$, in chemical free energy units (J mol^{-1}). The potential gradient may be plotted as a vector normal to the electrode-electrolyte interface. Since the charge-transfer *chemical* reaction takes place across this interface, the potential field may be simply depicted along a one-dimensional reaction co-ordinate of the chemical system. It is possible to depict diagramatically the consequences of this situation to the free energy of activation for the reacting system, and this is done in Figure 3.

Since only a portion of the potential gradient is required to do the necessary electrical work to raise the energy state of the ion to the peak of the free energy curve, a quantity β may be defined which is the fraction of the potential gradient required to do this activation operation. A physical depiction of the meaning of β may be gained from Figures 3 (a) and (b). We then write the electrical contribution to the free energy of activation as $\beta F\Delta\phi$. The total free energy of activation $\Delta\vec{G}^{\,o\ddagger}$ for the left-to-right reaction 2.1 may then be written

$$\Delta\vec{G}^{\,o\ddagger} = \Delta\vec{G}_c^{\,o\ddagger} + \beta F\Delta\phi \qquad 2.4$$

The factor β, known as the symmetry factor, is a fundamental quantity for all charge-transfer systems. It may be evaluated experimentally, but its physical meaning is not readily understood. Bockris and Reddy[31] have discussed its physical meaning at length and shown that it emerges naturally from Morse curve bond stretching making-and-breaking reasoning, and also from fundamental quantum mechanical reasoning for charge-transfer reactions. Substituting for $\Delta\vec{G}^{o\ddagger}$ from 2.4 in equation 2.3, we find for the reaction velocity $\vec{\nu}_c$ for reaction 2.1 under the influence of a potential field‡

$$\vec{\nu}_c = \frac{\kappa T}{h} C_{ox} e^{-\Delta\vec{G}_c^{o\ddagger}/RT} e^{-\beta F \Delta\phi/RT} \qquad 2.5$$

Substituting further from 2.2, 2.3, we find that

$$\vec{\nu}_e = \vec{\nu}_c e^{-\beta F \Delta\phi/RT} \qquad 2.6$$

$$= \vec{k}_c C_{ox} e^{-\beta F \Delta\phi/RT} \qquad 2.7$$

The rate $\vec{\nu}_e$ is the number of moles of OX ions reacting per second. Converting this rate to a current via the Faraday, we have :

$$\vec{i} = F \vec{\nu}_e \qquad 2.8$$

$$= F k_c C_{ox} e^{-\beta F \Delta\phi/RT} \qquad 2.9$$

If $\vec{\nu}$ has the units mol cm^{-2} s^{-1} and if F is expressed in A s mol^{-1}, $\vec{i}$ has the units A cm^{-2}.

‡ subscript e indicates that the reaction is under the influence of an electrical potential field.

2.2 Single electrode systems at equilibrium

Should reacting system 2.1 be considered as an equilibrium :

$$OX + ne \rightleftharpoons RED \qquad 2.1\ (a)$$

no net current will be flowing across the electrode-electrolyte interface. The forward and reverse charge-transfer reactions will be occurring at the same rates, and the currents corresponding to these rates will be equal in magnitude but opposite in direction.

$$\vec{i} = F\,\vec{k}_c\,C_{ox}\,e^{-\beta F\Delta\phi / RT} = \overleftarrow{i} = F\,\overleftarrow{k}_c\,C_{red}\,e^{(1-\beta)F\Delta\phi / RT} \qquad 2.10$$

The magnitude of current flowing at equilibrium is known as the exchange current density i_o, and

$$i_o = \vec{i} = \overleftarrow{i} \qquad 2.11$$

From 2.10, it follows that

$$e^{F\Delta\phi / RT} = \frac{\vec{k}_c}{\overleftarrow{k}_c}\,\frac{C_{ox}}{C_{red}} \qquad 2.12$$

and taking logarithms‡

$$\Delta\phi_e = \frac{RT}{F}\,ln\,\frac{\vec{k}_c}{\overleftarrow{k}_c} + \frac{RT}{F}\,ln\,\frac{C_{ox}}{C_{red}} \qquad 2.13$$

It is obvious from 2.13, that the two quantities on the right-hand side of the equation have the units of a potential, or voltage, and that the first of these quantities is equal to the potential across the interface when reactants and products are at unit concentration (or activity), i.e. in their thermodynamic standard states.

‡ $\Delta\phi_e$ denotes an equilibrium potential

We may thus re-write 2.13 as

$$\Delta\phi_e = \Delta\phi_e^o + \frac{RT}{F} \ln \frac{C_{ox}}{C_{red}} \qquad 2.14$$

Expressing $\Delta\phi$ values on the scale of standard electrode potentials, 2.14 takes the form :

$$E_e = E_e^o + \frac{RT}{F} \ln \frac{C_{ox}}{C_{red}} \qquad 2.15$$

This is the classical Law of Nernst, which may be derived also from strictly equilibrium thermodynamic reasoning.

E_e^o corresponds to the value of E_e at unit activity (or concentration) of the substance involved, and is known as the standard electrode potential for the system. It is accessible from thermodynamic data (such as standard chemical potentials μ^o), via well-known reasoning e.g.

$$\Delta G^o = -FE^o \qquad 2.16$$

for a single charge-transfer process, and

$$E^o = -\frac{\Delta G^o}{F} \qquad 2.17$$

The quantities E^o and i_o thus emerge as fundamental properties of a charge-transfer chemical system. E^o is accessible from thermodynamic data. i_o, however, being the magnitude of two equal and opposite currents defies direct measurement. It may be measured indirectly, however, by making the system depart from equilibrium and measuring certain quantities.

2.3 Single electrode systems not at equilibrium

The $\Delta\phi_e$ given by 2.13 is the equilibrium potential. Directly a net current flows in the system, it departs from equilibrium and

$$i = \overleftarrow{i} - \overrightarrow{i} \qquad 2.18$$

or,

$$i = F\overleftarrow{k}_c C_{red} e^{(1-\beta)F\Delta\phi / RT} - F\overrightarrow{k}_c C_{ox} e^{-\beta F\Delta\phi / RT} \qquad 2.19$$

where $\Delta\phi$, the non-equilibrium potential differs from $\Delta\phi_e$ by an amount η, the overpotential, such that

$$\Delta\phi = \Delta\phi_e + \eta \qquad 2.20$$

We may now write for the current flowing in the system

$$i = \left(F\overleftarrow{k}_c C_{red} e^{(1-\beta)F\Delta\phi_e / RT}\right) e^{(1-\beta)F\eta / RT} - \left(F\overrightarrow{k}_c C_{ox} e^{-\beta F\Delta\phi_e / RT}\right) e^{-\beta F\eta / RT} \qquad 2.21$$

We can see from 2.10 that the two bracketed terms are those for the exchange current density, i_o, at equilibrium. Thus

$$i = i_o \left(e^{(1-\beta)\eta F / RT} - e^{-\beta\eta F / RT}\right) \qquad 2.22$$

This central equation in electrode kinetics, named by Bockris and Reddy the Butler-Volmer equation[31], gives the i *versus* η relationships which enable us to determine the important quantity i_o experimentally.

There are two general forms of this equation which lend themselves particularly to experimental use. These arise out of approximations made as follows :

If we assume that the activation energy barrier is symmetrical, and the value of β is thus 0,5, we may write 2.22 as

$$i = i_o \left(e^{+F\eta/2RT} - e^{-F\eta/2RT} \right) \qquad 2.23$$

and since

$$\frac{e^{x} - e^{-x}}{2} = \sinh X \qquad 2.24$$

$$i = 2\, i_o \sinh \frac{F\eta}{RT} \qquad 2.25$$

Now the two approximate forms of the equation emerge as follows :

(i) <u>If the overpotential η is relatively small</u> :

the quantity $F\eta/2RT \ll 1$

and $$\sinh \frac{F\eta}{2RT} \simeq \frac{F\eta}{2RT} \qquad 2.26$$

and thus $$i = \frac{i_o F\eta}{RT} \qquad 2.27$$

This relationship is the *low-field approximation* of 2.22 and holds good in practice where $\eta < 0{,}010$ V for a single electron transfer process[31]. Within these overpotential limits, i is a linear function of η.

(ii) Where the overpotential η is relatively large :

the quantity $e^{F\eta/2RT} >> e^{-F\eta/2RT}$

and $$2\, sinh \frac{F\eta}{2RT} \simeq e^{F\eta/2RT} \qquad 2.28$$

and $$i \simeq i_o\, e^{F\eta/2RT} \qquad 2.29$$

the relationship $\eta = a + b \ln i$ 2.30
(a and b being constants).

holds, which is traditionally known as the Law of Tafel. Considering equation 2.21 diagramatically (Figure 4) it is apparent that i varies linearly with η at small overpotentials. If we write the *high-field* approximation 2.29 in the form

$$\eta = -\frac{RT}{(1-\beta)F}\, ln\, i_o + \frac{RT}{(1-\beta)F}\, ln\, i \qquad 2.31$$

we find that for the case $\eta = 0$, $ln\, i_o = ln\, i$ or in other words, the value of $ln\, i_o$ is accessible by extrapolation of the line $ln\, i$ *versus* η to the case of $\eta = 0$, at which i_o is the current. i_o is thus accessible to experimental measurement by causing the electrode system to depart from equilibrium and studying its current *versus* potential characteristics.

The application of this method to the determination of real i_o values will be described in the following section.

2.4 Real electrochemical reactions : multi-step reactions

The discussion hitherto has dealt with a single charge-transfer reaction which takes place in a single step. In this case, the influence of overpotential, η, on the rate of the reaction expressed as a current has been dictated by β, the symmetry factor for the system. A fraction of the overpotential, $\beta\eta$, influenced charge-transfer in one direction, while $(1-\beta)\,\eta$ influenced the reverse direction of the process. In practice, charge-transfer reactions usually occur in more than one step with, possibly, more than one charge-transfer step per overall occurrence of the reaction. Moreover, not all steps in the reaction are necessarily charge-transfer ones. For example, the reaction for the liberation of hydrogen from acid solution at an electrode surface

$$H_3O^+ + e \longrightarrow \tfrac{1}{2}H_2 + H_2O \qquad 2.32$$

usually occurs in at least two steps :

$$e + M + H_3O^+ \xrightarrow[\text{transfer}]{\text{charge}} MH + H_2O \qquad 2.33$$

Here, MH represents atomic hydrogen adsorbed on the metal surface (M). This step may be followed by

$$2MH \xrightarrow{\text{combination}} 2M + H_2 \qquad 2.34$$

As in all chemical reactions, charge-transfer electrochemical reactions have a rate-determining step.

A close examination[31,32] of the kinetics of multi-step charge-transfer reactions will reveal that they have the following characteristics.

(i) The rate-determining step occurs a fixed number of times, ν_n, per overall occurrence of the reaction. For example, if 2.33 is the rate-determining step of 2.32, ν_n for 2.32 is 2.

(ii) The overall reaction has a fixed number, η, of electron-transfer steps per overall occurrence of the reaction.

(iii) For every overall occurrence of the reaction, $\vec{\gamma}$ electron-transfer steps precede the rate-determining step, and $\overleftarrow{\gamma}$ electron-transfer steps follow it.

(iv) A number r is a further characteristic, which has a value 0 if the rate-determining step is not an electron-transfer reaction and the value of 1 if the rate-determining step is an electron-transfer reaction.

It is possible, in terms of these mechanism-determining quantities, to write a more general form of the fundamental electrode equation 2.22 :

$$i = i_o \left(e^{\overleftarrow{\alpha} F \eta / RT} - e^{\vec{\alpha} F \eta / RT} \right) \qquad 2.35$$

in which the quantities $\vec{\alpha}$ and $\overleftarrow{\alpha}$ determine the influence of overpotential, η, on the forward and reverse reactions respectively. The two quantities, $\vec{\alpha}$ and $\overleftarrow{\alpha}$ are defined as

$$\vec{\alpha} = \frac{\vec{\gamma}}{\nu_n} + r\beta \qquad 2.36$$

and

$$\overleftarrow{\alpha} = \frac{n-\vec{\gamma}}{\nu_n} - r\beta \qquad 2.37$$

and, as a result,

$$\vec{\alpha} + \overleftarrow{\alpha} = \frac{n}{\nu_n} \qquad 2.38$$

It is apparent, that if the reaction under consideration is a single-step, single electron-transfer reaction, $\nu_n = 1$, $n = 1$, $\vec{\gamma} = 0$, $\overleftarrow{\gamma} = 0$, $\nu_n = 1$, and r must be $= 1$. Consequently

$$\vec{\alpha} = \beta \qquad 2.39$$

and

$$\overleftarrow{\alpha} = 1-\beta \qquad 2.40$$

and 2.35 is identical to 2.22 in this case. The quantities α are known as the *transfer coefficients* for the reaction. These transfer coefficients become experimentally accessible when we apply the low- and high-field approximation reasoning to equation 2.35.

e.g., using the high-field approximation :

$$i = i_o \, e^{\overleftarrow{\alpha} F \eta / RT} \qquad 2.41$$

and

$$i = i_o \, e^{-\overrightarrow{\alpha} F \eta / RT} \qquad 2.42$$

Taking logarithms and re-arranging :

$$\eta = - \frac{2,303RT}{\overleftarrow{\alpha} F} \log i_o + \frac{2,303RT}{\overleftarrow{\alpha} F} \log i \qquad 2.43$$

$$= \frac{2,303RT}{\overrightarrow{\alpha} F} \log i_o - \frac{2,303RT}{\overrightarrow{\alpha} F} \log i \qquad 2.44$$

It is obvious from 2.43, 2.44, that when $\eta = 0$, $i = i_o$, and thus i_o values are accessible from extrapolation of log i *versus* η lines to $\eta = 0$.. Slopes of the log i *versus* η plots will yield a value of α, and are thus characteristic of the mechanism of the reaction. The results of a typical electrochemical polarization experiment in which an applied η causes current flow enabling determination of i_o and α, are given in Figure 5.

2.5 Corrosion systems : Systems of two or more interacting electrode processes

2.5.1 *A simple activation-controlled model*

The equations derived hitherto are basic to the study of electrochemistry. They enable electrochemical reactions to be described in formal physical chemical terms, and the experimental study of electrochemical kinetics depends upon them. The extension of fundamental electrochemical reasoning to corrosion processes has its roots in the recognition of a corroding metal surface as an electrode at which both anodic and cathodic electrochemical reactions are in process. An empirical graphical representation of corrosion rate theory incorporating these considerations was developed by Evans[25]. In 1938, Wagner and Traud[29] gave quantitative theoretical expression to this type of reasoning in studies of the corrosion of zinc amalgam in acid solution. The development of polarization techniques and, in particular, the potentiostatic technique gave impetus to the development of corrosion theory in attempts to interpret the results of polarization experiments. Stern[33-35], in developing electrochemical theory to explain quantitatively the nature of polarization curves obtained for corroding metals, made a valuable contribution to the study of the kinetics and mechanism of corrosion processes.

Those parts of electrochemical corrosion theory pertinent to the work performed in this investigation will be described here.

A corroding electrode system consisting of, at any one instant, a series of short circuited anodes (at which metal dissolution takes place) and cathodes will have no net current flowing to or from it. We shall assume that the anodic process is the dissolution of a metal, M to give its ions.

$$M^{n+} + ne \rightleftharpoons M \qquad 2.45$$

and that the cathodic process is the reduction of dissolved species (in the electrolyte) $Z^{n'+}$.

$$Z^{n'+} + n'e \rightleftharpoons Z \qquad 2.46$$

Process 2.45 at equilibrium at the electrode surface of metal M would have associated with it an equilibrium potential, E_m, which may be calculated from the Nernst relationship (equation 2.15), and an exchange current density, $i_{o,m}$, given by equation 2.10. Similarly, process 2.46 at equilibrium would have the characteristics E_z and $i_{o,z}$. We shall assume that anodic and cathodic areas are not necessarily equal, and thus that the effective anodic and cathodic exchange currents are given by

$$I_{o,m} = i_{o,m} A_a \qquad 2.47$$

and

$$I_{o,z} = i_{o,z} A_c \qquad 2.48$$

where $I_{o,m}$ and $I_{o,z}$ are anodic and cathodic exchange currents and A_a and A_c the respective anodic and cathodic surface areas. Points representative of these equilibrium potentials and exchange currents may be plotted on a set of potential (volts) *versus* log i (amperes/cm^2) axes as in Figure 6 (a).

Now if processes 2.45 and 2.46 were to interact such that a net transfer of electrons from metal M to ions Z^+ took place, the two systems would depart from equilibrium such that 2.45 would proceed in a net right-to-left direction, or

$$M \longrightarrow M^{n+} + ne \qquad 2.45\ (a)$$

and 2.46 in a net left-to-right direction, or

$$Z^{n'+} + n'e \longrightarrow Z \qquad 2.46\ (a)$$

The equilibrium potential of system 2.45 would have added to it an overpotential of $+\eta_m$ and the net current associated with the reaction would be given by an expression similar to 2.35.

$$I_m = I_{o,m} \left[e^{\overleftarrow{\alpha}_m \eta_m F/RT} - e^{-\overrightarrow{\alpha}_m \eta_m F/RT} \right] \qquad 2.49$$

Similarly, for system 2.46 a potential shift $-\eta_z$ would take place and a current

$$I_z = I_{o,z} \left[e^{-\overrightarrow{\alpha}_z \eta_z F/RT} - e^{\overleftarrow{\alpha}_z \eta_z F/RT} \right] \qquad 2.50$$

would flow.

The resulting corrosion system predicted by mixed potential theory, assuming that η_m and η_z are of sufficient magnitude to ensure Tafel dependence, and that no mass-transfer hindrances exist, is cases are those of concentration polarization at the cathode and passivity at the anode. These cases will now be presented.

2.5.2 *Concentration polarization at the cathode*

If we consider again the cathodic reaction in the corrosion case described above :

$$Z^{n'+} + n'e \longrightarrow Z \qquad 2.46\ (a)$$

the reaction being first order with respect to the concentration of $Z^{n'+}$, and if this concentration is C, the reaction rate expressed as a current density can be seen by the reasoning of Section 2.2 to 2.4 to be a function of that concentration.:

$$i_1 = k_1 C_1 \qquad 2.52$$

in which k_1 is a quantity incorporating $e^{-\vec{\alpha}_z F\eta/RT}$. We shall assume, to simplify the discussion, that η is sufficiently great for the high-field approximation of 2.35 to hold.

If the rate of the reaction is such that $Z^{n'+}$ ions are removed from the electrode-electrolyte interface at a rate greater than that at which they are replaced by diffusion of $Z^{n'+}$ towards the interface, a concentration differential between the bulk of the solution (in which the concentration is C_1^o), and the interface (at which the concentration is C_1) will exist. The flux of $Z^{n'+}$ ions from bulk to interface, expressed as a current may then be written

$$i_2 = k_2 (C_1^o - C_1) \qquad 2.53$$

This is predictable from Fick's First Law of Diffusion. When the rate of the charge-transfer reaction is constant, the flux associated with the removal of $Z^{n'+}$ ions from the interface by electrochemical discharge is equal to that associated with their diffusion from bulk to interface, and

$$i = i_1 = i_2 = \frac{k_1 k_2}{k_1 + k_2} C_1^o = kC_1^o \qquad 2.54$$

Now k_1 of equation 2.52 is composed of an overpotential-dependent and an overpotential-independent part, i.e.

$$k_1 = k_1' \exp(-\vec{\alpha}\eta) \qquad 2.55$$

When the overpotential $\eta = 0$, $i = i_o$ and

$$i_o = k_1' C_1^o \qquad 2.56$$

Now when the concentration at the electrode differs from that in the bulk of the solution, the current flowing at a given overpotential, i_{appl}, is given by

$$i_{appl} = i_o \frac{C_1}{C_1^o} e^{-\vec{\alpha}F\eta/RT} \qquad 2.57$$

The form of this equation most easily applicable to experimental results is obtained by considering the limiting case at which the concentration of $Z^{n'+}$ at the interface approaches zero, then

$$i_2 = i_d = k_2 C_1^o \qquad 2.58$$

where i_d is the diffusion-limited current density. From equations 2.53, 2.58

$$\frac{C_1}{C_1^o} = \left(1 - \frac{i_{appl}}{i_d}\right) \qquad 2.59$$

Equation 2.57 then becomes

$$i_{appl} = i_o \left(1 - \frac{i_{appl}}{i_d}\right) e^{-\vec{\alpha}F\eta/RT} \qquad 2.60$$

Taking logs and re-arranging 2.60,

$$\eta = \frac{RT}{\vec{\alpha}F} \left(ln\ i_o - ln\ i_{appl}\right) + \frac{RT}{\vec{\alpha}F}\ ln \left(1 - \frac{i_{appl}}{i_d}\right) \qquad 2.61$$

Now the first term on the right hand side of 2.61 is identical in form to equation 2.44, the purely activation-controlled relationship between η and applied current. We may thus re-write equation 2.61 as

$$\eta = \eta_a + \eta_{conc} \qquad 2.62$$

in which η_a is that part of the overpotential concerned with satisfying activation energy requirements for the cathodic reaction, and

$$\eta_{conc} = \frac{RT}{\vec{\alpha}}\ ln \left(1 - \frac{i_{appl}}{i_d}\right) \qquad 2.63$$

that part arising as a potential drop corresponding to changes in surface concentration produced by current flow.

The magnitude of the diffusion current i_d may be calculated from faradaically modified diffusion expressions. Formulations of this type have been presented by many authors, among them Delahay[36], Bockris and Reddy[31], and Makrides[37]. The case of the total current flowing to or from the electrode under control by the rate of diffusion of the reactant from bulk to interface may most simply be expressed by

$$i_{d,l} = nFD\,\frac{C^{o}}{\delta} \qquad 2.64$$

in which $i_{d,l}$ is the limiting diffusion current, n the number of electrons transferred during the reaction, F the faraday, D the diffusion coefficient of the reactant, C^{o} its bulk concentration and δ the thickness of the diffusion layer.

Concentration polarization detectable by cathodic polarization studies at a corroding electrode may be of two types :

(i) Diffusion control limits the rate of the cathodic corrosion reaction. In this case, diffusion control limits the overall corrosion reaction rate and $i_{corr} = i_{d,l}$.

(ii) Diffusion control limits the rate of the cathodic reaction only at potentials more negative than the corrosion potential. In this case, the corrosion reaction is under activation control and $i_{corr} < i_{d,l}$. The magnitudes of I_{corr}, $I_{o,z}$ are related to the respective current densities via the surface areas as in 2.47, 2.48.

These two extreme cases are depicted in Figures 7 (a) and (b). Intermediate cases resulting in mixed activation and concentration-polarization control may also arise.

2.5.3 *Anodic passivity*

Where the anodic process results in the formation of coherent solid reaction product films which prevent further corrosive attack, the metal is said to be passive. For any given circumstance in which a metal may passivate, a definite anodic current density, i_{crit}, must be surpassed before the process will occur. This i_{crit} may be attained at the metal surface to be passivated by two methods:

(i) The electrode to be passivated may be polarized anodically by a separate cathode connected to it *via* a suitable electrical power supply.

(ii) The metal may passivate spontaneously if a suitable cathodic reactant (usually a strong oxidising agent) is present. In this case, no externally applied current is required.

Figures 8 (a) and (b) depict the current-potential relationships of metals during passivation. The subject of anodic passivity has been reviewed by Hoar[163]. Reference will be made to passivity in discussing some of the experimental results of this work.

CHAPTER THREE

3 THE SULPHATE-REDUCING BACTERIA

The sulphate-reducing bacteria occupy a unique niche in the biological world, and increasing knowledge of their peculiar metabolism has led to speculation as to their possible role in such events as the evolution of life on this planet, and the genesis of petroleum. There is evidence, based on the distribution of stable sulphur isotopes in nature, that dissimilatory sulphate-reduction of the type exhibited by these organisms was occuring approximately $3{,}5 \times 10^{9}$ years ago, thus preceding the advent of free oxygen in our atmosphere which was approximately $1 - 2 \times 10^{9}$ years ago[59]. A number of mechanisms have been proposed to implicate sulphate-reducing bacteria both in petroleum genesis itself[43,59], and also in the release of oils from shale deposits during recovery operations[59]. The present chapter is intended to introduce those aspects of the ecology, taxonomy and nutrition of these organisms which bear directly on corrosion theory. Details of possible relationships between the biochemistry of the organisms and the electrochemistry of corrosion will be presented in the following chapter.

3.1 The sulphate-reducing habit

In all living organisms, the oxidation of chemical substances supplies energy which is channelled, *via* chemical energy carriers, to the energy-requiring processes of life. In most organisms visible to the naked eye, and many others, this chemical process of oxidation, known as respiration, proceeds down the oxidation-reduction energy gradient existing between the species oxidized (commonly known as foodstuff), and atmospheric oxygen, the foodstuff being oxidized with the release of chemical free energy, and oxygen being reduced according to

$$O_2 \quad + \quad 2H_2O \quad + \quad 4e \quad \longrightarrow \quad 4OH^- \qquad 3.1$$

The process of respiration thus requires a net transfer of electrons from the protoplasm to some part of its immediate environment. During this electron-transfer process, the synthesis of chemical energy-carriers used in life processes takes place. The multiplicity of intermediate electron-transfer reactions which take place between the first oxidative event at the foodstuff molecule and the final transfer of electrons to oxygen has led biochemists to describe oxygen as a "terminal electron acceptor" in this process. Respiratory processes where oxygen is the terminal electron acceptor are conventionally described as aerobic respiratory processes, and those where alternative terminal electron acceptors are used, as anaerobic respiratory processes.

Sulphate-reducing bacteria oxidise foodstuffs *via* an anaerobic respiratory pathway in which sulphates are used as terminal electron acceptors in respiration as follows :

$$SO_4^{=} \quad + \quad 10H^+ \quad + \quad 8e \quad \longrightarrow \quad 4H_2O \quad + \quad H_2S \qquad 3.2$$

This sulphate-reduction process has been described by Postgate[39] as "dissimilatory sulphate-reduction", thus distinguishing it from assimilatory sulphate-reduction practised by a large number of microorganisms in acquiring reduced sulphur compounds for the synthesis of cellular material. Dissimilatory sulphate-reduction processes, in organisms in which they occur, involve 10 to 100-fold greater metabolic turnovers of sulphur[39] than assimilatory processes.

While it is generally accepted that sulphate is the terminal electron acceptor in oxidative energy-yielding processes practised by the sulphate-reducing bacteria, the nature of the primary oxidative processes which provide energy are not clearly understood at present. It was once widely believed that the sulphate-reducing bacteria were facultatively autotrophic, and thus did not necessarily require reduced forms of carbon as nutrients. All of the energy requirements of the organisms would thus be satisfied by reaction 3.2. It was considered that reduced forms of carbon could be synthesized if the organism was supplied with CO_2 and hydrogen gas, and experimental evidence was quoted in support of this[40-43]. This experimental evidence is now considered invalid and, it seems firmly established that the organisms are heterotrophic[38, 44], thus requiring reduced forms of carbon in growth media. The energy relationships of the sulphate-reducing habit are of great importance to corrosion theory and will be discussed further in Chapter 5.

3.2 The ecology of the sulphate-reducing bacteria

It had been long known that anaerobic natural bacterial populations often emitted hydrogen sulphide when Beijerinck in 1895[45] first isolated a group of bacteria from an anaerobic environment, capable of reducing sulphates to sulphides. These organisms are now a well-defined physiological type, and appear ubiquitous in nature although they only become active where the oxygen supply is limited. The ecology of the organisms has been extensively reviewed by Zobell[46] and Bunker[47]. It is appropriate to discuss here those aspects of their ecology which circumscribe situations where they may grow actively in the proximity of structural metals.

Baas Becking, Kaplan and Moore[48] have described natural aqueous environments (soils and waters) in which microorganisms may grow, in terms of pH and oxidation-reduction potentials. Figure 9 depicts the pH and oxidation reduction coordinates of environments in which sulphate-reducing bacteria are found. This diagram is a compilation of published data on the distribution of sulphate-reducing bacteria superimposed upon the diagram giving the pH-potential coordinates of the thermodynamic stability of water. Water is stable in the zone between lines a and b of which a represents the equilibrium

$$O_2 + 4H^+ + 4e \rightleftharpoons 2H_2O \qquad 3.3$$

This is another form of equilibrium 3.1. The line follows the Nernst formulation

$$E = 1,23 - 0,059 \text{ pH} + 0,015 \log p_{O_2}$$

at 25°C. With oxygen in its standard state, the slope of the line is thus -0,059 volts per pH unit. Line b represents the equilibrium

$$2H_2O + 2e \rightleftharpoons H_2 + 2OH^- \qquad 3.4$$

which again with hydrogen in its standard state at 25°C, would have the Nernst slope of -0,059 volts per pH unit derived from $E = 0 - 0,059 \text{ pH} - 0,030 \log p_{H2}$ It is obvious that the environments favoured by the sulphate-reducing bacteria are far removed in redox potential from that of the oxygen-reduction reaction used by aerobic organisms in their energy-yielding respiratory reactions, and are relatively much closer to the hydrogen electrode potential. This diagram is similar in nature to the Pourbaix thermodynamic corrosion diagrams and will be used in discussing corrosion reactions in the presence of these organisms.

In addition to the pH and redox potential requirements given above, environments in which the sulphate-reducing bacteria will grow successfully should contain sulphates and organic matter. These are rarely absent from natural aqueous environments, and thus it is usual that where the oxygen supply is limited and the redox potential reaches a favourable value, sulphate-reducing bacteria will be active. Further environmental limits have been supplied by Zobell[46], and these are incorporated in Table 2.

3.3 The taxonomy of the dissimilatory sulphate-reducing bacteria

The existing theories of SRB corrosion mechanism depend upon the biochemical differences between the two major taxonomic groups of sulphate-reducing bacteria: the genera *Desulfovibrio* and *Desulfotomaculum*. *Desulfovibrio* species are not spore-forming, possess hydrogenase enzyme, and appear, from published experimental work to be corrosive, while *Desulfotomaculum* species are spore formers, do not possess hydrogenase enzyme, and do not appear to be corrosive[49-52].

Postgate and Campbell[53,54] reviewed the classification of the dissimilatory sulphate-reducing bacteria in 1966, and their system is universally accepted. Details of the classification of these organisms are given in Table 3. Of importance to corrosion theory is the type of respiratory pigment present in the two genera (cytochrome b in *Desulfotomaculum* and cytochrome c in *Desulfovibrio*). It is necessary to add to the information contained in the table that *Desulfovibrio* species usually display hydrogenase enzyme activity while *Desulfotomaculum* species do not.

3.4 The nutrition of dissimilatory sulphate-reducing bacteria

Since the statement by Booth[55] in 1964 that "the precise mechanism of the action of the sulphate-reducing bacteria in promoting metallic corrosion will only be fully elucidated by a complete investigation of the biochemistry of the organisms themselves", subsequent experimental work has confirmed that there is insufficient established biochemical knowledge to explain fully the apparent synergistic relationship of the organisms with corroding steel in anaerobic environments. That such a synergistic relationship exists is apparent from both field and laboratory investigations. Bunker[21] showed in a number of careful field investigations that sulphate-reducing bacteria are most active in the immediate vicinity of corroding metal pipes, the activity being highest at the corroding interface and progressively less at increasing distances from it. Butlin and Adams[41] described experiments in which the presence of corroding metal appeared to stimulate growth of the organisms in laboratory cultures. It is proposed to review here the major nutritional requirements of dissimilatory sulphate-reducing bacteria in order to investigate the basis of the relationship between organisms and corroding metal.

In 1930 Baars[56] published the results of the first careful stoichiometric investigation of the process of dissimilatory sulphate-reduction. This work invalidated the earlier results of van Delden, published in 1903, who considered that the sulphate-reduction process in the presence of organic nutrients such as lactate and malate proceeded by the following stoichiometry

$$2C_3H_5O_3Na \;+\; 3MgSO_4 \longrightarrow 3MgCO_3 \;+\; Na_2CO_3 \;+$$
$$2CO_2 \;+\; 3H_2O \;+\; 3H_2S \qquad 3.5$$

in the case of lactate, and

$$2C_4H_4O_5K_2 \quad + \quad 3MgSO_4 \longrightarrow 3MgCO_3 + 2K_2CO_3 + 3CO_2 + H_2O + 3H_2S \qquad 3.6$$

in the case of malate. Baars found that organic nutrients were not oxidized completely to CO_2 and that the stoichiometry of sulphate reduction coupled to organic nutrient oxidation approximated more closely to

$$2C_3H_5O_3Na \quad + \quad MgSO_4 \longrightarrow 2CH_3COONa + CO_2 + MgCO_3 + H_2S + H_2O \qquad 3.7$$

in the case of lactate, and

$$4\ CH_2OH.CHOH.CH\ OH \quad + \quad 3H_2\ SO_4 \longrightarrow 4CH_3COOH + 4CO_2 + 3H_2S + 8H_2O \qquad 3.8$$

in the case of glycerine, and

$$4COOH.CH_2.CH_2.COOH \quad + \quad 3H_2SO_4 \longrightarrow 4CH_3\ COOH + 8CO_2 + 3H_2S + 4H_2O \qquad 3.9$$

in the case of succinate. Baars thus considered that these organic compounds behaved as hydrogen donors for the sulphate-reduction process, being themselves incompletely oxidized. The primary role of the organic nutrient was thus as a hydrogen donor for sulphate-reduction.

It was found, however, by Butlin, Adams and Thomas[42], that in growth media containing only lactate and mineral salts, growth could be considerably improved by the addition of yeast extract, indicating that alternative forms of carbon to the "hydrogen donor" type may be required. This argument is not supported by the well-established fact that successful growth of sulphate-reducing bacteria has been demonstrated on innumerable occasions, in media containing only lactate and mineral salts, for example, in the media of Baars[56] and Starkey[57].

It has been claimed by a number of workers that dissimilatory sulphate-reducing bacteria are able to grow autotrophically in the presence of hydrogen (as H_2) with CO_2 as the sole carbon source. Butlin and Adams[41] claimed successful growth of *Desulfovibrio* cultures in media where $NaHCO_3$ was the sole carbon source, but only in the presence of hydrogen gas. When hydrogen was not supplied to the cultures, no growth occurred. However, when rods of mild steel were added to media inoculated with *Desulfovibrio* cells in the absence of hydrogen, growth occurred. It was considered possible that hydrogen formed at cathodic sites on the corroding metal was entering the sulphate-reduction pathway and supplying the energy necessary for HCO_3^- reduction and growth. The results of Mechelas and Rittenberg[58] and Postgate[39] do not support this proposed autotrophy. Postgate[39] indicated that the amount of CO_2 incorporated in cell carbon during growth under apparent autotrophic conditions never exceeded 15 - 25% of the total cell carbon, and usually differed only slightly from the amount of CO_2 assimilated during heterotrophic growth when other organic nutrients were supplied. Mechelas and Rittenberg[58] showed that H_2 *or* a non-assimilable organic compound, such as isobutanol may act as hydrogen sources during the sulphate-reduction reaction, but that neither labelled CO_2 nor isobutanol carbon was incorporated appreciably into the cell carbon. Carbon for cell synthesis was derived mainly from yeast extract in their experiments. Postgate[39] considered that the "autotrophic" growth reported by Butlin and Adams was in fact heterotrophic growth on organic impurities in the media. Quoting the data of Garvie and Strange *et al*, he pointed out that 0,067-M phosphate buffer could contain as many as 10^6 coliform bacteria per cm^3 and these would contribute organic impurities to media. The illusion of autotrophy probably arose because the presence of the H_2 allowed efficient use of these impurities by acting as an energy source *via* the sulphate-reduction reaction.

It is necessary to include at this stage, some comments on the iron requirements of the sulphate-reducing bacteria. It is by no means remarkable that these organisms require iron as a nutrient : Butlin, Adams and Thomas[42] demonstrated this, and Postgate[62] determined the optimal concentration in laboratory culture at 10 - 15μ mol of Fe per litre. Iron is a necessary part of cytochrome pigments, and also of hydrogenase enzyme systems. Apart from this absolute nutritional requirement for iron, it appears that this metal plays an important role in conditioning the environment for successful growth. Firstly, soluble iron enables sulphides to be precipitated as solids when they might otherwise reach toxic concentrations.

This role of iron has been discussed by Baas Becking, Kaplan and Moore[48]. Secondly, the presence of iron may play a role in control of the redox potential (E_H) of the environment. Baas Becking *et al*[48] have shown that the lower E_H limit of environments in which sulphate-reducing bacteria are active is too negative to be caused by H_2S or HS^- alone: their solubilities preclude this. The lower redox potential limits of such environments could be reproduced by titrating sodium sulphide (0,05M) with $FeSO_4$ (0,05M). In this ecological role, iron concentrations considerably greater than the 10 - 15 μmol per litre indicated by Postgate as a nutritional requirement, would be beneficial.

It is possible to summarize those aspects of the nutrition of the sulphate-reducing bacteria which are of importance to the corrosion theory which will be presented in the following chapter, as follows :

(i) Sulphate is required as a terminal electron acceptor in respiratory reactions. It has been shown[61] that fumarate may also fill this role in a few cases, but no evidence of this reaction in the natural environment has been reported.

(ii) Organic substances are required as sources of carbon for synthetic reactions. Inorganic carbon compounds cannot function as the sole source of cell carbon.

(iii) Hydrogen (as H_2) or organic hydrogen donors, such as lactate, are required for the sulphate-reduction reaction to proceed.

(iv) Iron is a nutrient essential for the formation of cytochrome and hydrogenase systems. Iron appears to play an additional important role in precipitating sulphides which would otherwise reach toxic concentrations and possibly, in regulating the redox potential of the environment in conjunction with sulphides.

The corrosion theory to be discussed in the following chapter is closely related to the biochemistry of sulphate-reduction. The details of this sulphate-reduction process will be introduced in assessing the validity of the corrosion theory.

CHAPTER FOUR

4 EXISTING THEORIES OF THE MECHANISM OF CORROSION CAUSED BY SULPHATE REDUCING BACTERIA

The fact that corrosion of ferrous metals occurs in the presence of sulphate-reducing bacteria is not, in itself, remarkable. Thermodynamic calculations give us the answer that corrosion of iron or steel will occur in environments favourable to the growth of sulphate-reducing bacteria. This may be demonstrated most simply from the superposition of the potential *versus* pH ecological diagram developed by Baas Becking, Kaplan and Moore[48] on the Pourbaix potential *versus* pH diagram for the corrosion of iron (Figure 10). It is apparent from this diagram that environments favourable to the growth of sulphate-reducing bacteria have coordinates of pH and redox potential within which iron metal is thermodynamically unstable. Horvăth and Novăk[63] (Figure 11) have developed a pH-potential diagram for iron in the presence of water and sulphides which shows that the domain of stability of iron metal is diminished by the presence of sulphides. It is obvious that the thermodynamic driving force of corrosion with hydrogen evolution as cathodic reaction is increased by the presence of sulphides.

Any satisfactory theory of SRB corrosion will have to explain the high *rates* of corrosion observed in the presence of these organisms. Ultimately it should be possible to express the corrosion rate as a current on an Evans-type corrosion diagram which describes accurately the kinetics of the individual anodic and cathodic processes.

Developments in the theory of the role of sulphate-reducing bacteria in metallic corrosion are best described in chronological order of their appearance. We shall divide the period over which existing theory was developed into two parts: the period before electrochemical polarization methods were applied to the study of the problem, and the period following the introduction of these methods. The second period extends from the year 1959 to the present date.

4.1 Theory developed before the introduction of polarization techniques (the period 1934 - 1959)

Von Wolzogen Kühr and van der Vlugt laid the basis of current theory of SRB corrosion with their paper "The graphitization of cast iron as an electro-biochemical process in anaerobic soils", published in 1934 [64]. This type of corrosion of cast iron was of frequent occurence in the anaerobic soils of Holland and consisted of the corroding away of the metal leaving a skeletal structure of graphite inclusions. Corrosion products consisted of the sulphides of iron, and this knowledge, coupled with the fact that sulphate-reducing bacteria were responsible for sulphide formation from sulphates in this type of soil, led the authors to study the relationship between corrosion and sulphate-reduction by the SRB in seeking a mechanism for graphitization.

In the anaerobic systems in which this type of corrosion took place oxygen was of course, not available as a cathodic reactant and von Wolzogen Kühr and van der Vlugt, aware that the absence of oxygen as a cathodic reactant usually resulted in very low corrosion rates, began to seek alternative cathodic reactions which might result in the high rates observed. Three years previous to their published investigation, Stephenson and Stickland had announced the existence of hydrogenase enzyme[65] and shown, that in sulphate-reducing bacteria, this enzyme catalysed the reduction of sulphate by molecular hydrogen[66] according to

$$H_2SO_4 \quad + \quad 4H_2 \quad \longrightarrow \quad H_2S \quad + \quad 4H_2O \qquad 4.1$$

It is necessary to introduce at this stage, some of the electrochemical corrosion theory invoked by these workers (1934)[64]. The corrosion of iron in a neutral aqueous environment was believed to take place as follows :

Water was known to be partly dissociated into hydrogen and hydroxyl ions

$$2H_2O \quad \rightleftharpoons \quad 2H^+ \quad + \quad 2OH^- \qquad 4.2$$

The ionization (corrosion) of metallic iron was believed to be accompanied by the discharge of hydrogen ions to give adsorbed atomic hydrogen (H_{ads}).

$$Fe + 2H \longrightarrow Fe^{++} + 2\,H_{ads} \qquad 4.3$$

This adsorbed hydrogen was believed to persist on cathodic metal surfaces, bringing about a condition known as "cathodic polarization" in which local cathodes were considered inactive, and the corrosion reaction would thus come to a standstill. However, if substances were present which could react chemically with the H_{ads}, this polarized condition would be removed, and the corrosion reaction would continue. For example, in the presence of oxygen,

$$4\,H_{ads} + O_2 \longrightarrow 2H_2O \qquad 4.4$$

This type of reasoning is not fully consistent with modern electrochemical knowledge as will be discussed in the following chapter, nevertheless it formed the basis of the von Wolzogen Kühr - van der Vlugt corrosion mechanism which is still accepted by a number of contemporary corrosion workers and authors.

Von Wolzogen Kühr and van der Vlugt[64], claiming support from Baars'[56] finding that hydrogen other than molecular hydrogen (e.g. hydrogen derived from lactate, malate or succinate) could be used in sulphate reduction by SRB, proposed that the reduction of sulphate by H_{ads} at cathodic sites on corroding iron constituted a "cathodic depolarizing" mechanism of the type illustrated by oxygen in reaction 4.4. The reaction scheme proposed was as follows :

$$8H_2O \rightleftharpoons 8H^+ + 8OH^- \qquad 4.5$$

$$4Fe + 8H^+ \longrightarrow 4Fe^{++} + 8H \qquad 4.6$$

$$H_2SO_4 + 8H \longrightarrow H_2S + 4H_2O \quad (\text{"depolarization"}) \qquad 4.7$$

$$3Fe^{++} + 6OH^- \longrightarrow 3Fe(OH)_2 \qquad 4.8$$

$$Fe^{++} + H_2S \longrightarrow FeS + 2H^+ \qquad 4.9$$

Summing 4.5 - 4.9 the overall corrosion reaction :

$$4Fe + H_2SO_4 + 2H_2O \longrightarrow 3Fe(OH)_2 + FeS \qquad 4.10$$

is obtained. It must be mentioned here that Stephenson and Stickland demonstrated that hydrogenase enzyme used *molecular* hydrogen to reduce sulphate to sulphide[65,66]. Von Wolzogen Kühr and van der Vlugt assumed that this was also true for H_{ads} at cathodic surfaces. The validity of this assumption in the light of present-day knowledge of the electrochemistry of corrosion and hydrogenase systems will be discussed in the following chapter.

From the stoichiometry of equation 4.10, it would appear that the ratio

$$\frac{\text{total iron corroded (mol)}}{\text{iron present as sulphide (mol)}}$$

would have the value of 4. Von Wolzogen Kühr and van der Vlugt found, from a number of analyses of corrosion products , that the ratio approximated 2,7 (see table 4). They ascribed this difference to the probability that dissimilatory sulphate reduction accompanying the oxidation of foodstuffs by the organisms resulted in greater yields of H_2S than would be produced by "cathodic depolarization" alone. This may result in a greater proportion of iron sulphide in the corrosion products than predicted by equation 4.10. Another possibility, not mentioned by these authors is that, if the corrosion product were iron sulphides alone (which it would be were the corrosion process due solely to H_2S as a corrodent) oxidation of some of the corrosion product by atmospheric oxygen during the analysis might account for the observed results.

During the period 1934 - 1959, the electrochemical techniques available at the time were applied to the study of the mechanism of SRB corrosion. Hadley[67] studied the potential-time behaviour of steel electrodes in cultures of SRB. His results were reported as follows (Figure 12) :

(i) A steady potential of approximately -465 mV (she) was reached in un-inoculated culture (portion AB of the potential-time curve).

(ii) On inoculation the potential rapidly fell to approximately -530 mV (she) as indicated by portion BC of the curve, which was interpreted by Hadley as being indicative of cathodic depolarization by actively growing SRB.

(iii) A relatively slow potential shift in the noble direction was taken by Hadley to represent loss of depolarizing activity (portion CD) by the bacteria, by then considered to have become inactive.

(iv) The final steady potential (DE) was considered to be a steady-state condition representative of the film-forming iron-sulphide reaction.

Wanklyn and Spruit[68] challenged Hadley's interpretation of his potential-time results. They indicated correctly that a shift in potential in the negative direction could only be interpreted, on the basis of the Evans-type corrosion diagram, as resulting from *anodic* depolarization (or cathodic inhibition: unlikely because of the increase in corrosion rate caused by these organisms). *Cathodic* depolarization would lead to positive shifts in potential (See Figure 13). Further potential-time studies by Wanklyn and Spruit showed the following (Figure 14) :

(i) The shift of potential in the negative direction found by Hadley could be reproduced, but the active organisms required the addition of lactate (resulting in sulphide production) in order to bring about this shift.

(ii) After the rise in potential to noble values, further additions of lactate or of H_2S-water caused further small negative shifts.

Wanklyn and Spruit concluded that the main contribution of the sulphate-reducing bacteria to high corrosion rates was caused by the production of sulphides leading to anodic depolarization and consequent increases in corrosion rates. These workers also performed quantitative studies of the stoichiometry of the SRB corrosion reaction[69]. If the mechanism proposed by von Wolzogen Kühr and van der Vlugt were correct, the ratio

$$N = \frac{\text{mol Fe corroded}}{\text{mol } SO_4^{=} \text{ reduced}}$$

would have the value of 4. Wanklyn and Spruit considered that if no organic hydrogen donors (such as lactate) were present and the von Wolzogen Kühr - van der Vlugt mechanism were correct, values of N lower than 4 would be obtained, as more sulphate would be reduced by the organisms than could be accounted for by equation 4.10 if active growth took place. If this were the case, their results would agree with those of von Wolzogen Kühr and van der Vlugt. In no case, however, should the value of N exceed 4, as this would imply that more iron corrosion would occur than corresponds to the amount of sulphate reduced by the mechanism of equation 4.10. Wanklyn and Spruit found that, where lactate was added to the culture medium in their corrosion experiments, values of N varied from 0,9 to 1,5. This was consistent with the reasoning of von Wolzogen Kühr and van der Vlugt. However, where lactate was omitted from the culture medium, values of N between 5 and 9 were obtained, indicating that the stoichiometry of 4.10 was not being followed. These authors also found, in experiments performed under an atmosphere of nitrogen and carbon dioxide, that appreciable quantities of hydrogen gas, detectable by mass spectrographic analysis, were evolved during corrosion.

The progress in the period 1934 - 1959 may be summarized as follows :

Von Wolzogen Kühr and van der Vlugt[64] proposed that the corrosion rate of ferrous metals which, in natural environments at neutral pH is usually controlled by the rates of cathodic reactions (e.g. oxygen reduction), is in the case of SRB corrosion, accelerated by an "electro-biochemical" effect in which the reduction of sulphate catalysed by SRB is the cathodic reaction of the corrosion cell. Stoichiometry was proposed for the corrosion reaction, and the stoichiometric evidence invoked by these authors agreed with this mechanism, although wide tolerances of error were allowed by the probability of dissimilatory sulphate-reduction by the organisms taking place in addition to biologically-catalysed cathodic sulphate-reduction. The experimental evidence quoted by von Wolzogen Kühr and van der Vlugt in favour of their mechanism was solely stoichiometric and no electrochemical proof of cathodic depolarization was presented.

Hadley[67] presented experimental evidence which was interpreted as supporting the von Wolzogen Kühr - van der Vlugt mechanism. Wanklyn and Spruit[68] presented similar results, but reasoned, correctly so, that these results were not consistent with cathodic depolarization mechanism, but with an anodic depolarization one. They also indicated that an appreciable amount of hydrogen evolution took place during corrosion[69].

4.2 The period following the introduction of polarization techniques (the period 1959 to the present date)

Since the publication of the results of the first electrochemical polarization studies in cultures of sulphate-reducing bacteria by Horvàth and Solti[70] in 1959, many results of similar studies have been presented. As yet, however, none of these has been subjected to analysis in terms of fundamental electrochemical corrosion theory. It is the opinion of the author that the results obtained have not been used to best advantage in characterising the influence of SRB on corrosion. Nevertheless they have been used as a basis for elaborate electrochemical theory. It is proposed to present, in this section the established experimental data together with the accepted theoretical interpretation, and to re-examine the data using a fuller theoretical interpretation, in the following chapter. Developments will be presented more or less in chronological order of appearance.

Horvàth and Solti[70] (1959) performed polarization experiments using steel electrodes in cultures of SRB and compared the polarization results with the potential-time behaviour of unpolarized electrodes. They found that potential-time behaviour agreed well with the results of Hadley[67], and Wanklyn and Spruit[68]. Their polarization results demonstrated that, coinciding with the initial drop in potential to a minimum value, both cathodic and anodic depolarization (characterized by an increase in current for a given potential shift) occured (Figure 15). These depolarization effects diminished during the following potential-rise phase (Figure 16). During the period of immersion of the electrodes, the pH of the cultures rose from values of between 7,0 - 7,2 to values between 7,8 - 8,0. Aeration of the cultures at this stage, for 24 hours, caused further diminution of the depolarization effects.

Booth and Tiller[71] published results of similar work in 1960, but took care to specify the organisms used in their experiments. They were able to draw a distinction between the influence of SRB possessing hydrogenase enzyme and those not possessing the enzyme on polarization characteristics of steel in their cultures. They found that both hydrogenase-positive and hydrogenase-negative organisms caused anodic depolarization. In addition to this, only the hydrogenase-positive organisms affected the cathodic behaviour, causing marked depolarization. Of the two effects, cathodic depolarization was considered to be the more important to corrosion mechanisms, since anodic depolarization was later followed by strong polarization (interpreted as corrosion inhibition) effects. Booth and Tiller considered that their results supported the cathodic depolarization theory of von Wolzogen Kühr and van der Vlugt and that sulphate reduction by cathodic H_{ads} catalysed by hydrogenase enzyme was the cause of the observed depolarization.

Booth and Wormwell[72] performed weight-loss experiments in batch cultures of SRB using mild steel and found a roughly linear relationship between the corrosion rate and the hydrogenase activities of the organisms used, as determined by hydrogen uptake in Warburg manometry (Table 5). This gave strong support to the von Wolzogen Kühr - van der Vlugt mechanism. The corrosion rates obtained in these experiments, however, were much lower than rates obtained under natural conditions (See Table 1).

Hoar and Farrer (1961)[73] published the results of electrochemical polarization studies in mixed cultures of soil organisms in which sulphate-reduction took place. They were unable to demonstrate any alteration to cathodic polarization characteristics by active sulphate-reducting bacteria. Cathodic characteristics were indistinguishable from those for the hydrogen evolution reaction, demonstrated in sterile electrolyte solutions such as sodium chloride solutions. Hoar and Farrer were able to demonstrate however, significant alterations in the *anodic* polarization behaviour caused by the addition of small quantities of sulphide to sterile electrolyte solutions, and by the growth of sulphate-reducing organisms in culture media. Lowering of the anodic Tafel slope from the usual 60 mV per decade of current to values as low as 35 mV per decade of current occured. This work of Hoar and Farrer has been severely criticised by Booth[74], mainly because of inadequate purification procedures where attempts were made to perform polarization experiments in a pure culture, nevertheless it is the only attempt that has been made to study the kinetics of SRB corrosion using formal quantitative electrochemical methods, and will receive further attention when the author's own results are discussed. It must be mentioned at this stage that the polarization methods used by Booth and his co-workers in demonstrating "cathodic depolarization" do not allow comments to be made on cathodic kinetics at or near the corrosion potential. Either the methods used were not sufficiently sensitive, or the interpretation of the results was not sufficiently detailed possibly because of ignorance of the theoretical background to the use of polarization methods in corrosion studies. In none of the work done by these authors has there been any attempt at a formal electrochemical interpretation of polarization curves.

Further polarization studies published by Booth and Tiller in 1962[75,76], were considered to give support to the von Wolzogen Kühr - van der Vlugt hypothesis. Of two strains of *Desulfotomaculum nigrificans* studied, one hydrogenase-positive, the other hydrogenase-negative, only the hydrogenase-positive one effected cathodic depolarization. Furthermore, a hydrogenase-negative strain of halophilic SRB (*Desulfovibrio desulfuricans* strain El Agheila Z NClB 8380) initially hydrogenase-negative and having no cathodic depolarizing influence, developed hydrogenase activity accompanied by cathodic depolarizing activity after storage under hydrogen for one year, with intermittent subculture.

By the year 1964, the introduction of polarization techniques had had the effect of demonstrating that both anodic and cathodic depolarization occured when steel corroded in the presence of SRB. Booth and his school considered that cathodic depolarization accompanying cathodic sulphate reduction by H_{ads} catalysed by hydrogenase enzyme was the major mechanism by which SRB accelerated corrosion. Cathodic depolarizing activity of an organism required the presence of hydrogenase, and this could be demonstrated and assayed by hydrogen uptake in Warburg manometry.

As yet, no laboratory corrosion experiment had given corrosion rates approaching those found in natural environments. The weight-loss experiments of Booth and Wormwell[72], had been performed in batch culture, and Booth, Shinn and Wakerley[77] performed further weight-loss experiments using semi-continuous culture techniques. The following significant points emerged :

(i) Corrosion rates under continuous culture conditions were significantly higher than those demonstrated by Booth and Wormwell in batch culture (Table 5), but were nevertheless considerably lower than rates observed in natural environments.

(ii) The linear correlation between hydrogenase activity of a culture and its corrosivity, demonstrated by Booth and Wormwell, disappeared completely, but nevertheless, the hydrogenase positive strains gave considerably higher rates than the single hydrogenase-negative strain tested.

(iii) The importance of the ferrous iron content of culture media to corrosion rates was demonstrated. The influence of increasing the Fe^{2+} content of the medium used for culturing *Dv. desulfuricans* (Hildenborough) from 25μ M (0,001% w/v $FeSO_4(NH_4)_2SO_4 6H_2O$) to 12 500 μM (0,5% w/v $FeSO_4(NH_4)_2 SO_4.6H_2O$) was to bring about an approximate 6-fold increase in corrosion rate (see Table 5) The influence of this added iron appeared to be to cause precipitation of any sulphide formed by the organisms as a loose FeS deposit around the metal specimen, rather than allowing the sulphide to form a tightly adherent corrosion product film on the metal surface as occurred in low-iron media.

Booth, Cooper and Wakerley[78] performed further weight loss experiments in semi-continuous culture to study this newly-established influence of dissolved ferrous iron on the corrosion process. They found that organisms which in previous experiments had no hydrogenase activity, developed it in iron-rich media (12 500 μM of Fe^{2+}), and this included *Desulfotomaculum* species. Under these conditions, no correlation at all existed between hydrogenase enzyme activity and corrosion rate. Organisms with manometrically assayed activities using methyl viologen as a reducible substrate, of between 80 and 1150 μl/hour/mg dry weight gave corrosion rates in the relatively narrow range of 15,3 - 27,4 mg/dm^2/day. By this time evidence of a correlation between hydrogenase activity and corrosion rates had become almost non-existent, nevertheless the authors found some support for the von Wolzogen Kühr - van der Vlugt mechanism in their results. The highest corrosion rates reported in laboratory cultures were found by Booth, Cooper and Cooper[79], using continuous culture techniques in culture media containing sufficient ferrous iron (0,285% w/v of Fe^{2+} = 51 000 $\mu\underline{M}$ was used) to precipitate all of the sulphide formed by the organisms as solid ferrous sulphide. Extremely high corrosion rates (95 - 221 mg/dm^2/day) of the mild steel specimens used, were found in cultures of sulphate-reducing organisms from various inocula, while the sterile controls gave a corrosion rate of 2,6 mg/dm^2/day. Corrosion products were loosely adherent black cocoons around each specimen consisting of a mixture of siderite ($FeCO_3$) and troilite (FeS), as evidenced by X-ray diffraction analysis. These rates of corrosion approached the values found in natural environments (Table 1).

Booth, Robb and Wakerley[80], examined the influence of varying the ferrous iron content of the culture medium on the electrochemical polarization characteristics of mild steel electrodes. They found that where sulphate reducing bacteria grew in the vicinity of mild steel electrodes in a "minimal iron medium" (i.e. of concentration 25 $\mu\underline{M}$ Fe^{++}), cathodic depolarization occurred but because a hard adherent sulphide film formed on the electrode surface, this effect did not persist, (Figure 17). However, where iron-rich medium (12 500 $\mu\underline{M}$ Fe^{++}) was used, persistent vigorous cathodic depolarization ensued (Figure 18). This was accompanied by the formation of a loose, bulky iron sulphide deposit on the electrode. The *Dv. desulfuricans* (Hildenborough) organism used in this work had a hydrogenase activity ($-Q_{H_2}^{SO_4^=}$) of 300 μl/hour/mg dry wt. *Desulfotomaculum orientis* (Singapore), which developed weak hydrogenase activity under these conditions ($-Q_{H_2}^{SO_4^=}$ = 20 μl/hour/mg. dry wt.) was nevertheless able to cause considerable cathodic depolarization (Figure 19).

By this stage (1968), it had become apparent that the presence of bulky, non-adherent deposits of iron sulphide in contact with steel surfaces in the presence of SRB had a pronounced stimulatory effect on both corrosion rates and cathodic depolarization. Booth, Elford and Wakerley[81], performed further polarization experiments in which attempts were made to study separately the influence of a hydrogenase-active sulphate-reducing organism and of ferrous sulphide deposits on the cathodic polarization characteristics of mild steel. Cultures of the hydrogenase-active organism *Desulfovibrio desulfuricans* Teddington R(NCIB 8312) were used. This organism is capable of using fumarate as terminal electron acceptor in dissimilatory reductive processes in the place of sulphate. By this means, sulphides could be eliminated from the system, and consequently the electrode surface could not be contaminated with solid sulphide reaction products. It was found that this organism was able to bring about cathodic depolarization, the effect being maximum at 2 days after inoculation, and diminishing with time after this (Figure 20). When sterile suspensions of FeS, prepared from ferrous chloride and sodium sulphate, in de-aerated 1% w/v NaCl solution were used in polarization experiments, vigorous cathodic depolarization was observed (Figure 21). The magnitude of the depolarizing effect appeared to depend upon the quantity of FeS in contact with the electrode surface. Horizontal electrodes were used, and polarization runs were performed when the ferrous sulphide in suspension had been allowed to settle on electrode surfaces. These results indicated to Booth and his co-workers that two separate cathodic depolarization mechanisms were operative in the corrosion process :

(i) Utilization of "polarizing hydrogen" by the hydrogenase systems of the organisms consistent with the von Wolzogen Kühr - van der Vlugt mechanism.

(ii) Depolarization by solid ferrous sulphide *via* some hitherto unknown mechanism.

This indicated that more detailed study of depolarization phenomena was necessary and, if possible, biological and non-biological effects should be studied separately. Attempts were made to do this, and the results will be presented separately in sections 4.2.1 and 4.2.2.

4.2.1 *Biological cathodic depolarisation mechanism studies*

If the von Wolzogen Kühr - van der Vlugt mechanism were operative, hydrogenase-catalysed sulphate-reduction by H_{ads} would take place at cathodic surfaces. This would, of course, result in sulphide formation and introduce the depolarizing effects (or inhibiting effects, depending upon the nature of the films formed) attendant upon the presence of FeS at the experimental electrode surfaces. Since the announcement of the existence of the hydrogenase of *Desulfovibrio* species by Stephenson and Stickland[65] in 1931, it was known that hydrogenase could catalyse the reduction of *a number* of reducible substrates by molecular hydrogen. Apart from the reduction of sulphate, Stephenson and Stickland showed that the reduction of methylene blue by molecular hydrogen could be catalysed. The hydrogenase activity of a particular sample of bacterial cells could be assayed by measurement of the rate of hydrogen gas uptake by the organism/reducible substrate system. Apart from sulphate and methylene blue, various other redox dyes had been used in *Desulfovibrio* hydrogenase assay : notably methyl viologen by Peck and Gest[82] and benzyl viologen by King and Winfield[83].

Iverson[84] (1966) published the results of experiments in which benzyl viologen was used to demonstrate the cathodic depolarizing properties of *Desulfovibrio* cells. A mild steel coupon was placed upon the surface of solid agar containing tris buffer and benzyl viologen. *Desulfovibrio* cells obtained from a separately-grown culture were placed under one portion of the coupon. After 17 hours incubation in a nitrogen atmosphere, a violet area of reduced benzyl viologen was found under the portion of the coupon in contact with the cells. The use of ferricyanide reagent disclosed that ferrous iron was present under the portion of the coupon remote from the cells, indicating that metal dissolution (corrosion) had taken place here. Using separate coupons one in contact with cells, the other not, a sustained current of 1 μA per square centimeter, with the coupon in contact with the cells cathodic, was detected between the two. Iverson was unsuccessful, however[85], in demonstrating similar cathodic depolarization currents, using sulphate as reducible substrate. He suggested that possibly some other electron acceptor (such as phosphate, since phosphides were found together with sulphides in some corrosion products[85]) was operative if von Wolzogen Kühr - van der Vlugt depolarization took place.

Booth and Tiller[86] attempted a study of hydrogenase-catalysed cathodic depolarization using resting-cell suspensions of *Desulfovibrio* and *Desulfotomaculum* species in tris buffer at pH 7 using benzyl viologen as a reducible substrate. Mild steel working electrodes were used, and great care was taken to exclude oxygen from the experimental system. It is necessary to describe the polarization cell used by Booth and Tiller in some detail.

A two-compartment polarization cell was used, each compartment being similar in design to a Hirsch-type gas scrubbing aparatus (Figure 22). Anodic and cathodic compartments were identical, and working electrode and counter-electrode were of the same metal (mild steel). The composition of the electrolyte in the anolyte (counter-electrode compartment) was maintained as tris buffer at pH 7,0 while that in the catholyte (working-electrode compartment) was varied. The following catholytes were used :

a) tris buffer (pH 7,0)

b) tris buffer containing 7,3 x 10^{-5} $\underline{M}$ benzyl viologen

c) suspension of *Dv. vulgaris* in tris buffer

d) suspension of *Dv. vulgaris* in tris buffer + 7,3 x 10^{-5} $\underline{M}$ benzyl viologen

e) suspension of *Dt. orientis* in tris buffer

f) suspension of *Dt. orientis* in tris buffer + 7,3 x 10^{-5} $\underline{M}$ benzyl viologen

A controlled potential range of -650 mV to-950 mV (she) was employed. No curves comparing results of (a) and (b) are reported with the results of this work, but the authors state that " at none of the potentials used in this work was benzyl viologen reduced at the cathode in the absence of either bacterial cells or cell fragments". If this was so, no detectable difference should have been observed between results of experiments with catholytes (a) and (b). Booth and Tiller were able to show, however, that cell suspensions of differing hydrogenase activities brought about depolarization, at the potentials used, of increasing magnitude with increasing hydrogenase activities (Figure 23). The hydrogenase-negative cells of *Dt. orientis* caused no such depolarizing effect (Figure 24). Analysis of the anolyte for dissolved iron showed that the amount of iron corroded from the steel counter-electrode was faradaically equivalent to the amount of current supplied by the potentiostat. It would have been most surprising if this were not the case, and the observation appears to be of no significance. Combined analyses of anolyte and catholyte indicated that the amount of iron that had passed into solution was electrochemically equivalent to the amount of benzyl viologen reduced. Booth and Tiller interpreted their results as being wholly consistent with a modified von Wolzogen Kühr - van der Vlugt depolarization theory in which hydrogenase enzyme brought about cathodic depolarization by catalysing the reduction of benzyl viologen by H_{ads}. A diagram of their reaction scheme is presented in Figure 25. In polarization experiments performed with disrupted cells and cell fragments of *Dv. vulgaris*, Booth and Tiller found that cell wall fragments and combinations of cell wall and cell membrane fragments were inactive in producing depolarization effects. Cell cytoplasm fractions did produce these effects and the authors considered that the active enzyme was situated here (i.e. within the cell wall and cell membrane).

Further cathodic depolarizing effects of hydrogenase-positive organisms have been demonstrated by Mara and Williams[87, 88]. Using methylene blue in the terminal electron acceptor role assigned to benzyl viologen by Booth and Tiller, they demonstrated that a wide range of hydrogenase-positive photosynthetic and non-photosynthetic bacteria as well as microalgae could bring about depolarization effects at iron cathodes of the type described by Booth and Tiller. Hydrogenase-negative organisms failed to demonstrate this effect (Figure 26). They concluded that, since most of the organisms were capable of nitrate reduction, von Wolzogen Kühr - van der Vlugt depolarization linked with nitrate reduction could be an additional cathode-depolarizing mechanism in natural environments.

Mara and Williams claimed support for this hypothesis from weight-loss experiments with mild steel in the presence of a hydrogenase-positive nitrate-reducing strain of *Escherichia coli* (NCIB 8666)[89]. They claimed that corrosion was consistent with the stoichiometry

$$Fe + H_2O + NO_3 \longrightarrow Fe(OH)_2 + NO_2^- \qquad 4.11$$

A hydrogenase-negative organism (*Pseudomonas stuzeri* : NCIB 9040) gave considerably lower corrosion rates and did not obey this stoichiometry. Ashton, King and Miller[90], in more careful work with the same hydrogenase-positive organism used by Mara and Williams, found no correlation between the amount of nitrate reduced and the amount of corrosion. They concluded that mechanisms other than enzymic depolarization were responsible for the corrosion observed.

4.2.2 *Studies of corrosiveness of the sulphides of iron*

It had become apparent[78 81] from the work of Booth *et al* that the presence of bulky ferrous sulphide precipitated by SRB from media containing large quantities of dissolved Fe (12 500 μM) brought about high corrosion rates and cathodic depolarization. King, Miller and Wakerley[91] demonstrated conclusively that high iron contents of this order were necessary for continued high corrosion rates in the presence of SRB. If the iron content of the medium, initially high and of the order required to give high corrosion rates in semi-continuous culture, were reduced to the level merely required to sustain growth, corrosion was almost completely arrested. Moreover, in these experiments, hydrogenase-positive and hydrogenase-negative SRB gave approximately the same corrosion rates, and $-Q_{H_2}^{SO_4^=}$ values bore no relationship to corrosion rates. King, Miller and Wakerley concluded thus, that the organisms' ability to produce FeS in contact with the corroding metal was their chief contribution to high corrosion rates.

Mara and Williams[92] performed polarization experiments with pure iron electrodes in contact with samples of various sulphide minerals and demonstrated differences in their polarization characteristics (Figure 27). Unfortunately, this work suffers from inadequate description of the experimental technique and also from lack of comparative figures for particle size or surface areas of the sulphides used, as cathodic activities would be best compared on the basis of current *densities*, rather than total currents as was done. The results indicate that greigite, mackinawite, pyrrhotite and marcasite were the most active in cathodic and anodic depolarization. King and Wakerley[93] selected mackinawite for particular study, since this is the first-formed iron sulphide during bacterial corrosion[94,95]. Weight-loss experiments in bacteria-free systems demonstrated a quantitative relationship between the amount of mackinawite in contact with the corroding steel surface and the amount of metal corroded. Mackinawite emerged as a corrosive agent which caused corrosion of steel roughly via first-order kinetics with about 10mg of iron corroded by 1 μmol mackinawite. Continued high corrosion rates could only be sustained where the ferrous sulphide was regularly replaced.

King, Miller and Smith[96], extended this work to compare the corrosive effects of various other sulphides of iron. Widely differing corrosion rates were found (Table 6), and it seemed that the corrosiveness of the sulphides increased with their sulphur content. The bright, etched appearance of the mild steel specimens suggested that they behaved as anodes in a galvanic cell with the sulphides acting as cathodes. The corrosivity of the sulphides did not follow the order suggested by the polarization results of Mara and Williams[92], pyrite being the most corrosive in weight-loss experiments while causing the smallest depolarization effects in polarization experiments. King and Miller[97] *et al* suggested that absorption of cathodically produced hydrogen by the sulphides may be responsible for their inactivation as cathodes (continuous replenishment of the sulphides was necessary for high corrosion rates). Differences in the rates of corrosion caused by the various sulphides were suggested to be due to different rates of absorption of cathodic hydrogen by these sulphides.

4.3 Summary of theory presented to date

(i) Von Wolzogen Kühr and van der Vlugt (1934) presented a theory of the mechanism of SRB corrosion based on cathodic depolarization caused by the hydrogenase-catalysed reduction of $SO_4^{=}$ by H_{ads}. Stoichiometric evidence presented by these workers was not at variance with their theory.

(ii) Potential-time studies by Hadley (1948) and by Wanklyn and Spruit (1952) indicated that anodic depolarization of steel took place during active growth of SRB, and Wanklyn and Spruit proposed that anodic depolarization was caused by biologically produced sulphides and that cathodic hydrogen evolution occurred.

(iii) Horvãth and Solti (1959) and Booth and Tiller (1960) used electrochemical polarization methods to demonstrate that SRB brought about both anodic and cathodic depolarization at mild steel electrodes.

(iv) Booth and Tiller (1960 - 1962) demonstrated a correlation between hydrogenase activity and cathodic depolarizing activity of SRB cultures, and Booth and Wormwell (1961) demonstrated, by means of weight-loss experiments, that corrosivity of various strains of SRB depended upon their manometrically determined hydrogenase activity. In subsequent electrochemical work in actively growing SRB cultures using sulphate as terminal electron acceptor, and weight-loss corrosion determinations, this correlation between hydrogenase activity, cathodic depolarization activity and corrosivity disappeared.

(v) Hoar and Farrer (1961) demonstrated, admittedly in cultures of SRB that might not have been pure, by means of formal electrochemical polarization studies, that cathodic depolarization appeared to play no role in the corrosion mechanism, but that a lowering of the anodic Tafel slope definitely took place in the presence of SRB.

(vi) Booth, Elford and Wakerley (1968) indicated that apart from von Wolzogen Kühr - van der Vlugt depolarization by bacteria, solid ferrous sulphide at the mild steel surface could cause considerable cathodic depolarization. Ferrous sulphide causing depolarization was a loose bulky precipitate likely to be formed by reaction of bacterially-produced H_2S with dissolved Fe in the medium, rather than an adherent corrosion product film formed at the metal surface.

(vii) Booth and Tiller (1968) demonstrated a correlation between cathodic depolarizing activity of resting cells of SRB with benzyl viologen as "terminal electron acceptor" in the place of sulphate, and the hydrogenase activity of the cell suspension. This was taken as direct proof of von Wolzogen Kühr - van der Vlugt depolarization. Mara and Williams (1971 - 1972) demonstrated this type of depolarization effect in cultures of a number of other hydrogenase-positive organisms, using methylene blue as "terminal electron acceptor", and suggested that von Wolzogen Kühr - van der Vlugt depolarization with nitrate as reducible substrate might take place in a wide range of corrosion situations in natural environments.

(viii) King, Miller *et al* (1971 -) have demonstrated that the formation of corrosive ferrous sulphide is the main contribution made by SRB to corrosion rates of mild steel. They have shown that cathodically active FeS loses this activity after a time and have proposed a model SRB corrosion system in which von Wolzogen Kühr - van der Vlugt depolarization of FeS cathodes occurs allowing continuous high corrosion rates (Figure 28).

CHAPTER FIVE

5 A CRITICAL APPRAISAL OF THE VON WOLZOGEN KÜHR - VAN DER VLUGT MECHANISM

A detailed examination of cathodic depolarization phenomena recorded in the presence of sulphate-reducing bacteria may be justified by a number of reasons. The phenomenon of corrosion caused by these organisms represents a considerable gap in corrosion theory. The answer to the question of whether or not an enzyme system can catalyse electrode reactions may have profound significance to electrochemical and biological science and technology. Suggestions have been made that SRB cultures may act as useful electrode reagents in biological fuel cells[99]. The putative sulphate-reduction cathodic reaction will be examined against the background of existing knowledge of cathodic processes and the biochemistry of sulphate reduction. The scope of the experimental section of this thesis will then be outlined.

The sulphate reduction metabolic pathway of SRB may be of use to the organisms in two possible ways:

(i) The sulphate reduction system serves as a sink for electrons liberated during oxidative metabolic processes.

(ii) Energy fixation, such as phosphorylation resulting in the formation of ATP, may occur during the process.

If either or both of these alternatives are true, cathodic hydrogen (or electrons) must compete for the sulphate reduction system with hydrogen or electrons liberated during metabolic processes. This proposed competition is expressed diagramatically in Figure 29. If there is a yield of biologically useful compounds such as ATP during sulphate reduction this competitive intervention of cathodic processes may be of benefit to the organisms. If no such yield results, cathodic processes catalysed by the organisms would merely be wasteful of sulphate required as an electron acceptor. The organisms would, in such a case, not be expected to flourish in the vicinity of corroding metal. It is well known, however, that the organisms grow vigorously in association with corroding steel and this indicates the possibility of a biologically catalysed cathodic sulphate-reduction reaction of some benefit to the organisms.

An alternative possible relationship between organisms and corroding metal is that a suitable environment for growth of the organisms is generated by corroding steel, for example, by supplying the high dissolved iron content favourable to growth as indicated by Baas Becking *et al*[48], and that metabolic waste products liberated during growth, e.g. sulphides, are themselves corrosive. The role of the organism in corrosion in this case would be a secondary one. We may thus define the following possible roles for SRB in corrosion processes :

(i) *Primary role*: The organisms intervene directly in corrosion reactions, accelerating them, and derive some benefit, possibly ATP, by doing so.

(ii) *Secondary role*: The environment generated by the corroding metal is favourable to growth, possibly in its iron content, and the metabolic waste products of the organisms stimulate corrosion.

A detailed examination of cathodic corrosion processes and sulphate-reduction processes is necessary before deciding upon either of these alternatives or enlarging upon them.

5.1 Thermodynamic considerations

Standard thermodynamic data for some of the pertinent reactions are given in Table 7. Values of ΔG^o used in these calculations and their sources are given in the table. These results indicate the following:

(i) The reduction of sulphate by molecular hydrogen taken up by the hydrogenase system of SRB is thermodynamically possible and may yield energy to the organisms if suitable energy-fixing systems such as phosphorylation systems are present.

(ii) The reduction of sulphate with lactate as hydrogen donor, as practised by SRB (reaction ii) is thermodynamically possible and may result in a yield of energy to the organism.

(iii) The von Wolzogen Kühr - van der Vlugt reaction (reaction iii) is thermodynamically possible when the substances involved are in their standard states. Hoar and Farrer[59] have calculated, using the Nernst relationship with values of $a_{SO_4^{2-}} = 10^{-3}$ $\underline{M}$, $a_{S^{2-}} = 10^{-12}$ $\underline{M}$ and pH = 7 (reasonable values for an agressive natural environment) that the equilibrium potential for

$$SO_4^{2-} + 4H_2O + 8e \rightleftharpoons S^{2-} + 8OH^- \qquad 5.1$$

would be -0,200V (she) making it a thermodynamically possible cathodic reaction (the published value of 0,002V[59] is probably a printing error).

(iv) and (v). During the reduction of sulphate to sulphide, which is known to take place stepwise via sulphite in organisms[39,59], no significant free energy change takes place during the reduction (iv) of sulphate to sulphite. The free energy change accompanying the reduction of sulphite to sulphide (v) is relatively large and is close to the value of that for the overall sulphate reduction process (i).

5.2 The kinetics of cathodic reactions on iron and the von Wolzogen Kühr - van der Vlugt Mechanism

We shall consider here the individual steps of this mechanism (steps 4.5 to 4.9) against the background of established knowledge of cathodic reactions in order to evaluate its validity.

5.2.1. *Can reaction 4.5 supply sufficient hydrogen ion to permit the von Wolzogen Kühr - van der Vlugt depolarization reaction to proceed at current densities observed in polarization experiments?*

The step in question is

$$8H_2O \longrightarrow 8H^+ \quad + \quad 8OH^- \qquad 4.5$$

Can it supply sufficient hydronium ion to support current densities observed during "cathodic depolarization". (e.g. Booth and Tiller[71, 75, 76] report values of up to 100 μA cm^{-2} for this phenomenon and the pH at which these values were reported is close to neutrality, i.e. pH 7). Delahay[102] has provided a means of answering this question by calculations of the maximum current densities associated with the discharge of hydronium ions formed by water dissociation. The calculation is for a plane electrode at which hydronium ion is reduced to hydrogen and water. The possible sources of hydronium ion at the electrode are :

(i) the ions transferred from the bulk of the electrolyte to the interface and,

(ii) hydronium ions formed by water dissociation in the vicinity of the electrode.

The expression derived is

$$i_{cd} = F\, D_{H_3O^+}^{1/2}\; k_r^{1/2}\; C^o_{H_3O^+}\; C_{OH}^{-1/2} \qquad 5.2$$

where k_r is the rate constant for the recombination of H_3O^+ and OH^- ions according to

$$2H_2O \rightleftharpoons H_3O^+ + OH^- \qquad 5.3$$

and has the value 7,85 x 10^{13} (moles per $cm^3)^{-1}$ sec^{-1} at 25° C. The diffusion coefficient, $D_{H_3O^+}$ of hydronium ion is 9,34 x 10^{-5} cm^2 sec^{-1}. Using the value of Onsager[103] for the ionic product of water $C_{H_3O^+}$ x C_{OH^-} of 10^{-20} (moles per $cm^3)^2$, 5.2 simplifies to

$$i_{cd} = 0{,}83 \quad C_{H_3O^+}^{1/2} \qquad 5.4$$

in which i_{cd} is in amp cm^{-2} and $C_{H_3O^+}$ in mol cm^{-3}.

Using this relationship, we may calculate that the maximum possible current density allowed for the discharge of hydronium ion produced by water dissociation, i_{cd}, at pH = 7,0 is 8,3 μA cm^{-2}. This is in the absence of overpotential limitations on the process and must be considered as an absolute maximum. It may thus be concluded that step 4.5 cannot supply hydrogen ion in sufficient quantity to support the current densities observed during "cathodic depolarization" at more or less neutral pH values. The species most likely to feed H_{ads} to the cathodic depolarizing system is water, which is present at pH 7,0 at much higher concentration than is H_3O^+.

Assuming then, that water is the source of cathodic hydrogen, we may write an equation equivalent to 4.5, 4.6 as follows :

$$4Fe + 8H_2O \longrightarrow 4Fe^{++} + 8\,H_{ads} + 8OH^- \qquad 4.6a$$

Now, if the von Wolzogen Kühr - van der Vlugt mechanism were true, any reaction removing this H_{ads} in the absence of catalysis (i.e. in the absence of its being enzymically removed for SO_4^{2-}- reduction) would be a rate determining step in the cathodic hydrogen evolution reaction, and thus in the cathodic corrosion reaction. This is an important point upon which hinges the validity of the von Wolzogen Kühr - van der Vlugt mechanism. The questions to be answered are as follows :

Is the removal of H_{ads} a rate-determining step in the attack of neutral de-aerated water on metallic iron? In a system in which iron is corroding by an electrochemical mechanism at neutral pH, can the overall process be accelerated by accelerating the removal of H_{ads} ? This is a question in electrochemical kinetics and we may draw an answer from established data.

5.2.2 *The kinetics of cathodic reactions on iron*

We know the rate of corrosion of iron in neutral de-aerated water to be extremely small. If the von Wolzogen Kühr - van der Vlugt mechanism is true, iron in neutral de-aerated water corrodes at an infinitesimally slow rate because of very slow rate-determining reactions involving the removal of cathodically-formed H_{ads} . An examination of the electrode kinetics of the hydrogen evolution reaction on iron will tell us if this is so.

It is necessary here, to introduce the possible mechanism schemes for the h.e.r. in order to discuss depolarization reactions any further. The possible mechanisms are well-documented in electrochemical textbooks[31,104], and one of these is described in reactions 2.32 - 2.34. The possible steps are as follows :

$$M + H_3O^+ + e \longrightarrow MH_{ads} + H_2O \text{ (acid solution)} \qquad 5.5a$$

$$M + H_2O + e \longrightarrow MH_{ads} + OH^- \text{ (neutral and alkaline solutions)} \qquad 5.5b$$

M represents the metal surface at which the reaction takes place. Subsequent to the formation of MH_{ads}, the reaction may proceed along two possible paths :

(i) Chemical desorption may occur :

$$2\,MH_{ads} \longrightarrow 2M + H_2 \qquad 5.6$$

(ii) Electrodic desorption may occur

$$MH_{ads} + H_3O^+ + e \longrightarrow M + H_2O + H_2 \text{ (acid solution)} \qquad 5.7a$$

$$MH_{ads} + H_2O + e \longrightarrow M + OH^- + H_2 \text{ (neutral and alkaline solutions)} \qquad 5.7b$$

Electrochemical criteria, some of which are described in section 2.4, may be used to diagnose which of the above steps is rate-determining. If the removal of H_{ads} is not rate-determining, increasing its removal rate will have no influence on the overall cathodic charge transfer rate. Three groups of experimental data will be invoked in discussing the kinetics of the h.e.r. on iron.

(i) *Differences between heats of activation for H_2O discharge and H_3O^+ discharge*

Parsons and Bockris[105] using the reasoning of absolute rate theory, calculated the difference in heats of activation of reactions 5.5 (a) and 5.5 (b). Standard enthalpy data for the reacting species and products were used as follows :

For 5.5 (a)

$$H^o_{5.4(a)} = H^o(H_{ads})_{5.4(a)} - H^o(H_3O^+) - H^o(e) \qquad 5.8(a)$$

and for 5.4 (b)

$$H^o_{5.4(b)} = H^o(H_{ads})_{5.4(b)} + H^o(OH^-) - H^o(H_2O) - H^o(e) \qquad 5.8(b)$$

The standard enthalpy of dissociation of water into its ions, $H^o(H_3O^+) + H^o(OH^-) - H^o(2H_2O)$, was taken as 13,6 k cal. The difference of the standard heat content of adsorbed hydrogen for the two reactions was taken as arising from the interaction energy difference between the couples H_{ads} and H_2O and H_{ads} and OH^-. Calculation of these interaction energies from potential energy-distance reasoning enabled the difference in activation energies to be calculated:

$$H^{o}_{5.4(b)} - H^{o}_{5.4(a)} = (H^{o}(H_{ads})_{5.4(b)} -$$

$$H^{o}(H_{ads})_{5.4(a)} + H^{o}(H_3O^{+}) + H^{o}(OH^{-}) - H^{o}(2H_2O) \qquad 5.9$$

as being between 7 and 14 k cal.

Bockris, Drazic and Despic[106], examined experimental electrode kinetic data for hydrogen evolution from H_3O^+ and H_2O species on iron electrodes. From the exchange current densities for hydrogen evolution from H_3O^+ and H_2O they calculated that the heat of activation for hydrogen evolution on iron from H_2O was 9,6 k cal mol^{-1} greater than that from H_3O^+. This was within the range predicted by Parsons and Bockris and was considered to be consistent with the argument that charge-transfer reactions forming H_{ads}, i.e. reactions 5.5 (a) and 5.5 (b), were rate determining steps in the hydrogen evolution reaction on iron.

(ii) *Transfer coefficients, α, for the hydrogen evolution reaction on iron*

Using the reasoning developed in Chapter 2, we may calculate from

$$\vec{\alpha} = \frac{\vec{\gamma}}{\nu} + r\beta \qquad 2.36$$

(using the values of γ,ν and r, appropriate to the various mechanisms and assuming a symmetry factor, β, of 0,5) what the anticipated values of α for the mechanisms 5.5 - 5.7 would be. Calculated values with the consequent Tafel slopes are given in Table 8. Most observed Tafel b values for the h.e.r. on iron approximate to 0,12V in both acidic and neutral solutions[104,106-108]. This indicates a transfer coefficient,α of 0,5 allowing us to eliminate

(i) discharge followed by rate-determining chemical desorption.

(ii) discharge followed by rate-determining electrodic desorption.

as possible reaction mechanisms.

It is apparent, thus, that this type of experimental data indicates also, that the discharge of H_3O^+ or of H_2O species is the rate-determining step in the h.e.r. on iron.

(iii) *Hydrogen pressure/Hydrogen overpotential data*

Enyo[109,110] has introduced a diagnostic method for hydrogen evolution mechanisms based upon the relationship between hydrogen pressure and hydrogen overpotential. The reasoning used by Enyo was that, if the discharge step (i.e. 5.5) were rate-determining, the other step (5.6 or 5.7) would be in quasi-equilibrium. This would mean that, were the ambient hydrogen pressure kept constant, the activity of the adsorbed intermediate H_{ads} would remain constant, irrespective of overpotential. Conversely, if steps 5.6 or 5.7 were rate-determining, the discharge step (5.5) would be in quasi-equilibrium and $a_{H_{ads}}$ would be dependant upon η. Considering hydrogen pressures p_{H_2} hypothetically in equilibrium with H_{ads}, Enyo concluded that if 5.5 is rate-determining p_{H_2} should be independent of η. Where 5.6 or 5.7 is rate-determining p_{H_2} should vary with η according to

$$- \eta = (\tfrac{1}{2} f) \ln p_{H_2} / p_{H_2 eq} \qquad 5.10$$

where $f = F/RT$ and $p_{H_2 eq}$ is the value of p_{H_2} where the overall reaction is at equilibrium. Hydrogen pressure/ hydrogen overpotential data studied by Enyo were consistent with the discharge step (5.5) being rate-determining.

We must conclude, therefore, that most established electrochemical data indicate that the slow discharge - rapid desorption mechanism is the most likely one for the h.e.r. on iron. Plotting the available electrochemical data on an Evans type of corrosion diagram (Figure 30), we may evaluate the statement that H_{ads} causes "cathodic polarization", and that its removal will accelerate corrosion rates. It is evident, from the reasoning accompanying this diagram, that this is not the case.

Since it seems unlikely from the foregoing reasoning that catalysed sulphate reduction using H_{ads} formed at cathodes on iron would affect the rate of cathodic charge transfer, we must next consider whether the reaction

$$SO_4^{2-} + 4H_2O + 8e \longrightarrow S^{2-} + 8OH^- \qquad 5.1$$

proceeding by *any* mechanism whatsoever is likely to be the cathodic reactions in SRB corrosion.

5.3 Is reaction 5.1 the cathodic reaction in SRB corrosion?

Thermodynamics (Table 7) gives the answer that reaction 5.1 is an energetically possible reaction. Existing literature on the cathodic reduction of sulphate tells us that it is an extremely difficult reaction to perform. Cathodic formation of sulphide can be achieved, but has only been reported in concentrated sulphuric acid at high voltages and at temperatures greater than 160° C[111]. We have indicated above that it is unlikely that this reaction, proceeding *via* the mechanism of von Wolzogen Kühr and van der Vlugt, is the cathodic reaction of SRB corrosion. In considering the possibility of its occurence *via* some other mechanism catalysed by SRB, we shall examine the nature of hydrogenase enzyme and the details of the sulphate-reduction reaction as performed by SRB.

Hydrogenase enzyme, as first described by Stephenson and Stickland in 1931[65 66], was an enzyme catalysing the reduction of substances by *molecular* hydrogen. For example, in the case of sulphate reduction :

$$SO_4^{=} \quad + \quad 4H_2 \quad \longrightarrow \quad S^{=} \quad + \quad 4H_2O$$

is the reaction catalysed. The relatively large number of experimental investigations that have been made of hydrogenase systems since that date have all described the enzyme as catalysing the reduction of some species by molecular hydrogen (dissolved H_2 or H_2 gas)[112-129]. Moreover the accumulation of biochemical knowledge since that date has allowed a more precise definition of the role of hydrogenase to be given. The systematic name of hydrogenase enzyme as defined by the International Union of Biochemistry (1964)[130] is, "Hydrogen : ferredoxin oxidoreductase"(classification number 1.12.1.1.) It is a catalyst for the reduction

$$H_2 \quad + \quad 2 \quad \text{ferredoxin} \longrightarrow 2 \text{ Reduced ferredoxin} \qquad 5.11$$

The reduced ferredoxin thus produced may be directed into various biological reduction pathways, such as the reduction of sulphate, nitrate, fumarate, or redox dyes. It must be emphasised that the function of hydrogenase is to catalyse equilibria involving *molecular* hydrogen and a reducible species. Green and Stickland demonstrated the equivalence of hydrogenase of *Escherichia coli* with colloidal palladium in catalysing the reduction of methyl viologen by molecular hydrogen[131]. Hydrogenase-catalysed sulphate reduction using cathodically-liberated hydrogen would seem unlikely, as no molecular hydrogen would be available (apart from the extremely small quantities formed by h.e.r. during corrosion in neutral solutions, see Figure 30) to feed the hydrogenase enzyme system. Were H_{ads} presented to the enzyme system, a reaction more consistent with the properties of hydrogenase would be :

$$2H_{ads} \xrightarrow{\text{hydrogenase}} H_2 \qquad 5.12$$

but we have indicated above (section 5,2) that this would be unlikely to affect the cathodic charge transfer rate. If this argument is correct, there must be another explanation for the correlation between hydrogenase activity and depolarizing activity, observed by Booth and Tiller[86], than that advanced by the authors themselves. An alternative explanation will be advanced in discussing the experimental results of this thesis.

A careful examination of the biochemistry of the dissimilatory sulphate reduction system allows a further evaluation of 5.1 as a possible cathodic reaction during corrosion, on biochemical grounds. The question to be answered is : Is the reduction of sulphate to sulphide gainful to the organisms in terms of energy? If this were so, reduction of sulphate by cathodic charge *via* the organisms' enzyme systems might be advantageous to them. The most likely biological energy-fixing system in this case would be a phosphorylation system leading to the formation of ATP. We shall exmmine the possibility of phosphorylation taking place during sulphate reduction. A comprehensive review of the biochemistry of dissimilatory sulphate reduction has been published by Roy and Trudinger[59]. A schematic summary of the sulphate reduction pathway presented by these authors is given in Figure 31. If hydrogenase-catalysed cathodic charge transfer to the sulphate reduction system is unlikely (for reasons given above), it is possible that ferredoxin or cytochrome C_3 may be reduced directly at the cathode, and the reduced substances fed into the sulphate reduction pathway, as indicated. Such a process would interfere with the rôle of sulphate as a sink for electrons liberated during oxidative metabolic processes. The only possible benefit of such a process (cathodic reduction of ferredoxins or cytochrome C_3 and, ultimately, of sulphate) to the organisms would lie in the production of biologically useful compounds such as ATP. The turnover of high-energy phosphate bonds during dissimilatory sulphate reduction has been investigated by Peck[128,129,132-135], and as a result, it is possible to divide the sulphate reduction process into two parts which will be described separately.

(i) *The reduction of sulphate to sulphite*

Peck[132] isolated a cell-free system which could reduce sulphate to sulphite under an atmosphere of hydrogen. During this process, one mole of ATP was consumed for each mole of H_2 taken up. Peck postulated the existence of an ATP sulphurylase system catalysing the formation of adenosine phosphosulphate (APS) as the primary step in sulphate reduction.

$$ATP + SO_4^{=} \longrightarrow APS + PP_i \qquad 5.13$$

(PP_i represents inorganic metaphosphate)

Subsequent studies of this system have confirmed that this is the case. It has further been demonstrated[135] that this APS is subsequently reduced to sulphite and adenosine monophosphate in the presence of molecular hydrogen, the reaction being catalysed by an APS - reductase enzyme system :

$$\text{APS} \quad + \quad H_2 \xrightarrow[\text{reductase}]{\text{APS}} \text{AMP} \quad + \quad SO_4^{2-} \quad + \ 2H^+ \qquad 5.14$$

Thus far, the reduction of sulphate to sulphite has involved the consumption of one mole of ATP per mole of sulphate reduced.

(ii) *The reduction of sulphite to sulphide*

The intermediate steps in this process are not as well known as those in the reduction of sulphate to sulphite. The presence of a sulphite-reductase system catalysing

$$SO_3^{2-} \quad + \quad 3H_2 \longrightarrow S^{2-} \ + \ 3H_2O \qquad 5.15$$

has been demonstrated[136], but the details of the process are not yet clearly established. Peck[134] has reported evidence of phosphorylation coupled with sulphite reduction, but as yet this has not been satisfactorily confirmed. For the sulphate to sulphide reduction as a whole to be gainful in ATP production, at least two moles of ATP must be produced per mole of sulphite reduced, since the sulphate-sulphite reduction consumes one mole of ATP. Thermodynamics (Table 7) tells us that the production of 2 moles of ATP is energetically possible. The free energy yield of sulphite reduction (31,27 k cal) would be sufficient to allow 2 moles of ATP to be formed per mole of sulphite reduced. The basis for this statement is that the calculated value of Burton and Krebs[101] of 9,4 k cal mol^{-1} for pH 7,5 and the unit activity of ATP (and 13 - 16 k cal mol^{-1} for the concentrations found in animal tissues), for the free energy change associated with the hydrolysis of ATP to ADP and inorganic phosphate.

While it is possible energetically for the sulphate-reduction process to be gainful in energy to the organisms, for example, via phosphorylations, Khosrovi, MacPherson and Miller have demonstrated that hydrogenase-catalysed sulphate-reduction (accompanying hydrogen uptake) competes against organic substrate oxidation (e.g. lactate oxidation) for the sulphate-reduction system[137]. They have demonstrated that in actively growing cultures where H_2 is supplied, the organic substrate oxidation process competes successfully against H_2 oxidation for the sulphate-reductase system. In manometric experiments they found that H_2 oxidation was completely inhibited during active growth. Only in resting-cell suspensions (i.e. non-growing cells suspended in buffer solutions) did hydrogen uptake coupled with sulphate-reduction occur. This indicates that, were hydrogenase-catalysed depolarization accompanied by sulphate-reduction to take place in the presence of SRB, this would not be achieved by actively growing cells and that no apparent benefit in terms of energy uptake would accrue to the organisms.

5.4 Summary of chapter 5 and outline of scope of experimental work to be conducted

In this chapter, the von Wolzogen Kühr - van der Vlugt mechanism has been examined against the background of established thermodynamic, electrode kinetic, and biochemical data. Thermodynamics indicates that the overall reaction scheme is possible, but kinetic data, particularly regarding the presence of H_{ads} at electrode surfaces as a rate-limiting factor, indicates that the steps postulated by von Wolzogen Kühr and van der Vlugt (Steps 4.5 - 4.9) are unlikely. Other possible mechanisms of cathodic depolarization accompanied by sulphate-reduction have been examined. The utilisation of cathodic electrons for sulphate-reduction by the SRB may have advantages for the organisms in ATP formation. Energetically, the use of cathodic electrons for sulphate-reduction by the SRB may hold advantages for the organisms but as yet, no direct experimental evidence of this exists. Indirect experimental evidence (i.e. that of Khosrovi, MacPherson and Miller[137]) indicates that the organisms would not benefit by such a process.

The objectives of the experimental work conducted by the author were as follows :

(i) To study the mechanism of the cathodic depolarization phenomenon exhibited by *Desulfovibrio* species. In particular, it is proposed to demonstrate whether or not sulphate reduction catalysed by these organisms is the cause of observed depolarization phenomena.

(ii) To investigate the mechanism of corrosion of ferrous metals by the organisms.

CHAPTER SIX

6 THE ORGANISMS STUDIED : THEIR ENRICHMENT-, ISOLATION, AND MAINTENANCE CULTURING

Pure cultures of *Desulfovibrio* species were required for the electrochemical experimental work, and these were isolated from a number of sources. Inocula were subjected to enrichment culturing, purification, and contaminant-testing prior to identification and the growth of experimental cultures.

6.1 Enrichment culture

Soil-, mud-, or corrosion-product samples were inoculated into the medium of Baars[56],(see appendix to Chapter 6) supplemented with 0,1% (W/V) of yeast extract, with Na_2S added to a concentration of 0,5 m M for poising of the redox potential. Where marine inocula were used, 2,5% (W/V) NaCl was added to this and to all subsequent media used before sterilizing. 30 cm^3 screw-capped bottles were used for enrichment culturing. Bottles were filled to the brim with sterile medium to displace all air after inoculation of the 25cm^3 of medium previously added, sealed, and incubated at 30°C. For reference purposes, pure cultures for *Desulfovibrio vulgaris* (Postgate and Campbell) were raised from freeze-dried material obtained in ampoules from the National Collection of Industrial Bacteria, Torrey, Aberdeen (reference number of culture used : NCIB 8303). In this case, the contents of ampoules were incubated under hydrogen in Baars' medium, in McIntosh and Fildes' anaerobic jars. When blackening of the medium, caused by the precipitation of FeS, indicated that sulphate reduction was occurring cultures were examined microscopically. The time required for blackening varied from 2 or 3 days for mud samples to 12 days for freeze-dried material. When staining and microscopy indicated a preponderance of gram-negative vibrioid organisms, the purification procedures were followed.

6.2 Purification procedures

Three purification procedures were assayed and the last of these found to be the best.

6.2.1 *The "Stab tube" method of Postgate*

The medium (see appendix to Chapter 6) and isolation method of Postgate[138] was used. The sterile, molten medium was dispensed into 150 x 10 mm soda glass tubes with spring-locking aluminium sterile caps. The medium was maintained in molten condition by immersion of the tubes in a water bath at 40°C. Inoculation of these isolation tubes was by dipping a closed, flamed Pasteur pipette into the enrichment culture to be purified and then successively into each of six tubes of isolation medium. The isolation tubes were then incubated at 30°C until the appearance of black colonies suitable for isolation. Figure 32 shows a series of tubes containing successive dilutions with colonies of this type. Tubes were broken open aseptically at the point of colony growth, and colonies removed with sterile Pasteur pipettes for inoculation into fresh, sterile Baars' medium. The resultant cultures were subjected to purity checking as in Section 6.3. This purification method was successful in a few cases, but too often insufficient colony-isolation was achieved, and consequently alternative methods were attempted.

6.2.2 *The agar plate method of Iverson*

Iverson[139] had reported successful growth of colonies of *Desulfovibrio* species on the surface of media containing Trypticase Soy Agar (TSA). It was considered possible that serial dilutions of enrichment cultures in Iverson's TSA + lactate medium (appendix to Chapter 6) poured as plates in Petri dishes might provide suitable colonies for isolation : dilutions of cultures (10^{-1} to 10^{-3} times) were made in TSA + lactate medium, plates poured, and incubated under hydrogen in McIntosh and Fildes' anaerobic jars. Well-isolated colonies were produced, but growth of the organisms on transference to liquid medium seldom occurred, probably because of over-much exposure of the colonies to atmospheric oxygen during manipulation.

6.2.3 *The dilution method of Sisler and Zobell*

Ultimately, a modification of the method used by Sisler and Zobell[140] was the most successful. The method used can best be described by reference to Figure 33.

The isolation medium of Postgate[138] was prepared and dispensed in 4,5 cm^3 quantities into each of 4 of 20 cm^3 sterile test tubes fitted with aluminium caps. The medium was maintained in the molten state by storage of the tubes in a water bath at 45°C. The medium of MacPherson and Miller[98], supplemented with 0,1%(W/V) of yeast extract and treated with $FeSO_4$ to 25 µ<u>M</u> and Na_2S to 0,5 <u>m M</u> (appendix to Chapter 6) was dispensed in 0,9 cm^3 quantities into each of 4 sterile 5 cm^3 screw-capped bottles. The isolation procedure was then conducted as in Figure 33.

A 0,1 cm^3 quantity of enrichment culture was transferred to the first of the four tubes containing liquid medium (Stage I), giving a dilution factor of 10^{-1}. The operation was completed for each successive tube, giving serial dilutions of 10^{-1} to 10^{-4}. A 0,5 cm^3 quantity of each of these dilutions was transferred to the agar-containing medium in the tubes in the water bath, and the resulting dilution mixed well by rolling the tubes between the palms (Stage II). By this means, dilutions of 10^{-2} to 10^{-5} were produced. Finally, the contents of each of these tubes were drawn up into a sterile glass tube of 2 mm diameter and 600 mm in length (Stage III). The ends of the tubes were sealed with sterile molten paraffin wax and the tubes incubated at 30°C. Well-isolated black colonies were usually produced in the 10^{-4} and 10^{-5} dilutions (see Figure 34). Tubes were broken open aseptically at the points at which colonies were observed and colonies transferred into freshly prepared sterile Baars' medium with sterile Pasteur pipettes. The Baars' medium was contained in aluminium-capped 20 cm^3 test tubes in this case, and incubation was at 30°C under hydrogen in McIntosh and Fildes' anaerobic jars.

6.3 Criteria of purity

6.3.1 *Contaminant-testing*

Contaminant-testing was carried out for both aerobic and anaerobic contaminants, using the procedures of Postgate[138]. These are described in the appendix to this chapter.

6.3.2 *Microscopy*

Cultures were examined microscopically for purity. After initial isolation from enrichment cultures as above, stock cultures were examined as follows.

(a) Droplets of culture were examined for vibrioid motion and the presence of contaminants apparent while cells were motile.

(b) Gram-staining was performed.

(c) Specimens were prepared for electron microscopy. Figures 35 - 37 show some of the results of this examination.

6.3.3 *Periodic checks for purity*

Cultures were checked for purity microscopically by methods (a) and (b) whenever a batch of inoculations for electrochemical experiments were performed. Great difficulty was not experienced in maintaining cultural purity. When contaminants were observed, cultures were re-purified. This was required on one occasion for each of three of the stock cultures used over a period of three years (four cultures, each from different inocula were maintained as stocks).

6.4 Taxonomic criteria

For the purpose of the present experimental investigation, it was considered necessary to identify cultures only as far as the genus. The accepted taxonomic criteria for the genus *Desulfovibrio* are clearly stated by Postgate and Campbell[53, 54]. All organisms used in the experimental work were assigned to the genus *Desulfovibrio* on the following grounds :

(a) Anaerobic growth in media containing lactate and sulphate took place, with concomitant reduction of sulphate to sulphide.

(b) Polar flagellation was observed in all cases.

(c) Desulfoviridin was present in all cases, as evidenced by positive fluorescence tests, using the method of Postgate[141] (see appendix to this chapter).

(d) Hydrogenase enzyme was present in all cases, as evidenced by benzyl viologen reduction under a hydrogen atmosphere in Thunberg tubes.

6.5 The organisms used in the experimental work and their sources

Each enrichment culture was accorded a name indicating its origin, before positive identification as a species of *Desulfovibrio*. All cultures used were shown to be species of *Desulfovibrio* but differed slightly in morphology and hydrogenase activity. The source names were used throughout the experimental work to distinguish between the cultures.

These were as follows :

6.5.1 "*Black River*" (abbreviated: BR)

This was isolated from an enrichment culture prepared from an inoculum of bottom mud from the Black River in Mowbray, Cape.

6.5.2 "*Durban*" (abbreviated: DB)

The corrosion product of a water pipe beneath a hospital in Durban was the inoculum in this case.

6.5.3 "*Hildenborough*" (abbreviated: HIL)

This was raised from freeze-dried material acquired from the National Collection of Industrial Bacteria, Torrey, Aberdeen, and has been positively identified as *Desulfovibrio vulgaris* (Postgate and Campbell) (NCIB 8303). The source name "Hildenborough" has been used frequently in the literature describing experiments with this organism.

6.5.4 *"Walvis Bay"* (abbreviated:WVB)

This was isolated from an enrichment, in Baars' medium, of an inoculum of inter-tidal mud from Walvis Bay, South West Africa. The culture was maintained throughout the work in media supplemented with 2,5% (W/V) sodium chloride.

6.6 Maintenance culture and the growth of experimental cultures

Liquid media were considered most suitable for electrochemical experimental work. Baars' medium, which contains a precipitate was suitable for enrichment and for initiating growth after isolation of colonies during purification procedures. When this had been achieved, the organisms were sub-cultured into the medium of MacPherson and Miller[125], supplemented with 0,1% (W/V) of yeast extract. Immediately, prior to inoculation, sterile $FeSO_4.7H_2O$ solution was added to give a concentration of 25 $\mu\underline{M}$ and sterile $Na_2S.9H_2O$ solution to a concentration of 0,5 $m\underline{M}$.

Using this medium it was found possible to grow all of the cultures to any volume required in screw-capped bottles with rubber seals provided that all air was displaced by filling bottles to the brim with medium. Figure 38 shows the incubator used, and a variety of the cultures. Stocks were maintained in 30 cm^3 screw-capped bottles, sub-culturing weekly into fresh medium.

CHAPTER SEVEN

7 ELECTROCHEMICAL METHODS

7.1 Electronic equipment

The theoretical background to potentiostatic studies is described in detail in chapter 2. Electronic methods of achieving potentiostatic control are well-documented and do not require detailed discussion here. The basic layout of a potentiostatic circuit, including the electrochemical cell is given in Figure 39. Three different electronic instruments were used at various stages of this work to achieve potentiostatic control. These were :

(a) A Wenking 61 TR potentiostat

(b) A Tacussel model PRT 100 potentiostat

(c) An operational amplifier-controlled system constructed for the purpose, the electrical circuit for which is given in Figure 40.

The characteristics of these instruments are given in the appendix to this chapter. In some cases during this work, automatic variation of the reference voltage (and thus the controlled voltage) was achieved by means of motor-driven potentiometers and in others manual operation was used. The method employed and the scanning speeds will be described where necessary. Where other electronic equipment was used, the relevant data will be given where required.

7.2 The polarization cell and electrodes used

The polarization cell assembly used throughout this work is depicted in Figure 41. The cell was basically a 5-necked flask, water jacketed for temperature control, with an outlet tube at the bottom. The auxiliary electrode was a circumferential loop of platinum wire attached to the inside of the cell. The necks of the flask contained the following :

(i) A cylindrical mild steel working electrode assembly, the details of which are described in section 7.3.

(ii) A reference electrode probe consisting of a Luggin capillary bridge of bore size, 0,2mm at the tip, and set at a distance of 0,5mm from the working electrode surface. This was filled with a 3% (W/V) agar gel, 0,02 M in NaCl. The reference electrode used throughout was a saturated KCl-calomel electrode (sce).

(iii) A gas-purging device consisting of 2 concentric tubes, the inner one of 2mm bore, the outer slightly larger, independently connected to the purging gas supply by means of a tap such that the purging gas could be passed through either inner or outer tube. The inner tube projected below the surface of the electrolyte during operation : the outer one ending approximately 2cm above the surface, being used for head-space purging.

(iv) A pH electrode assembly consisting of a Jena type micro-electrode inserted through a PTFE seal in a B "Quickfit" cone assembly.

(v) The fifth neck, of diameter 4mm, was usually left free to allow gas outlet during purging. It was also used to add reagents to the electrolyte by means of micro- or Pasteur pipettes, and to withdraw samples of solutions for analysis where required.

7.3 The working electrode assembly

Considerable attention was devoted towards developing a working electrode assembly which would be reproducible and would give quantitatively meaningful results. Basically the electrode was a compression-gasket assembly similar to that of Stern and Makrides[142]. The gasket was of PTFE, and compression was achieved by torque on the nut at the top of the glass holder. (see Figure 42).

The mild steel specimen was a cylinder of diameter approximately 8mm and length 15mm, of EN 3A mild steel of nominal composition : C 0,15 - 0,25%; Si 0,05 - 0,33%; Mn 0,40 - 0,90%; P 0,060% max; S 0,060% max; balance Fe. Stages in the preparation of this assembly before each experimental run were as follows :

7.3.1 The cylinder, which was threaded internally, was mounted on a threaded chuck and rotated on the shaft of an electric motor. Wet grinding with successively finer grades of metallurgical grinding papers to grade 600, was then performed.

7.3.2 After this grinding the steel cylinder was rinsed in distilled water, dried in acetone with an airblast, and mounted in the compression gasket holder.

7.3.3 The electrode was then electropolished in a 5% perchloric acid in glacial acetic acid solution. This operation was performed, using a spiral of platinium wire as counter-electrode, in a beaker with a cooling-water jacket. During polishing, the solution was agitated vigorously using a PTFE-coated magnetic stirrer. It was found that 1,0 to 1,5 A at 60V for 10 - 15 seconds gave a suitable smooth specular surface.

7.3.4 The polished electrode was again rinsed in distilled water and dried using A.R. acetone. A coating of nitrocellulose lacquer was then applied as in Figure 43, using a strippable polythene foil for masking, such that the exposed electrode surface area was a band of width 5mm. The diameter of the electrode was checked with vernier callipers after each experiment to enable accurate calculation of electrode surface area.

7.3.5 Immediately before use, the electrode was lightly etched in 1,0 M HCl for 5 seconds, rinsed well with distilled water, and inserted into the experimental electrolyte. It was found that this method of preparation gave a specular, lightly-etched surface, a photomicrograph of which is given in Figure 43(a).

7.4 Calibration of the polarization system using hydrogen ion diffusion currents

Since the polarization work to be performed during this investigation was to be done in electrolytes of unknown electo-chemical nature, it was decided to test the experimental methods using a known electrochemical polarization system. The hydrogen ion/hydrogen gas system was chosen, as this has been extensively studied by Stern[33 34].

7.4.1 *The procedure*

(i) The pH-electrode was calibrated against standard 7,00 pH buffer and the experimental cell assembled. Water was circulated through the jacket from a thermostatically controlled bath, to maintain the temperature at 25 $\pm$ 0,2^{o}C.

(ii) 100 cm^3 of a 0,02 M solution of sodium chloride was introduced into the cell, and vigorous gas-purging of the electrolyte with high-purity ("white-spot") nitrogen commenced.

(iii) During nitrogen-purging, the pH of the electrolyte was adjusted to the required value by means of dropwise additions of dilute HCl or NaOH solutions.

(iv) Gas-purging was continued for a period of 2 hours, during which time the working electrode was suspended above the electrolyte surface. After this period the electrode was immersed and purging continued for a further 10 minutes, after which the tap on the gas-purging assembly was adjusted to permit nitrogen to pass through the head-space of the cell without agitating the electrolyte.

(v) The working electrode was allowed to reach a steady potential, and cathodic polarization commenced at a rate of 500 mV per hour.

7.4.2 *Results*

The results compare well with those obtained by Stern[33] in similar studies using pure iron electrodes in a 4% W/V NaCl electrolyte. The following points are notable :

(i) At values close to the corrosion potential, large current changes accompanied small shifts in potential (Figure 44). These currents represent cathodic charge transfer accompanying the reaction

$$2H_3O^+ + 2e \longrightarrow H_2 + 2H_2O \qquad 7.1$$

(ii) At current densities dependent upon pH, the curves adopt a slope parallel to the potential (vertical) axis, representative of a diffusion limitation on the rate of 7.1. In these zones, the rates of 7.1 are dependent upon the rates of diffusion of H_3O^+ ions from the bulk of the solution to the electrode/electolyte interface. Using the expression derived from Fick's First Law by Stern[33] (This expression is similar to 2.64 described previously).

$$i_{dl} = \frac{nFD}{\delta} (10^{-3}) \quad a(H_3O^+) \qquad 7.2$$

(where i_{dl} = limiting diffusion current in amp / cm^2, n = the number of electrons taking part in the reaction, F = the Faraday constant, δ = the thickness of the diffusion layer (cm), $a(H_3O^+)$=the activity of H_3O^+ ions in mol/litre and D = the diffusion coefficient for H_3O in cm^2/sec). With D = 7,39 (10^{-5}) cm^2/sec, and δ = 0,05 cm, (a value commonly assumed for δ) i_{dl} becomes

$$i_{dl} = 0,143 \; a(H_3O^+) \qquad 7.3$$

or

$$\log i_{dl} = 0,845 - pH \qquad 7.4$$

Plots of log i_{dl} *versus* pH (Figure 45) show that a linear relationship was closely followed over the range of pH studied. The slope of the experimental plot is given by

$$\log i_{dl} = 1,15 - pH \qquad 7.5$$

The constant (-1,15) was somewhat greater than that obtained by Stern[164] for pure iron, but agreement was considered sufficiently good to give validity to quantitative interpretations of subsequent cathodic studies.

(iii) At current density values greater than i_{d1} the curves followed Tafel dependence, the slope being -0,178 log i, agreeing well with the value of -0,174 log i, obtained by Stern[33] for pure iron. This latter, linear portion of the curves corresponds to the activation-controlled reduction of water according to

$$2H_2O \quad + \quad 2\,e \longrightarrow H_2 \quad + \quad 2OH^- \qquad 7.6$$

C H A P T E R   E I G H T

8 CATHODIC POLARIZATION STUDIES IN CULTURES OF SRB: DEMONSTRATION OF CATHODIC DEPOLARIZATION ACTIVITY, AND PRELIMINARY OBSERVATIONS REGARDING THE ACTIVE SPECIES

The first requirement of the experimental study of cultures was to reproduce the cathodic depolarization effects demonstrated by Booth and Tiller[72,75,76], and by Horvàth and Solti[70]. The experimental technique used in these early experiments required that the cultures under study be grown in the experimental polarization vessel in continuous contact with the mild steel electrodes, and requirements of sterility-maintenance prevented the preparation of a fresh electrode at the time of each polarization experiment. It was decided not to employ this technique for two reasons.

i) The experimental electrode would corrode and thus its surface area would change.

ii) Preliminary experiments showed that films of iron sulphide developed on the metal surface, and the presence of these films introduced additional depolarization effects to those demonstrated by the cultures alone (See section 4.2).

Accordingly, cultures were grown separately in screw-capped bottles and added to the polarization cell immediately before polarization experiments were performed. It was considered that the time required to perform an experiment (a maximum of three hours after opening the culture bottle) precluded the possibility of any contaminant organism arising from accidental exposure to the air or lack of sterility of the polarization cell, being responsible for observed effects.

8.1 Demonstration of cathodic depolarizing activity in experimental cultures

Experimental cultures were grown for this purpose in 1,3 dm^3 screw-capped bottles in the medium of MacPherson and Miller[98] (appendix to chapter 6). The medium was sterilized in the bottles by autoclaving leaving a 100 cm^3 head-space. Before inoculation, the necessary pH and redox potential adjustments were made. A 30 cm^3 inoculum of active culture was added, and the bottle filled to the brim with sterile medium. The stopper was tightened and the culture incubated at 30^{o}C. When cultures became turbid and microscopic examination showed the presence of large numbers of active, motile organisms, the polarization experiments were performed as follows :

8.1.1 *Experimental method*

(i) The polarization cell was assembled and water at a temperature of $30 \pm 0{,}2\,^{\circ}C$, from a thermostatically controlled bath circulated through the water jacket.

(ii) A brisk stream of high-purity nitrogen was passed through the cell, at present empty of electrolyte, for a period of twenty minutes.

(iii) The culture was added against this stream of nitrogen, and when sufficient had been added to allow a polarization plot to be recorded (approximately 100 cm^3), the nitrogen purging device was adjusted to the head-space purging position. It was found that passing the purging gas through the active cultures for excessively long periods (five minutes or more) caused a rise in pH, from values of approximately 6, to alkaline pH values of 9 or more, and any results obtained with the cultures in this condition would not necessarily be representative of the behaviour of undisturbed cultures. It was thus decided to limit the period of exposure to purging gases to the shortest possible. The redox potential values of the active cultures (see section 8.3) precluded the possibility of dissolved oxygen being responsible for any observed electrochemical effects.

(iv) The mild steel working electrode was prepared as in section 7.3 and lowered into position in the culture.

(v) When the potential of the working electrode became steady (an interval of 10 - 15 minutes was required), cathodic polarization was commenced at a rate of 500 mV per hour.

(vi) The polarization runs were repeated on sterile growth medium with pH adjusted to the value at which cultures had been inoculated (7,2). It was found that the set pH remained constant during purging with nitrogen, and sterile media were purged for one hour before performing polarization runs.

8.1.2 *Results*

The results of polarization experiments performed in cultures "Black River", "Durban", "Hildenborough" and "Walvis Bay", (these names will be abbreviated in future references, to BR, DB, HIL, and WVB, respectively) are reflected in Figure 46. It is evident that the cultures of the four organisms examined exhibit the phenomenon described as "cathodic depolarization" by Booth and Tiller[71, 75, 76].

8.2 Retention of depolarizing activity in cell-free centrifugates

Cell-free centrifugates of the cultures were examined for cathodic depolarizing activity. Active cultures were centrifuged at 5×10^4 g for 10 minutes, and the polarization experiments performed using the clear, cell-free centrifugates. The results are given in Figure 47. Clearly a large proportion of the depolarizing activity of cultures was retained in cell-free centrifugates.

This observation was clearly of some importance since Booth and Tiller[86], as a result of their experiments with redox dyes, had concluded that for SRB to cause depolarization, physical contact of bacterial cells with the electrode surface was necessary.

The retention of depolarizing activity in cell-free centrifugates was characteristic of all cathodically active cultures used during this investigation, and indicated that whatever the nature of the depolarizing species (an enzyme or simpler organic or inorganic substance) it is soluble, and is produced by the organisms during growth. The simplest species in solution likely to bring about cathodic depolarization are dissolved oxygen and hydrogen ion. The possibility of contributions from these two causes to the observed results will now be examined.

8.3 The influence of hydrogen ion and dissolved oxygen on cathodic polarization curves.

8.3.1 *Hydrogen ion*

The influence of hydrogen ion on cathodic polarization curves has been described in the preliminary experimental work of section 7.4. However, since the growth medium represents a more complex electrolyte than that used in this preliminary work, further experiments were performed.

Cultures of the organisms HIL and BR were prepared by inoculating 0,5 cm^3 of active culture into each of 10 of 60 cm^3 screw-capped bottles containing sterile growth medium, with the appropriate pH and redox-potential adjustments, and incubated at 30°C. At intervals, individual bottles were removed from the incubator and pH values of the cultures determined. The results are reflected in Figure 48.

The lowest pH value of an SRB culture recorded in this, or any other experiment, was 6,10. Irrespective of the age of the culture (up to 35 days), pH values never dropped below this level. Figure 49 illustrates the influence of pH on the cathodic polarization characteristics of mild steel in de-aerated growth medium. These experiments were performed using the growth medium with pH adjusted by additions of conc HCl, A.R. A polarization curve recorded in a cathodically-active culture of organism HIL, 8 days old, and having a pH of 6,35 is superimposed. The following may be concluded from these results :

i) Sterile media of greater hydrogen ion content than the active cultures showed a depolarizing effect at cathodic potentials close to the corroding potential, but this influence is limited to considerably less than that of active cultures, at more extreme potentials, by the rate of hydrogen ion diffusion to the electrode (see section 7.4).

ii) The culture of pH 6,35 had a considerably greater depolarizing effect at extreme cathodic potentials than did sterile medium at pH 4,20.

iii) Evidently the cathodic depolarizing influence of the culture was not attributable to its hydrogen ion concentration. Some other cathodically active species is present.

8.3.2. *Dissolved oxygen*

Cathodic polarization of mild steel in oxygen-containing solutions would be expected to yield the following type of curve :

i) At potentials close to the corrosion potential, a rapid rise in current would be expected until, at a current approximately equal to the corrosion current, the rate of diffusion of oxygen to the electrode surface becomes rate-limiting on the overall cathodic charge-transfer rate.

ii) This limiting diffusion current would be expected to remain constant with further cathodic shifts in potential, until the activation-controlled water-reduction rate becomes significant, as it does at extreme cathodic voltages in the experiments performed in acid solution, described in section 7.4.
The anticipated magnitude of the diffusion current for

$$O_2 \quad + \quad 2H_2O \quad + \quad 4e \quad \longrightarrow \quad 4OH^-$$

may be calculated from an expression derived from Fick's first Law, similar to 2.64

$$i_{d\ell} = n \; F D_{o_2} \frac{a(o_2)}{\delta} \qquad 8.1.$$

For the purposes of this calculation, the value of D_{o_2} was calculated using the expression of Wilke and Chang[143]. Values thus obtained were $2{,}02 \times 10^{-5}$ cm^2 S^{-1} at $30^\circ C$. (The value quoted in International Critical Tables[144] for a measured value at $18^\circ C$ is $1{,}8 \times 10^{-5}$ cm^2 S^{-1}). A value of $2{,}24 \times 10^{-7}$ (mol cm^{-3}) for $a(o_2)$ was calculated using the method given by Uhlig[145]. Substituting these values together with $n = 4$, $F = 96\,500$ As mol^{-1}, and $\delta = 0{,}05$ cm for an unstirred solution[146] in expression 8.1.

$$i_{d\ell} = 45{,}7 \;\; \mu A \; cm^{-2}$$

The value of this diffusion current would probably be modified, in the case of the bacterial growth medium, by a diminished oxygen activity (the value used above is that for distilled water in equilibrium with air at $25^\circ C$), and by the presence of other air-oxidizable, cathodically-active substances in the medium.

Sterile culture medium was placed in the polarization cell, its pH adjusted to 7,2 and saturated with a stream of air through the gas-purging device from a small electrical diaphragm-type pump for a period of 3 hours. At the end of this time the air stream was turned off and a polarization experiment performed on the quiescent solution, using a freshly-prepared mild steel working electrode. Thereafter, a fresh sample of the medium of the same pH was purged of oxygen by passing a stream of high-purity nitrogen through it for a period of one hour after which the tap of the gas purging assembly was turned to the head-space purging position, and a further polarization plot was performed. The results are recorded in Figure 50. The polarization curve for an active HIL culture is superimposed. It is evident that, while dissolved oxygen does cause a detectable amount of cathodic depolarization, the effect is considerably smaller in magnitude than that observed in active cultures.

The magnitude of the apparent oxygen diffusion current (Figure 51) obtained from the log i *versus* E plot is approximately 50 μA cm^{-2}. This is somewhat greater than the calculated value of 34, the discrepancy probably being caused by the presence of air-oxidizable cathodically active substances in the medium.

It was mentioned earlier (8.1.1. (iii)) that redox potentials recorded in active cultures precluded the possibility that dissolved oxygen was responsible for any observed depolarization effects. Redox potentials of active cultures were measured *in situ* in the polarization cell using a bright platinum wire electrode. The electrode was cleaned in hot alcoholic KOH solution, rinsed with distilled water and dried with A.R. acetone before insertion into the polarization cell in the place of the working electrode. The potential was then measured against the satd.KCl-calomel/agar bridge reference electrode system. Results are recorded in Table 9.

The redox potentials measured, while not as strongly reducing as those found by some authors, nevertheless serve to indicate that the cultures were within the range usually known as "anaerobic". Baas Becking, Kaplan and Moore[48] report that SRB are active within pH limits of 4,15 and 9,92 and over a redox potential (Eh) range of +0,115 V to -0,450 V. The values in the last column of the above table, indicate the extremely low oxygen activities (calculated from the Nernst relationship for the oxygen reduction equilibrium) of the active cultures.

8.4 Conclusions drawn from preliminary experiments

The results reported thus far have afforded the following information :

i) The pure cultures of the organisms under study gave cathodic depolarization effects similar to those reported by Horvàth and Solti[70], and by Booth and Tiller[71,75,76].

ii) The cathodic depolarizing activity is largely retained in cell-free centrifugates of active cultures and thus is likely to be caused by some soluble species produced during growth of the cultures.

iii) It was shown to be impossible that these depolarization effects were attributable to either dissolved oxygen or hydrogen ion.

CHAPTER NINE

9 THE CATHODICALLY-ACTIVE SPECIES

Further investigations of cathodic depolarization phenomena, at the stage reached at the end of the previous chapter, could have taken two possible courses :

(i) An accurate electrochemical description of the depolarization phenomenon could have been obtained from further experimental work, and an attempt made to characterize the active species from its electrochemical properties.

(ii) The depolarization phenomenon could have been used as an assay of cathodic activity, and attempts made to isolate, purify and characterize chemically the active species.

All experimental studies of the depolarization phenomenon, particularly those of Booth and Tiller[71,75,76,85], Iverson[84] and Mara and Williams[87,88], were considered to lend support to the hypothesis of von Wolzogen Kühr and van der Vlugt[64], namely that cathodic depolarization was caused by an enzyme (hydrogenase) which removed adsorbed atomic hydrogen thought to be responsible for the sluggishness of the cathodic charge-transfer reaction (although the arguments of chapter 5 have indicated that this is not so), and caused the reduction of substrates in the electrolyte, the overall effect being an increase in the cathodic charge-transfer rate and, ultimately, the corrosion rate.

It was decided to follow course (i) above as far as allowed by the experimental technique employed in this investigation, and subsequently to follow course (ii) assuming for the time being, that an enzyme was responsible for the observed activity.

9.1 The electrochemical characteristics of the depolarizing species

It was decided to study the onset of cathodic depolarization and its development with age of experimental culture. The culture HIL was used in most of this work, since this material had been used extensively by Booth and Tiller in electrochemical studies of the depolarization phenomenon[71 86], and its behaviour appeared representative of that of the other organisms.

9.1.1. *Experiments with culture HIL*

Samples of culture of different ages were required. To ensure homogeneity of the cultures studied and minimize possible errors arising from differences in inoculum size and activity, a large volume of sterile medium was inoculated, dispensed into a number of sterile bottles, and the bottles incubated for varying lengths of time before examination. Cultures were prepared as follows :

(i) A 30 cm^3 culture was used as inoculum for 1,3 dm^3 of sterile medium prepared, as before, with appropriate pH and redox potential adjustments. The inoculated medium was mixed well by inverting the screw-capped 1,3 dm^3 container a large number of times.

(ii) The inoculated medium was then dispensed into sterile 60 cm^3 capacity screw-capped bottles, filling them to the brim and tightening the screw-cap. This operation was conducted in a sterile cabinet using the method depicted in Figure 52.

(iii) The bottles were placed in an incubator at 30°C, and single cultures removed for examination at intervals of time.

(iv) When removed for examination, cultures were added to the polarization cell as in section 8.1. Their pH values were determined using the pH micro-electrode assembly in the cell. Small samples (5 cm^3) were removed from the cell with Pasteur pipettes for determination of the relative optical density of the culture. This determination was performed in an EEL absorptiometer colorimeter using un-inoculated medium as a blank.

(v) The mild steel working electrode was prepared and inserted into the cell, and cathodic polarization experiments performed. The potential was set accurately at the potential value of zero applied current (the corrosion potential) and cathodic polarization commenced. A true potentiostatic method was used here, increments in potential being set manually and the current allowed to reach a constant value before adjusting the potential further.

The results are presented in Figures 53 to 55. Figure 53 indicates that the corrosion potential tends to shift in a positive direction with age of the culture. This is accompanied by a shift in pH to more acidic values. During this time the relative optical density of the cultures rose to the upper limit of 1,5. The shift of corrosion potential in the positive direction may be interpretable in terms of mixed-potential theory, as being caused by anodic inhibition or cathodic depolarization. That cathodic depolarization is responsible for these shifts is demonstrated by the polarization results of Figure 54, in which the cathodic depolarization phenomenon is clearly visible. The results of the polarization experiments are given as plots of log current density *versus* potential in Figure 55. These curves indicate two different potential regimes within which cathodic depolarization takes place :

(i) At cathodic values close to the corrosion potential, the first depolarization effect is observed. This is manifested by positive shifts in the corrosion potential itself, and by increasing magnitude, with age of culture, of the depolarization effect. The behaviour in this regime is indicative of the appearance of a depolarizing species whose effect on the cathodic reaction rate is limited by the rate of diffusion of the species to the electrode.

(ii) At greater cathodic potentials (-730 to -1100 mV *versus* she) another depolarization effect becomes operative. This is the effect mainly responsible for the depolarization observed in chapter 8, and probably, in the early experiments of Booth and Tiller[71,75,76]. This effect also appears to be subject to a concentration polarization limitation at extreme cathodic potentials.

It was observed that at cathodic potential settings at which effect (ii) began to be observed, the current-time behaviour altered from a decrease with time to a constant value at a potentiostatically fixed potential at the end of regime (i), to an increase with time to a constant value at the beginning of regime (ii). At the higher current densities, depolarization was accompanied by a visibly much-increased rate of gas evolution from the electrode surface.

9.1.2 *Other Cultures*

Similar experiments performed with the other experimental cultures (BR, DB, and WVB) demonstrated the same effects : two depolarization regimes became apparent as cultures became more active, and this was accompanied by a decrease in pH, and a much-increased rate of gas evolution at extreme cathodic potentials. Typical results are given in Figures 56 - 58.

9.2 The identity of the depolarizing species.

If cathodic depolarization were attributable to enzyme-catalysed reduction of sulphate by cathodic charge, this would not be likely to lead to increased rates of gas evolution at the voltages at which this effect occurs. The major depolarization effects caused by the SRB cultures studied occurred within the potential range of the hydogen evolution reaction and were accompanied by increased rates of gas evolution from the polarized electrodes. This indicated that stimulation of the hydrogen evolution reaction is caused by active cultures. This stimulation could occur in two possible ways :

(i) Catalysis of the electrochemical reduction of hydrogen ion or water by some chemical species produced by the organisms.

(ii) The electrochemical reduction of some hydrogen-containing species other than water or hydrogen ion (produced by the organisms) to produce hydrogen gas.

9.2.1 *Theoretical considerations concerning the possibility of catalysis of the hydrogen evolution reaction by hydrogenase enzyme.*

It was indicated in section 5.3 that hydrogenase enzyme could catalyse reactions between *molecular* hydrogen and reducible substrates. Mention was made of the demonstration by Green and Stickland[131] of the equivalence in the effect of hydrogenase and colloidal palladium in catalyzing hydrogen evolution from reduced substrates. It was indicated that, for hydrogen adsorbed on an electrode,

$$2H_{ads} \rightleftharpoons H_2 \qquad 5.12$$

may possibly be catalysed by the enzyme, but according to the argument of chapter 5, this effect would be unlikely to affect the overall cathodic charge transfer rate on iron, as the rate-determining step in the h.e.r. on iron at near-neutral pH is the water electronation step.

$$M + H_2O + e \longrightarrow MH_{ads} + OH \qquad 5.5(b)$$

There is, however, an alternative mechanism which may make it possible for a substance produced by the bacteria (an enzyme) to catalyse the hydrogen evolution reaction at an iron surface. This is the type of mechanism exhibited by the group of soluble substances responsible for the "catalytic hydrogen waves" observed in polarography most commonly, at the dropping mercury electrode. Delahay has described the principle in general electrochemical terms[146], and the subject has been reviewed in detail by Kolthoff and Lingane[147], Heyrovsky[148], and Mairanovskii[149]. The phenomenon appears to have been explored in very few areas of electrochemistry other than in polarography at the dropping mercury electrode.

Delahay[146] has described the term catalytic current as being "applicable to all types of currents in which the product being consumed in the electrochemical reaction is partially regenerated by some chemical process involving a product of the electrochemical reaction". He gives the scheme

$$O \quad + \quad ne \longrightarrow R \qquad 9.1$$

$$R \quad + \quad Z \longrightarrow O \qquad 9.2$$

the net reaction being the reduction of Z catalysed by O. Catalytic hydrogen evolution was first observed in the presence of proteins at a dropping mercury electrode. It is now recognised[147-149] that several classes of organic compounds, particularly thiols and amines, can behave as hydrogen evolution catalysts, and that the catalytic action is heightened by coordination of these compounds with metal ions, particularly cobalt.

Using Delahay's scheme, the catalytic influence of a compound containing a thiol group in neutral aqueous solution may be described :

$$RSH \quad + \quad e \longrightarrow RS \quad + \quad \tfrac{1}{2}H_2 \qquad 9.3$$

$$RS \quad + \quad H_2O \qquad\qquad\qquad RSH \quad + \quad OH^- \qquad 9.4$$

the net reaction being the electronation of water :

$$H_2O \quad + \quad e \longrightarrow \tfrac{1}{2}H_2 \quad + \quad OH^- \qquad 9.5$$

Sulphur-containing amino acids exhibit this effect both as the free acids, and also when present as part of a protein molecule. The effect is heightened in both cases by coordination of the thiol group with metal ions, notably cobalt. The hydrogenase enzyme of *Desulfovibrio vulgaris* (strain Hildenborough) has been shown by Ackrell, Asato and Mower[127] to exist in a number of different forms, solid or soluble. A soluble form, examined by Haschke and Campbell[126] had six cysteine residues and one mole of ferrous iron per mole of enzyme. The ultraviolet absorption spectrum indicated that the iron was present in a nonheme-type configuration. Activation and inhibition studies on hydrogenase enzyme preparations from *Dv. vulgaris*[121–124, 126], have shown that thiol groups and iron are necessary for activity of the enzyme. It was considered possible that the enzyme could behave as a hydrogen-evolution catalyst of the type described in reactions 9.3 – 9.5. If the enzyme were present in the cell-free centrifugates of cultures used in the electrochemical studies, it may have been the species responsible for the observed depolarization (accompanied by increased hydrogen evolution) effects. Accordingly, attempts were made to isolate the enzyme from cell-free centrifugates of active cultures.

9.2.2. *Dialysis experiments*

750 cm^3 of cathodically-active cell-free centrifugates of cultures HIL, BR and DB were dialyzed for 12 h against each of four changes of distilled water in a 2,5 dm^3 vessel. At the end of this period, the dialysates were assayed for depolarizing activity by bubbling them with nitrogen for 1 h, adjusting the pH to the original value of the culture and performing polarization plots as before. The result of such an experiment for BR is given in Figure 59. Clearly, depolarizing activity was lost during dialysis. This loss of activity could be a consequence of the following :

(i) Diffusion of the active species through the dialysis tubing may have occurred. The tubing used was 4,8 x 10^{-3} μM pore diameter cellulose tubing supplied by the Fisher Scientific Co., which retains substances of molar mass greater than 12 000.

(ii) Inactivation of the cathodically-active species by the dialysis treatment, possibly by dissolved oxygen in the dialysis water may have occurred. If this were the case, and a cathodically active protein were present, it may have been possible to re-activate it by treatment with hydrogen gas and sulphydryl reagents such as cysteine. This technique had been successfully used before[116,118,121,124,150] to re-activate hydrogenase preparations of *Desulfovibrio* inactivated in this way. A dialysate of an active HIL centrifugate was treated with hydrogen and cysteine as follows : the inactive dialysate was bubbled for 1 h with hydrogen, 0,02 M of cysteine being added (the cysteine was added as L-cysteine hydrochloride, supplied as "Laboratory Reagent" by Hopkins and Williams Ltd., Essex, England) and the pH adjusted to the original value of the active centrifugate (7.2). The results (Figure 60) of polarization experiments performed in this case showed a rise in cathodic activity caused by this treatment. The depolarization effects were accompanied by increased rates of gas evolution from the electrode surface. This rise in activity may have been caused either by the re-activation of some oxygen-inactivated species, or by cathodic activity of the cysteine itself. This observation was tested by treating the sterile culture medium with cysteine, with the result, shown in Figure 61, that the cysteine itself was cathodically active the gas evolution phenomenon being exhibited as before.

The results of these experiments performed on cathodically-active centrifugates of SRB cultures had indicated the following :

(i) The cathodic depolarization phenomenon was accompanied by increased rates of gas evolution from polarized electrodes, and may have been a type of catalytic hydrogen evolution in which hydrogenase enzyme or some other soluble product of bacterial growth acted as a catalyst.

(ii) Dialysis of the active centrifugates caused a loss of cathodic activity.

(iii) Cysteine additions to the dialysates appeared to stimulate cathodic activity, but it was found that the cysteine added itself caused cathodic activity in the absence of any product of bacterial growth.

Since cysteine is well-known as a hydrogen evolution catalyst[147-149], it was decided to examine the possibility of it being the active substance in the centrifugates. Being of low molar mass (121), cysteine would have been removed from centrifugates by the dialysis process.

9.2.3. *The possible role of cysteine in depolarization*

A brief experimental investigation was conducted into the hydrogen evolution catalysis phenomena exhibited by cysteine. The phenomena are well-documented in work done with dropping mercury electrodes, but virtually no information is available on possible catalysis at other metal electrodes. At the dropping mercury electrode, cobalt or nickel ions are required in the electrolyte for the phenomenon to be demonstrated[147-149]. It appears that the catalytically active species is a coordination complex of organic catalyst with metal ion. Toropova and Elizarova[149], have reported that complexes of sulphur compounds with iron (as Fe^{++}) also possess catalytic activity. In the cathodically active cultures used in the present investigation, only slight traces of cobalt and nickel may be present as impurities at the levels present in analytical reagent grade chemicals and in the small amount of yeast extract in the media. Iron however, is added at 25 $\mu\underline{M}$ to the media. The relative catalytic activities of complexes of iron,cobalt and nickel were compared at the dropping mercury electrode. A Metrohm model E 261 "Polarecord" was used in this investigation. The electrolyte used was Brdickas'[147] buffer (appendix to chapter 9) containing 0,002 $\underline{M}$ concentrations of the appropriate metal ion, and cysteine at concentrations between 10^{-5} and 10^{-3} $\underline{M}$. Solutions were purged of oxygen using high purity ("white spot") nitrogen before addition of the cysteine. The results are recorded in Figures 62 - 64. It is evident that, while the cobalt and nickel complexes (olive-green and pink in colour respectively) gave typical catalytic hydrogen waves, the iron complexes (purple in colour) exhibited no such effects.

The influence of cysteine on the cathodic polarization characteristics of iron was nevertheless investigated using the potentiostatic techniques already described. In Brdickas' buffer solution containing 0,002 $\underline{M}$ Fe^{++} as used in the polarographic investigation, cysteine, over a concentration range of 10^{-4} to 10^{-2} $\underline{M}$ appeared to behave as a cathodic inhibitor (Figure 65). In a 0,2 $\underline{M}$ NaCl solution containing 25 $\mu\underline{M}$ Fe^{++}, cysteine exhibited depolarization effects (Figure 66) but these were only significant at concentrations greater than 10^{-3} $\underline{M}$.

A search for cysteine in the cathodically-active centrifugates of culture used in this investigation, using the thin layer chromatography techniques of Brenner, Niederwieser and Pataki[151], showed that free cysteine (or any other sulphur-containing amino acid) was not present at the levels detectable by the techniques used (i.e. a concentration of 10^{-8} M in the original solution or centrifugate)[152].

9.2.4. *Inhibition experiments*

The foregoing results had indicated that it was unlikely that proteins or amino acids were responsible for cathodic depolarization pheonema observed at mild steel electrodes in cultures of sulphate-reducing bacteria. It was still considered possible, however, that some unstable hydrogen evolution catalyst may have been present in the active cultures, and the possibility of an inorganic iron-sulphide species complex (possibly with SH^-) being responsible for depolarization was next investigated. Baas Becking, Kaplan and Moore[48] had indicated that such a complex may be responsible for the redox potential "poising" activity of iron and sulphide additions. When media were prepared for inoculation by additions of ferrous iron and sulphide, the medium acquired a pale green colour. As cultures grew and became cathodically active, this colour intensified. On dialysis and inactivation of the depolarizing species the cell-free centrifugates became straw-yellow in colour, the colour of un-inoculated inactive medium. It was decided to perform inhibition studies to characterize the cathodically active species assuming that an iron-SH^- complex was responsible for observed effects. Possible methods of approach would have been the use of complexing reagents likely to remove iron from the active complexes, and also reagents likely to react with the SH^- group, thus inactivating the complex. Unfortunately, the reagents usually used in this type of study (substances such as cyanide and o-phenanthroline which are iron complexants, and mercury and silver compounds : sulphide reagents) are also likely to behave as cathodic inhibitors, thus interfering with the assay of the effect.

Air was the first inactivator tested. The centrifugate of an active 1,3 dm^3 BR culture was prepared and its depolarization activity tested and shown to be present. A 100 cm^3 quantity of this centrifugate was added to the polarization cell and bubbled with air for a period of 8 h using a small electrical diaphragm pump. The pH rose from 7,28 to a value of 10,5 during this period, at the end of which the air supply was removed and nitrogen bubbling commenced for a period of 1 h during which the pH was adjusted to the original value of 7,28 by means of dropwise additions of dilute HCl from a Pasteur pipette. The colour of the solution had, by this time, changed from a deep green to straw yellow, and the subsequent potentiostatic polarization plot (Figure 67) showed that the cathodic activity had been removed.

It was found that bubbling the active centrifugate with nitrogen alone caused the rise in pH to 10,5 and on adjustment of the pH back to the original value, the solution became straw-yellow and cathodically inactive. This type of behaviour was found to be typical of all cultures used in this work. These results indicated that the oxidizing action of oxygen was not necessary to remove the effect : purging with an inert gas had the same influence, and therefore that a gaseous species removable by nitrogen-purging was responsible for observed depolarization effects. The most obvious gaseous species likely to be involved was H_2S. The cathodic activity of dissolved sulphide species was investigated as the next stage of this work.

9.3 The cathodic activity of dissolved sulphide species

9.3.1 *The species present and possible cathodic reactions*

It is well-known that dissolved sulphide species (designated $S^{\ddagger}$ for the present purposes) exist mainly as H_2S, HS^- and S^{2-}, the relative quantities of these depending upon pH *via* the equilibria

$$H_2S \rightleftharpoons H^+ + HS^- \qquad 9.6$$

$$HS^- \rightleftharpoons H^+ + S^{2-} \qquad 9.7$$

The molarity of the total sulphide species $\{S^{\ddagger}\}$ present in any of the experimental cultures used in this investigation would be, at maximum, equimolar to the quantity of sulphate originally present in the medium (0,05 $\underline{M}$). The $\{S^{\ddagger}\}$ of active cultures used in this investigation was determined by a volumetric (iodometric) method (see appendix to chapter 9), and the results recorded in Table 10.

It is evident (see Figure 68) that an increasing depolarization effect is observable as the $\left[S^{=}\right]$ of the culture increases. The quantity of each sulphide species at any given pH and $\left[S^{=}\right]$ may be calculated, using the relevant equilibrium constant data. The results of calculations for a $\left[S^{=}\right]$ of 10^{-2} are recorded in Figure 69. The equilibrium constant data used were[100]

$$9{,}1 \times 10^{-8} \text{ for } \frac{\left[H^{+}\right]\left[HS^{-}\right]}{\left[H_2S\right]} \text{ and}$$

$$1{,}1 \times 10^{-12} \text{ for } \frac{\left[H^{+}\right]\left[S^{2-}\right]}{\left[HS^{-}\right]} .$$

It is apparent that at a pH of 6,0 (the approximate pH of the most active cultures) sulphides present exist almost entirely as H_2S. If this is the species causing the observed depolarization effects, the cathodic reaction

$$2H_2S \quad + \quad 2\,e \quad \longrightarrow \quad 2HS^{-} \quad + \quad H_2 \qquad 9.8$$

may be operative. The calculated E^{o} for this reaction from the relevant thermodynamic data[100] (μ^{o} of $H_2S_{(aq)}$ = 27 400 J mol^{-1}, μ^{o} of $HS^{-}_{(aq)}$ = 12 600 J mol^{-1}) is $-0{,}415$ V (hydrogen scale), and the Nernst formulation is

$$E \quad = \quad -0{,}415 \quad - \quad \frac{2{,}303RT}{F} \quad \log \frac{\left[H_2S\right]}{\left[HS^{-}\right]} \qquad 9.9$$

At pH = 6,0 and $\left[S^{=}\right]$ = 0,02 (common values for cathodically-active cultures) $\left[H_2S\right]$ would be approximately 0,0183, $\left[HS^{-}\right]$ would be 0.0017 and the equilibrium potential of 9,8 would be -0,353 V (she), This is a reasonable value for the species responsible for the major depolarization effects observed in the experimental work.

The possible cathodic activity of dissolved sulphides has received scant attention from corrosion workers. Bolmer[154] has described reaction 9.8 in polarization experiments performed with iron in sulphide solutions, and Iofa and Phan Luong Cam have described a possible catalytic rôle for H_2S in hydrogen evolution reactions on iron in acid solution[155]. A comparison of the bond strength data (378 000 J mol^{-1} for H-SH, and 500 000 J mol^{-1} for H-OH)[153] indicate that the liberation of hydrogen from H_2S may be energetically easier than its liberation from H_2O. These values are quoted merely as a rough indication of anticipated cathodic behaviour. The ease of discharge of hydrogen from the substances concerned will be dependent upon a number of factors, among them the possible catalytic rôle of the metal electrode. Exchange current density data for the two reactions (9.9 and water electronation) enable their activation energies to be compared, and of course, their relative efficiencies as cathodic reactions in corrosion. Table 10 gives some of the established data for the two reactions. It is evident that under certain conditions, hydrogen sulphide may be very effective as a cathodic reactant on iron.

9.3.2 *Cathodic polarization experiments*

The object here was to show that hydrogen sulphide reduction could be responsible for the depolarization effects observed in cathodically-active *Desulfovibrio* cultures. It was decided to study these effects firstly in sodium chloride solutions with sulphide additions and, having described the effects in this simple system, to proceed to demonstrate them in bacterial culture media.

Initally, a 0,02 M NaCl solution was used as electrolyte. A series of sodium sulphide solutions was made up from $Na_2S.9H_2O$, A.R. in 0,02 M NaCl made with de-aerated (by N_2 purging) boiled out distilled water. The concentrations were adjusted such that the addition of 1 cm^3 of these solutions to 100 cm^3 of de-aerated 0,02 M NaCl in the polarization cell gave a series of $[S^{=}]$ concentrations in the range of 10^{-6} to 10^{-2} M. The first polarization experiment was performed on 0,02 M NaCl without sulphide additions. The pH of all solutions subsequently studied was adjusted to the value of this first solution (7,4) by means of dropwise additions of dilute HCl rendered oxygen-free by bubbling with nitrogen. Additions of sulphide and acid to the polarization cell were made under conditions of nitrogen bubbling to ensure adequate mixing, and as soon as additions were complete, the gas purging device was adjusted to the head space purging position. The working electrode was inserted, and cathodic polarization experiments performed using a true potentiostatic technique (increments in controlled potential were made only when the current

(i) That hydrogen sulphide is a powerful reactant.

(ii) The rate of any cathodic corrosion reaction of iron in which hydrogen sulphide is reduced is under diffusion control at concentrations below a certain level. The experiments quoted here indicate that diffusion control prevails up to a concentration of approximately 3×10^{-3} M.

(iii) At concentrations greater than 3×10^{-3} M, activation limitations (characterized by Tafel interdependence of current and potential) control the rate of any cathodic corrosion reaction, and diffusion control commences where high current densities are reached, and at high over-potentials. This occurs in a similar current and potential range to the diffusion control on the major depolarization effects reported in sections 9.1.1., 9.1.2, for cathodically active cultures.

(iv) H_2S is the cathodic reactant likely to be responsible for the major cathodic depolarization effects demonstratable in cultures of sulphate-reducing bacteria.

Iofa and Phan Luong Cam[155] as a result of polarization experiments using a rotating iron disc electrode in sulphuric acid solutions containing hydrogen sulphide, concluded that cathodic stimulatory effects exhibited by hydrogen sulphide were a result of catalysis of the hydrogen evolution reaction from H_3O^+ ions by a Fe (H-S-H) species formed at the electrode surface. The main evidence quoted in favour of this was the fact that, at a given acid concentration, alterations in the H S concentration between 10^{-4} and 10^{-2} M did not alter the limiting cathodic diffusion current, i_{dl}. Altering the H_3O^+ ion concentration at a fixed H_2S concentration, however, did affect the magnitude of i_{dl}. Unfortunately quantitative i_{dl} *versus* $[H_2S]$ data were not presented and it not possible to analyse the validity of this.

The results of the present investigation are more consistent with those of Bolmer[154], i.e. that as $[H_2S]$ is increased, i_{dl} increases. Bolmer found, in addition to this that increasing the stirring rate of the solution increased i_{dl}. It is suggested, as a result of the experiments described above, that the influence of H_2S on the corrosion rate of steel is that of a cathodic reactant which at concentrations below approximately 10^{-3} M is subject to diffusion control, and at high concentrations is subject to activation control. These are the two types of control depicted in Figures 7a and 7b for a cathodic reactant under diffusion control.

The form of the cathodic polarization curves recorded in cathodically-active cultures (Figures 53 to 58) is probably modified by the presence of other electrochemically active substances in the growth medium (proteins, amonium ions and phosphates). Substances of this nature would probably not be present at these concentrations in the electrolyte in which corrosion takes place in a natural environment. Nevertheless, it can be shown that all of the depolarization effects demonstrated in cathodically-active cultures can be reproduced by the addition of purely inorganic substances to the growth medium.

Cathodic polarization curves were recorded in the growth medium employed, with the iron and sulphide additions usually made prior to inoculation, and also with the addition of sulphide to give a concentration of 3 x 10^{-3} M. It is evident (Figure 72) that these additions successfully reproduce cathodic depolarization as recorded in both depolarization regimes in cathodically-active cultures. The depolarization regime at higher currents and overpotentials appears certain to be ascribable to the influence of cathodic reaction 9.9., the reduction of hydrogen sulphide. Depolarization effects in the regime of small currents and overpotentials may be ascribable to cathodic reactions involving an iron-HS^- complex or some other substance modified by the presence of sulphides in the growth medium.

All experimental work conducted during this investigation has indicated, therefore, that cathodic depolarization effects recorded in cultures of sulphate-reducing bacteria are ascribable solely to the presence of inorganic sulphide species in the electrolyte. The relationships of this fact to the von Wolzogen Kühr - van der Vlugt hypothesis and experimental demonstrations of cathodic depolarization effects ascribed to enzymes will be dealt with in the following chapter.

CHAPTER TEN

10 AN EXPERIMENTAL INVESTIGATION OF THE VON WOLZOGEN KÜHR - VAN DER VLUGT HYPOTHESIS

As indicated in Chapter 4, the majority of recent investigators of mechanisms of SRB corrosion favour the von Wolzogen-Kühr - van der Vlugt depolarization mechanism. The only evidence supporting this mechanism has been derived from two types of electrochemical polarization experiments :

i) Cathodic polarization experiments in actively-growing cultures of the organisms in sulphate-containing growth media as performed by Booth and Tiller[71 75 76] and Horvàth and Solti[70]. These experiments were taken to indicate that cathodic depolarization in which sulphate reduction according to reaction 5.1 took place was the major charge-transfer reaction. Similar polarization experiments performed during the present investigation have indicated that the cathodic reduction of dissolved hydrogen sulphide is responsible for all such effects observed in this type of experiment.

ii) Cathodic polarization experiments performed in suspensions of hydrogenase-active bacterial cells from which sulphate was omitted, and in which redox dyes were substituted as "terminal electron acceptors". Experiments of this type have been performed by Iverson[84], Booth and Tiller[86] and Mara and Williams[88 89]. The results of these experiments were taken as evidence that cathodic reduction of the dye was catalysed by hydrogenase enzyme systems.

It was decided to perform experiments of type (i) above, in order to test directly the von Wolzogen Kühr - van der Vlugt hypothesis in the light of the knowledge given in the previous chapter.

10.1 Cathodic polarization experiments performed in cultures and in cell suspensions in the presence of sulphates

The object of these experiments was to test the hypothesis that reaction 5.1 catalyzed by hydrogenase-active bacterial cells, could be responsible for the type of depolarization effects observed in cultures of these organisms. Figure 73 is a diagramatic description of the experimental procedure which was as follows :

(i) Cultures of organisms HIL, and WVB of volume 1,3 dm^3 were prepared as in section 8.1, using an inoculum of 30 cm^3 of actively-growing culture.

(ii) After incubation at 30°C for four days, the cultures were removed from the incubator and stored for 14 h at 0°C.

They were then examined as before for cathodic depolarizing activity at mild steel electrodes at 30°C using a polarizing speed of 500 m V per hour.

(iii) Having satisfactorily demonstrated this activity, the cultures were centrifuged at 5 x 10^4 g for 10 minutes and depolarization activity demonstrated in the centrifugates as before.

(iv) The centrifugates were purged with high-purity nitrogen as before, adjusting the pH values to the original value of the culture. During this process, cell suspensions were prepared by washing the cells and re-centrifuging with three changes of de-aerated tris buffer at pH = 7,0, and re-suspending the cells in 15 cm^3 of tris buffer. These suspensions, containing the cells of approximately 1 dm^3 of active culture, were bubbled vigorously with hydrogen for 20 minutes and stored under hydrogen in anaerobic jars at 0°C until required (periods of 2 - 3 hours).

(v) Cathodic polarization runs were repeated using the purged centrifugates, at the pH value of the active culture.

(vi) Quantities of 1,5 cm^3 of cell suspensions were then added to 100 cm^3 of de-aerated sulphate-containing growth medium in the polarization cell under conditions of vigorous nitrogen purging, at the original pH values of the active cultures. This gave suspensions of cell in fresh de-aerated growth medium of cell concentrations approximately equal to those of the original cultures. Purging was continued for a further 30 minutes, and cathodic polarization data determined as before.

(vii) The cell suspensions were then assayed manometrically for hydrogenase enzyme activity, using benzyl viologen as reducible substrate, according to the technique given in the appendix to Chapter 10, and their hydrogen absorption coefficients determined.

Each set of experiments with an active culture occupied a period of approximately 8 hours, the manometric hydrogenase assay being the last operation performed. The results of the polarization experiments are given in Figures 74 - 76 and the hydrogen absorption coefficients are given in Table 12.

It is evident from these results that depolarization effects demonstrated in cultures were largely retained in cell-free centrifugates, and that the activity could be removed by purging the centrifugates with an inert gas. This is consistent with the argument that dissolved hydrogen sulphide is the species responsible for observed depolarization effects. The cells, while demonstrably hydrogenase active (as shown in manometry), when resuspended in fresh, de-aerated, sulphate-containing medium, failed to show any depolarizing activity. These cell suspension experiments were performed in solutions de-aerated by nitrogen purging at pH = 7,0, the conditions used by Booth and Tiller[78] to demonstrate the depolarization effects caused by the putative hydrogenase-catalysed dyestuff reduction reaction. If the von Wolzogen Kühr - van der Vlugt depolarization step (reaction 5.1) were responsible for observed depolarization effects in these cultures, hydrogenase-active cell suspensions in fresh, de-aerated sulphate-containing medium would have been likely to demonstrate these effects as well.

These results have been published[156] and support strongly the argument that all observed depolarization effects are attributable to the cathodic activity of hydrogen sulphide, and not to enzyme-catalysed depolarization reactions. The experimental results of Booth and Tiller[86], Iverson[84], and Mara and Williams[88,89] recorded in systems containing redox dyes still require explanation, however, and some effort was directed towards studying the type of systems in which these results were produced.

10.2 Experiments performed in media containing redox dyes.

In reports of experimental investigations of cathodic depolarization phenomena in the presence of redox dyes, no mention is made of the cathodic activities of the dyes themselves. The cathodic activities of the dyes used in these experiments, methylene blue and benzyl viologen, were examined in de-aerated tris buffer solution at pH = 7,0, at mild steel electrodes, using polarization speeds of 500 m V per hour. The results are presented in Figures 77 and 78.

It is evident that both dyestuffs are cathodically active.

The behaviour of mild steel electrodes in a series of methylene blue solutions was as follows :

(i) The "rest" or "freely corroding" potential was displaced to more positive values by increasing concentrations of the dye, indicating that cathodic depolarization may be occuring.

(ii) At cathodic polarization values close to the "rest" potential, methylene blue behaved as a cathodic simulator, increasing the amount of cathodic charge passed for a given shift in potential.

(iii) At more negative values, methylene blue behaved as a cathodic inhibitor, the inhibitive effect increasing with methylene blue concentration

Benzyl viologen (Figure 78) produced similar behaviour of the mild steel electrode, the inhibitive effect at extreme cathodic voltages not being evident at the scanning speeds used, but this effect was readily demonstratable at high concentrations of the dye, using a true potentiostatic technique (Figure 79).

It is notable that at the concentration used by the workers mentioned above in enzyme depolarization experiments ($7{,}5 \times 10^{-5}$ $\underline{M}$) the dyes exhibited no strong inhibitive effects and, in fact were mild cathodic stimulators over most of the potential ranges studied. It was noted that where marked inhibition occured during a cathodic run, the electrode on removal from the cell was observed to be coated with a thin film of reduced dye. At the concentration $7{,}5 \times 10^{-5}$ $\underline{M}$, no such film was visible. It is possible that this concentration is insufficient to form complete inhibitive coating (possibly a monolayer thick) on the electrode.

10.3 An alternative explanation for cathodic depolarization effects observed in the presence of redox dyes and enzyme preparations

It is necessary to refer to some of the published data on the redox dyes concerned. The known pH *versus* potential behaviour of the dyes is presented in Figure 80. The line drawn for methylene blue consists of data presented by Clark[157], while the data for the viologen dyes was obtained from Michaelis and Hill[158].

The viologens, first described by Michaelis and Hill[158], were considered to have the unique property among redox dyes that their equilibrium oxidation-reduction potentials were independent of pH. This view has since been challenged by Green and Stickland[131] as a result of experiments in which "Degrees of reduction" found in experiments with bacterial and metal catalysts over a pH range of 7,3 to 8,9 enabled a slope of potential *versus* pH to be calculated which was very close to the theoretical pH *versus* potential hydrogen equilibrium slope. Nevertheless, Peck and Gest[120] have used the constancy of the methyl viologen potential over a pH range as the basis of a method of hydrogenase assay in which hydrogen evolution from reduced methyl viologen (which in neutral and acid solution has a potential more negative than that of hydrogen : see Figure 80) by the enzyme was the measured quantity. Benzyl viologen was found to behave similarly, although hydrogen evolution from its reduced form was not as pronounced.

Methylene blue, although frequently used as a reducible substrate in hydrogenase assay techniques is at least 400 m V more positive in oxidation reduction potential, over the entire pH range betweeen 1 and 12, than the hydrogen ion-hydrogen gas equilibrium. Consequently, it is likely to be reduced very early in the chain of redox events of which the action of hydrogenase is the climax. Figure 81 contains some of the known reactions in this redox chain, together with the oxidation-reduction potentials concerned. Clearly, hydrogenase which is systematically known as hydrogen : ferredoxin oxidoreductase can be more directly assayed by benzyl viologen than by similar processes with methylene blue which will only be active where several co-factors are present. This was pointed out in 1956 by Sadana and Jagannathan[121].

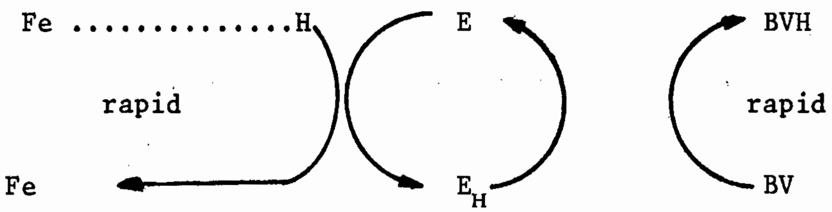
The experimental systems used by Booth and Tiller[86] and by Mara and Williams[88 89] to demonstrate enzyme-catalyzed depolarization consisted of three main parts :

(i) Cathodically polarized mild steel electrodes in the potential ranges at which the redox dyes used would be expected to be in the reduced condition.

(ii) Hydrogenase-active bacterial cells in suspension.

(iii) A redox dye which can be oxidized or reduced by the hydrogenase enzyme system present.

The mechanism proposed by Booth and Tiller[86] to explain depolarization phenomena observed in such systems (this was supported by Mara and Williams[88, 89]) was that the enzyme removed "polarizing hydrogen" from the electrode surface and that this hydrogen was used to reduce the dye according to



The arguments of chapter 5 have indicated that, if this process does occur, it is unlikely to affect cathodic charge transfer rates. Booth and Tiller also state[86] that : "At none of the potentials used in this work was benzyl viologen reduced at the cathode in the absence of either bacterial cells or cell fragments". During the work with benzyl viologen described in section 10.2, reduction of the benzyl viologen certainly did occur in buffer solutions at mild steel electrodes in the absence of any biological material, within a similar potential range to that used by Booth and Tiller. This may be diagnosed, both from the nature of the polarization curves produced and also by the production of the purple reduced form of the dye in the vicinity of the electrode. This was particularly obvious in benzyl viologen solutions of high concentration ($>10^{-4}$ M)

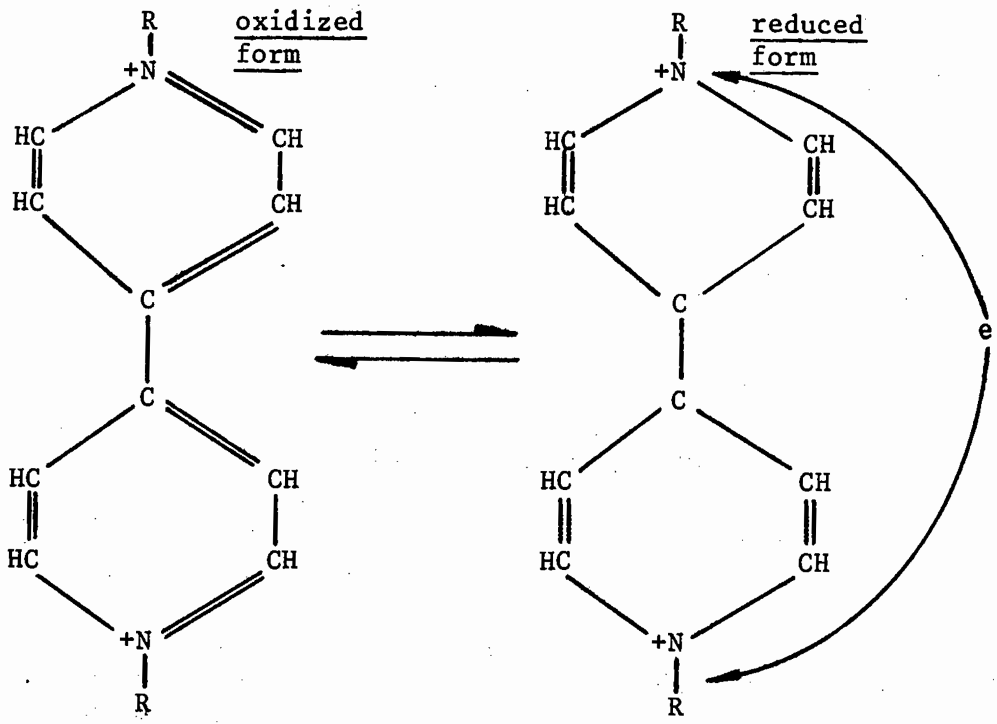
The results of Booth and Tiller[86] may be given the following alternative explanation :

(i) Rapid reduction of the redox dye by cathodic charge occurs. This is probably by direct electron transfer from electrode to dye molecule and not via "polarizing hydrogen". The oxidation-reduction potential of the dye at pH = 7,0 (-0,359V she) makes this easily possible. The organisms or enzymes play no role in this reduction.

(ii) The dye is present at a very low concentration ($7{,}5 \times 10^{-5}$ M) and the rate of cathodic charge transfer would, in the absence of any other influence, be controlled by the rate of diffusion of the oxidized dye molecules from the bulk of the solution to the electrode/electrolyte interface.

(iii) Hydrogenase enzyme systems, however, can increase the effective interfacial concentration of oxidized dye by re-oxidizing the reduced form with, possibly, the liberation of hydrogen gas.

The type of behaviour described in (iii) is well-known for systems containing viologen dyes and hydrogenase. The viologen dyes, first described by Michaelis and Hill[158] are dipyridyl derivatives of the form.



R = benzyl or methyl group

Michaelis and Hill showed that when palladium catalyst was added to a system containing methyl viologen reduced with chromous chloride, hydrogen evolution took place. Viologen dyes appeared to have the property of bringing about the production of hydrogen from water in the presence of a sufficiently strong reducing agent and a catalyst.

Peck and Gest[120] showed that hydrogenase enzyme could serve as a catalyst in this type of system, with hydrosulfite as the reducing agent. Methyl viologen and, to a lesser extent benzyl viologen in their reduced forms would then be used to assay hydrogenase activity by causing hydrogen evolution at a rate dependent upon catalyst (hydrogenase) activity.

In the type of experimental system used by Booth and Tiller[86], the cathode of the electrochemical cell would have behaved as reducing agent for the dye which was then re-oxidized by the hydrogenase enzyme system in the vicinity of the electrode and made available again for electrochemical reduction. This sequence would constitute a catalytic electrochemical system of the type described in the previous chapter, and would result in cathodic currents of magnitude dependent upon hydrogenase enzyme activity.

The direction in which any enzyme-catalysed dyestuff oxidation or reduction would be expected to proceed would be expected to depend upon the redox gradient existing between the polarized cathode and the electrolyte. The experiments of Booth and Tiller[86] were commenced at redox potentials measured with platinium electrodes (at pH = 7,0) of -0,25 V (she) in the bulk of the electrolyte, and were continued until this value reached -0,32 V (she), the potential at which 50% reduction of the dye would be expected. The polarized potentials of the cathodes employed, however, were in the range -0,65 to -0,95 V (she). Under these conditions, the thermodynamic tendency exists for substances reduced at the electrode surface to be re-oxidized. Oxidation of reduced benzyl viologen catalysed by the enzyme (probably accompanied by hydrogen evolution) is a possibility under such circumstances.

A similar catalytic system may explain the results obtained by Mara and Williams[88 89] using methylene blue as redox dye. In this case, some part of the electron transport system associated with hydrogenase and effective in the redox potential range of methylene blue may be operative. In such a potential range, the hydrogen evolution reaction would not be the overall reaction catalyzed. The enzyme-catalyzed re-oxidation of electrodically reduced methylene blue would have to be associated with some ultimate electron acceptor, other than water, active in this potential range.

CHAPTER ELEVEN

11 THE MECHANISM OF THE CORROSIVE EFFECT OF SULPHATE REDUCING BACTERIA

It has been shown, by reasoning based on established information on the cathodic kinetics of hydrogen ion and water electronation at iron electrodes, that enzyme-catalyzed depolarization reactions frequently hypothesized in the literature, in which atomic hydrogen is removed from "polarized" cathodes, are unlikely to play a role in the corrosion of iron. Direct experimental testing has supported this inference and has shown that all observed cathodic depolarization effects in SRB cultures are attributable to the action of hydrogen sulphide. Established electrochemical corrosion theory has been successful in quantitatively describing most aqueous corrosion systems in chemical thermodynamic and kinetic terms. The case of SRB corrosion has hitherto eluded such quantitative explanation. Table 1 lists some of the high corrosion rates observed in the presence of these organisms. The corrosion current densities i_{corr}, in the right-hand column, were calculated as the current densities, i_a, required at a metal surface where metal dissolution according to

$$Fe \longrightarrow Fe^{2+} + 2e \qquad 11.1$$

is proceeding, for these rates of penetration to have occured. The overall corrosion process is assumed to have taken place in a system where

$$I_{corr} = I_m = -I_z \qquad 2.51$$

and

$$I_m = i_m A_a \qquad 2.47\ (a)$$

$$I_z = i_z A_c \qquad 2.47\ (b)$$

(i_m and i_z represent current densities at anodic and cathodic areas and A_a and A_c the respective areas).
It is intended to propose a mechanism of corrosion which is consistent both with the experimental results accumulated during this investigation, and with the high corrosion rates observed in practice (Table 1).

All experimental results presented hitherto have concerned the cathodic corrosion reaction; it is necessary to present some data concerning the anodic reaction before proceeding with a corrosion mechanism discussion.

11.1 The anodic behaviour of mild steel in SRB cultures

Booth and Tiller[71, 75, 76] in their early polarization studies of the behaviour of steel in cultures of SRB demonstrated that, as the age of the culture increased, anodic depolarization manifested by a decrease in slope of the anodic polarization curve occured and this was followed, at greater culture ages, by a stepping up of the slope of the curve. Unfortunately in these experiments all consecutive anodic and cathodic curves for any one culture were recorded using the same mild steel electrode which was left in the growing culture. The results are likely to have been affected by accumulations of corrosion products, some of which were probably created artificially by anodic treatment, and also by changes in effective electrode surface area. Hoar and Farrer[73] found that culture media inoculated with mixed soil cultures containing sulphate-reducing bacteria caused a diminishing of the anodic polarization slope, and indicated that this polarization behaviour was similar to that recorded in solution containing small quantities of sulphides (10^{-4}M or less).

It was decided to follow the course of the onset of anodic activity of cultures with age in the same way as cathodic activity was studied in section 9.1. Cultures of HIL were prepared as before, and the anodic polarization characteristics of freshly-prepared mild steel electrodes in cultures of different ages studied. Increments in set potential were made when the current reached a constant value at any given setting. The results are given in Fig. 82. It is evident that anodic inhibition appeared to arise with age of the culture. This was accompanied by a drop in pH and an increase in turbidity of the culture, as in the experiments described previously in Section 9.1. The apparent anodic inhibition demonstrated here was accompanied by the formation of black ferrous sulphide films on the electrode surface during polarization experiments. No initial diminution of the slope of the curves was detected in the youngest cultures.

11.2 The anodic behaviour of mild steel in sulphide solutions

A series of solutions of $Na_2S\ 9H_2O$, A.R. in 0,02 M NaCl was made as before with $[S^=]$ values between 10^{-6} and 10^{-2} M. Anodic polarization curves were recorded for mild steel electrodes in these solutions adjusted to pH 7,4 as before. These curves together with the corresponding cathodic curves for solutions of the same concentration are given in Fig. 83. It is evident from these results that sulphides do affect the anodic process. Anodic Tafel slopes recorded in the presence of various quantities of sulphides are given in Table 13.

These results are consistent with those of Hoar and Farrer in showing that small amounts of sulphide (10^{-5} M or less) in solution lower the anodic Tafel slope, and that larger amounts ($>10^{-5}$ M) tend to increase it (with respect to the slope obtained in the absence of sulphides). At the higher sulphide concentrations, visible films were formed.

A solution of sulphide of molarity, $[S^=]$,0,014 (determined by titration using the iodometric method described in the appendix to Chapter 9) was obtained by treating 0,02 M NaCl solution with hydrogen sulphide from a Kipp's generator. Its pH was adjusted to 6,65 with additions of NaOH solution before analysis and use in polarization experiments. An anodic polarization experiment was performed using a freshly-prepared mild steel electrode at a scanning speed of 500 mV per hour. The curve produced (Fig. 84) was typical of the type produced in an electrolyte in which passivity occurs. If freshly-prepared electrodes were left in this solution for 30 to 40 minutes the potential rose to within the passive zone, this process being accompanied by the formation of an adherent black sulphide film. This apparent passivity is similar to a phenomenon reported by Bolmer[154] for iron in solutions containing 10^{-2} to 10^{-1} M HS^-.

It is apparent, thus, that in the presence of dissolved sulphide species at near-neutral pH, the polarization results indicate the following corrosion behaviour of mild steel.

(i) *At low sulphide concentrations (below approximately 10^{-3} M).*
The presence of small quantities of sulphide appears to alter the mechanism of the anodic reaction, lowering the Tafel slope, and possibly increasing the corrosion rate. The cathodic process at these low concentrations is likely to be

$$H_2S + e \longrightarrow HS^- + \tfrac{1}{2}H_2$$

and is under diffusion control in quiescent conditions, as is the overall corrosion process.

(ii) *At high sulphide concentrations (above approximately 10^{-3} M).*
The same cathodic reaction as above occurs, but at the corroding potential is under activation control, measurable Tafel slopes

being obtained. The anodic Tafel lines become steeper at higher sulphide concentrations, indicating anodic inhibition, and at a concentration of 0,014 M passivation by a reaction product film appears to occur.

11.3 The properties of iron sulphide corrosion products

That corrosion rates of steel in the presence of SRB are profoundly influenced by the type of sulphide film formed on the metal was clearly shown by Booth and co-workers, as described in Chapter Chapter 4[77,81]. Culture media of high iron content gave considerably higher corrosion rates than those of low iron content (Table 5), and these high corrosion rates were associated with loosely-adherent, bulky iron sulphide films. Polarization experiments with mild steel electrodes in cultures grown in media of varying iron contents[80] showed that where the iron content was high (12 500 μM), bulky, non adherent deposits formed, and this was accompanied by vigorous, sustained cathodic depolarization. Where iron contents were low (25 μM), hard, adherent films, accompanied by diminished cathodic depolarization effects, were formed. These conclusions regarding the rôle of dissolved iron in the medium were supported by the results of King, Miller and Wakerley[91] and it was concluded that, whatever the hydrogenase activities of the sulphate-reducing organisms (these observations included *Desulfotomaculum* species), provided sufficient iron is present, the organisms could cause cathodic depolarization and corrosion.

While it has been recognized that loosely-adherent FeS deposits have a pronounced stimulatory effect on corrosion and on the cathodic charge-transfer rate in polarization experiments, no attempts have been made to interpret these effects in quantitative electrochemical terms. Polarization experiments have been performed with iron or steel electrodes in contact with various forms of FeS [81,92,93,96,97] in the absence of any bacteria, and it has been shown clearly (Fig. 21) that the presence of solid iron sulphide does have the property of greatly increasing the current required to polarize an electrode of mild-steel.

These observations were tested experimentally during the present investigation. 0,5 M solutions of each of $FeSO_4.7H_2O$ A.R. and $Na_2S.9H_2O$ A.R. were made up in 1 dm^3 quantities of distilled water, de-aerated by nitrogen purging. The sodium sulphide solution was added to the iron sulphate, stirring the mixture with the nitrogen stream. A dense, black FeS precipitate was produced. The pH of the mixture was adjusted to 7,0 by additions of dilute HCl and NaOH solutions. The precipitate was then filtered off, using a Sartorius SM pressure filtration apparatus fitted with a Sartorius 1,5μm pore-diameter membrane filter. The filtration was conducted under nitrogen pressure and the precipitate was washed with 1 dm^3 (added in 200 cm^3 quantities) of 0,02 M NaCl solution, previously de-aerated by nitrogen purging. A portion of the precipitate was transferred to a 5 cm^3 capacity porcelain crucible which was placed in the bottom of the polarization cell immediately above the outlet tube. The top of the cell was placed in position and a freshly-prepared mild steel electrode inserted until the metal portion was completely immersed in the FeS precipitate. 0,02 M sodium chloride solution was added against a stream of nitrogen gas until crucible and electrode assembly were completely immersed. The reference electrode probe was inserted such that its tip was just clear of the surface of the FeS precipitate (see Fig. 84). A steady stream of nitrogen was passed through the head-space of the cell, and cathodic polarization commenced at a rate of 500 mV per hour. The polarization curve recorded, together with the curve for the mild steel electrode in de-aerated 0,02 M - NaCl are presented in Fig. 85.

It is evident that the presence of the FeS precipitate has the influence of altering the corroding potential of the mild steel electrode by approximately 100 mV in the anodic direction. In addition to this effect, a very large current is required to polarize the mild steel/FeS composite cathodically. This behaviour conforms to that demonstrated by Booth, Elford and Wakerley for a similar system[81].

The fact that the corroding potential of steel is shifted in the anodic direction by the presence of FeS indicates (from mixed-potential reasoning) that the FeS behaves as a cathode towards mild steel. King *et al*[93,96,97] have proposed that this is so, but have indicated, as a result of weight loss experiments, that its rôle as a cathode (when prepared chemically in the laboratory) is short-lived, and that continuous high corrosion rates can only be sustained where the supply of FeS in contact with corroding metal is regularly replenished with fresh precipitate. As stated previously, no quantitative electrochemical interpretation of the polarization characteristics of this FeS corrosion product has yet been proposed.

The Butler-Volmer equation was derived in Chapter 2 as the fundamental relationship governing the polarization characteristics of an electrode. This equation was derived in two convenient forms for expressing polarization behaviour : the low-field and high-field approximations. According to the low-field approximation

$$i = \frac{i_o F\eta}{RT} \qquad 2.27$$

or

$$\eta = \frac{i\,RT}{i_o F}$$

Only when $i > i_o$ will η become appreciable. When η exceeds 10-15 mV, i.e. when $i >> i_o$, the high-field approximation.

$$\eta = \frac{2{,}303RT}{\vec{\alpha}\,F} \log i_o - \frac{2{,}303\;RT}{\vec{\alpha}\;F} \log i \qquad 2.44$$

becomes applicable, and η becomes proportional to log i. Now we have indicated, for the cathodic component of a corrosion reaction, that

$$I_z = i_z \; A_c \qquad 2.48(a)$$

Where I_z is the total cathodic current and is the product of the cathodic current *density* i_z and the cathodic surface area, A_c. The results of cathodic polarization experiments with FeS have indicated that very large values of I_z are produced for small overpotentials. Therefore, by 2,27, $I_{o,z}$ must also be appreciable. The reason for this must be that either i_{oz} or A_c is unusually large for a corrosion system.

An attempt was made to determine the specific surface area of some of the FeS corrosion product from one of the pits in the pipe corrosion case depicted in Fig. 1. A description of the technique is given in the Appendix to this chapter. The method used was the measurement of nitrogen adsorption by a differential technique and calculation of the surface area from the Brunauer-Emmett-Teller relationship. The mean specific surface area determined by this method was 13,3 ($\pm$ 3%) $m^2 g^{-1}$. The total contents of the pit (weighing 1,0222g) therefore had a surface area of 13,6 m^2. This corrosion product was removed from a pit of approximately 1 cm^3 volume.

11.4 A proposed corrosion mechanism

The essential question to be answered by any proposed corrosion mechanism is : how is it possible for anodic current densities of the magnitude calculated for actual corrosion rates (using Faraday's Laws) to be sustained at the corroding metal surface? For the model proposed in section 2.5.1. at the corroding potential

$$I_{corr} = I_m = -I_z \qquad 2.51$$

and also

$$I_m = i_m A_a \qquad 2.47(a)$$

$$I_z = i_z A_c \qquad 2.48(a)$$

In other words, at the corroding potential, the magnitude of the corrosion current is equal to those of each of the individual currents flowing across the charge-transfer interfaces of anodic and cathodic components of the corrosion cell. The corroding (or anodic) surface in this case is usually the interior of a pit in the metal concerned. We shall select case 11 of Table 1 as an example of a case where a high corrosion current caused pitting.

From Faraday's Laws, we may calculate that a current density of 441μA cm^{-2} would have been required within the pit to cause the observed rate of penetration. The pit involved had a volume of approximately 1 cm^3. If we assume that the approximate dimensions of the pit to be a cylinder of diameter = 1,50 cm, depth = 0,57 cm, the internal surface area ($\Pi Dh + \Pi r^2$) would be 4,44 cm^2. Now, assuming further that (i) the current density of 441μA cm^{-2} is uniformly distributed over the internal surface of the pit, and (ii) that the whole of the internal surface is a corroding anode,

$$\begin{aligned} I_m &= i_m A_a \\ &= 441 \times 4{,}44\mu A \qquad 2.47(a) \\ &= 1958\mu A \end{aligned}$$

This current would be supported by the mass of FeS corrosion product filling the pit, acting as cathode. In this case, with a cathode of surface area 13,6 m , the current density required is

$$i_z = \frac{I_z}{A_c}$$

$$= \frac{1958}{136\ 000}\ \mu A\ cm^{-2}$$

$$= 0{,}014 \mu A\ cm^{-2}$$

It is possible that not all of the surface area of the corrosion product is available as a cathode. However, even if the effective area were reduced by a factor of 10^2, the cathodic current density required would be only $1{,}4 \mu A\ cm^{-2}$.

We must next ask the nature of the cathodic reaction responsible for this current density. The experimental results of chapters 8 and 9 have indicated that the reaction

$$H_2S + e \longrightarrow HS^- + \tfrac{1}{2} H_2 \qquad 9.8$$

is the most likely. The corrosion product mass within the pit would contain cells of sulphate-reducing bacteria, continuously producing the cathodic reactant. The absence of this continuous supply of cathodic reactant may have been responsible for the non-permanence of the cathodic behaviour of FeS precipitates in the experiments of King *et al*[93,96,97]. The rate of sulphate-reduction within a pit would be expected to depend upon the following :

(i) *The growth of the bacterial colony*

As indicated by Khosrovi, MacPherson and Miller[137], actively growing cells would be expected to carry on sulphate reduction by organic substrate oxidation, provided these substrates were present.

(ii) *The utilisation of molecular hydrogen, produced by the cathodic reaction, in sulphate-reduction by the hydrogenase systems of resting-stage cells*

i.e. by cells not actively growing by organic substrate oxidation as in (i). This effect has been demonstrated by Khosrovi, MacPherson and Miller[137]. Such consumption of hydrogen would not be of the type proposed by the von Wolzogen Kühr - van der Vlugt mechanism, but it may play some part in determining the rate of the corrosion reaction by removing *molecular* hydrogen (probably present as bubbles) from the cathode surface and supplying further H_2S for the cathodic reaction. The presence of gas bubbles may also have a supporting influence on the cathodic reaction rate by decreasing the thickness of the diffusion layer (if the reaction is diffusion-controlled) as shown by Bockris and Cahan[159] for porous electrodes.

(iii) *The rate of transfer of organic nutrients and sulphates from the external aqueous environment to the bacterial cell/FeS mass.* In this respect, natural convection due to density gradients and also to evolved gas bubbles may play a rôle. At peripheral parts of the culture/FeS mass, i.e. where it may come in contact with traces of dissolved oxygen (as indicated by Copenhagen[160] for harbour conditions), re-oxidation of H_2S or HS^- to $SO_4^=$ may occur. Species of *Thiobacillus* may be involved in such a reaction[3]. In this way, a considerable economy in sulphate consumption may occur by its cyclic use.

The type of corrosion situation in which a mechanism of the type described above may be operative is expressed in Fig 86(a) and (b). Fig. 86 (a) expresses the electrochemical situation at any given instant during corrosion of the type taking place in case 11, Table 1. The cathodic reaction rate may be either activation- or concentration-polarization controlled. Over an extended period of time I_{corr} would ultimately depend upon factors (i), (ii) and (iii) above. The physical situation proposed is depicted in Fig. 86(b).

It has emerged from laboratory experiments and ecological observations that media of high iron content (higher contents than the minimum nutritional requirement) are suitable for the growth of sulphate-reducing bacteria and consequent corrosion. On initial lodgement of a sulphate-reducing organism on a metal surface and hydrogen sulphide production, the presence of sufficient Fe^{++} in the medium would cause the formation of FeS precipitates around the bacterial cell thus creating a cathode for the initial corrosion reaction. Within this cathode mass, the cathodic reactant would be generated which, on electrochemical discharge, would cause the dissolution of iron as Fe^{++} from the metal surface. The Fe^{++} ions, in migrating away from the anode surface, would encounter H_2S or HS^- ions and would cause further precipitation of FeS, thus enlarging the cathodic surface area. Under such conditions, the amount of H_2S or HS^- reaching the corroding anode surface would be small, probably insufficient to cause anodic inhibition by film-formation as described earlier, but possibly sufficient to lower the anodic Tafel slope as shown in section 11.2.

This model may be elaborated by incorporating particle size *versus* surface area data for the FeS corrosion product, or by morphological data on the *Desulfovibrio* cell and its geometrical relationship to the FeS corrosion product and FeS particles said to exist in cell walls[97], but in the absence of firmly established data, such elaboration would be conjecture.

11.5 Recommendations for further research

The experimental results accumulated during this investigation as well as those published by other investigators have been used as a basis for a proposed mechanism of SRB corrosion. Two particular types of experimental data have been found lacking in the published literature and, in the author's opinion, have been insufficiently investigated during the course of his own experimental work. These are proposed as topics for future research which may have consequences of far wider significance than developments towards a satisfactory theory of SRB corrosion mechanism. These topics are the phenomena of catalytic hydrogen evolution and the electrochemical behaviour of the sulphides if iron.

11.5.1 *Catalytic hydrogen evolution*

This phenomenon, relatively well-documented in studies performed at the dropping mercury electrode, has been sufficiently described for other electrode systems. The possibility of its occurence during corrosion of iron in sulphide-containing media, as indicated by Iofa and Phan Luong Cam[155], should be afforded a full experimental investigation with as complete a theoretical analysis as possible in fundamental electrochemical terms. The development of a comprehensive theory of catalytic hydrogen evolution would enable a more complete investigation of the rôle of enzyme systems in electrochemical reactions to be made, in particular, that of hydrogenase systems in cathodic reactions. It is also possible that the phenomenon of catalysis of the hydrogen evolution reaction by substances adsorbed at the electrode may have considerable technological importance, for example, in the electrolytic production of hydrogen.

11.5.2 *The electrochemical properties of the sulphides of iron*

Data is available on the thermodynamic (Nernstian) properties of the sulphides of iron. Sato[161] has given a particularly comprehensive thermodynamic treatment to metal sulphides. Electrochemical kinetic data, however, are lacking. Ward[162] has indicated that mackinawite (the first-formed sulphide during corrosion) has a most unusual structure, making it metallically conducting along one crystallographic plane and semiconducting along another. Further experimental effort should be devoted towards the study of the cathodic characteristics of this, and of other metal sulphides. Experimental systems such as used in studying these sulphides in contact with metal electrodes (as in section 11.3) could be used to better advantage. More effort should be devoted towards determining the actual nature of the cathodic reaction observed in such systems and its fundamental electrochemical characteristics (such as i_0 and α).

GLOSSARY OF SYMBOLS AND ABBREVIATIONS USED

(i) Symbols

α	the transfer coefficient for an electrochemical reaction.
β	the symmetry factor for an electrochemical reaction.
γ	the number of electron transfer steps preceding ($\vec{\gamma}$) or following ($\overleftarrow{\gamma}$) the rate-determining step of an electrochemical reaction.
η	the overpotential of an electrode system (the difference in potential between the polarized and the equilibrium state of an electrode system).
κ	the Boltzmann Constant ($1{,}38 \times 10^{-23}$ JK^{-1}).
μ_A	the chemical potential ($J\ mol^{-1}$) of substance A.
ν	the rate of an electrochemical reaction ($mol\ cm^2\ S^{-1}$).
ν_n	the stoichiometric number of an electrochemical reaction (the number of times the rate-determining step occurs per overall occurrence of the reaction).
ϕ	the potential difference (volts) across an electrode-electrolyte interface.
a(A)	the thermodynamic activity of species A in liquid solution.
C_A	the molar concentration of species A in solution.
D_A	the diffusivity ($cm^2\ S^{-1}$) of species A.
E	the potential of an electrode on the standard hydrogen electrode (she) scale.

E_H the oxidation-reduction (redox) potential of an electrolyte measured at a platinum electrode relative to the redox potential of the hydrogen ion - hydrogen gas system at the same pH.

F the Faraday Constant ($9{,}648 \times 10^4$ C mol^{-1}).

$\Delta G^{\circ\ddagger}$ the standard free energy of activation (J) of a reacting chemical system.

H^o the standard enthalpy (J) of a chemical species.

p_A the partial pressure of gaseous species A.

$S^{\ddagger}$ the sulphide species (includes S^{2-}, HS^-, H_2S).

(ii) Abbreviations

APS adenosine phosphosulphate

ATP adenosine triphosphate

she the standard hydrogen electrode (the hydrogen ion - hydrogen gas system with the chemical species present in their thermodynamic standard states).

SRB the dissimilatory sulphate-reducing bacteria.

REFERENCES CITED

1. Lehman, G. In : *Korrosion und Korrosionschutz*, F. Todt, Editor. de Gruyter, Berlin, 122 (1961).

2. Postgate, J. In : *Corrosion*, L.L. Shrier, Editor. Newnes, London, Vol I, 251 (1965)

3. Costello, J.A. *International Biodeterioration Bulletin*, 5 (3), 101 (1969)

4. Miller, J.D.A., and Tiller, A.K. In : *Microbial Aspects of Metallurgy*, J.D.A. Miller, Editor. Medical and Technical Publishing Co., Aylesbury (1971).

5. Wessel, C.J. *News Report*, National Acad. Sci. USA NRC, 7 53 (1957)

6. Starkey, R.L. *Prod. Mon.* 22 (9), 12 (1958).

7. Booth, G.H. *J. Appl. Bact.* 27 (1), 174 (1964)

8. Council for Scientific and Industrial Research (S.A.), Pretoria: *Technical Information for Industry*, 8 (2), 1 (1970)

9. Copenhagen, W.J. *Brit. Corros. J.* 1 (9), 344 (1966)

10. Costello, J.A. *Proceedings : 2nd South African Corrosion Conference*. CSIR, Pretoria. GA_{CSIR} 23D 1642*, 172 (1972)

11. Blanchard, G.C., and Goucher, C.R. *Electrochem. Technol.*, 5 (3-4), 79 (1967)

12. Churchill, A.V. *Mater. Prot.* 2 (6), 18 (1963)

13. Foley, R.J. *Electrochem. Technol.*, 5 (3-4), 72 (1967)

14. Hedrick, H.G., Crum, MG., Reynolds, R.J., and Culver, S.C. *Electrochem. Technol.*, 5 (3-4), 75 (1967)

15. Iverson, W.P. *Electrochem. Technol.*, 5 (3-4), 77 (1967)

16. Iverson, W.P. *Science*, 151 (3713), 986 (1966)

17. Ward, C.B. *Mater. Prot.*, 2 (6), 10 (1963)

18. Cox, G.L., and Roetheli, B.E. *Ind. Engng. Chem.*, 23, 1012 (1931)

19. Booth, G.H., Cooper, A.W., and Cooper, P.M. *Chem. Ind.*, 49, 2084 (1967)

20. LaQue, F.L. In : *Corrosion Handbook*, H.H. Uhlig, Editor. Wiley, New York, 383 (1963)

21. Bunker, H.J. *J. Soc. Chem. Ind.*, 58, 93 (1939)

22. Copenhagen, W.J. *Trans. Roy. Soc. of S.A.*, 22, 2 (1934)

23. Doig, K., and Wachter, A. *Corrosion*, 7 (7), 212 (1951)

24. Uhlig, H.H. In : *Corrosion Handbook*, H.H. Uhlig, Editor. Wiley, New York, 133 (1963)

25. Evans, U.R. *The corrosion and oxidation of metals*, Arnold, London (1960)

26. Evans, U.R., and Hoar, T.P. *Proc. roy. Soc.* (A) 137, 343 (1932)

27. Agar, J.A. work described by U. R. Evans in *J. Iron St. Inst.*, 141, 219 (1940)

28. Glasstone, S., Laidler, K.J., and Eyring, H. *The theory of rate processes*, McGraw-Hill, New York (1941)

29. Wagner, C., and Traud, W. *Z. Elektrochem*, 44, 391 (1938)

30. Pourbaix, M. *Thermodynamics of dilute aqueous solutions*, Arnold, London (1949)

31. Bockris, J. O'M., and Reddy, A.K. *Modern Electrochemistry*, Vol 2. Plenum Press, New York (1970)

32. Vetter, K.J. *Elektrochemische Kinetik*, Springer-Verlag, Berlin (1961)

33. Stern, M. *Corrosion*, 13, 775t (1957)

34. Stern, M., and Geary, A.L. *J. Electrochem. Soc.*, 104, 56 (1957)

35. Stern, M. *Corrosion*, 14, 440t (1958)

36. Delahay, P. *New Instrumental Methods in Electrochemistry*, Interscience, New York (1954)

37. Makrides, A.C. *Corrosion* 18, 338t (1962)

38. Hoar, T.P. *The anodic behaviour of metals*. In *Modern Aspects of Electrochemistry*, No. 2, J. O'M. Bockris, Editor. Butterworth, London (1959)

39. Postgate, J. *Bact. Reviews*, 29 (4), 425 (1965)

40. Wight, K.M., and Starkey, R.L. *J. Bact.* 50, 238 (1945)

41. Butlin, K.R., and Adams, M.E. *Nature*, Lond. 160, 154 (1947)

42. Butlin, K.R., Adams, M.E., and Thomas, M. *J. Gen. Microbiol.* 3, 46 (1949)

43. Sisler, F.D., and Zobell, C.E. *J. Bact.* 62, 117 (1951)

44. Postgate, J.R. *Z. Allgem. Mikrobiol.* 1, 53 (1960)

45. Beijerinck W.M. *Centr. Bakteriol. Abt.* 1, 1, 49, 104 (1895)

46. Zobell, C.E. *Prod. Mon.* 22 (7), 12 (1958)

47. Bunker, H.J. *DSIR Chemistry Research. Special Report No. 3*, HMSO (1936)

48. Baas Becking, L.G., Kaplan, J.R., and Moore, D. *J. Geol.* 68 (3), 243 (1960)

49. Booth, G.H., and Tiller, A.K. *Trans. Faraday Soc.* 56, 1689 (1960)

50. Tiller, A.K., and Booth, G.H. *Trans. Faraday Soc.* 58, 110 (1962)

51. Booth, G.H., and Tiller, A.K. *Trans. Faraday Soc.* 58, 2510 (1962)

52. Booth, G.H., and Tiller, A.K. *Corrosion Sci.* 8, 583 (1968)

53. Campbell, L.L., and Postgate, J.R. *Bact. Rev.* 29, 359 (1965)

54. Postgate, J.R., and Campbell, L.L. *Bact. Rev.* 30, 732 (1966)

55. Booth, G.H. *J. Appl. Bact.* 27, 174 (1964)

56. Baars, J.K. "*Over Sulfaatreductie Door Bacterien*". Doctoral Thesis. Naamloze Vennootschap W.D. Meinema, Delft (1930)

57. Starkey, R.L. *Arch. Mikrobiol.* 9, 268 (1938)

58. Mechelas, B.J., and Rittenberg, S.C. *J. Gen. Microbiol.* 31, 365 (1960)

59. Roy, A.B., and Trudinger, P.A. *The Biochemistry of Inorganic Compounds of Sulphur*, Cambridge University Press (1970)

60. Zobell, C.E. *J. Sedimentary Petrology* 22, 42 (1952)

61. Miller, J.D.A., and Wakerley, D.S. *J. Gen. Microbiol* 43, 101 (1966)

62. Postgate, J.R., *J. Gen. Microbiol.* 15, 186 (1956)

63. Horváth, J., and Novák, M. *Corrosion Sci.* 4, 159 (1964)

64. Von Wolzogen Kühr, C., and van der Vlugt, L. *Water*, Den Haag, 16, (1934)

65. Stephenson, M., and Stickland, L. *Biochem. J.* 25, 205 (1931)

66. Stephenson, M., and Stickland, L. *Biochem. J.* 25, 215 (1931)

67. Hadley, R. *Corrosion by micro-organisms in aqueous and soil environments.* In Uhlig, H.H., (editor), *The corrosion handbook*, Wiley, New York, 466 (1948)

68. Wanklyn, J., and Spruit, C. *Nature*, Lond, 169, 928 (1952)

69. Wanklyn, J., and Spruit, C. *Nature*, Lond, 168, 951 (1951)

70. Horváth, J., and Solti, M. *Werkstoffe und Korrosion* 10, 624 (1959)

71. Booth, G., and Tiller, A. *Trans. Faraday Soc.* 56, (455), 1689 (1960)

72. Booth, G., and Wormwell, F. *1st Int. Congr. Metall. Corros.*, Butterworth, London (1961)

73. Hoar, T., and Farrer, T. *Corrosion Sci.* 1, 49 (1961)

74. Booth, G. *Corrosion Sci.* 2, 227 (1962)

75. Tiller, A., and Booth, G. *Trans Faraday Soc.* 58 (469), 110 (1962)

76. Booth, G., and Tiller, A. *Trans Faraday Soc.* 58 (480), 2510 (1962)

77. Booth, G., Shinn, P., and Wakerley, D. *Congrés International de la Corrosion Marine et des Salissures*, Cannes, C.R.E.O., Paris (1965)

78. Booth, G., Cooper, P., and Wakerley, D. *British Corrosion J.* 1, 345 (1966)

79. Booth, G., Cooper, A., and Cooper, P. *Chem. Ind.* 49, 2084 (1967)

80. Booth, G., Robb, J., and Wakerley, D. *3rd int. Cong. metall. Corros.*, Moscow, 1966 2 (5), 542

81. Booth, G., Elford, L., and Wakerley, D. *British Corrosion J.* 3, 242 (1968)

82. Peck, H., and Gest, H. *J. Bacteriol.* 71, 70 (1956)

83. King, N., and Winfield, M. *Biochim. Biophys. Acta* 18, 431 (1955)

84. Iverson, W. *Science* 151, (3713), 986 (1966)

85. Iverson, W. *Nature*, Lond, 217, 1265 (1968)

86. Booth, G., and Tiller, A. *Corrosion Sci.* 8, 583 (1968)

87. Mara, D., and Williams, D. *Corrosion Sci.* 11, 895 (1971)

88. Mara, D., and Williams, D. *Corrosion Sci.* 12, 29 (1972)

89. Mara, D., and Williams, D. *Chem. Ind.*, May 1971, 566 (1971)

90. Ashton, S., King, R., and Miller, J. *British Corrosion J.* 8, 132, (1973)

91. King, R., Miller, J., and Wakerley, D. *British Corrosion J.* 8, 89, (1973)

92. Mara, D., and Williams, D. *British Corrosion J.* 7, 94 (1972)

93. King, R., and Wakerley, D. *British Corrosion J.* 8, 41 (1973)

94. Berner, R. *J. Geol.* 72, 293 (1964)

95. Milton, C. *Corrosion* 22, 191 (1966)

96. King, R., Miller, J., and Smith, J. *British Corrosion J.* 8, 137 (1973)

97. King, R.A., and Miller, J.D.A. *Nature* 233, 491 (1971)

98. MacPherson, Rhona, and Miller, J.D.A. *J. Gen. Microbiol* 31, 365 (1963)

99. Sisler, F.D. *New Sci.* 12 (256), 110 (1961)

100. *Handbook of Chemistry and Physics*, R.C. Weast, Editor. Chemical Rubber Company, Cleveland (1971)

101. Burton, K., and Krebs, H.A. *Biochem. J.* 54, 94 (1953)

102. Delahay, P. *J. Amer. Chem. Soc.* 74, 3497 (1952)

103. Onsager, L. *J. Chem. Phys.* 2, 599 (1934)

104. Potter, E.C. *Electrochemistry : principles and applications*, Cleaver-Hume, London (1961)

105. Parsons, R., and Bockris, J. O'M. *Trans. Faraday Soc.* 47, 916 (1951)

106. Bockris, J. O'M., Drazic, D., and Despic, A.R. *Electrochimica Acta* 4, 325 (1961)

107. Barnartt, S. *J. Electrochem. Soc.* 119, (7), 812 (1972)

108. Parsons, R. *Handbook of electrochemical constants*, Butterworth, London (1959)

109. Enyo, M. *Electrochimica Acta* 18, 155 (1973)

110. Enyo, M. *Electrochimica Acta* 18, 163 (1973)

111. Mellor, J. *A Comprehensive treatise on inorganic and theoretical chemistry*, Longmans, Green and Co, London (1952)

112. Hoberman, H.D., and Rittenberg, D. *J. Biol. Chem.* 147, 211 (1943)

113. Joklik, W.K. *Australian J. exptal. Biol. Med. Sci* 28, 321 (1950)

114. Joklik, W.K. *Australian J. exptal. Biol. Med. Sci.* 28, 331 (1950)

115. Sisler, F.D., and Zobell, C.E. *J. Bact.* 60, 747 (1950)

116. Krasna, A., and Rittenberg, D. *J. Amer. Chem. Soc.* 76, 3015 (1954)

117. Gest, H. *Bact. Rev.* 18, 43 (1954)

118. Sadana, J.C., and Jagannathan, V. *Biochim. Biophys. Acta* 14, 287 (1954)

119. King, N.K., and Winfield, M.E. *Biochim. Biophys. Acta* 18, 431 (1955)

120. Peck, H.D., and Gest, H. *J. Bact.* 71, 70 (1956)

121 Sadana, J.C., and Jagannathan, V. *Biochim. Biophys. Acta* 19, 440 (1956)

122. Sadana, J.C., and Morey, A.V. *Biochim. Biophys. Acta* 32, 592 (1959)

123. Sadana, J.C., and Morey, A.V. *Biochim. Biophys. Acta* 50, 153 (1961)

124. Sadana, J.C., and Rittenberg, D. *Proc. Nat. Acad. Sci. U.S.A.* 50, 900 (1963)

125. Yagi, T., Honya, M., and Tamiya, N. *Biochim. Biophys. Acta* 153, 699 (1968)

126. Haschke, R.H., and Campbell, L.L. *J. Bact.* 105, 249 (1971)

127. Ackrell, B., and Asato, R.N., and Mower, H. *J. Bact.* 92, 828 (1966)

128 Peck, H.D. *J. Bact.* 82, 933 (1961)

129 Peck, H.D. *Proc. Nat. Acad. Sci., U.S.A.,* 45, 701 (1959)

130. *Recommendations (1964) of the International Union of Biochemistry on the nomenclature and classification of enzymes,* Elsevier, Amsterdam (1965)

131. Green, D., and Stickland, L.H. *Biochem. J.* 28, 898 (1934)

132. Peck, H.D. *Bact. Rev.* 26, 67 (1962)

133. Peck, H.D. *J. Biol. Chem.* 237, 198 (1962)

134. Peck, H.D. *Biochem. Biophys. Res. Commun.* 22, 112 (1966)

135. Peck, H.D., Deacon, T.E., and Davidson, J.T. *Biochim. Biophys. Acta* 96, 429 (1965)

136. Postgate, J.R. *Ann. Rev. Microbiol.* 13, 505 (1959)

137. Khosrovi, B., MacPherson, Rhona, and Miller, J.D.A. *Arch. Mikrobiol.* 80, 324 (1971)

138. Postgate, J.R. In : *Anreicherungskultur und Mutantenauslese*, Schlegel, H.G. and Kröger, E., Editors. Fischer Verlag, Stuttgart.

139. Iverson, W.P. *Applied Microbiol.* 14, 529 (1966)

140. Sisler, F.D., and Zobell, C.E. *J. Bact.* 62, 117 (1951)

141. Postgate, J.R. *Nature*, Lond, 183, 481 (1959)

142. Stern, M., and Makrides, A.C. *J. Electrochem. Soc.* 107, 782 (1960)

143. *Chemical Engineers' Handbook*, R.H. Perry, Editor. McGraw-Hill, New York (1963).

144. *International Critical Tables*, National Research Council, U.S.A. Vol V, McGraw-Hill, New York (1929)

145. Uhlig, H.H, In : *Corrosion Handbook*, H.H. Uhlig, Editor. Wiley, New York, General Tables (1963)

146. Shrier, L.L. In : *Corrosion*, L.L. Shrier, Editor. Newnes, London, 1.45 (1965)

147. Kolthoff, I., and Lingane, J. *Polarography Vol. 2*, Interscience, New York (1952)

148. Heyrovsky, J., and Kuta, J. *Principles of polarography*, Publishing House : Czechoslovak Acadamy of Sciences, Prague (1966)

149. Maironovskii, J. *J. Electroanal. Chem.*, 6, 77 (1963)

150. Fisher, H.F., Krasna, A.I., and Rittenberg, D. *J. Biol. Chem.*, 209, 569 (1954)

151. Brenner, M., Niederwieser, A., and Pataki, G. In : *Thin-layer chromatography*, E. Stahl, Editor. Acadamic Press, New York (1962)

152. Unpublished work, communicated by D.A. Sanan.

153. Harvey, K.B., and Porter, G.B. *Introduction to physical inorganic chemistry*, Addison-Wesley, London (1965)

154. Bolmer, P.W. *Corrosion*, 21, 69 (1965)

155. Iofa, Z.A., and Fan Lyong Kam, *Zascita metallov*, 10, 17 (1974).

156. Costello, J.A. *South Afr. J. Sci.*, 70, 202 (1974)

157. Clark, W.M. *Oxidation-reduction potentials of organic systems*, London, Baillière, Tindall and Cox (1960)

158. Michaelis, L., and Hill, E.S. *J. Gen. Physiol.*, 16, 859 (1933)

159. Bockris, J. O'M., and Cahan, B., *J. Chem. Phys.*, 50, 1307 (1969)

160. Copenhagen, W.J. *South Afr. Industrial Chemist*, 8, 32 (1954)

161. Sato, M. *Electrochimica Acta* 11, 361 (1966)

162. Ward, J.C. *Rev. Pure and Appl. Chem.*, 20, 175 (1970)

163. Hoar, T.P. *The anodic behaviour of metals*, In : *Modern Aspects of Electrochemistry*, No.2, J. O'M. Bockris, Editor. Butterworth, London (1959)

164. Stern, M. *J. Electrochem. Soc.*, 102 (11), 609 (1955)

TABLE I

Rates of penetration of iron and steel in various corrosive environments

Case No.	Author	Incidence of corrosion	Observed rate: mm per year	Calculated i_{corr} ($\mu A\ cm^{-2}$)
1.	Booth, Cooper and Cooper[19]	Mild steel. Sterile de-aerated bacterial culture medium 30°C.	0,012	1,03
2.	Cox and Roetheli[18]	Mild steel. Water in equilibrium with air. 23°C.	0,220	18,9
3.	LaQue[20]	Mild steel. Sea water 21°C.	0,254	21,3
4.	Booth, Cooper and Cooper[19]	Mild steel. Continuous culture *Dv. vulgaris* (Hildenborough) 30°C.	0,636	54,6
5.	Booth, Cooper and Cooper[19]	Mild steel. Continuous culture *Dv. vulgaris* (Canvey Island) 30°C.	0,957	82,2
6.	Bunker[21]	Steel water main in soil SRB present	1,129	96,9
7.	Copenhagen[22]	Steel sheet piling. Harbour water. SRB present	1,270	109
8.	Doig and Wachter[23]	Steel oil-well casing SRB present	2,078	178
9.	Copenhagen[9]	Steel hull of trawler in bilge water. SRB present	4,00	343

Case No.	Author	Incidence of corrosion	Observed rate: mm per year	Calculated i_{corr} ($\mu A\ cm^{-2}$)
10.	Uhlig[24]	Iron in 1-$\underline{M}$ HCl SRB absent	5,1	436
11.	Costello[10]	Mild steel suction pipe. Cape Town Harbour SRB present	5,14	441
12.	Costello[10]	Mild steel condenser tube, using Cape Town Harbour water. SRB present	7,62	654
13.	Uhlig[24]	Iron in 1-$\underline{M}$ HNO_3. SRB absent	50,8	4362

TABLE 2

Environmental limits for growth of the sulphate-reducing bacteria

pH	Redox Potential Eh	Temperature C	Salinity % w/v N Cl	Hydrostatic Pressure Atmospheres
4,2 - 10,4		0 - 65°C		
	-0,050 to -0,300			
Most commonly 6,5 - 7,5		Most commonly: 25 - 45°C	0,1 - 30%	1 - 1000

It should be noted that the salinity and pressure tolerances of individual strains of the organisms are usually restricted to rather narrow limits.

TABLE 3

CLASSIFICATION OF DISSIMILATORY SULPHATE-REDUCING BACTERIA[53,54]

Genus *Desulfovibrio* (Kluyver & van Niel, 1936; Postgate & Campbell, 1966)

Non sporulating, Gram-negative vibrios, sometimes sigmoid or spirilloid; occasionally straight. Obligate anaerobes with polar flagella showing progressive motility. Mesophilic, sometimes halophilic. Contain cytochrome-c_3 and desulphoviridin and generally hydrogenase. Facultative or obligate sulphate reducers; sulphate reduction is their respiratory dissimilatory process. Habitats, sea water, marine mud, fresh water and soil.

Species

Dv. desulfuricans (type species); *Dv. vulgaris; Dv. salexigens* (salt water species); *Dv. gigas; Dv. africanus*

Genus *Desulfotomaculum* (Campbell & Postgate, 1965)

Gram-negative, straight or curve rods, usually single or sometimes in chains; thermophilic types show lenticulate and otherwise swollen forms. Terminal or subterminal sporulation, slightly swelling the cells. Motile, with peritrichous flagella. Obligate anaerobes which reduce sulphate to sulphide. Habitats, fresh water, soils geothermal regions, certain spoiled foods, intestines of insects and in rumen contents of ruminant animals.

Species

Dv. nigrificans (thermophilic, opt. temp. 55^{o}), *Dv. orientis* (mesophilic, opt. temp. 30 - 37^{o}); *Dv. ruminis* (mesophilic, opt. temp. 37^{o}).

TABLE 4

ANALYTICAL DATA OF VON WOLZOGEN KÜHR AND VAN DER VLUGT[64] QUOTED IN SUPPORT OF THEIR PROPOSED CORROSION MECHANISM

NO	TOTAL IRON CORRODED (mg)	QUANTITY OF IRON AS AS IRON SULPHIDE (mg)	RATIO $\frac{\text{TOTAL Fe}}{\text{Fe as FeS}}$
1	10,6	3,9	2,7
2	9,8	3,0	3,3
3	12,6	3,8	3,3
4	13,6	4,0	3,4
5	39,3	16,0	2,5
6	26,6	11,1	2,4
7	52,3	19,7	2,7
8	5,7	1,8	3,2
9	6,5	2,4	2,7
10	4,6	1,8	2,6

TABLE 5

CORRELATION OF HYDROGENASE ACTIVITY WITH CORROSION RATE FOUND BY BOOTH AND WORMWELL[72]

ORGANISM	HYDROGENASE ACTIVITY µl/hr/mg dry wt	CORROSION RATE mm per year
Dv. desulfuricans strain America	30	0,009
Dv. desulfuricans strain Teddington R	140	0,019
Dv. desulfuricans strain Hildenborough	240	0,025
Dv. desulfuricans strain Llanelly	340	0,031

TABLE 6

DATA OF KING, MILLER AND SMITH[96] FOR CORROSIVITIES OF VARIOUS SULPHIDES OF IRON

COMPOUND	CORROSION PER MOL SULPHUR mg Fe/m mol	CORROSION PER MOL IRON SULPHIDE mg Fe/m mol	MOLAR % SULPHUR
Pyrite, FeS_2	61,53	123,06	67
Greigite, Fe_3S_4	12,53	50,12	57
Smythite, Fe_3S_4	19,51	78,04	57
Mackinawite, $Fe_{1+x}S$	10,08	10,08	ca 50
Pyrrhotite, $Fe_{1-x}S$	6,39	6,39	ca 50

TABLE 7

Reaction	Description	ΔG^o
(i) $SO_4^= + 4H_2 \rightarrow S^= + 4H_2O$	Reduction of sulphate by hydrogen taken up by hydrogenase system of SRB	-123,1 kJ
(ii) $2CH_3CHOHCO_2^- + SO_4^= \rightarrow 2CH_3CO_2^- + 2CO_2 + S^= + 2H_2O$	Reduction of sulphate with lactic acid as hydrogen donor, practised by SRB	-130,8 kJ
(iii) $4Fe + SO_4^= + 4H_2O \rightarrow 3Fe(OH)_2 + FeS + 2OH$	The von Wolzogen Kühr - van der Vlugt equation (4.10)	-525,6 kJ
(iv) $SO_4^= + H_2 \rightarrow SO_3^= + H_2O$	Reduction of sulphate to sulphite by molecular hydrogen	+7,74 kJ
(v) $SO_3^= + 3H_2 \rightarrow S^= + 3H_2O$	Reduction of sulphite to sulphide by molecular hydrogen	-130,9 kJ

ΔG_f^o values of all chemical species above other than $CH_3CHOHCO_2^-$ (aq) and $CH_3CO_2^-$ (aq) were obtained from Handbook of Chemistry and Physics (1970)[100]. Values for $CH_3CHOHCO_2^-$ (aq) and $CH_3CO_2^-$ (aq) used are those of Burton and Krebs[101].

TABLE 8

TRANSFER COEFFICIENTS, α, FOR VARIOUS HYDROGEN EVOLUTION MECHANISMS AND CORRESPONDING ANTICIPATED TAFEL SLOPES[31]

MECHANISM	TRANSFER COEFFICIENTS α ASSUMING $\beta = 0,5$	TAFEL SLOPE, $\frac{RT}{\alpha F}$, AT 25°C
1. Discharge step rate-determining, followed by chemical desorption	0,5	0,120 V
2. Discharge followed by rate-determining chemical desorption	2,0	0,030 V
3. Coupled discharge-chemical desorption	0,5	0,120 V
4. Discharge step rate-determining followed by electrodic desorption	0,5	0,120 V
5. Discharge followed by rate-determining electrodic desorption	1,5	0,040 V
6. Coupled discharge-electrodic desorption	0,5	0,120 V

<u>TABLE 9</u>

Culture	Age (days)	Em V	Eh V	pH	Eh (pH=7,0) V	p_{O_2}
WVB	5	-0,380	-0,138	6,48	-0,169	5×10^{-66}
WVB	8	-0,312	-0,070	6,85	-0,079	5×10^{-60}
BS	6	-0,330	-0,088	6,65	-0,109	5×10^{-62}
BS	6	-0,405	-0,163	6,50	-0,193	$2,5\times10^{-67}$
BS	7	-0,338	-0,096	6,70	-0,114	5×10^{-62}

Em = potential (V) *versus* satd. KCl - calomel

Eh = potential on hydrogen scale (satd. KCl - calomel at $25^{o}C = 0,242V$)

Eh(pH 7,0) = calculated potential at pH = 7, assuming $\frac{RT}{nF} = 0,060V$.

p_{O_2} = partial pressure of O_2 calculated from the Nernst relationship ($E = 1,\ 23 - 0,060\ pH + 0,015 \log p_{O_2}$) for the equilibrium

$$O_2 + 4H^+ + 4e \rightleftharpoons 2H_2O, \text{ at pH } = 7,0$$

TABLE 10

Total sulphide species content $\left[S^{=}\right]$ and pH characteristics of cultures of *Desulfovibrio* sp (BR), cathodic polarization curves for which are given in Fig. 68.

Culture	pH	$\left[S^{=}\right]$
A	6,80	0,0086
B	6,40	0,0236
C	6,50	0,0250
D	6,45	0,0263

TABLE 11

A comparison of data for the cathodic discharge on pure iron of the species H_2S, H_3O^+ and H_2O.

Species	pH	b_c (V)	i_o ($\mu A\ cm^{-2}$)
p_{H_2S} = 0,23 atm; $[HS^-] = 10^{-1}$	7,34	0,065	$1,8 \times 10^{-2}$
p_{H_2S} = 0,34 atm; $[HS^-] = 10^{-2}$	6,17	0,070	5×10^{-2}
p_{H_2S} = 0,28 atm; $[HS^-] = 10^{-3}$	5,25	0,120	4
p_{H_2S} = 0,16 atm; $[HS^-] = 10^{-3}$	5,50	0,115	3
p_{H_2S} = 0,10 atm; $[HS^-] = 10^{-3}$	5,69	0,110	8×10^{-1}
p_{H_2S} = 0,058 atm; $[HS^-] = 10^{-3}$	5,94	0,120	$5,5 \times 10^{-1}$
H_3O^+	1	-	10
H_3O^+	3,6	0,113	1
H_3O^+	4,5	-	3×10^{-1}
H_2O	3,6	0,140	1×10^{-2}

Data for sulphide-containing solutions are those of Bolmer[154], and were determined in a 0,5 M Na_2SO_4 supporting electrolyte. Partial pressure (p_{H_2S}) values are those of H_2S in a mixture with pure nitrogen gas in equilibrium with the electrolyte. The Tafel b_c values indicate differences in reaction mechanism for the various concentrations of sulphide species.

Data for H_3O^+ and H_2O species are those of Bockris, Drazic Despic[106] for pure iron in 0,5 M Na_2SO_4.

TABLE 12

Hydrogen absorption coefficients of cells of *Desulfovibrio* separated from cathodically active cultures (Figs. 74 - 76).

Culture	$-Q_{H_2}^{BZV}$ µl/h/mg dry wt of cells
Desulfovibrio vulgaris (strain Hildenborough NCIB 8303)	259
Desulfovibrio sp (strain ND)	661
Desulfovibrio sp (strain WVB)	201

TABLE 13

Anodic Tafel slopes (b_a) for mild steel in 0,02 M NaCl with sulphide additions at pH 7,40.

$[S^=]$	b_a (V)
0	0,045
10^{-6}	0,029
10^{-5}	0,035
10^{-4}	0,051
10^{-3}	0,057

APPENDIX : CHAPTER 6

All growth media listed below were supplemented with 2,5% W/V of NaCl, A.R. for the growth of culture *Desulfovibrio* sp. "WVB".

6.1. Sterilization Procedures

All media and some of the dry glassware used were sterilized by autoclaving in steam at 103 kPa (15 pounds per square inch) for 15 minutes. Other dry glassware was oven-sterilized at 160°C for 2 hours.

Transference of colonies from solid to liquid media was conducted in a hood sterilized by ultra-violet irradiation from a mercury vapour lamp. Irradiation was switched off during the operation.

6.2. Baars' Medium[56]

	g per dm^3 of distilled water
KH_2PO_4, A.R.	0,5
NH_4Cl, A.R.	1,0
$CaSO_4$, A.R.	1,0
$M\ SO_4.7H_2O$, A.R.	2,0
Sodium lactate (70% solution)	5,0
Mohrs' salt, $(NH_4)_2\ SO_4\ FeSO_4 \cdot 6H_2O$, A.R.	0,5

Immediately before inoculation, sterile $Na_2S.9H_2O$ solution was added to give a concentration of 0,5 m M and the pH adjusted by dropwise addition of sterile 10% W/V sodium hydroxide solution, to values between 7,2 and 7,4.

6.3. Postgate's Isolation Medium[138]

	g per dm³ of distilled water
KH_2PO_4,, A.R.	0,5
NH_4Cl, A.R.	1,0
Na_2SO_4, A.R.	1,0
$MgSO_4 . 7H_2O$, A.R.	2,0
Sodium lactate (70% solution)	5,0
$CaCl_2 .2H_2O$, A.R.	0,1
Mohr's salt $(NH_4)_2SO_4FeSO_4.6H_2O$, A.R.	0,5
sodium ascorbate	0,1
sodium thioglycollate	0,1
yeast extract	1,0
agar, "Bacto"	10,0

The medium was always prepared freshly on the day of use, and immediately before inoculation, its pH was adjusted to values between 7,2 and 7,4 with sterile 10% W/V sodium hydroxide solution.

6.4. Iverson's Agar Medium[139]

Iverson's "TSA plus salts" medium containing lactate was employed.

	g per dm^3 of distilled water
Trypticase Soy Agar	40
Agar "Bacto"	5
sodium lactate (70% solution)	5
$MgSO_4 \cdot 7H_2O$, A.R.	2
Mohr's salt, $(NH_4)_2SO_4FeSO_4 \cdot 6H_2O$, A.R.	5

The medium was prepared freshly on the day of use and its pH was adjusted to values between 7,2 and 7,4 after autoclaving.

6.5. Contaminant Testing

(a) *Aerobic tests*

Postgate[138] recommends any nutrient agar containing glucose and peptone for this purpose. A medium recommended by Miller* was used.

* Personal communication : J.D.A. Miller

	g per dm^3 of distilled water
Bacto-peptone	5
Yeast extract	4
Glucose	10
Agar	15
Na_2SO_4	1,4
M $SO_4.7H_2O$	1,2

Plates of this medium were poured in Petri dishes and inoculated from the purified cultures using a platinum wire loop.

(b) *Anaerobic tests*

The medium recommended by Postgate[138] was employed :

	g per dm^3 of distilled water
Bacto-peptone	4
Glucose	10
Na_2SO_4, A.R.	2
$MgSO_4.7H_2O$, A.R.	1
$(NH_4)_2$ $SO_4FeSO_4.6H_2O$,A.R.	0,5

The medium, with its pH adjusted to values between 7,0 and 7,6 was dispensed in 10 cm^3 quantities into 20 cm^3 test tubes. Inoculation was performed before setting of the agar (at a temperature of 40°C). A loopful of the culture to be tested (in

2 mm diameter platinum wire loop) was plunged to the bottom of the agar in a tube and then successively into 3 other tubes, thereby diluting the inoculum.

Tubes and agar plates thus inoculated were incubated at room temperature, at 30°C, and at 37°C.

6.6. Postgate's Test for *Desulfoviridin*[141]

A 10 cm^3 quantity of culture was centrifuged and the cells resuspended in 1 cm^3 of distilled water. One drop of 20% W/V of sodium hydroxide solution was added and the suspension inspected at once under ultra-violet irradiation at 365 m μ in a darkened room. All of the putative *Desulfovibrio* cultures were examined in this way. A brilliant red fluorescence was exhibited in all cases (varying in intensity for the different cultures), confirming the presence of desulfoviridin pigment and the identity of the organisms as *Desulfovibrio* species.

6.7. The Medium of MacPherson and Miller[98]

The medium of MacPherson and Miller, modified by the addition of 0,1% yeast extract was used :

	g per dm^3 of distilled water
Sodium lactate (70% solution)	16,0
KH_2PO_4, A.R.	0,340
NH_4Cl, A.R.	0,535
Na_2SO_4, A.R.	7,100
$MgSO_4.7H_2O$, A.R.	0,062
$CaCl_2$, A.R.	0,055
Yeast extract	1,000

Immediately prior to inoculation, sterile $FeSO_4.7H_2O$ solution was added to give a concentration of 25 μ<u>M</u>, and sterile $Na_2S.9H_2O$ solution to a concentration of 0,5 m<u>M</u>. The pH was adjusted to values between 7,2 and 7,4 by addition of sterile 10% w/v sodium hydroxide solution.

APPENDIX : CHAPTER 7

Wenking

Control voltage range	± 2 V
Response time	10^{-6} S
Circuit voltage	± 15 V
Current output	± 0,5 A

Tacussel

Control voltage range	± 5 V
Circuit voltage	± 20 V
Response time	2 - 3 x 10^{-6} S
Current output	2 A
Control	2 - 3 mV

Opamp

Control voltage range	± 1,5 V
Rise time	10^{-12} S
Circuit voltage	± 15 V
Current output	0,5 A
Control sensitivity	< 1 mV

APPENDIX : CHAPTER 9

The determination of sulphides in experimental cultures

A method, based on that described by Belcher and Nutten* using standard arsenite and iodine solutions, was used. The reactions are

$$As_2O_3 + 3H_2S \longrightarrow As_2S_3 + 3H_2O$$

and

$$As_2O_3 + 2I_2 + 5H_2O \longrightarrow 2HAs_2O_4^{2-} + 4I^- + 8H^+$$

A 50 cm^3 quantity of 0,025 M As_2O_3 solution was mixed with 50 cm^3 of centrifugate of the culture in a 250 cm^3 volumetric flask. The solution was well-mixed, acidified with dilute HCl and made up to the mark. The excess arsenious oxide was back-titrated by filtering the resultant solution to remove the As_2S_3 precipitate and titrating 100 cm^3 aliquots with 0,1 M iodine solution using starch indicator.

* Belcher, R., and Nutten, A.J., *Quantitative Inorganic Analysis*, Butterworth's, London (1960), p 277.

APPENDIX : CHAPTER 10

The determination of hydrogen absorption coefficients

Hydrogen absorption coefficients were determined by measuring hydrogen uptake in the Warburg respirometer. The instrument used was a rotary-shaking assembly (model V85) manufactured by B. Braun Apparatebau, Melsungen, equipped with factory calibrated manometers and flasks. Experiments were conducted at 30 $\pm$ 0,01 °C, and the reducible substrate was benzyl viologen.

The 15 cm^3 cell suspension was removed from the anaerobic jar (in which it had been stored under hydrogen at 0° C) and bubbled with hydrogen for a period of 5 minutes. Duplicate manometers were prepared for each cell suspension. 0,5 cm^3 of cell suspension was placed in the body of the flask and 0,3 cm^3 of benzyl viologen solution (0,1 M in tris buffer, pH 7,0) in the side arm. A solution of 20% w/v of KOH (0,2 cm^3 on a filter paper support) was used in the centre well as absorbent for any CO_2 or H_2S formed. The manometer/flask assemblies were completed and the whole swept with H_2 gas for 20 minutes. Thereafter the system was sealed and placed in the water bath.

After a 30-minute equilibration period, manometers were tilted to mix cell suspension and reducible substrate, and shaking commenced. Readings were taken at intervals of one minute. The mean of duplicate hydrogen uptake values for each culture (with thermobarometer corrections) was plotted against time for each cell suspension, and hydrogen absorption coefficients determined from the steepest portion of the resultant curve. Hydrogen uptake curves for the four cell suspensions prepared are given in the diagram overleaf.

Cell suspension dry-weight determinations were performed by washing the cells in the remaining 14 cm^3 of cell suspension by re-centrifuging and washing six times with distilled water, re-suspending the cells in 14 cm^3 of distilled water and drying 5 cm^3 aliquots of the suspension to constant weight at 105°C.

APPENDIX : CHAPTER 11

DETERMINATION OF THE SURFACE AREA OF CORROSION PRODUCTS

Experimental Method

Sulphide corrosion products are sensitive to oxidation on exposure to the air, and care was taken to prevent this during the determination. The corroded pipe had been freshly removed from the harbour when the corrosion product was sampled. The corrosion product was removed from the pit using a clean nickel spatula, and was placed under a solution of 0,01 M sodium sulphide in a screw-capped bottle with a rubber seal. The sample was then transferred to the laboratory. The corrosion product was separated off from the solution and washed using nitrogen pressure filtration in a Sartorius SM pressure filtration apparatus fitted with a 1,5 μm pore-diameter membrane filter. The distilled water used for washing was contained in a separating funnel and was continuously purged with a stream of nitrogen. The water was added to the filtration chamber against a positive pressure of nitrogen. In this way, the precipitate was washed with 1 dm^3 of distilled water in 100 cm^3 quantities.

The wet sample was transferred to the 100 cm^3 size flask of a Ströhlein Surface Area Meter and dried by heating in a nitrogen stream at 150° C for 2 hours. The nitrogen adsorption principle is based on the use of two glass adsorption vessels of the same volume, one containing the sample while the other remains empty. Both vessels are filled with nitrogen at ambient temperature and pressure. They are then connected to the different manometric apparatus and cooled by immersion in liquid nitrogen. The adsorption of nitrogen on the sample results in a pressure difference between the flask containing the sample and the empty one. This pressure difference is indicated on the differential manometer which is filled with coloured dibutyl phthalate.

The principle of calculation of the surface area from this reading on the Ströhlein apparatus (Ströhlein and Co., Dusseldorf) is that developed by Haul and Dümbgen*. The following data are required

m	=	sample mass (g)
Δh	=	pressure difference on manometers (mm dibutyl phthalate)
p_β	=	filling pressure (atmospheric pressure, mm Hg)

From Δh and p_β, the equilibrium pressure p is first calculated :

$$p = C_1 p_\beta - C_2 \Delta h$$

where C_1 and C_2 are apparatus constants. Using p, the specific surface area (sg) is then obtained as

$$Sg = \frac{2,1 \times 10^{-3} \; (785-p)\Delta h \; (K_1 + K_2 . p)}{m}$$

where K_1 and K_2 are apparatus constants. For simplicity, this expression may be transformed to

* *Chemie* - Ing. - Tech. 32 (5), 349 (1960)

$$Sg = \frac{A.\Delta h}{m}$$

where A is a constant which may be obtained from a nomogram supplied with the apparatus

$$A = (785- \ )(K_1 + K_2 .p) \times 2,1 \times 10^{-3}.$$

For the purposes of the present determination, the apparatus was calibrated using a sample of carbon black of known specific surface area supplied by the manufacturer (specific surface area = $106 \pm 2\ m^2 g^{-1}$).

<u>Results</u>

	1	2	3	4
Sample	Carbon Black	Carbon Black	Corrosion Product	Corrosion Product
Mass (g)	0,3462	0,3697	1,0222	1,0222
p_β (mm Hg)	648,97	648,71	648,20	651,26
Δh (mm)	253	271	96	90
A	0,1486	0,1489	0,1467	0,1465
Sg(m^2g^{-1})	108,6	109,1	13,78	12,90

Mean surface area of corrosion product = $13,3\ m^2g^{-1}$.

FIGURE 1

Perforated mild steel intake pipe from condenser system in which harbour water was used as a coolant.

FIGURE 2

Section of condenser tube

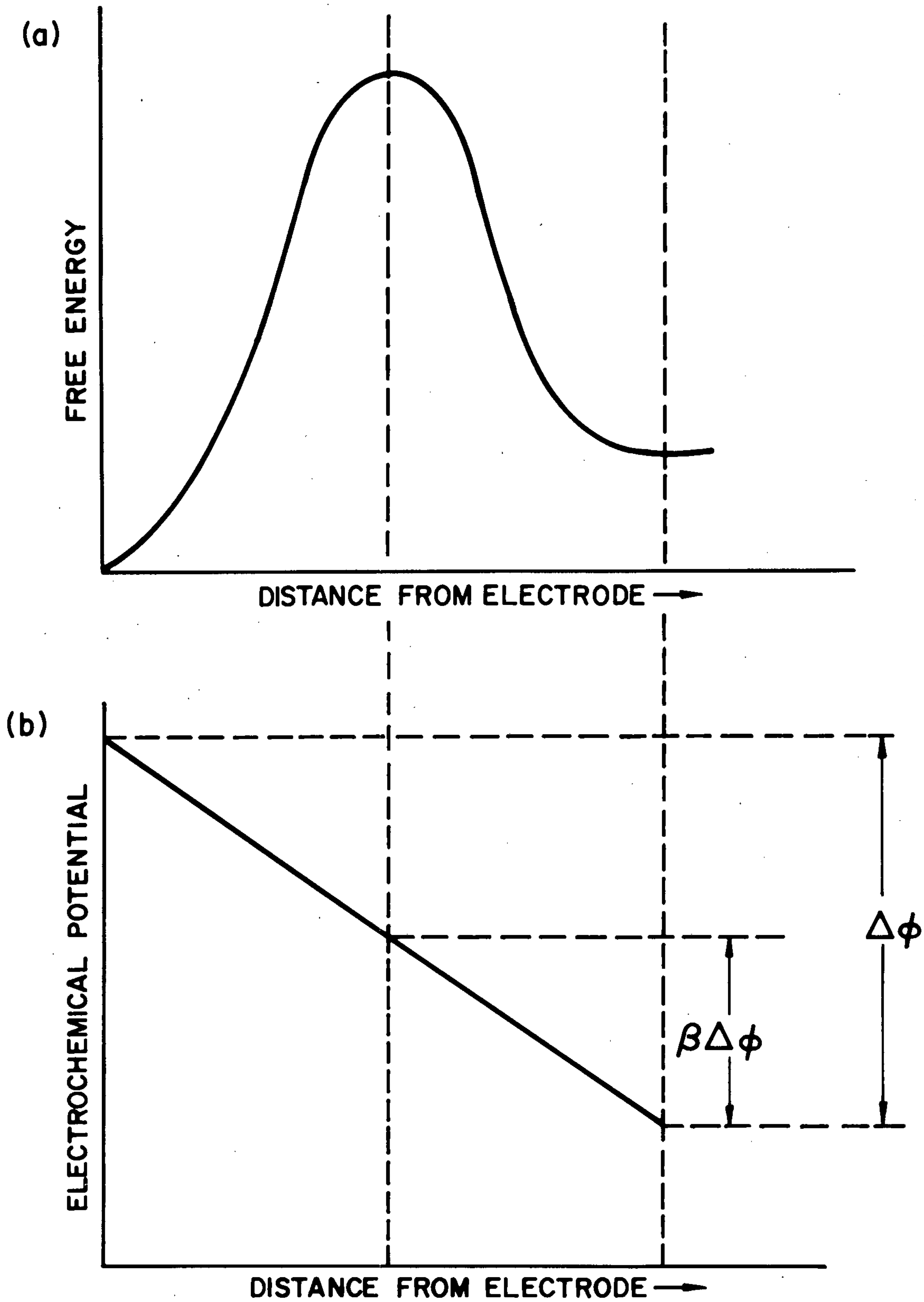


FIGURE 3

Schematic depiction of the anticipated free energy vs. distance and potential vs. distance relationship for an electrode/electrolyte interface. The electrical work of activating the ion is determined by the potential difference across which the ion has to be moved to reach the top of the free energy vs. distance relationship

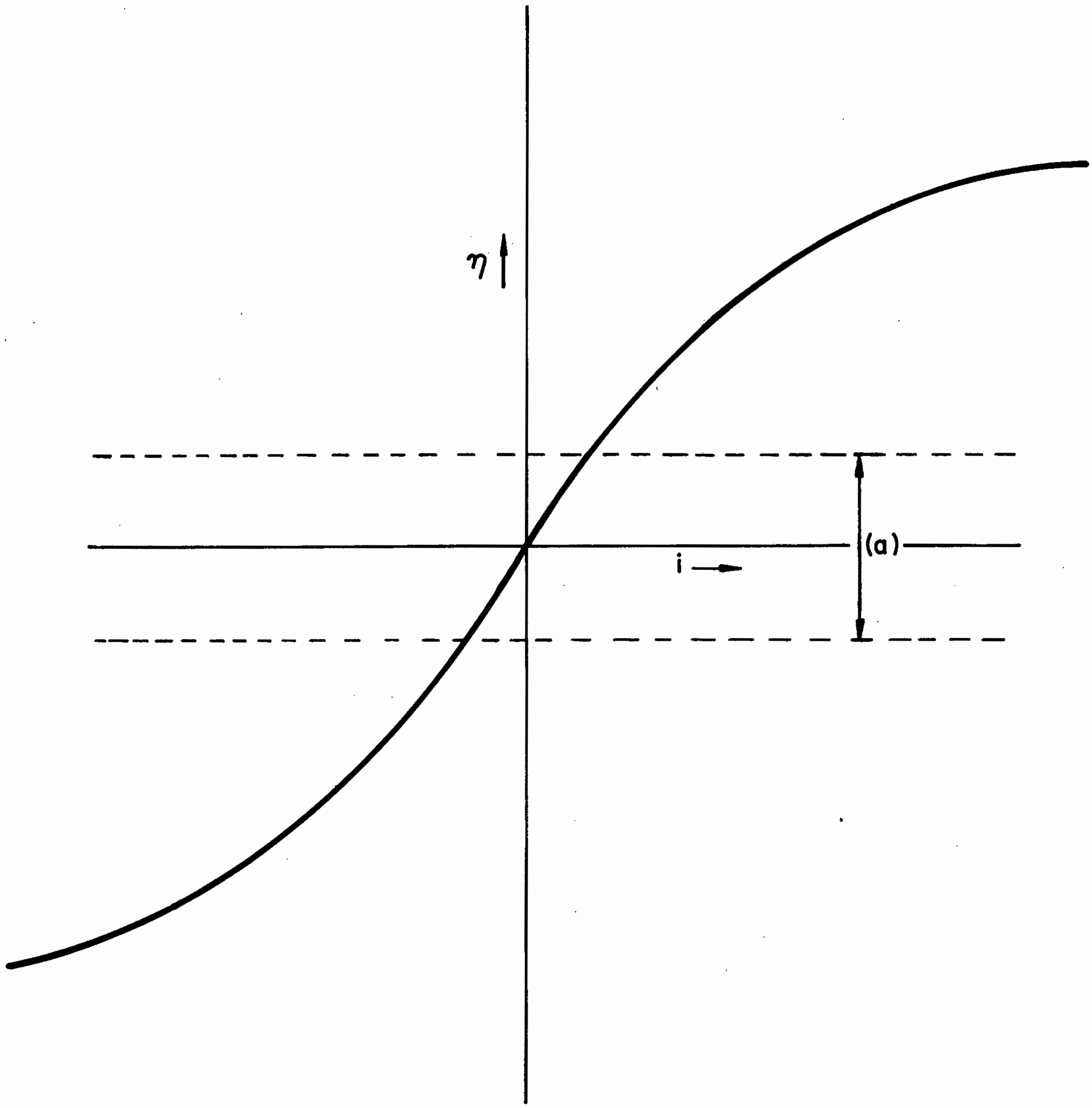


FIGURE 4

i <u>vs</u> η relationships for an electrode system undergoing excursions from equilibruim state. The low-field approximation resulting in a linear i <u>vs</u> η relationship holds at small η values (between dotted lines, marked (a)). At larger values of η the exponential high-field approximation holds

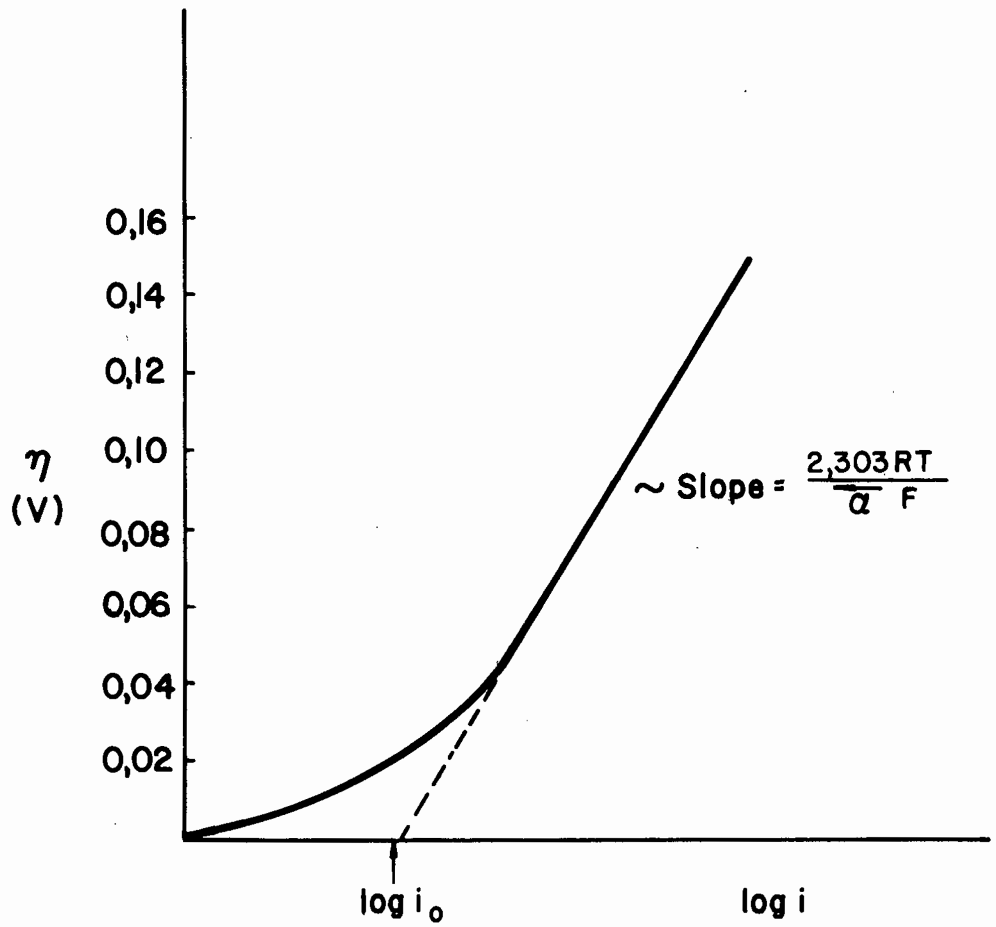


FIGURE 5

Results of an electrochemical polarization experiment enabling i_o and α to be determined

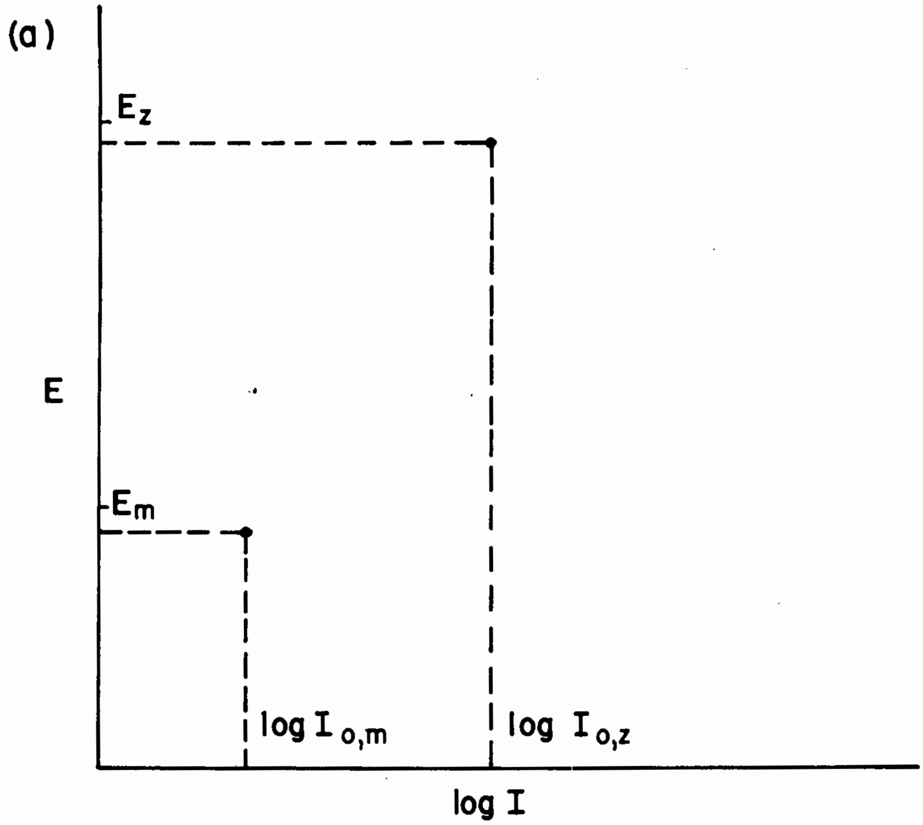


FIGURE 6(a)
Critical coordinates of electrode systems $Z^{n'+} + n'e \rightleftharpoons Z$ and $M^{n+} + ne \rightleftharpoons M$

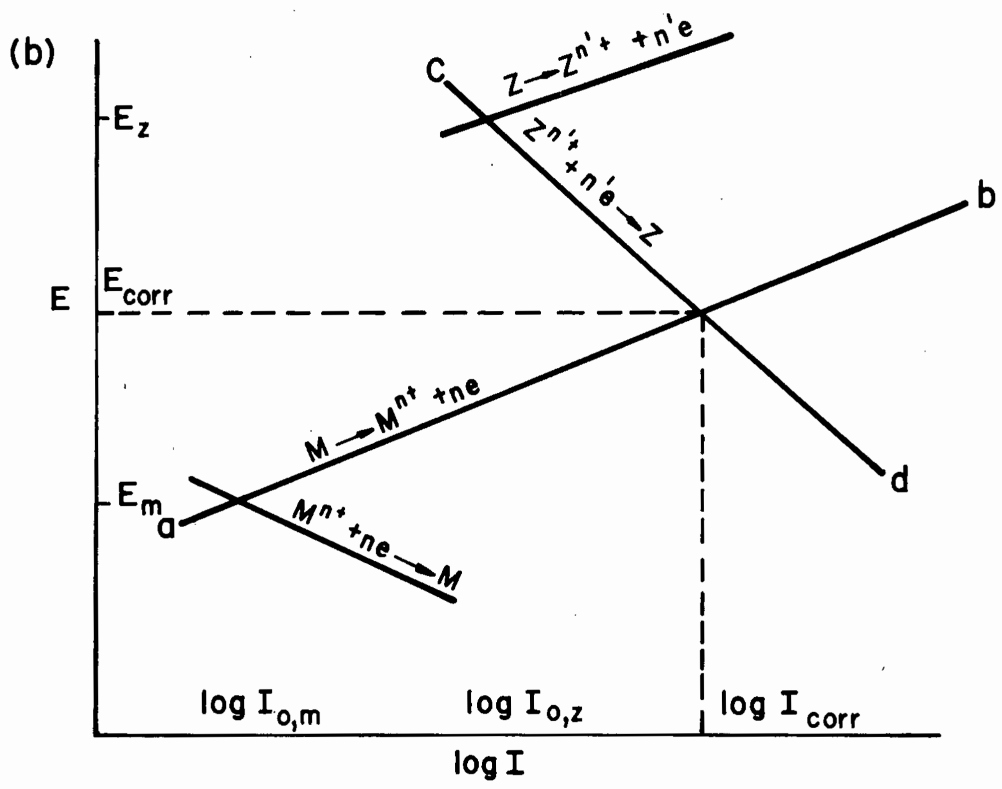


FIGURE 6 (b)
Interaction of the anodic dissolution of M with the cathodic reduction of $Z^{n'+}$. Lines a b and c d may be found by polarization experiments and E_{corr}, i_{corr} may thus be determined

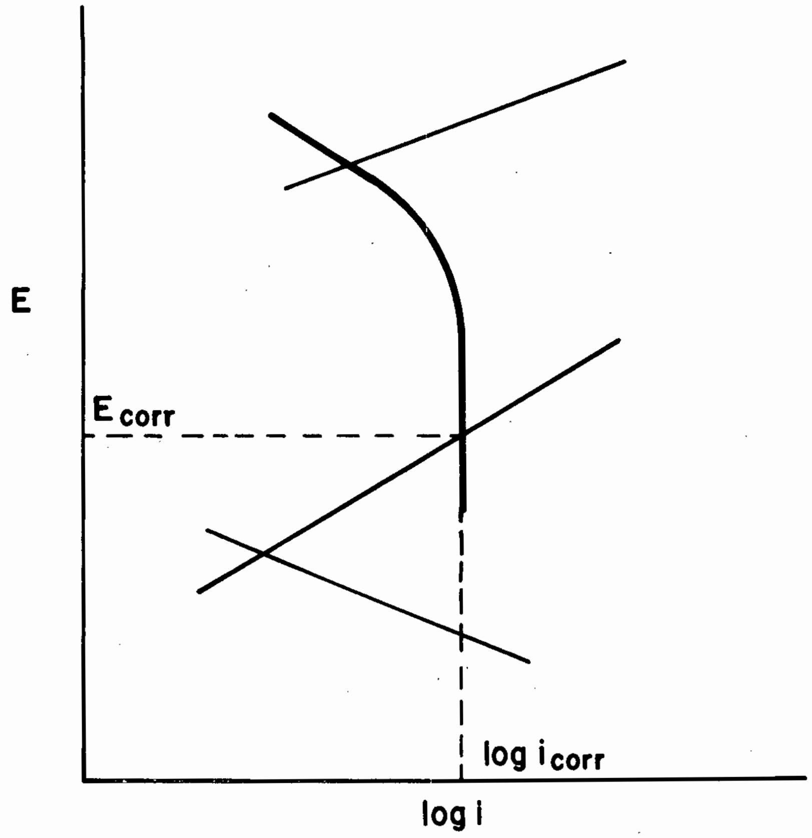


FIGURE 7(a)
Concentration polarization at the cathode where
$i_{corr} = i_{d,l}$

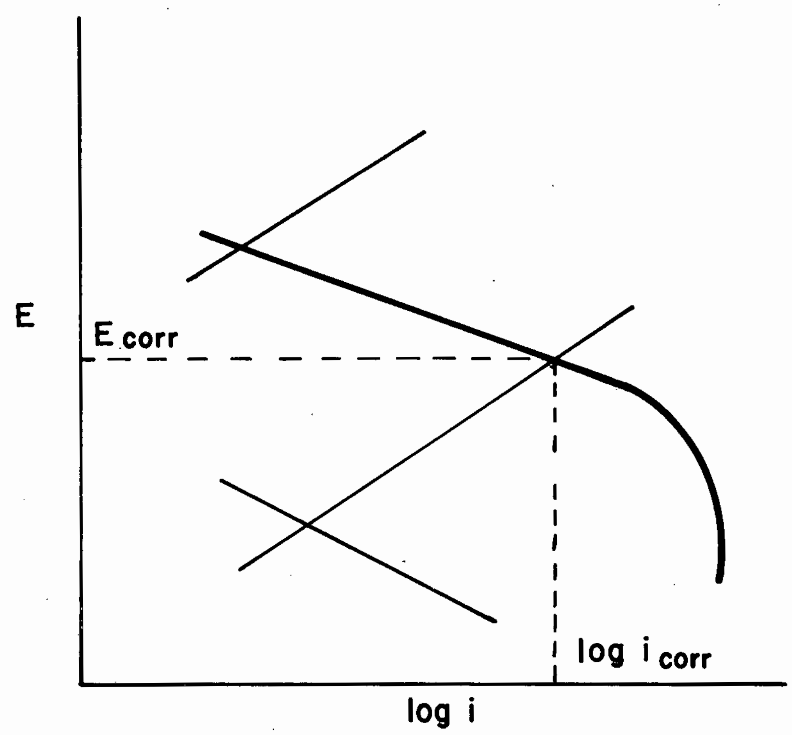


FIGURE 7(b)
Concentration polarization at the cathode where
$i_{corr} < i_{d,l}$

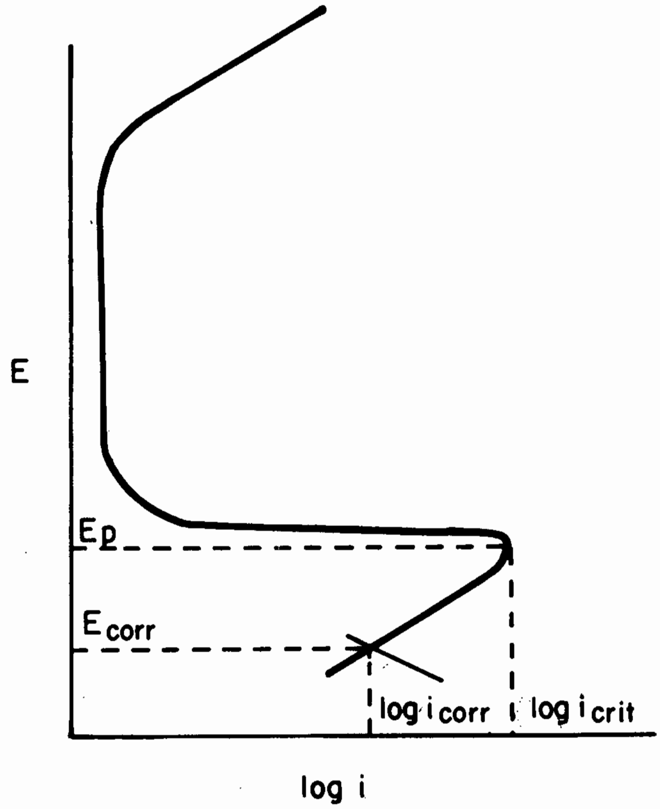


FIGURE 8(a)

Anodic polarization of metal to be passivated beyond coordinates i_{crit} and Ep causes passivity characterized by very low anodic current, constant over a range of passive potentials. Far anodic polarization causes a current increase caused by the anodic oxidation of water, liberating oxygen.

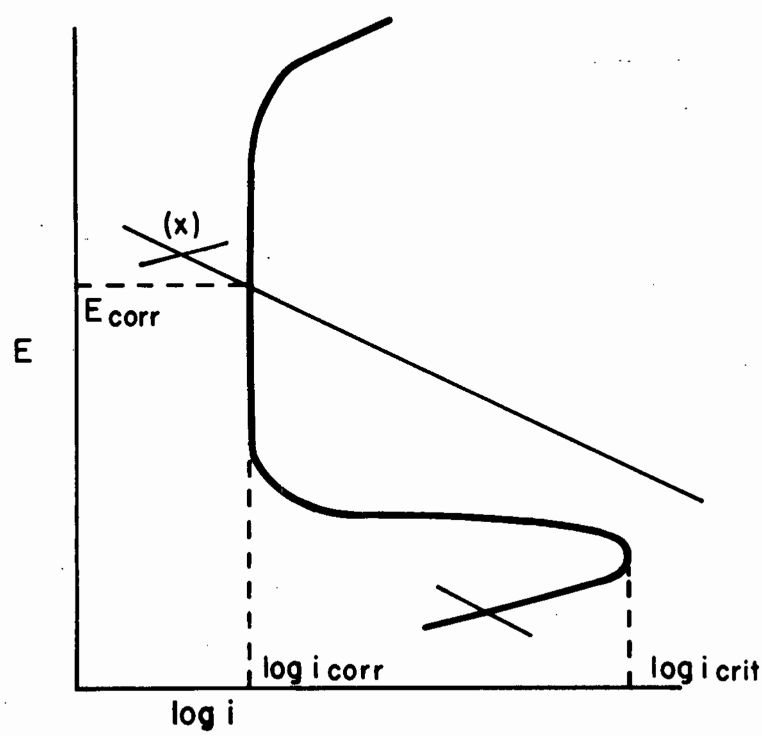


FIGURE 8(b)

A chemical oxidizing agent active as a cathodic reagent (with critical coordinates, $E°, i_0$, at point x) may polarize the corroding metal to potentials at which passivity occurs.

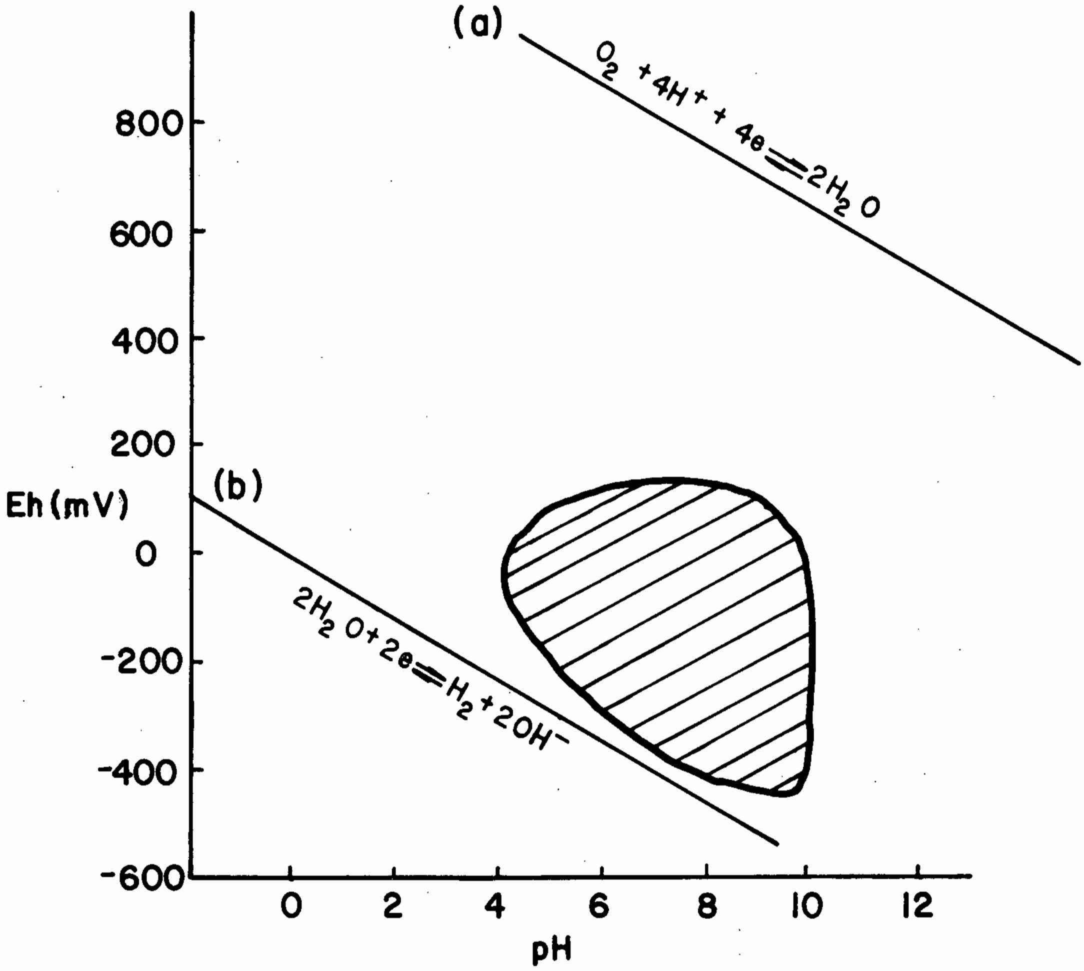


FIGURE 9

pH vs potential data for environments favourable to SRB activity. Shaded zone represents coordinates of pH and redox potential within which sulphate-reducing bacteria are active

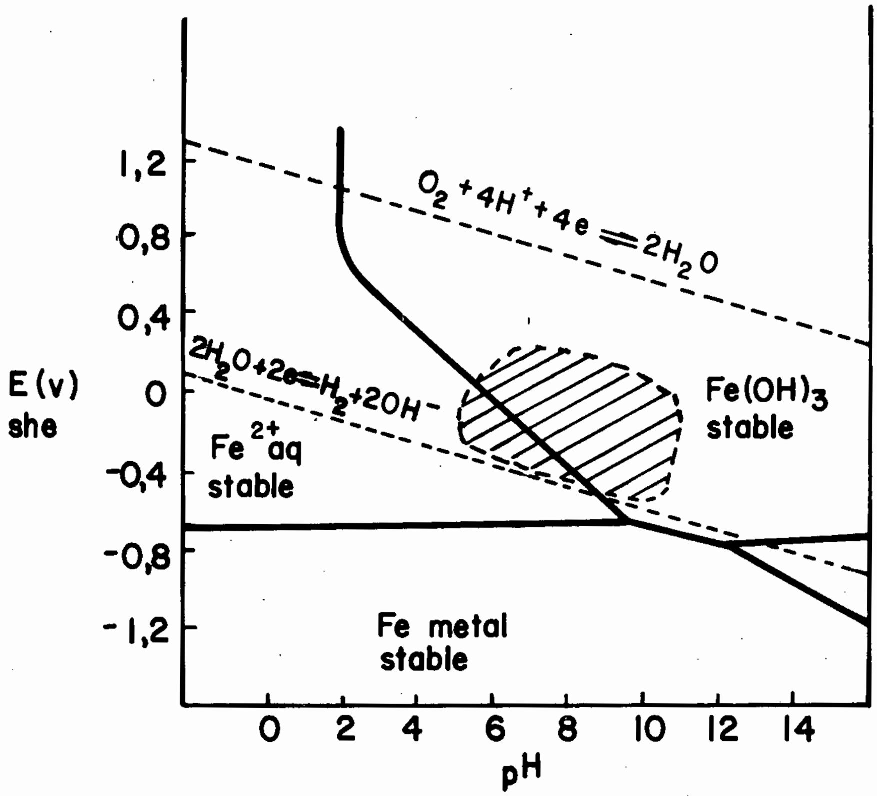


FIGURE 10

pH – potential regime (shaded area) favourable to SRB growth superimposed upon Pourbaix pH vs potential diagram for the Fe – H_2O system. Lines on Pourbaix diagram are for eqiulibria involving 10^{-6} M metal ions

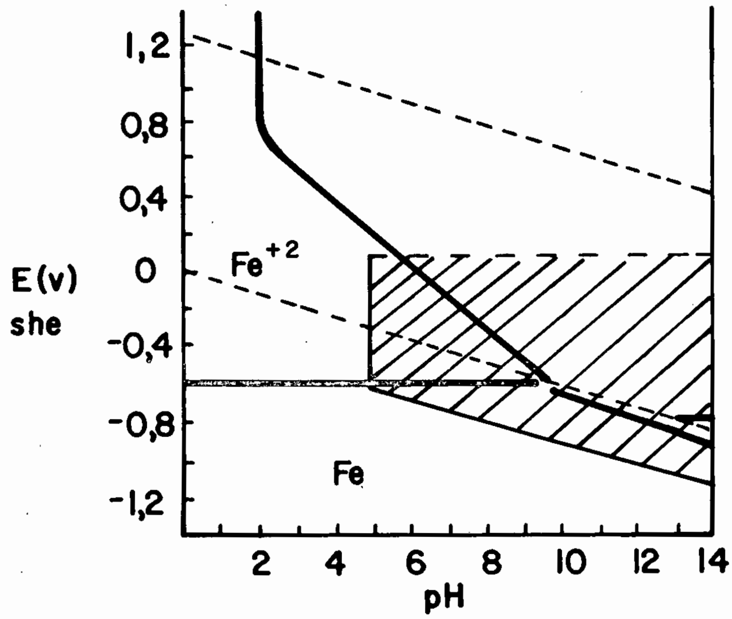


FIGURE 11

Combined Pourbaix diagrams for systems $Fe-H_2O$ and $Fe-S-H_2O$. Hatched area corresponds to stability domain of FeS

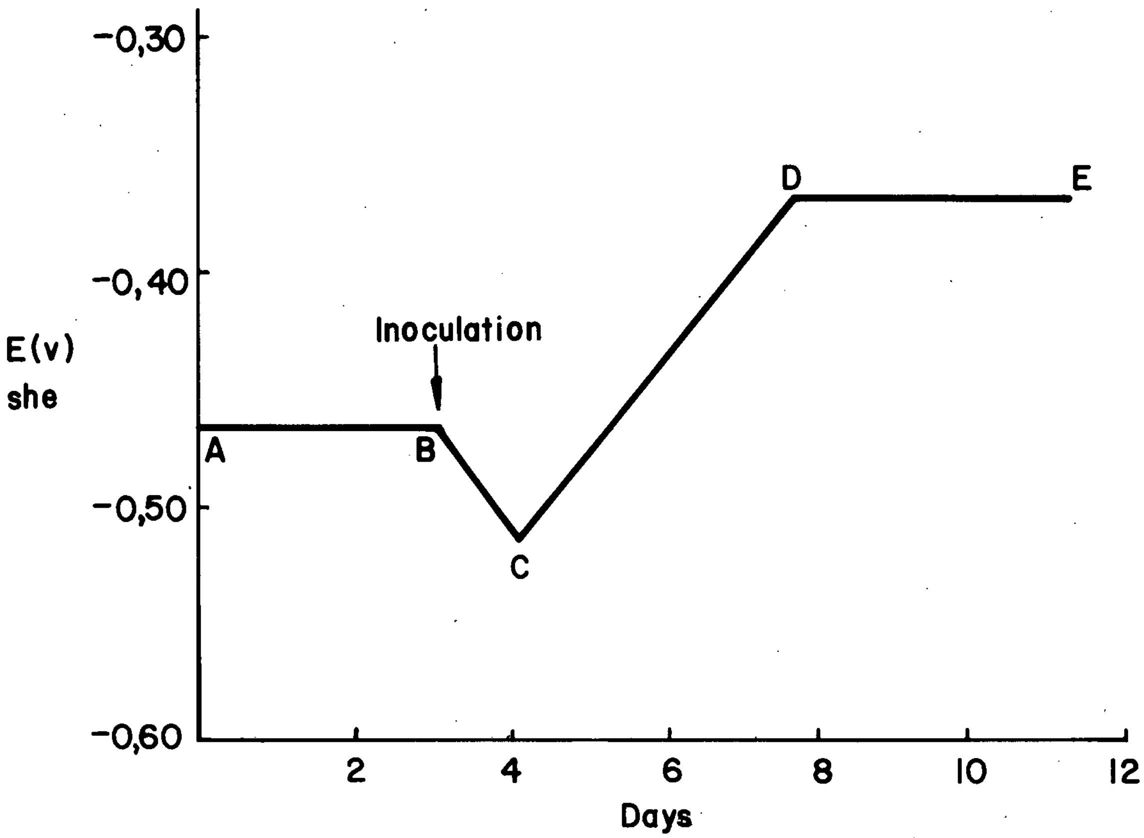


FIGURE 12
Potential-time results of Hadley[67]

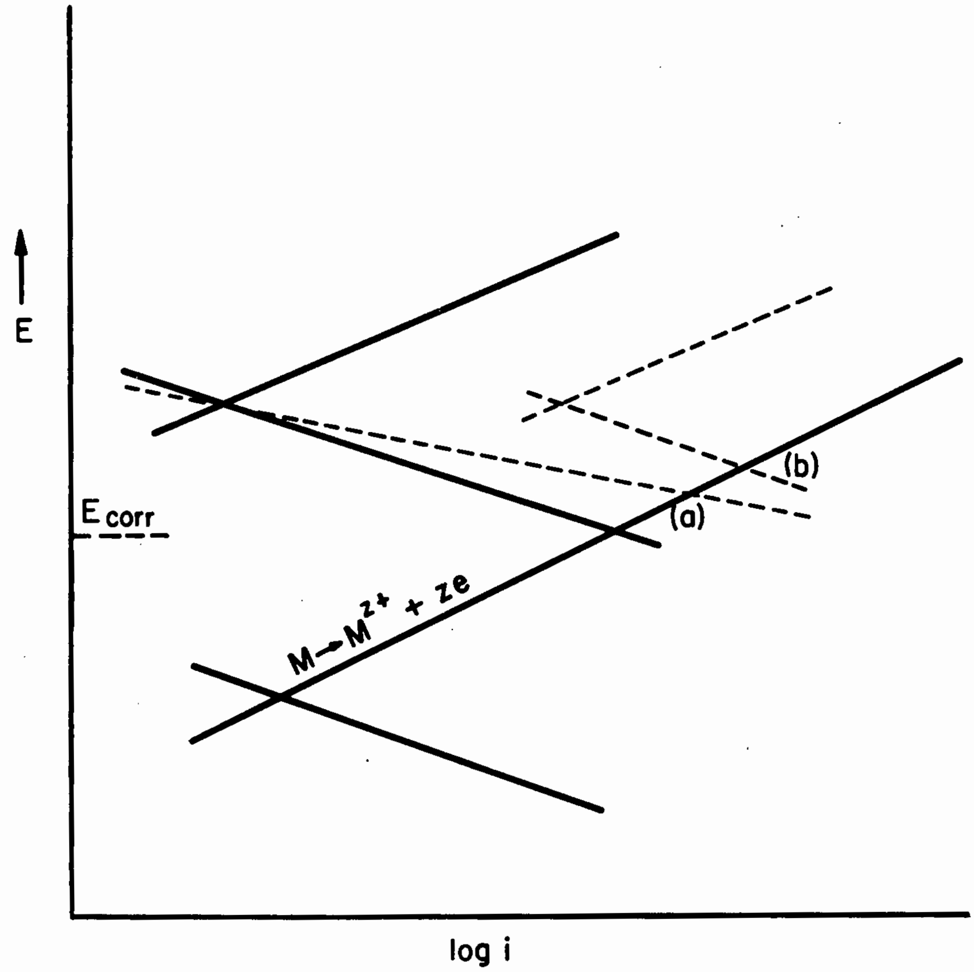


FIGURE 13

Positive shifts in corrosion potential (E_{corr}) will result from either a change in mechanism of the cathodic reaction giving a diminished Tafel slope, intersection point (a), or from an increase in i_o for the cathodic reaction, intersection point (b). Negative shifts in corrosion potential can only result from an increased (steeper) Tafel slope for the cathodic reaction, or from accelerating influences on the anodic reaction (a decreased Tafel slope or an increased i_o)

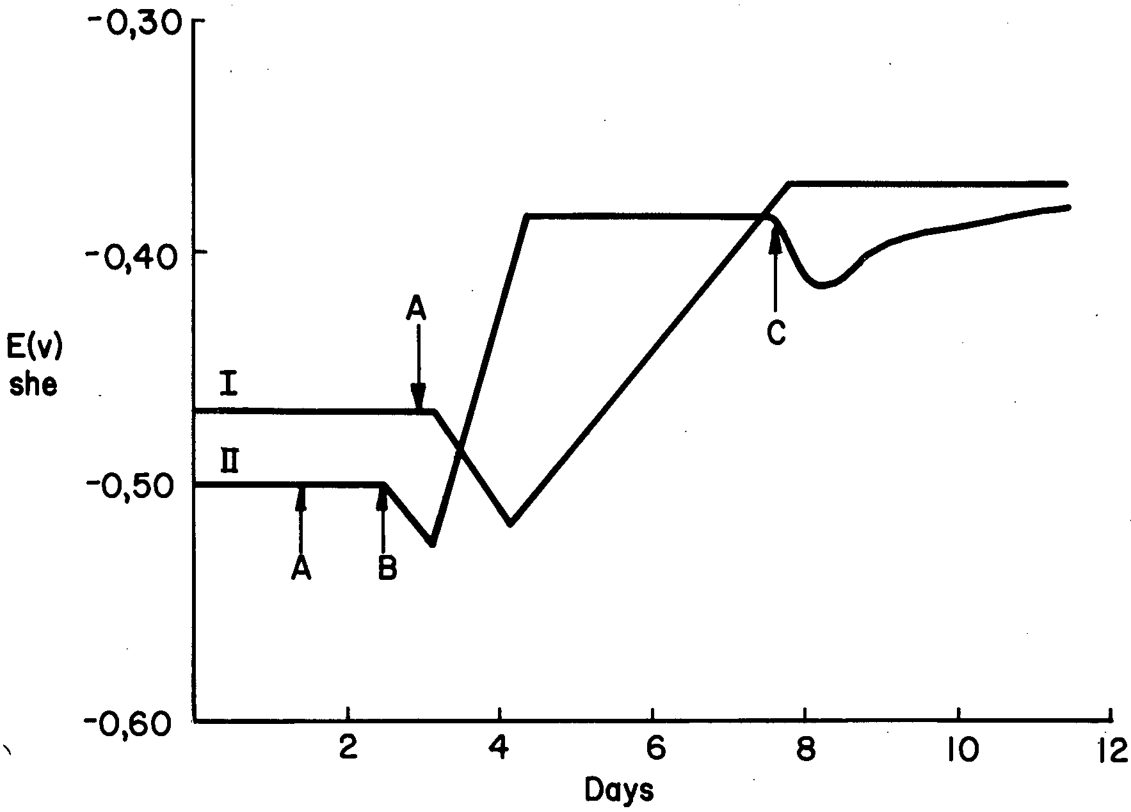


FIGURE 14

Potential - time results of Wanklyn and Spruit[68] (II) and those of Hadley (I)[67]

A inoculation time

B time of addition of lactate

C time of addition of lactate or hydrogen sulphide

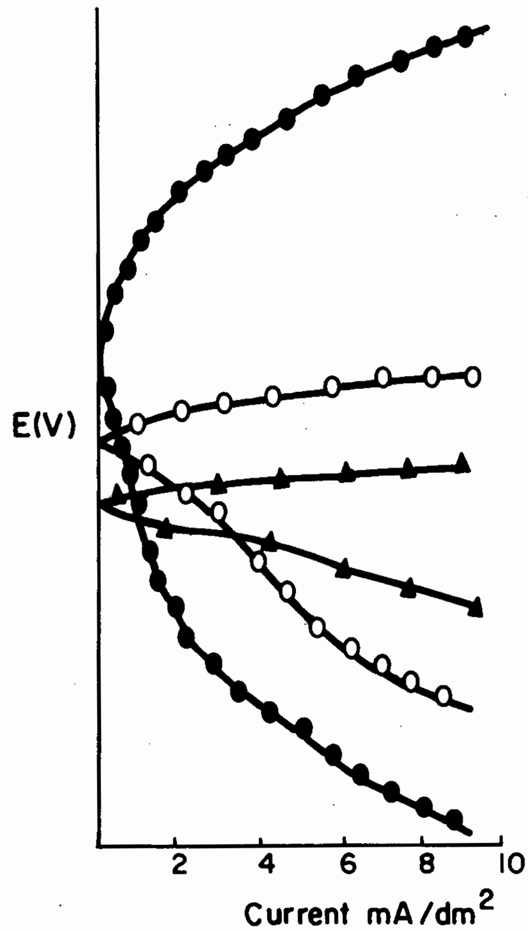


FIGURE 15

Anodic and cathodic polarization curves recorded by Horváth and Solti [70] for mild steel electrodes in SRB cultures

●-●-●-● sterile medium

○-○-○-○ 4 days after inoculation

▲-▲-▲-▲ 6 days after inoculation

During the corrosion potential drop phase (as recorded by Hadley) depolarization activity increases

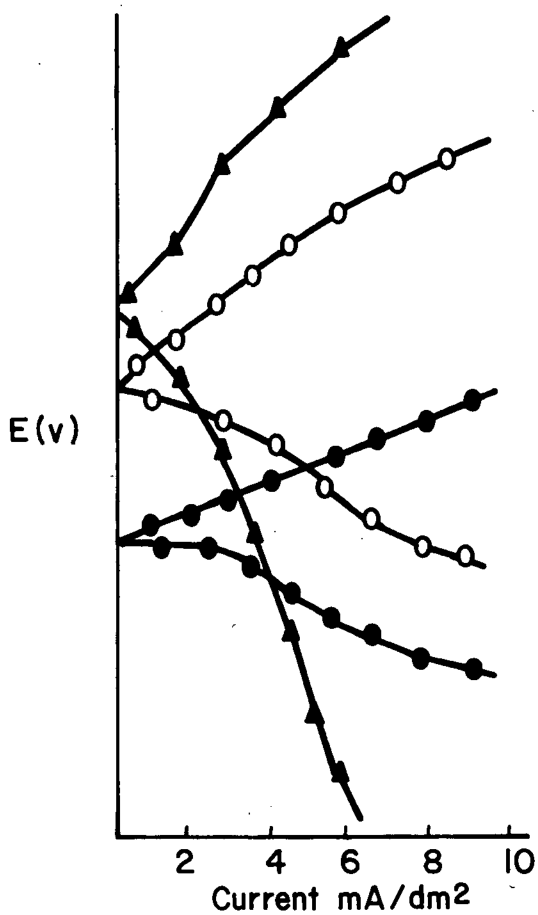


FIGURE 16

Anodic and cathodic polarization curves recorded by Horváth and Solti[70] for mild steel electrodes in SRB cultures.

—●—●—●— 8 days after inoculation
—o—o—o— 20 days after inoculation
—▲—▲—▲— As above, after 24 hours aeration

During the corrosion potential rise phase (Hadley), cathodic depolarization effects decrease.

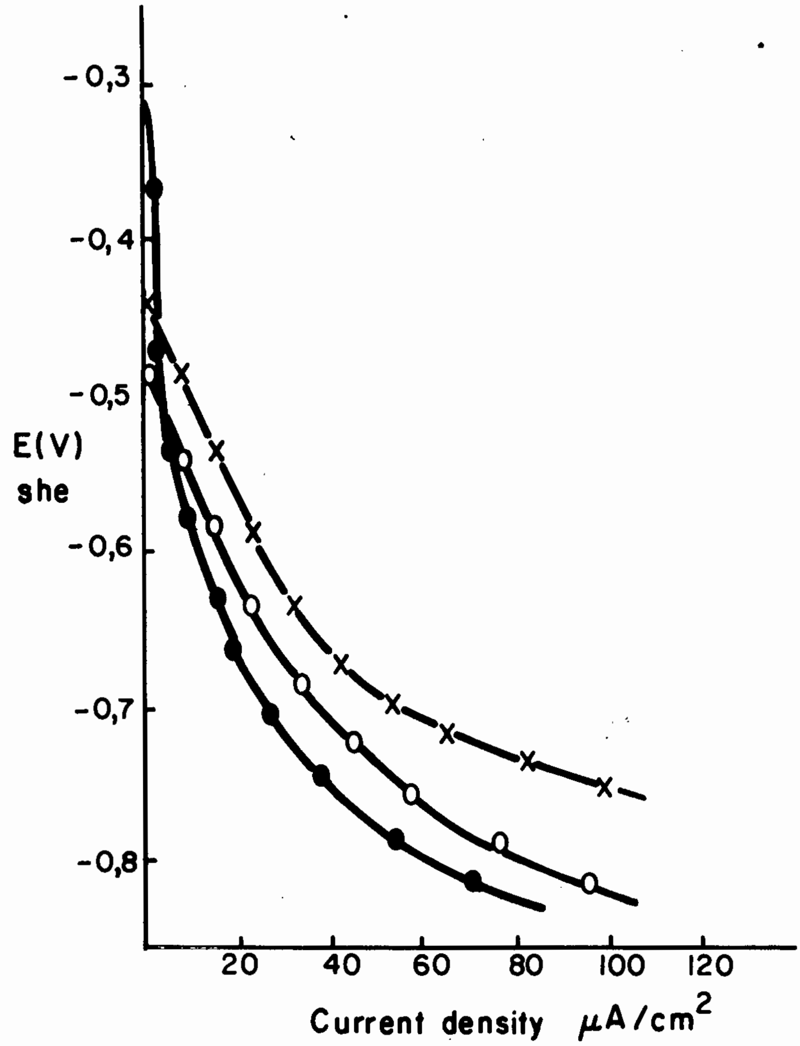


FIGURE 17

Cathodic polarization curves recorded by Booth, Robb and Wakerley[80] for mild steel in Dv. desulfuricans (Hildenborough) culture in minimal iron

O-O-O-O-O initially
X-X-X-X-X 1 day after inoculation
●-●-●-●-● 15 days after inoculation

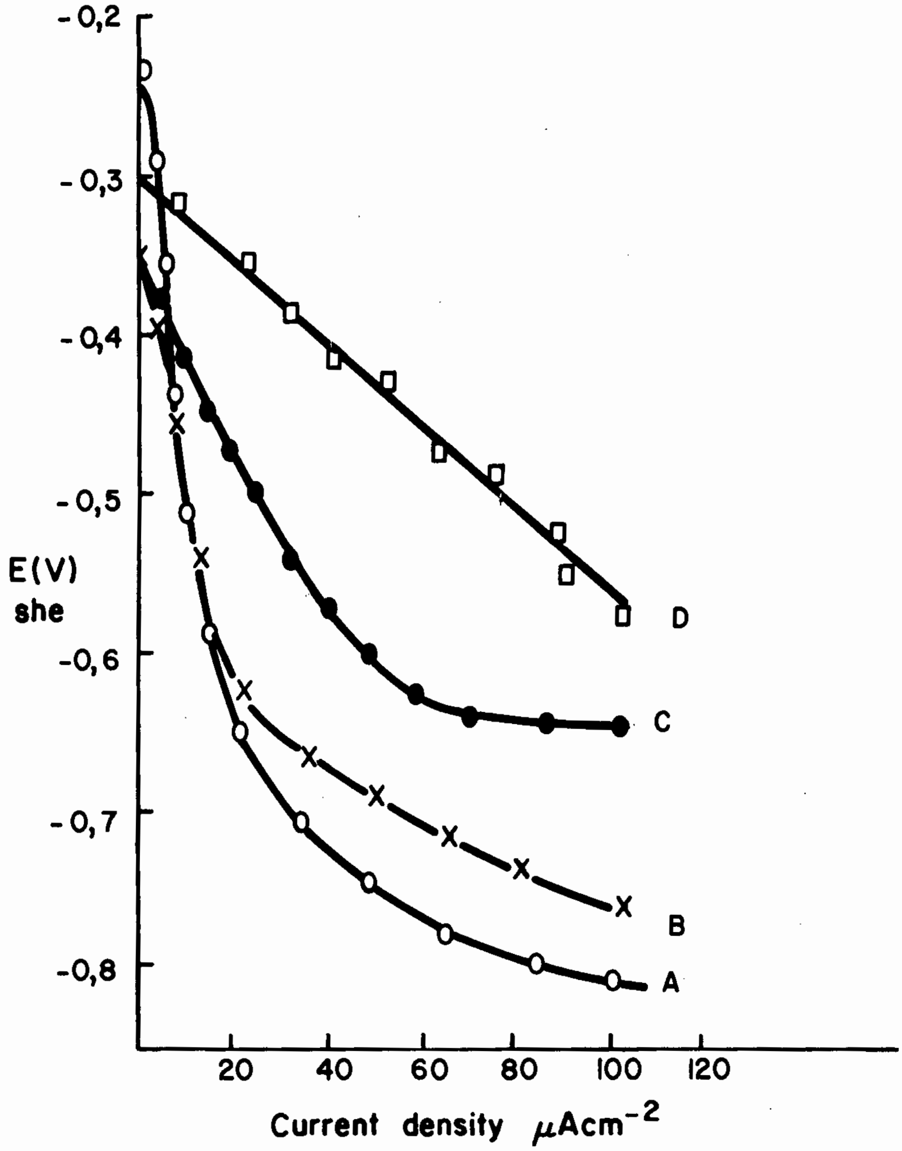


FIGURE 18

Cathodic polarization curves recorded by Booth, Robb and Wakerley[80] for mild steel in Dv. desulfuricans (Hildenborough) culture. Minimal iron medium changed to iron-rich medium during experiment

A	19	days minimal iron
B	2	days after changing medium to iron-rich
C	7	days after changing medium to iron-rich
D	33	days after changing medium to iron-rich

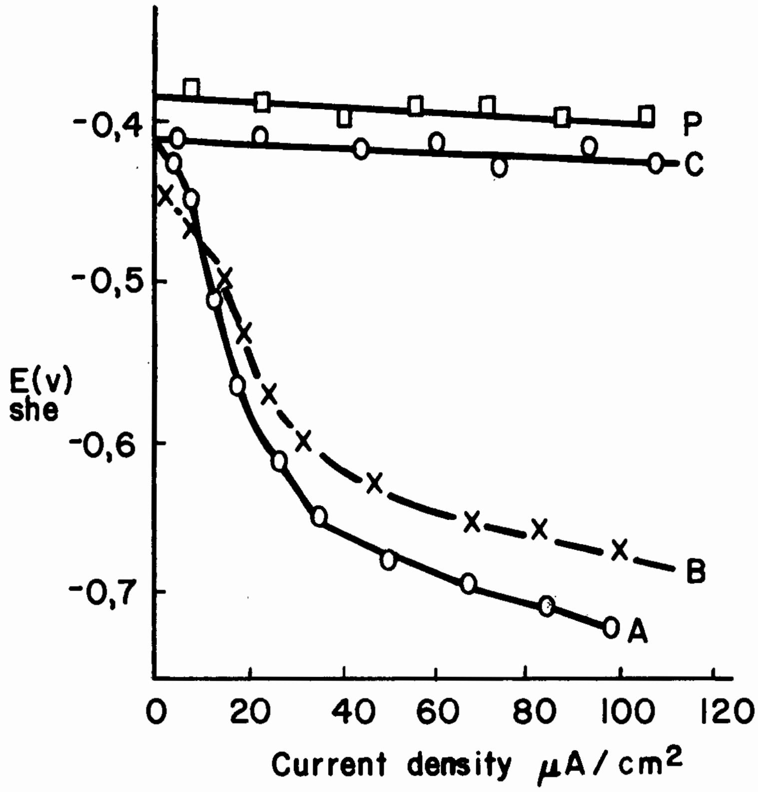


FIGURE 19

Cathodic polarization curves recorded by Booth, Robb and Wakerley[80] for mild steel in Dt. orientis culture in iron-rich medium.

A initially
B after 1 day
C after 5 days
D after 29 days

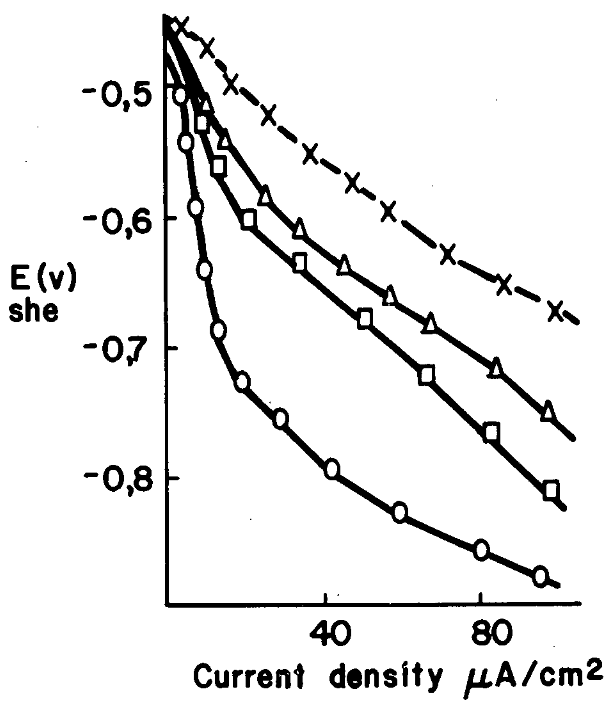


FIGURE 20

Cathodic polarization curves recorded by Booth, Elford and Wakerley[81] for mild steel in a culture of Dv. desulfuricans (Teddington R) in sulphate-free, fumarate-containing medium

- o initially
- x after 2 days
- Δ after 7 days
- □ after 15 days

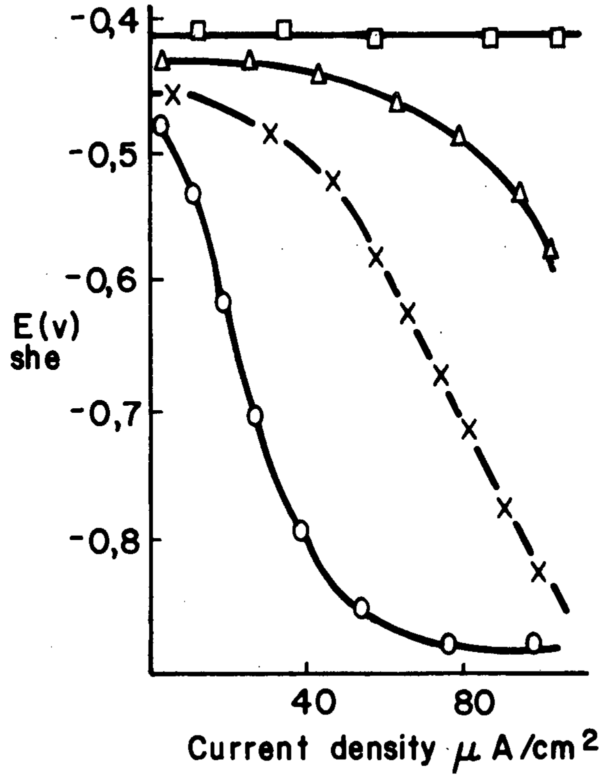


FIGURE 21

Cathodic polarization curves for mild steel recorded by Booth, Elford and Wakerley[81] with electrode in static sterile suspensions of FeS in 1% (w/v) NaCl

O without FeS
X with 0,6m M FeS
Δ with 1,2m M FeS
□ with 5m M FeS

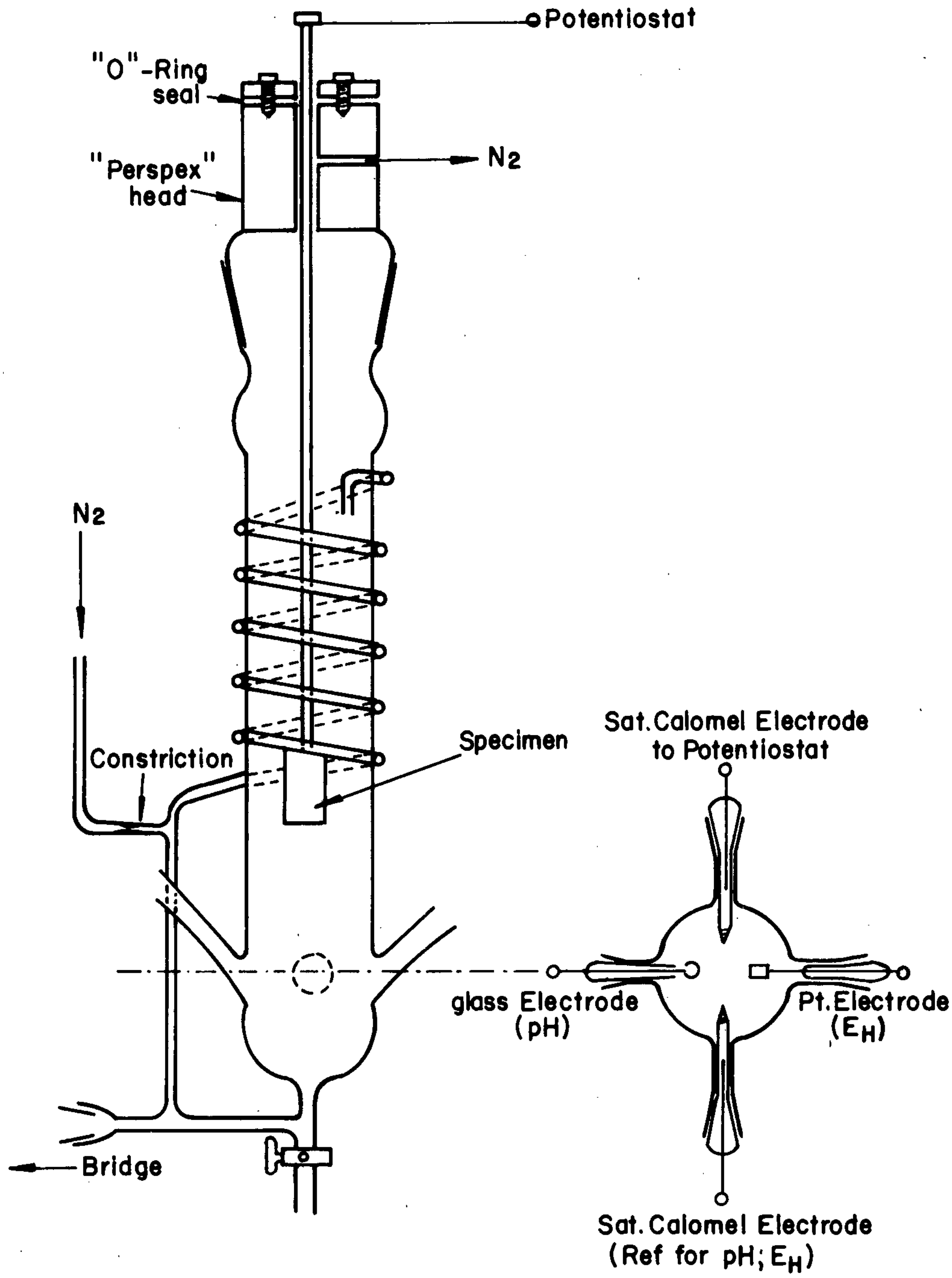


FIGURE 22.

Sketch of the cathodic compartment of the polarization cell used by Booth and Tiller[86]

DESULFOVIBRIO VULGARIS (HILDENBOROUGH) with BENZYL VIOLOGEN

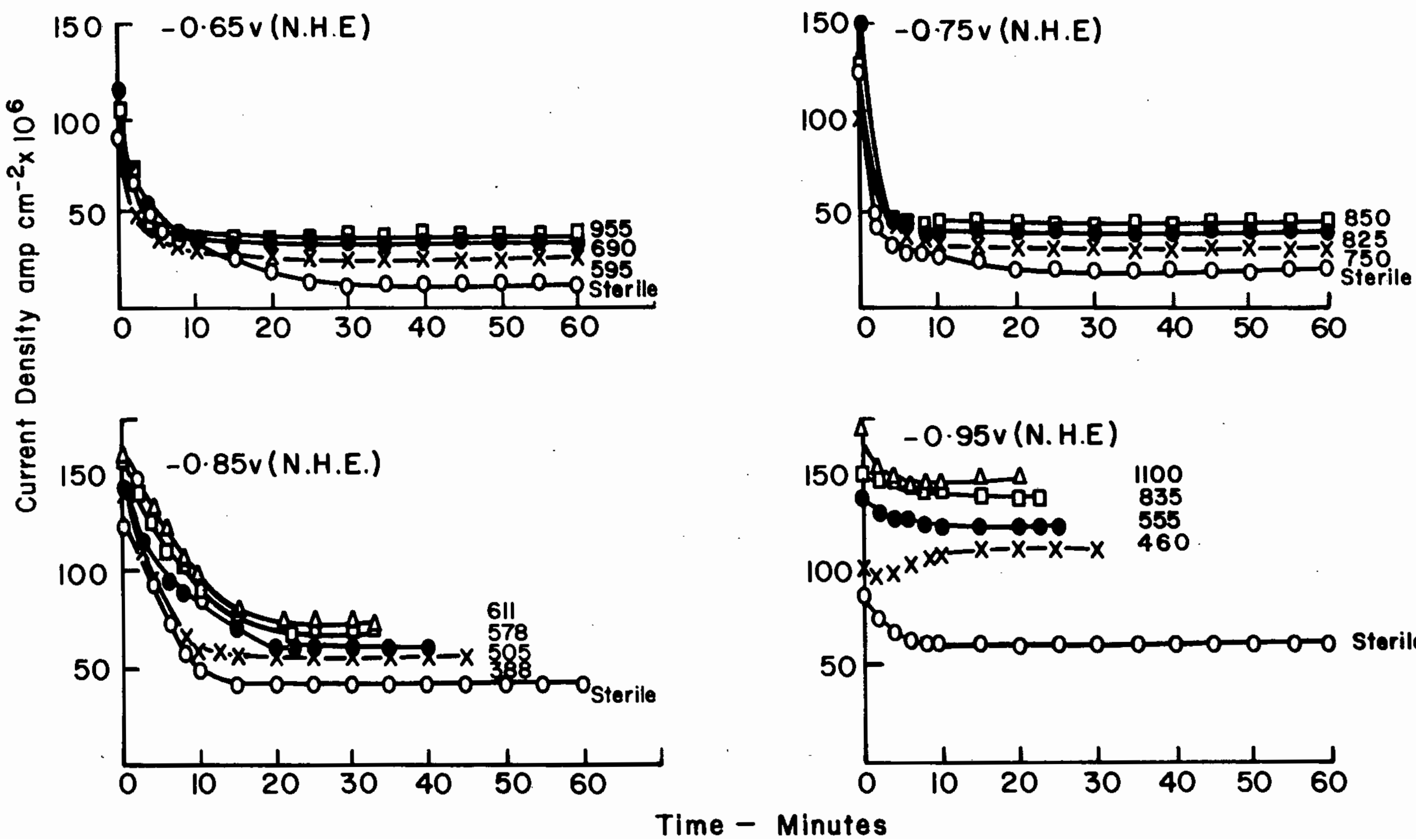


FIGURE 23.

Current density vs time curves, produced by Booth and Tiller[86], for mild steel electrodes held at potentials of -0,65V, -0,75V, -0,85V, and 0,95V (she) in suspensions of Desulfovibrco cells of various hydrogenase activities, containing benzyl viologen

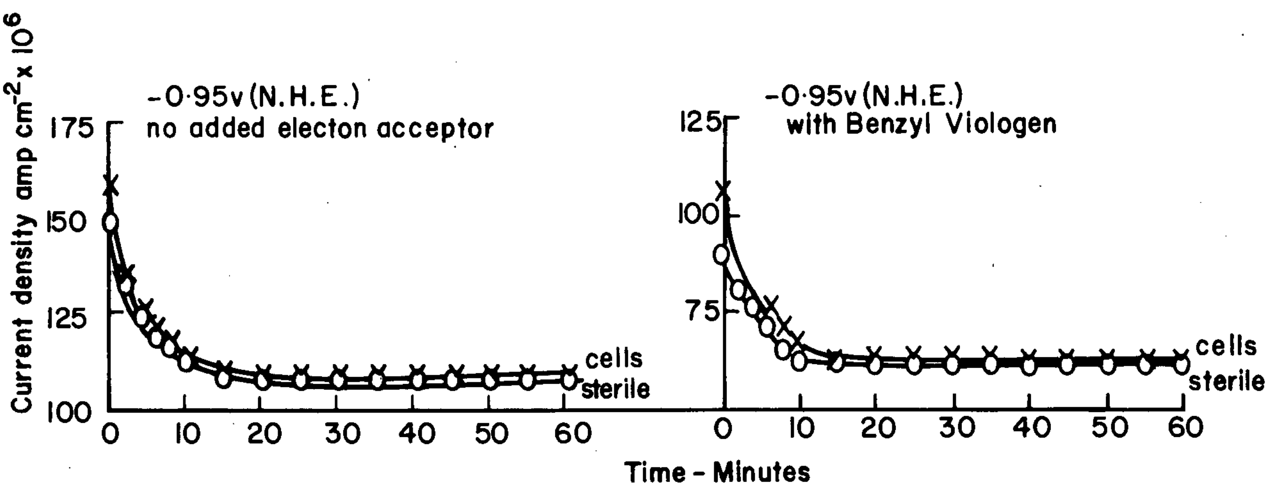


FIGURE 24.

Current *vs* time curves recorded by Booth and Tiller[86] for mild steel electrodes held at a potential of -0,95 V(she) in suspensions of *Desulfotomaculum* cells (hydrogenase negative) with and without benzyl viologen additions

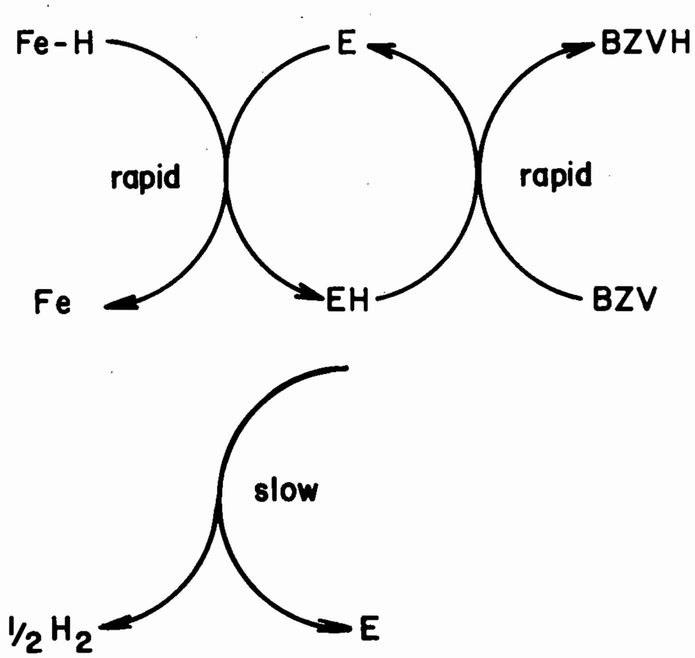
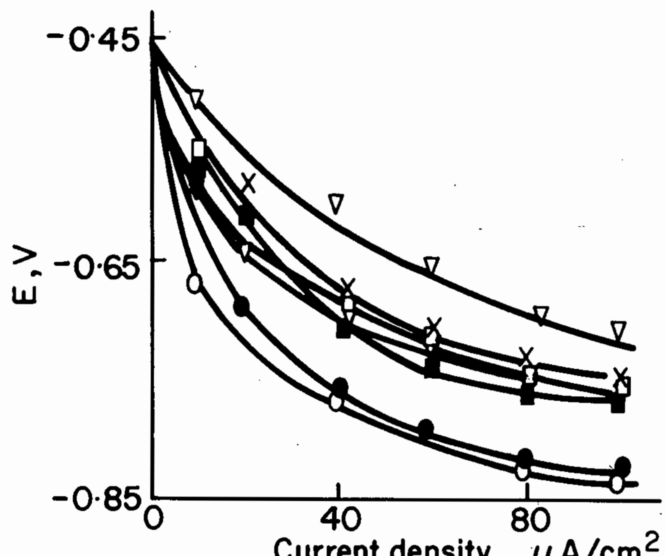


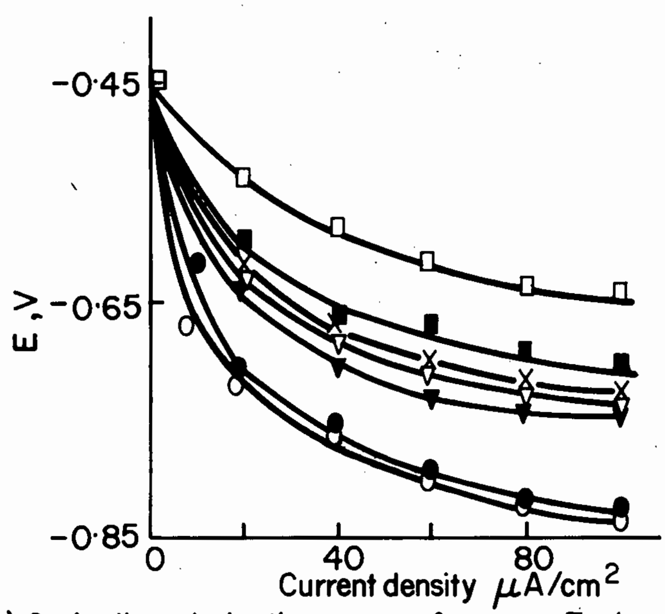
FIGURE 25

Mechanism of depolarization proposed by Booth and Tiller[86], in which the enzyme removes atomic hydrogen from the polarized electrode and reduces benzyl viologen

66-00-4-206/1



a) Cathodic polarization curves for pure Fe in suspensions of clostridia. O, Sterile buffer control; ●, Cl. roseum; ∇, Cl. pasteurianum; ▼, Cl. butylicum; □, Cl. sporogenes; ■, Cl. butyricum; X, Cl. felsineum



b) Cathodic polarization curves for pure Fe in suspensions of nonphotosynthetic bacteria
O, Sterile buffer control; ●, Ps. stuzeri; □, Pa. denitrificans; ■, Pa. halodenitrificans; ∇, Ps. maltophilia; ▼, Ps. aeruginosa; X, Chr. violaceum

FIGURE 26

Cathodic polarization curves recorded by Mara and Williams[87] for pure iron in suspensions of various bacteria in the presence of methylene blue. All organisms were hydrogenase-positive excepting Cl. roseum and Ps. stuzeri.

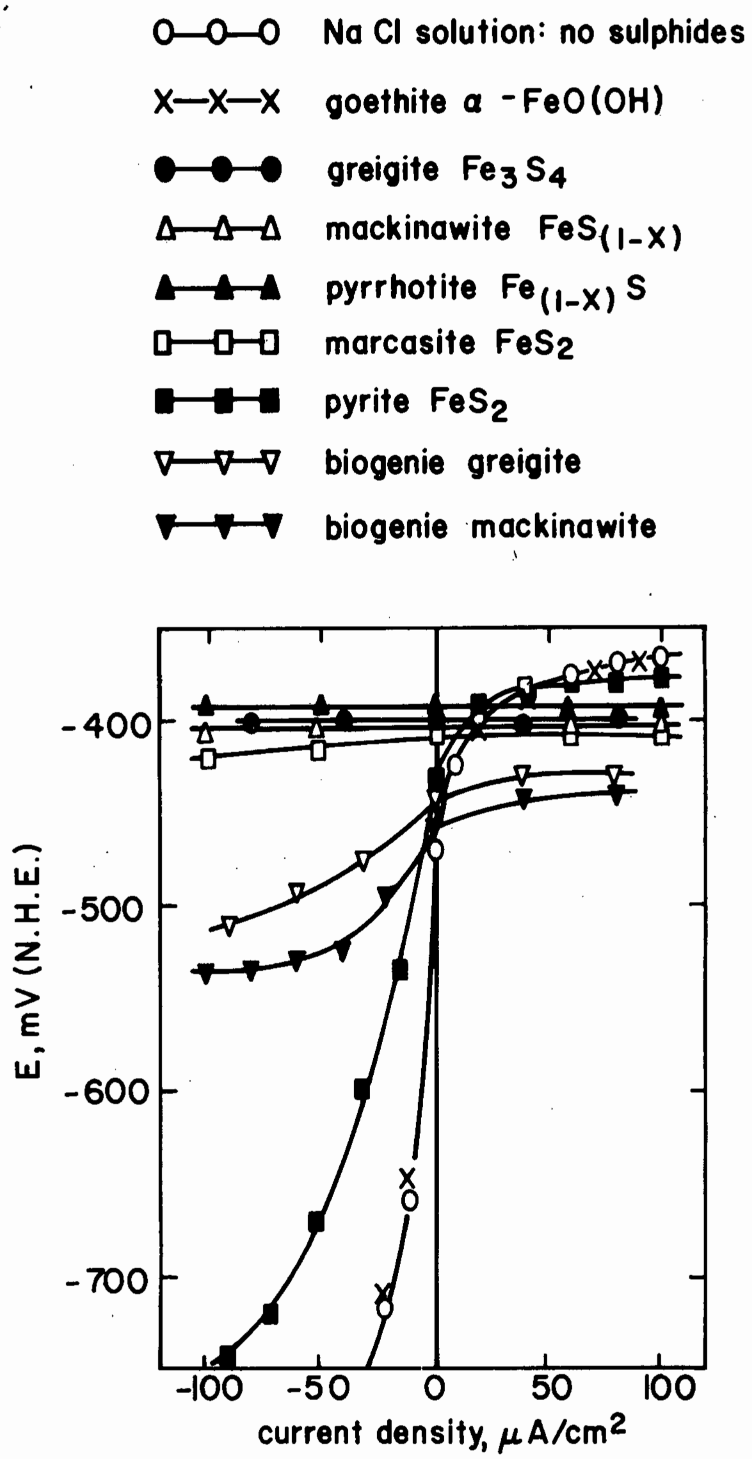
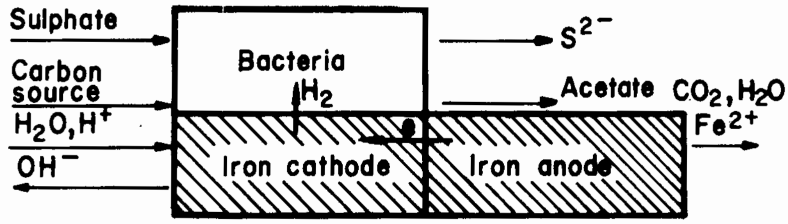
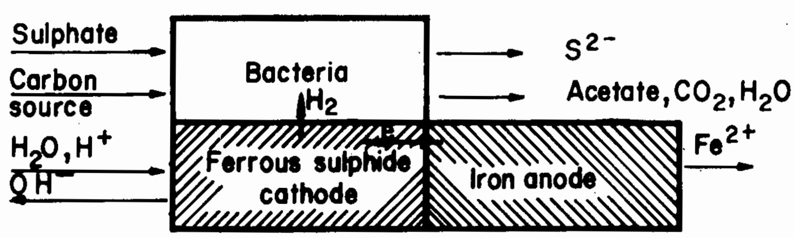


FIGURE 27.

Polarization curves recorded by Mara and Williams[92] for pure iron electrodes in contact with samples of various sulphides of iron



a) Scheme illustrating the classic theory of cathodic depolarization by the sulphate-reducing bacteria



b) Scheme illustrating the proposed mechanism of corrosion by the sulphate-reducing bacteria

FIGURE 28

Models proposed by King and Miller[97] to describe classic mechanism of cathodic depolarization and their own scheme

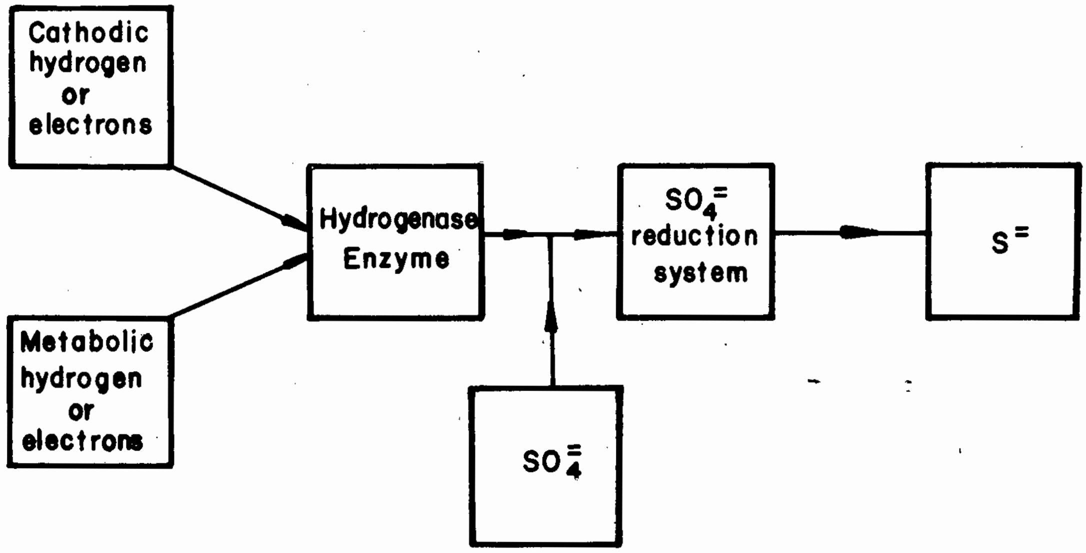


FIGURE 29

If sulphate acts as a sink for both cathodic charge and metabolic charge, cathodic depolarization and oxidative metabolism will compete with one another for the sulphate-reduction system

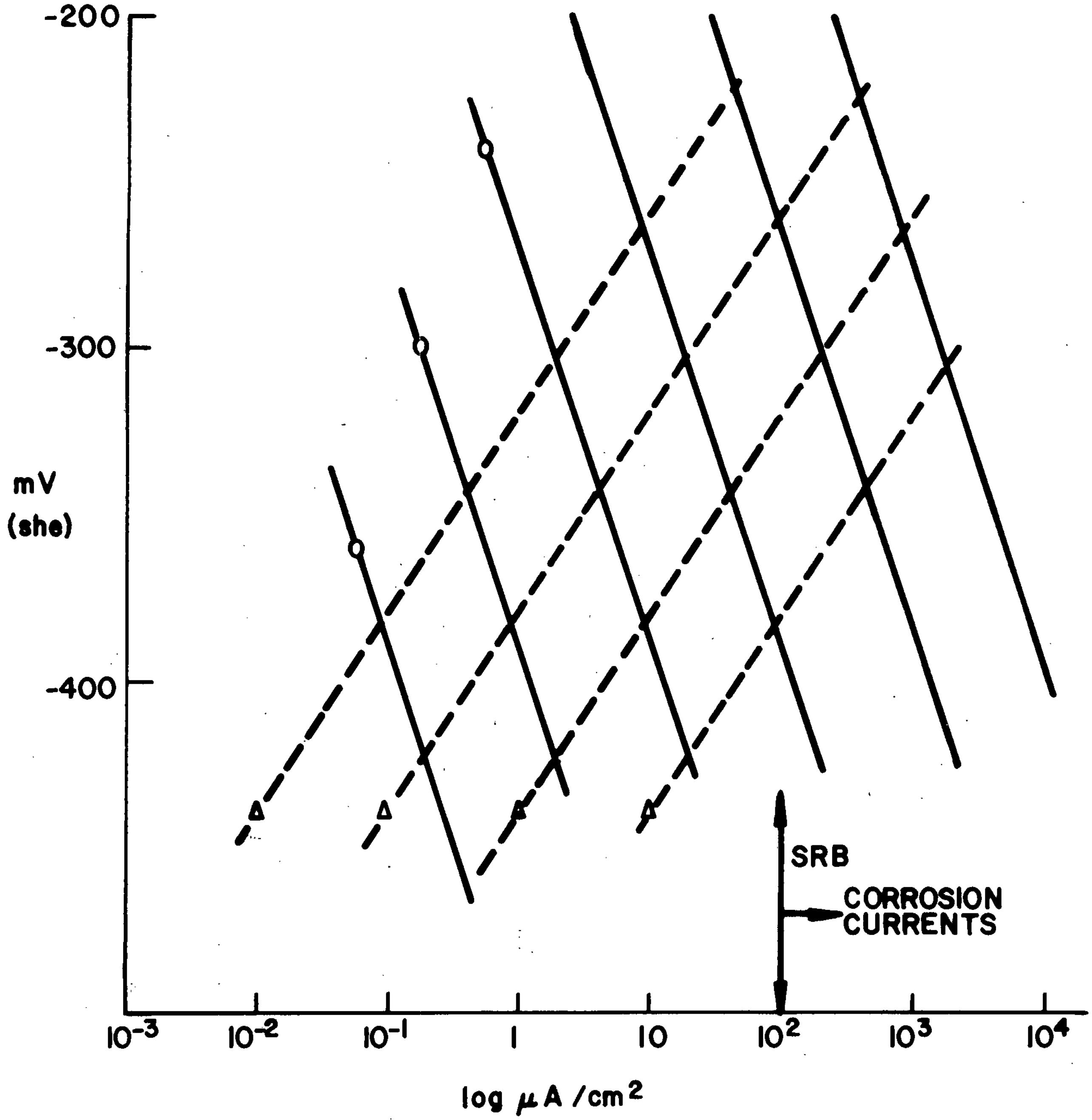


FIGURE 30

Evans-type corrosion diagram for iron corroding in water in the presence of hydrogen ion. Points ⊙ are exchange current densities for $H_3O^+ + e = \frac{1}{2}H_2$ equilibrium determined by Bockris, Drazic and Despic [106] for pure iron. Cathodic Tafel (solid) lines have a slope of 0,12 V per decade of current. Hypothetical exchange current densities for $Fe^{2+} + 2e = Fe$ (marked Δ) are within the range established by Bockris, Drazic and Despic and plotted at the E^o for this reaction. An anodic Tafel slope of 60 mV per decade, consistent with their results is employed (dotted lines). The currents associated with SRB corrosion are typically greater than 10^2 μ Acm^{-2} and are thus only likely, for iron corroding with hydrogen evolution as cathodic reaction, at a pH of 3 or less. The rate-determining step in the cathodic part of the corrosion reaction is the discharge of H_3O^+ or H_2O to produce H_{ads}. At neutral pH, where SRB are active "H_{ads} removal" mechanisms will not affect the corrosion rate. Some other mechanism must be responsible for the corrosion rates caused by SRB.

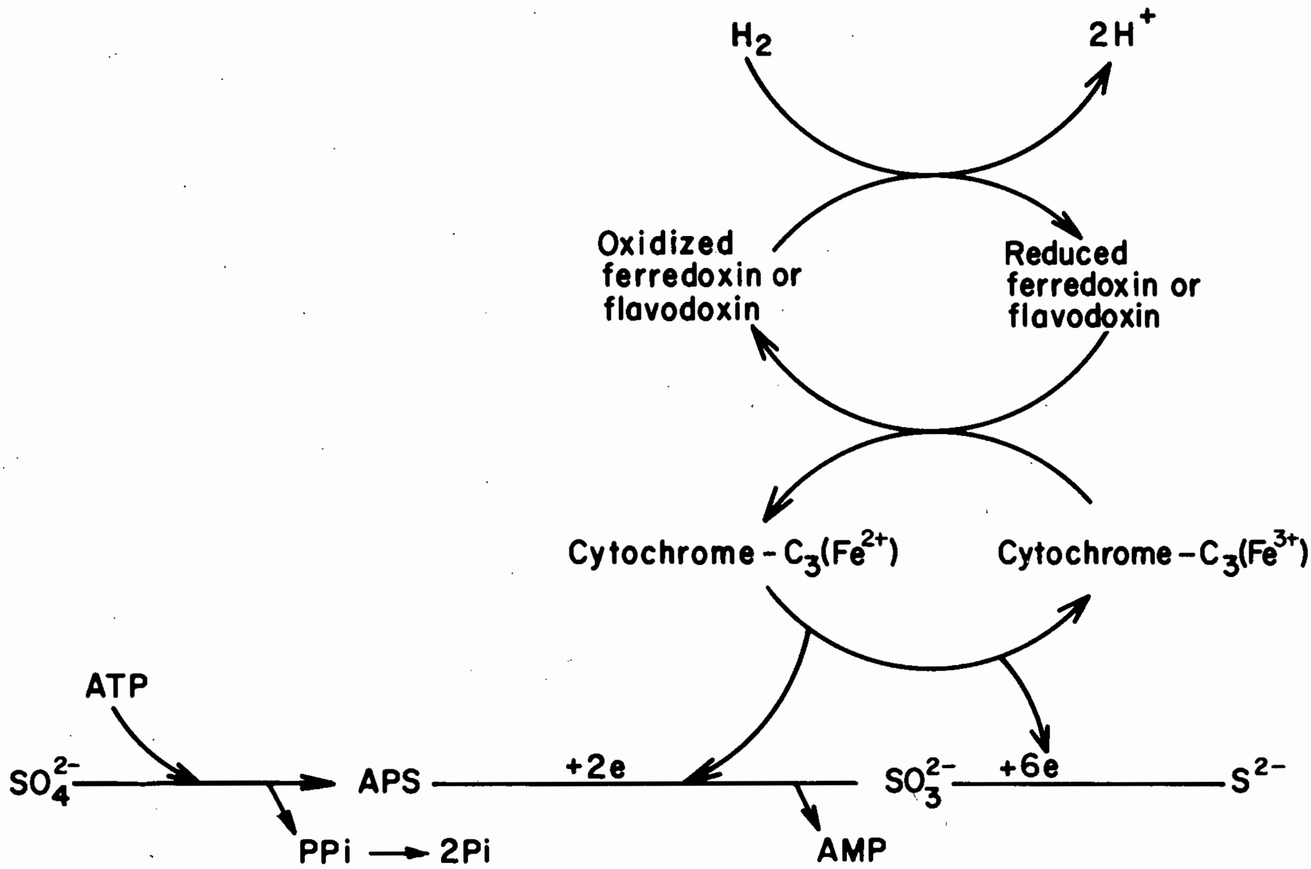


FIGURE 31

Schematic summary of sulphate-reduction pathways produced by Roy and Trudinger[59]. Possibly ferredoxin or cytochrome-C_3 may be directly reduced at corrosion cell cathodes and sulphate reduction may accompany this, but such a process would interfere with the metabolic electron sink role of the sulphate-reduction system

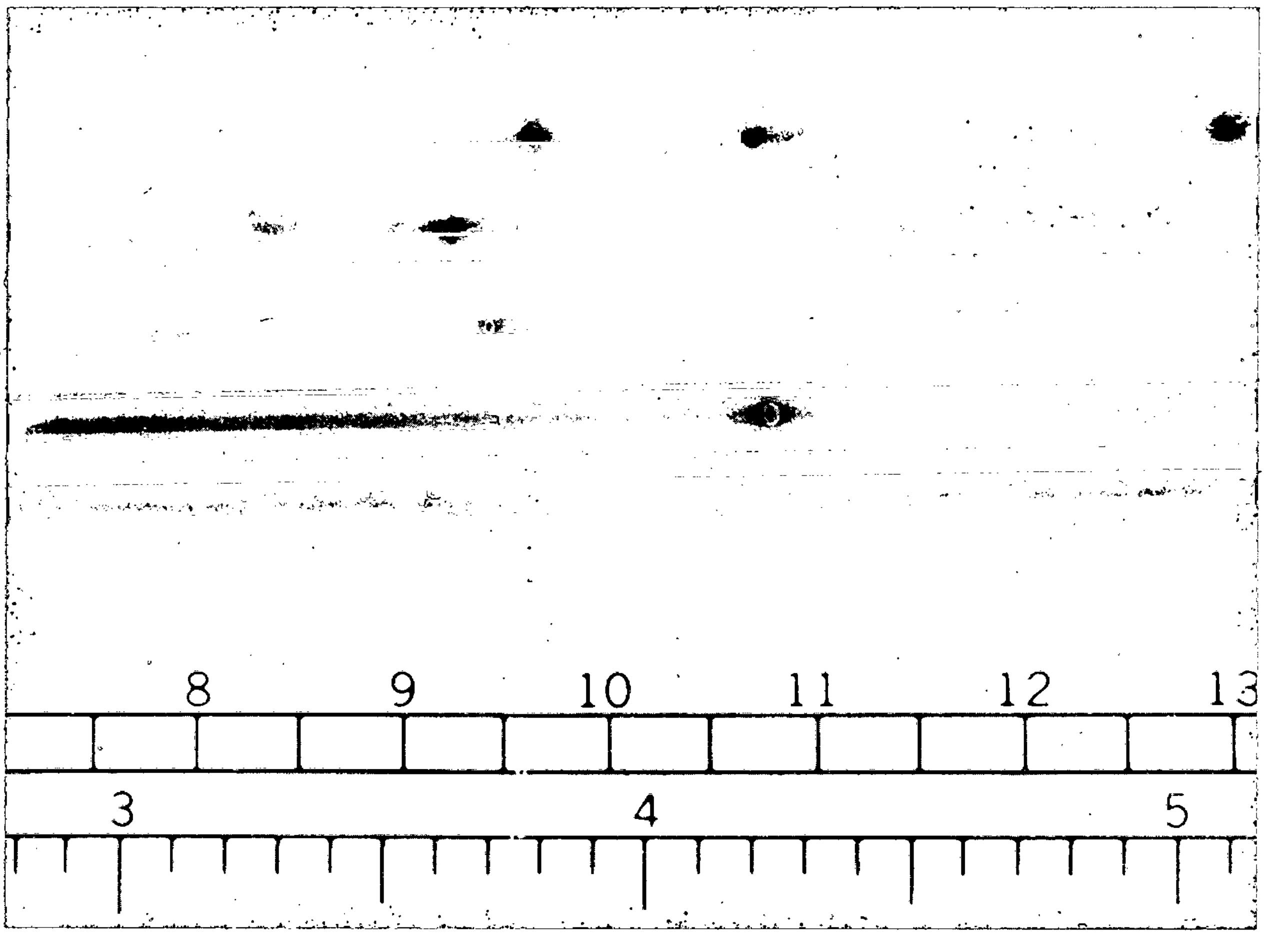


FIGURE 32

Series of stab tubes containing successive dilutions of colonies.

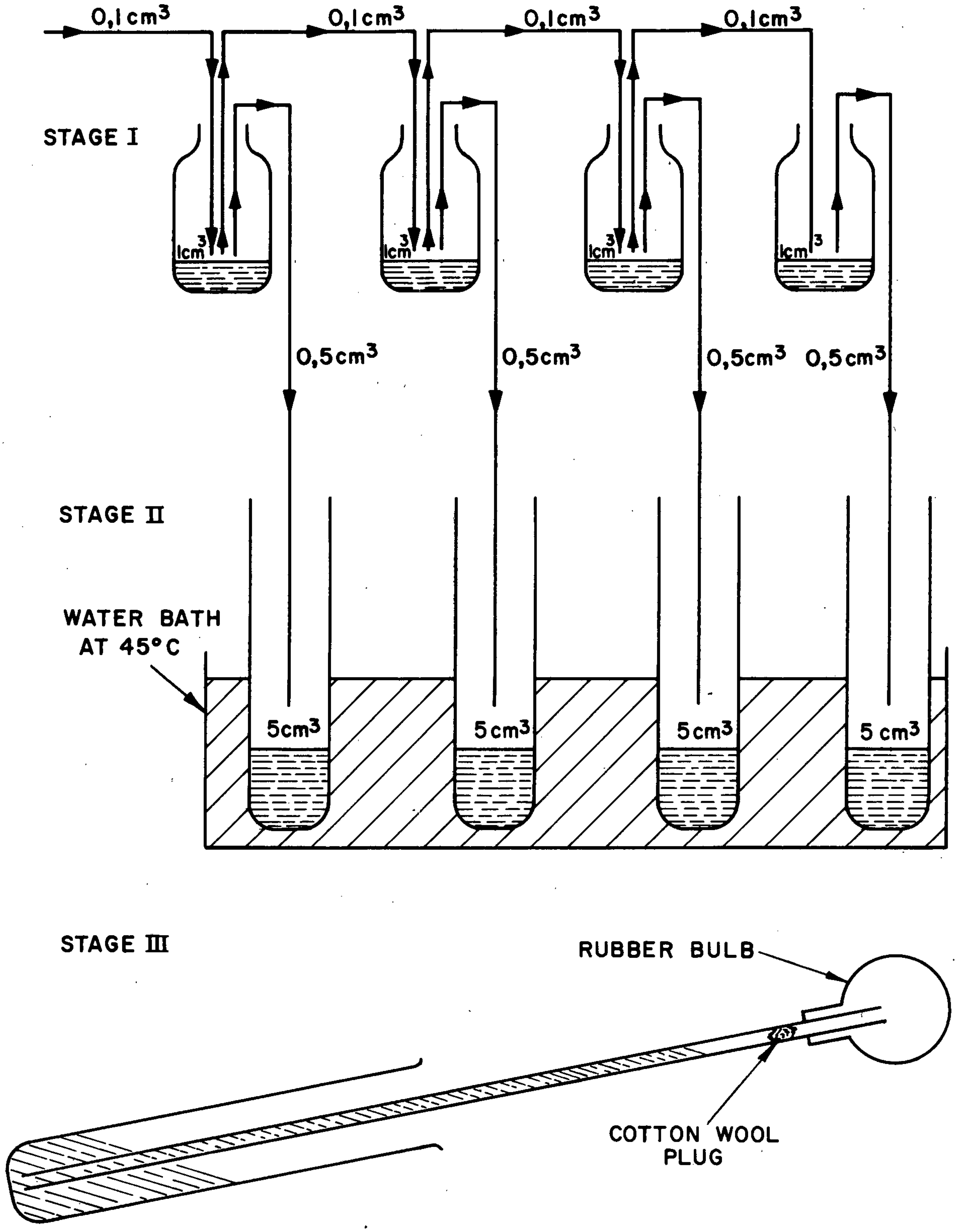


FIGURE 33

The method used for isolation of pure cultures

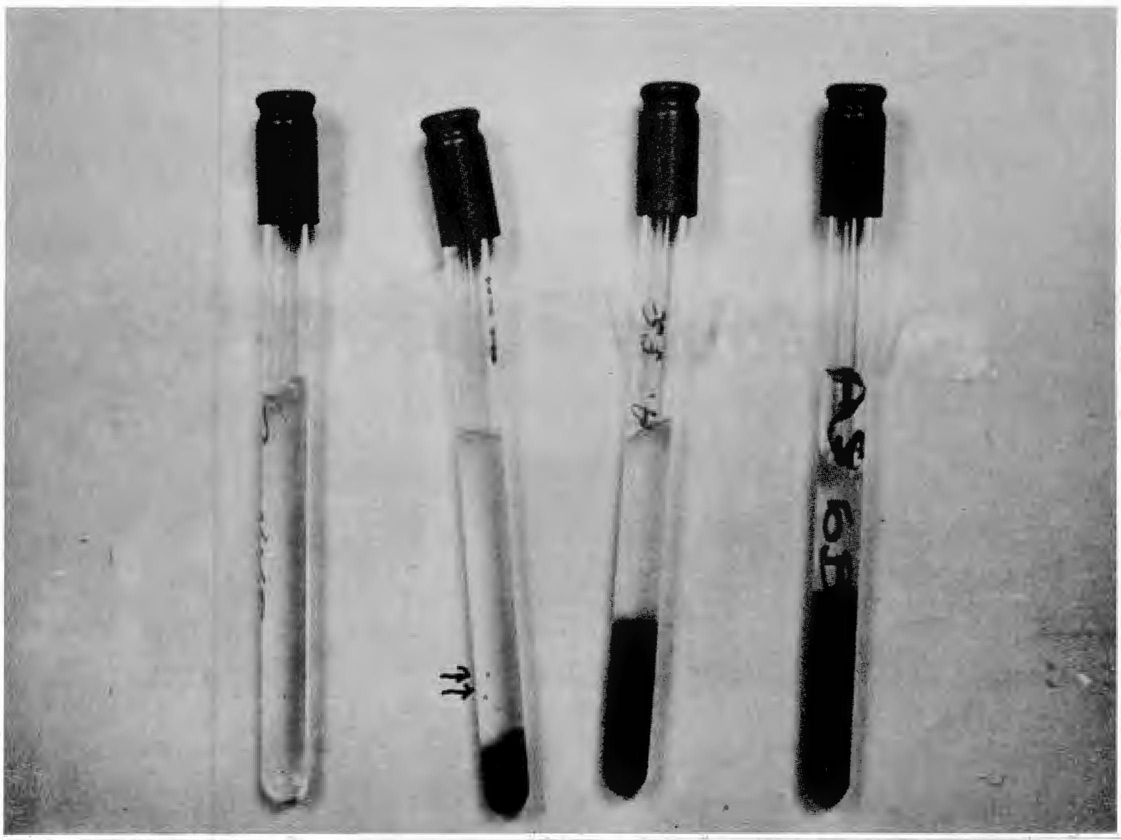


FIGURE 34
Sislar and Zobell tubes

FIGURE 35

Culture WVB. Cells magnified x 78,660. Polar flagellum clearly visible.

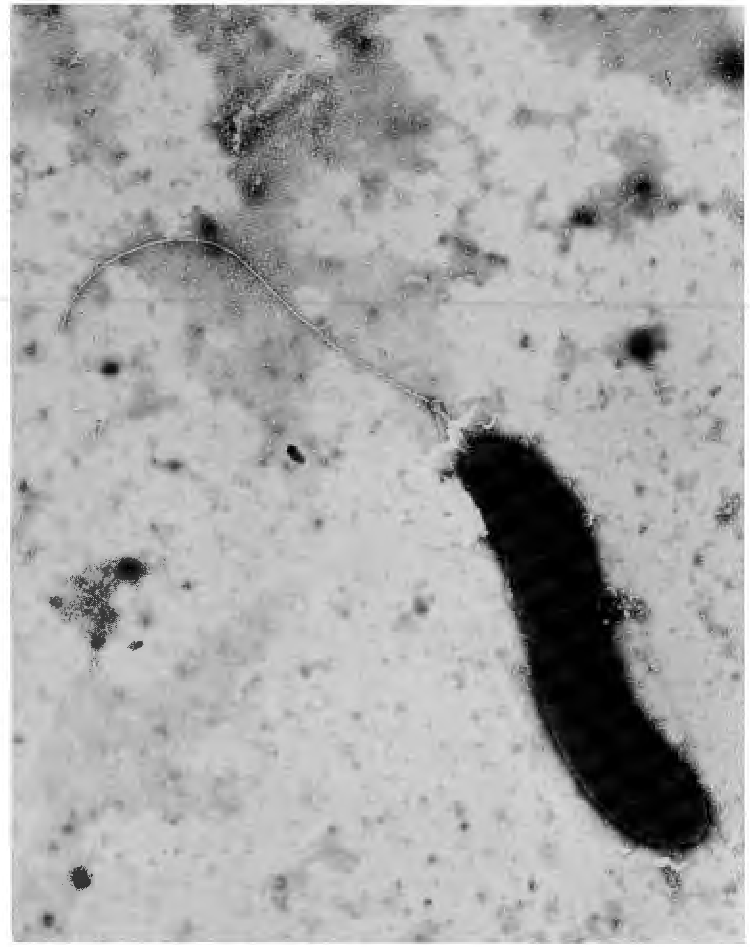


FIGURE 36

Culture HIL. Cell magnified x 36,660. Typical sigmoid cell shape with polar flagellum.

FIGURE 37

Culture WVB. Total magnification 34,830. Cells harvested from active culture.

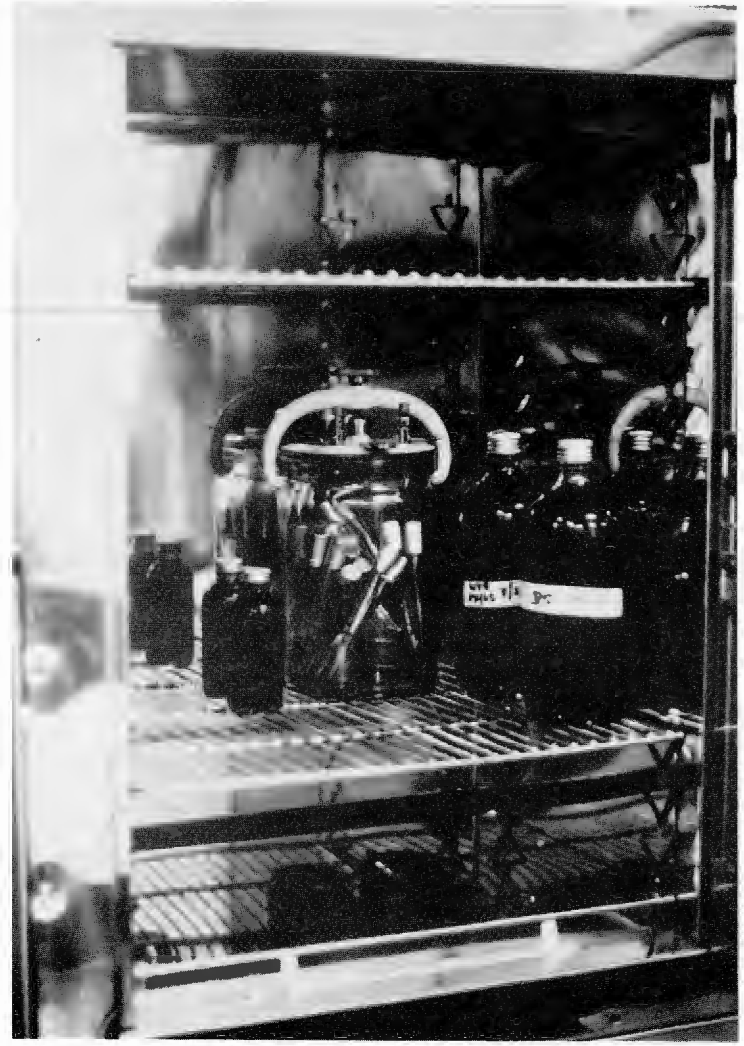


FIGURE 38

Photograph showing incubator and variety of cultures used.

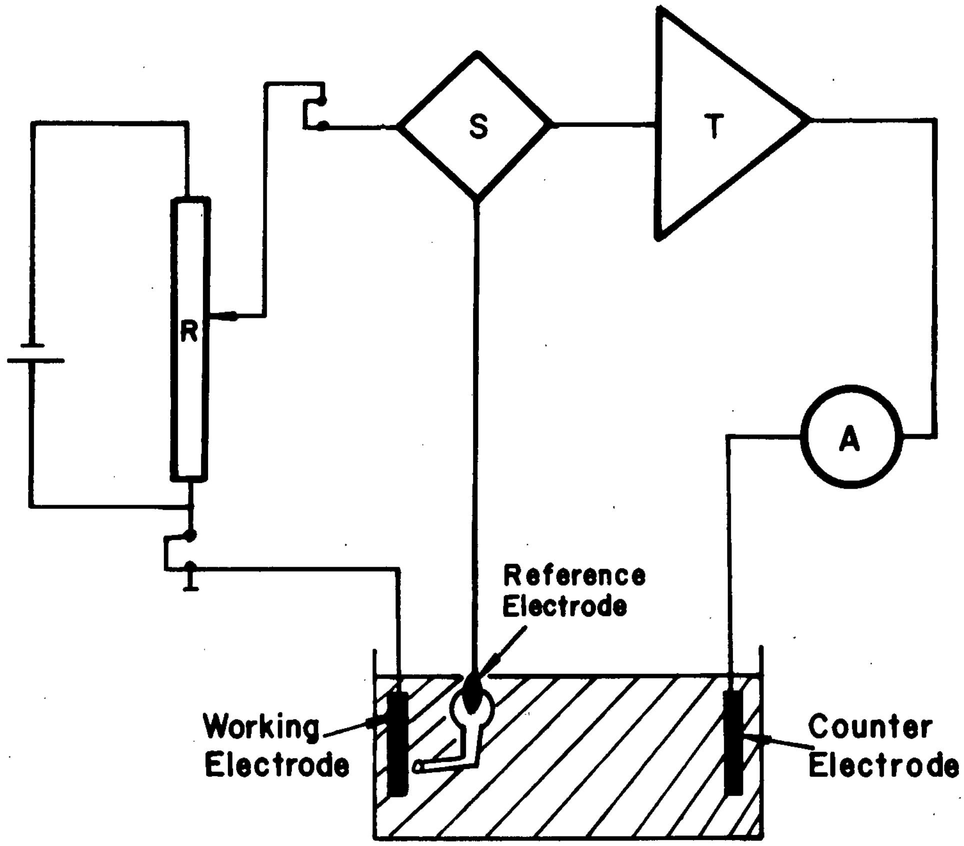


FIGURE 39

Diagram of the basic components of a potentiostatic circuit. The potential between reference and working electrodes is compared (S) with the potential tapped from a reference supply (R). The error signal is amplified (T) and generates a current measurable at (A) between counter electrode and working electrode such as to minimise that error

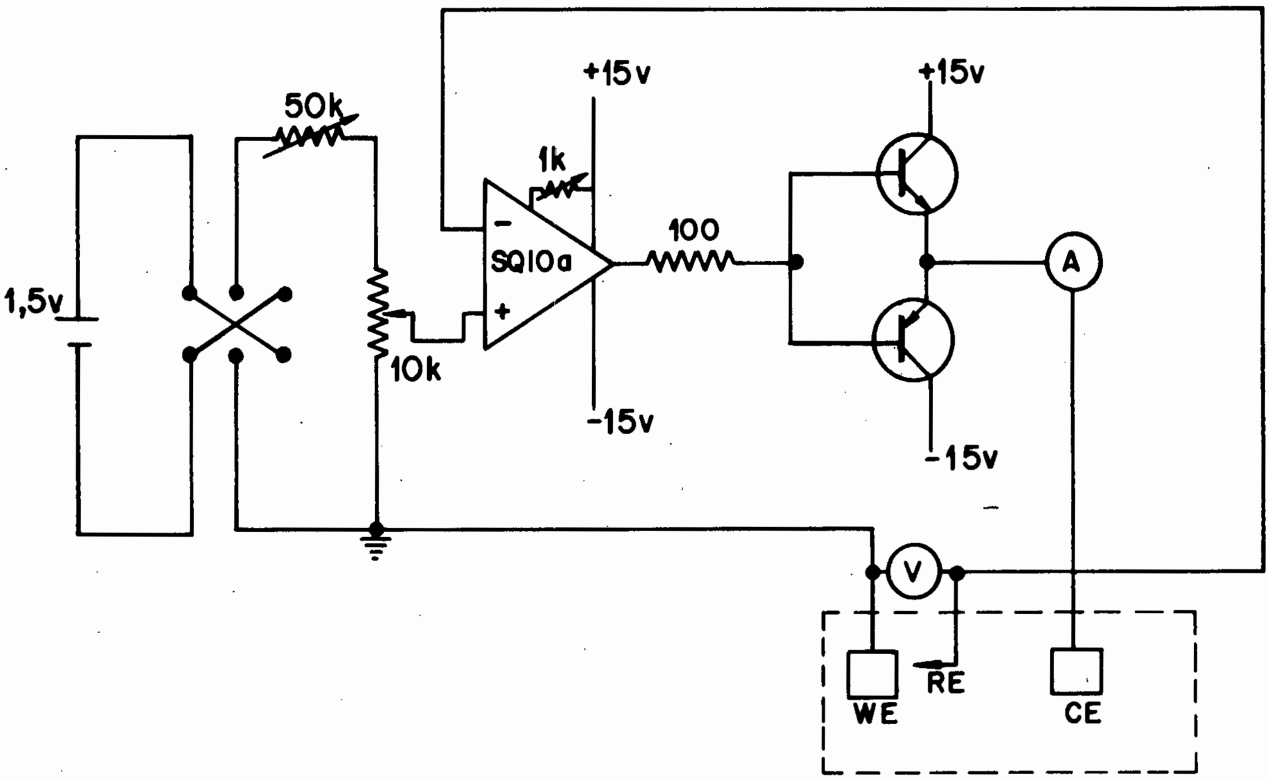


FIGURE. 40

Potentiostatic control circuit. The operational amplifier employed was a Philbrick Nexus SQ10a. Dotted area represents electrochemical cell containing working electrode (WE), reference electrode (RE), and counter electrode (CE).

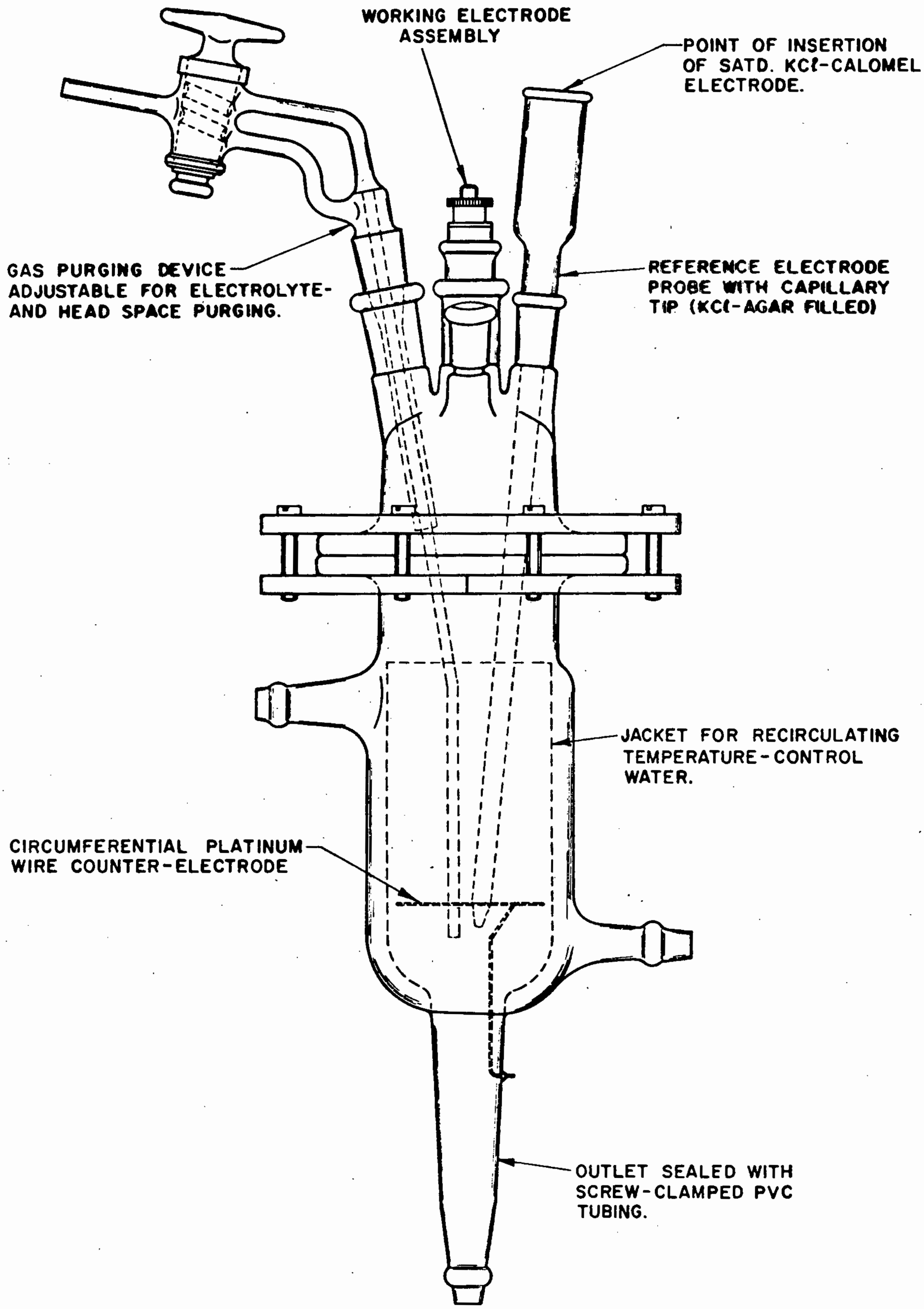


FIGURE 41 POLARIZATION CELL

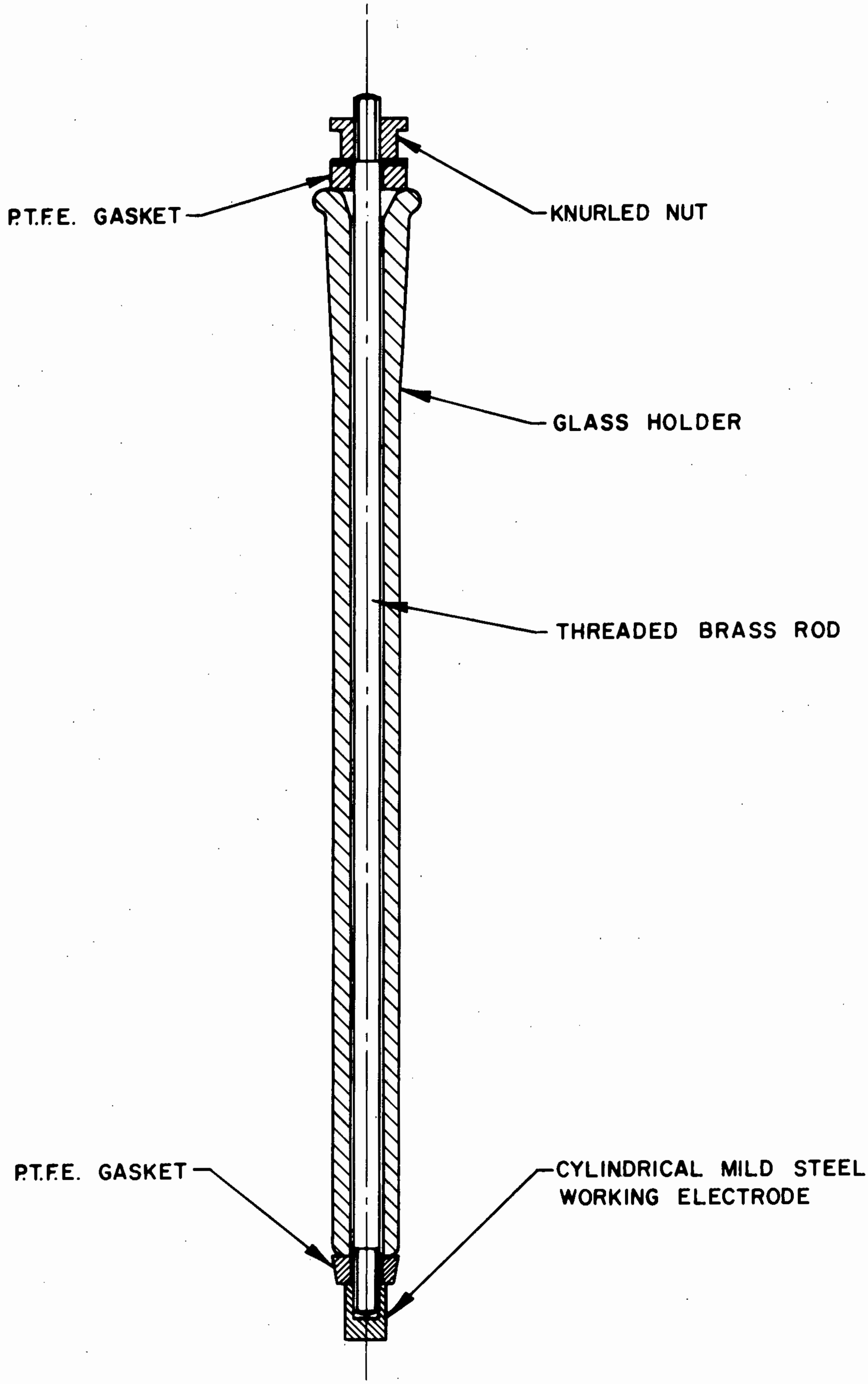


FIGURE.42 WORKING ELECTRODE ASSEMBLY

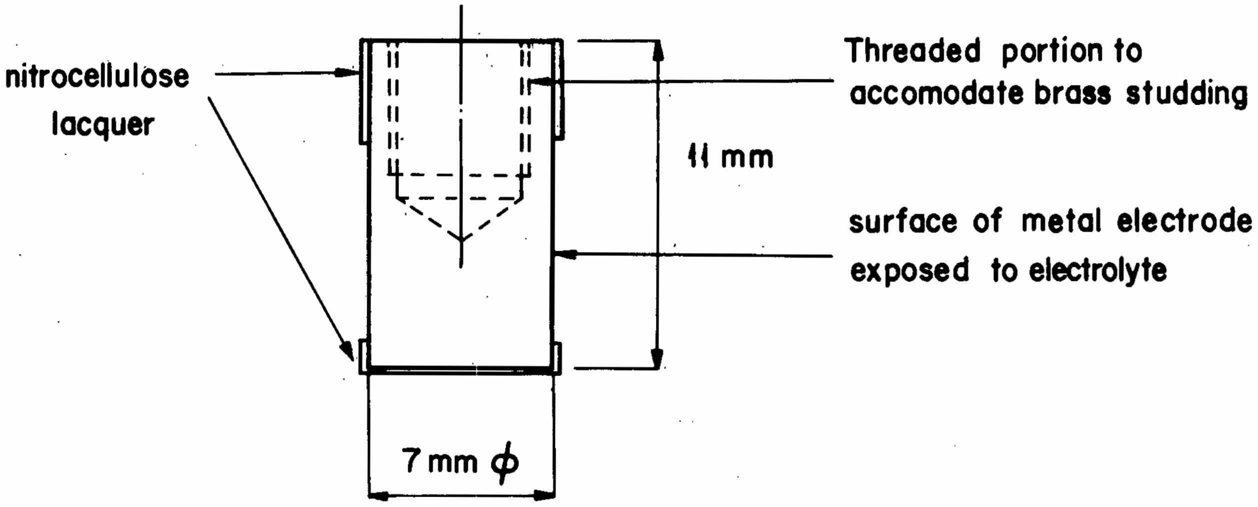


FIGURE 43

Working electrode showing application of nitrocellulose lacquer (not to scale)

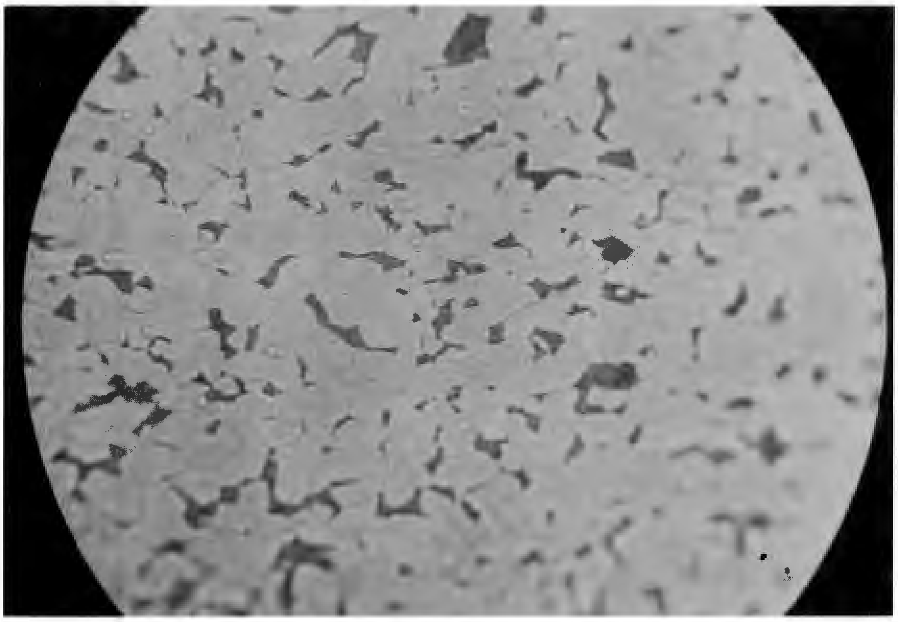


FIGURE 43(a)

Photomicrograph (x250) of electropolished mild steel working electrode surface.

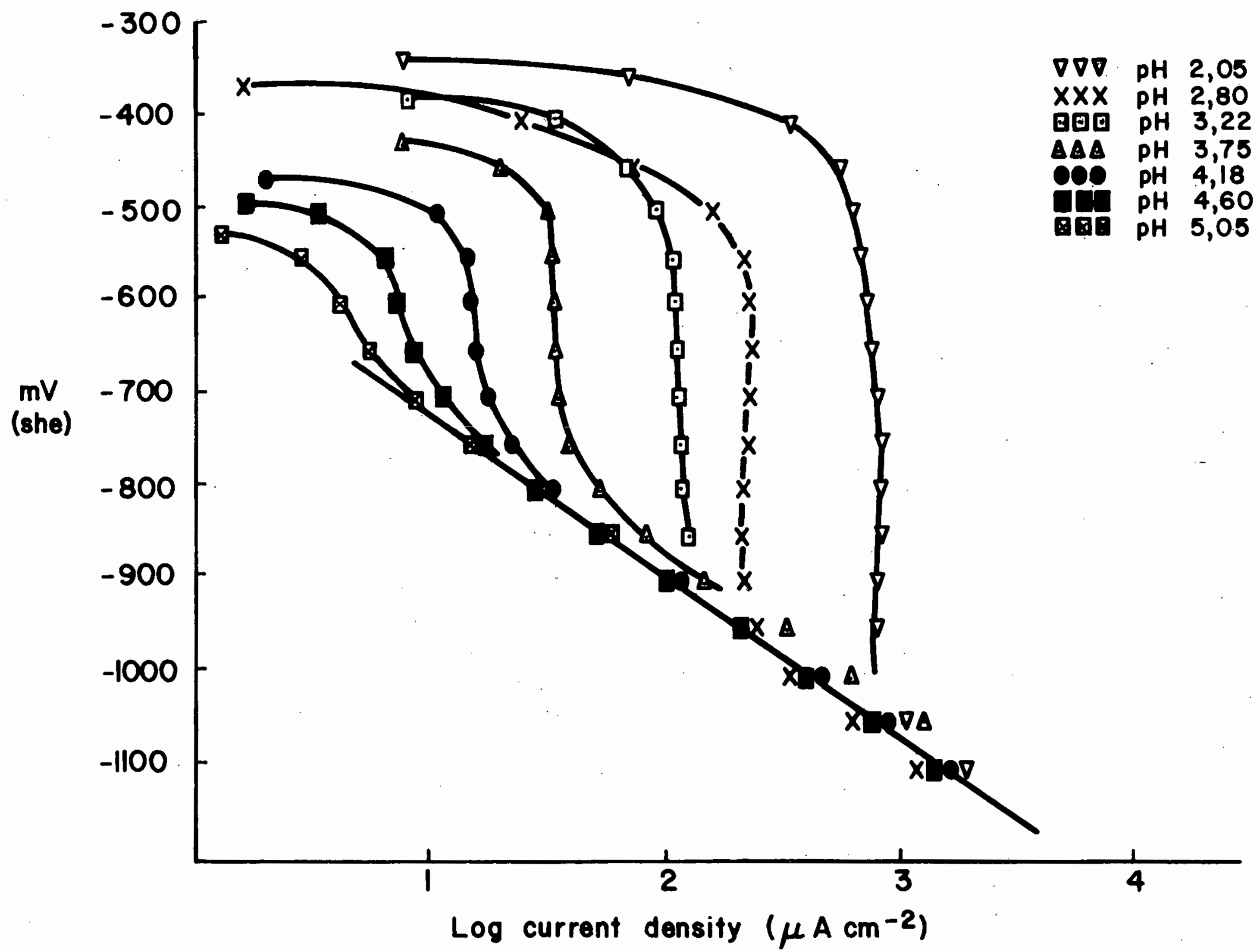


FIGURE 44

Cathodic polarization curves recorded in 0,02M Na Cl solution at various pH values

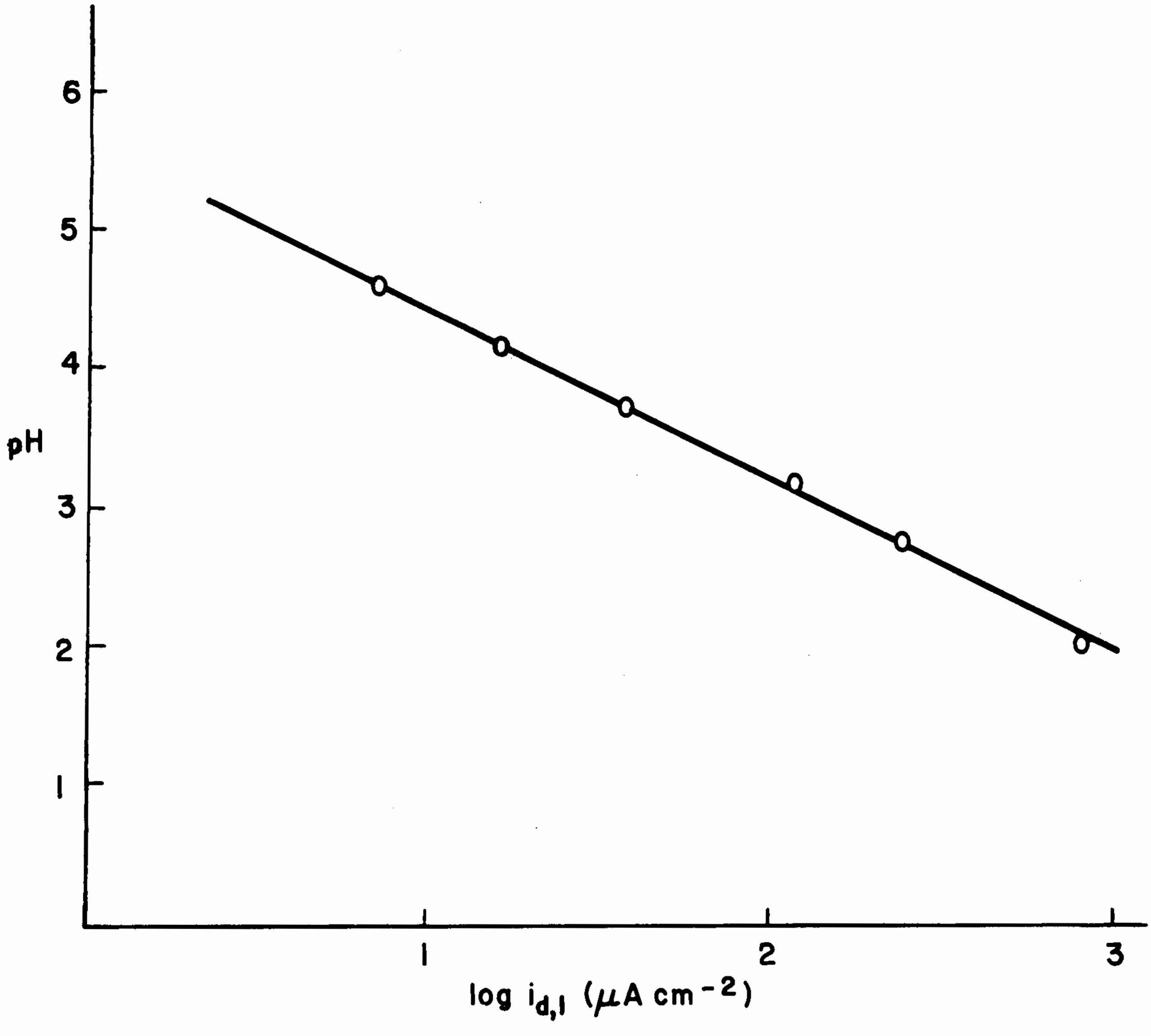


FIGURE 45

Log $i_{d,l}$ data from figure 44 plotted against pH.
Slope = 0,845 - pH

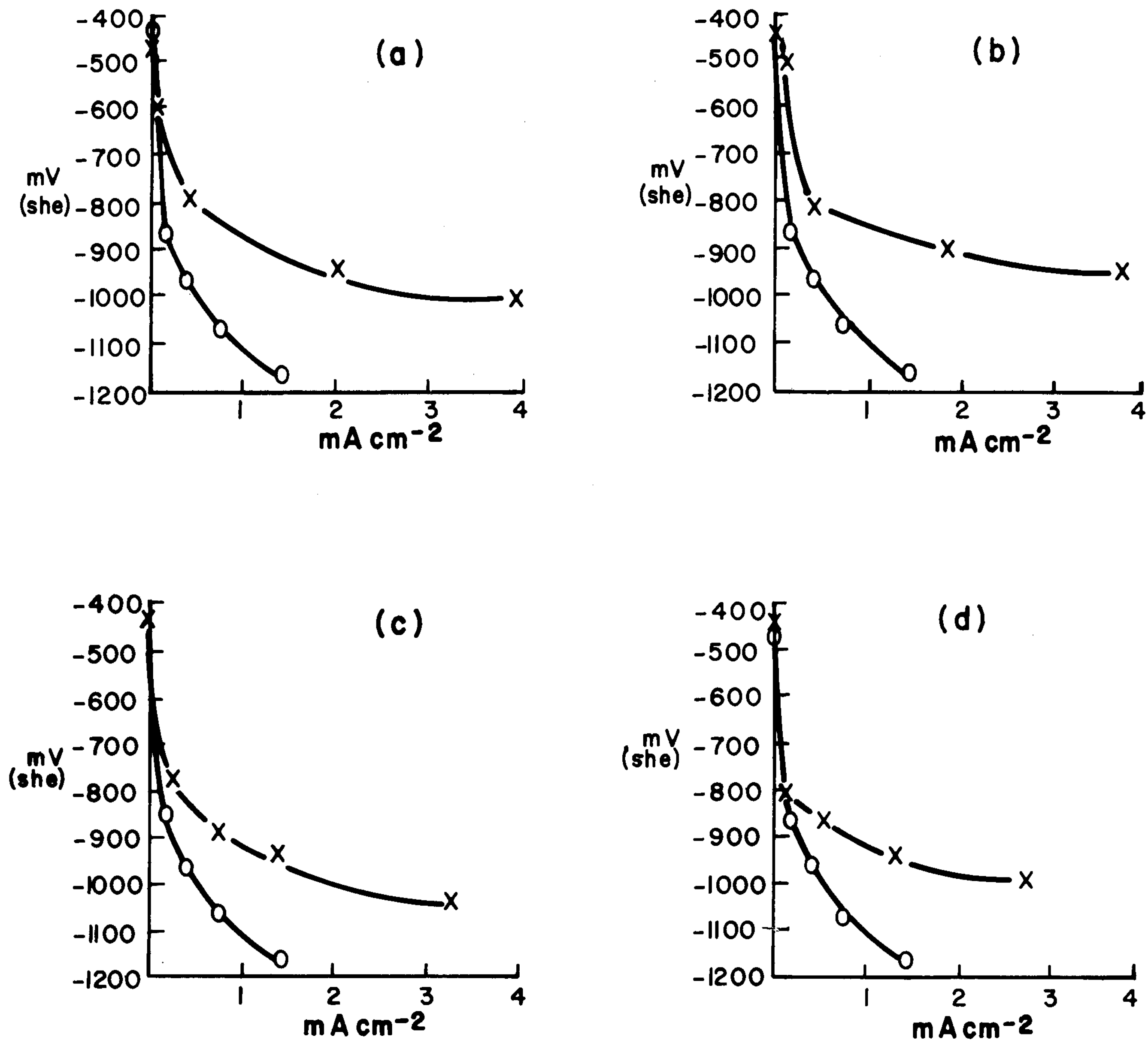


FIGURE 46.

Preliminary cathodic polarization curves for cultures

(a) BR 6 day old

(b) WVB 4 day old

(c) DB 8 day old

(d) HIL 8 day old

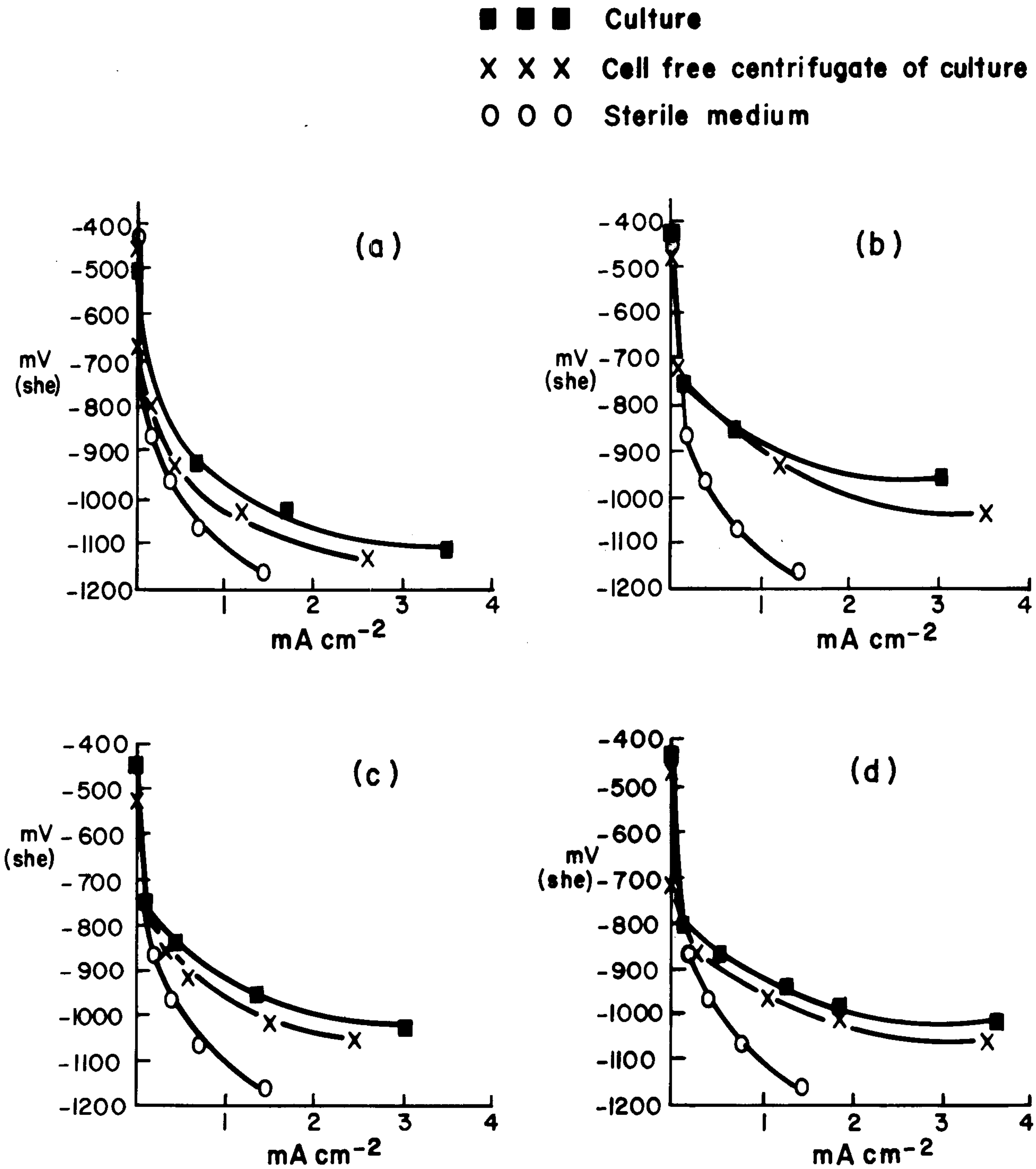


FIGURE 47.

Cathodic polarization curves showing retention of depolarizing activity of cultures in cell-free centrifugates.

a BR Culture 12-day old.
(b) WVB Culture 8-day old.
(c) DB Culture 4-day old.
(d) HIL Culture 4-day old

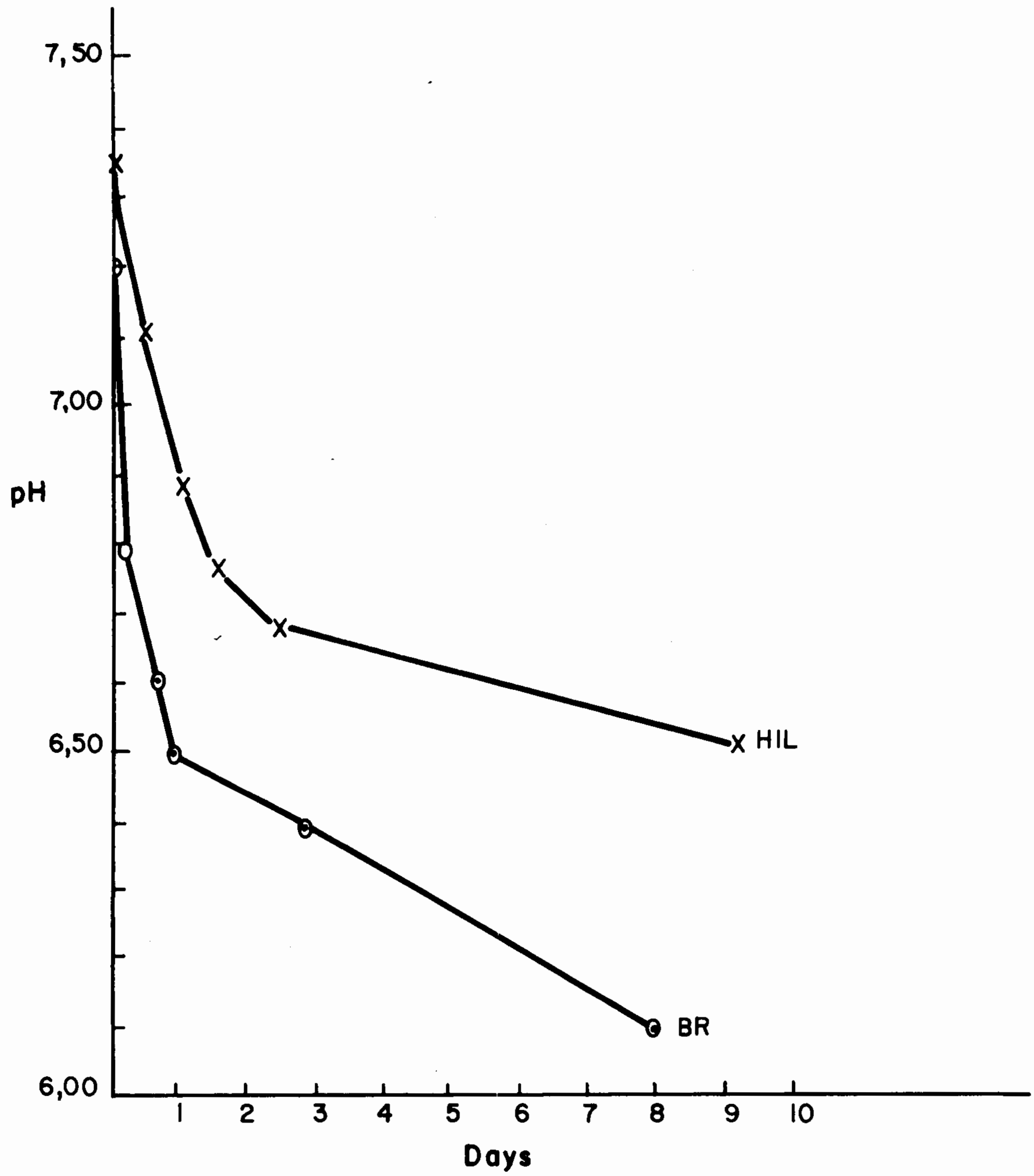


FIGURE 48
Time dependence of culture pH values

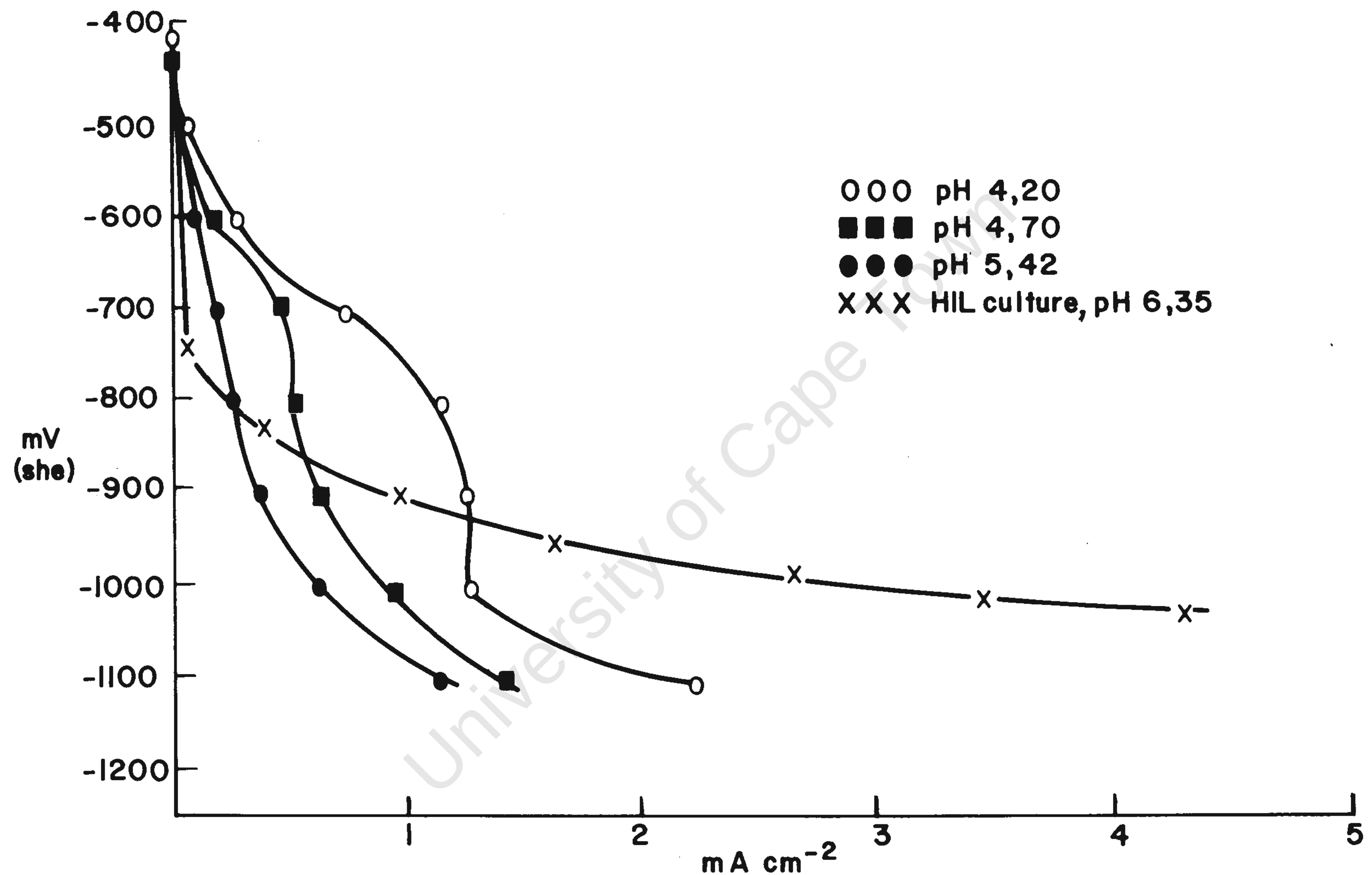


FIGURE 49

Cathodic polarization curves recorded in sterile medium at various pH values and in HIL culture

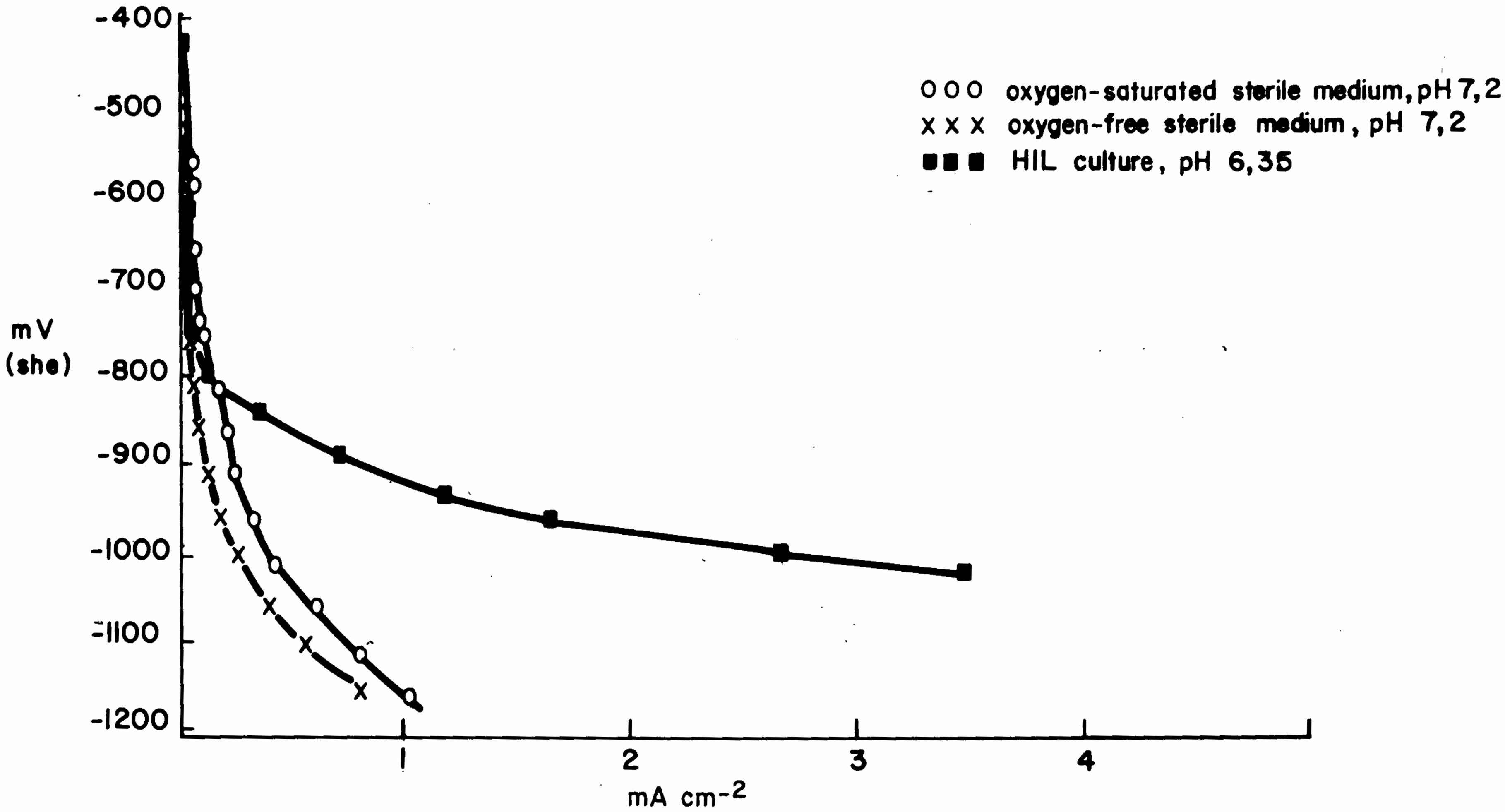


FIGURE 50

Cathodic polarization curves recorded in an active culture and in sterile medium, in presence and absence of oxygen

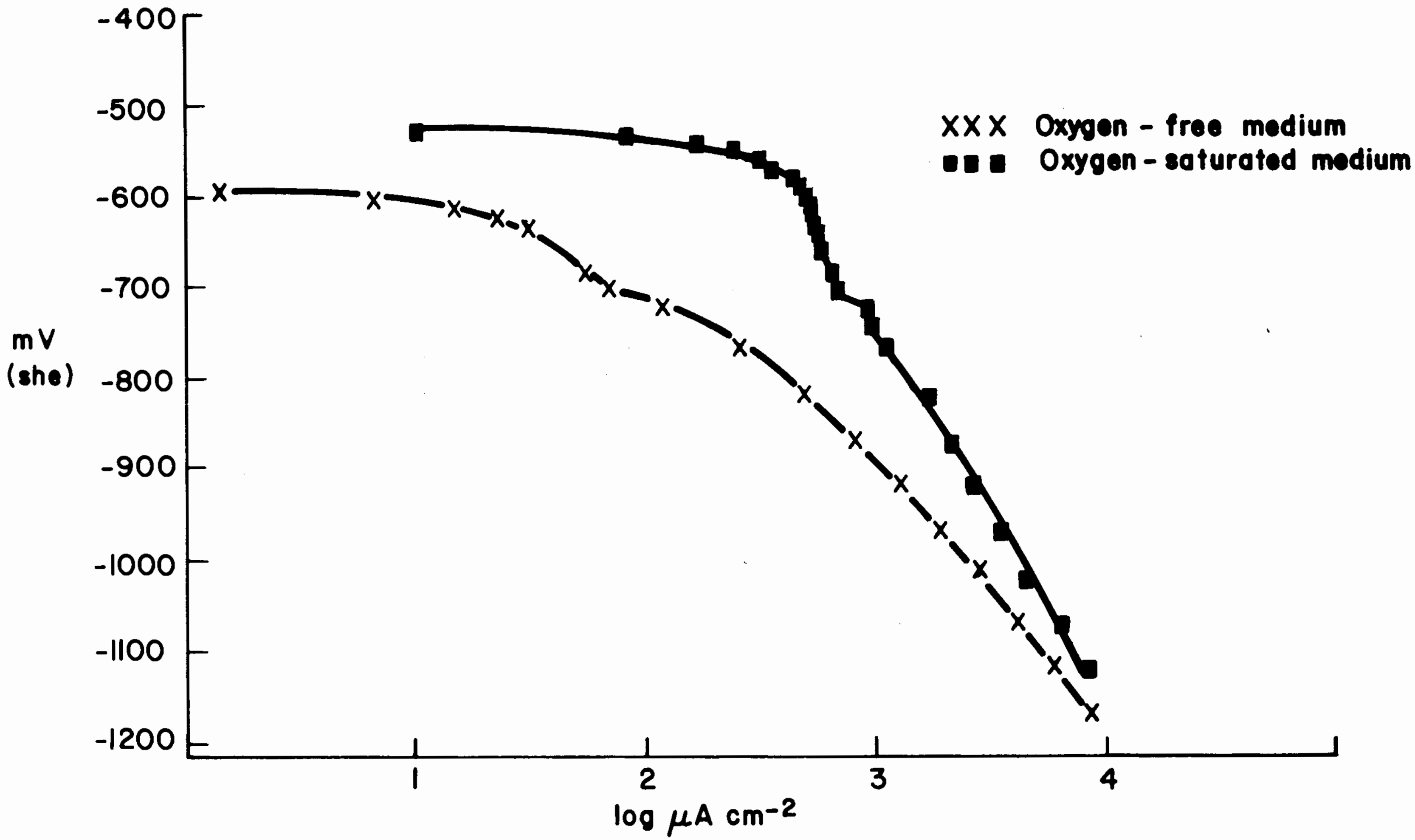


FIGURE. 51.

Semilogarithmic plots of cathadic polarization curves for mild steel recorded in growth medium

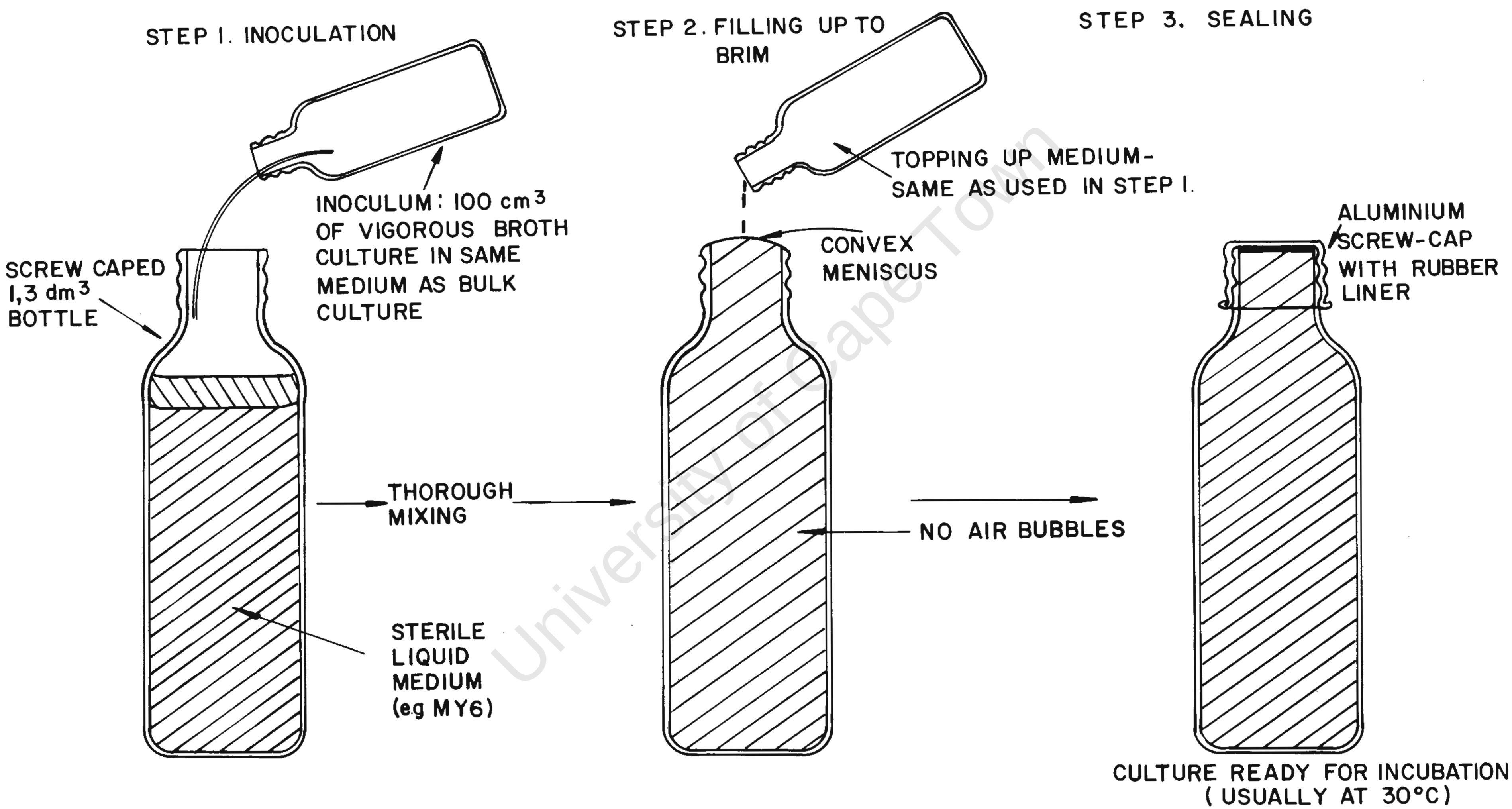


FIGURE 52

Method of inoculating bulk cultures (1,3 dm^3) of sulphate reducing bacteria. All steps conducted in a bacteriological hood, using sterile glassware, gas flaming orifices and sterile media.

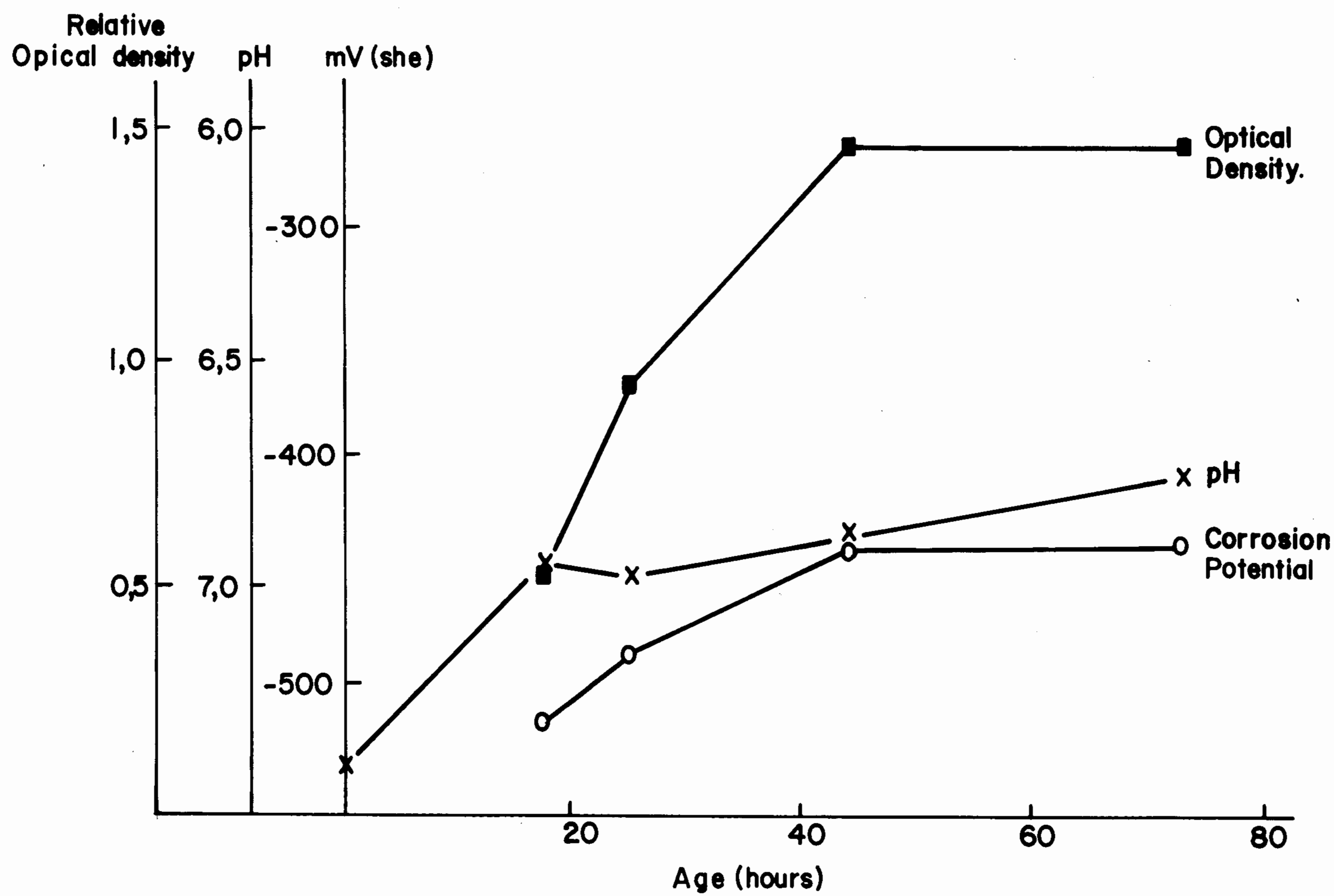


FIGURE 53.

Variation with age of the Relative Optical Density, pH, and Corrosion Potential of Mild Steel in HIL culture.

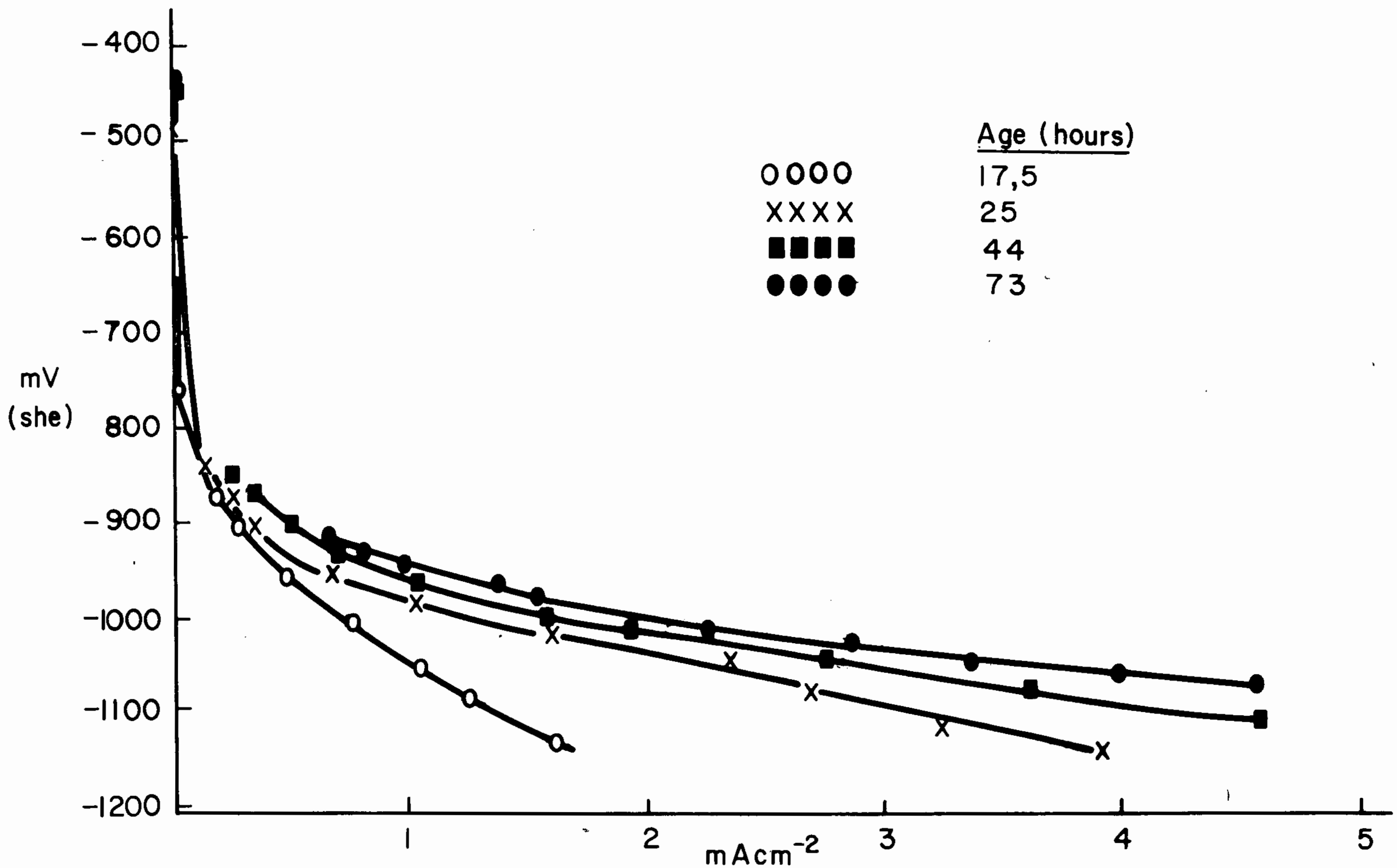


FIGURE 54

Cathodic polarization curves recorded for cultures of HIL of different ages (optical density, corrosion potential and pH data for these are recorded in Fig. 53)

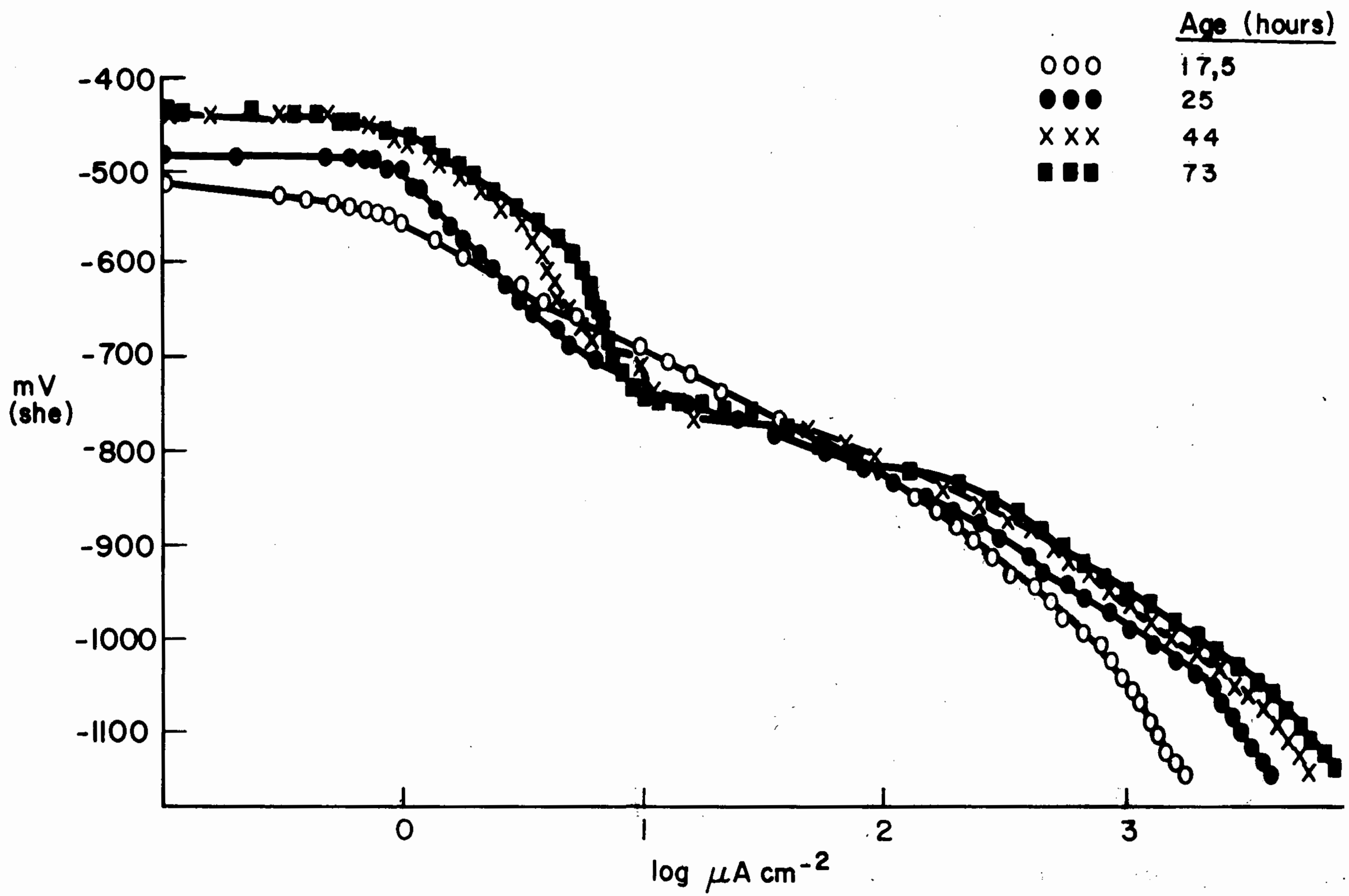


FIGURE 55

Semilogarithmic plots of cathodic polarization curves recorded for cultures of HIL of different ages, as in Figure 54

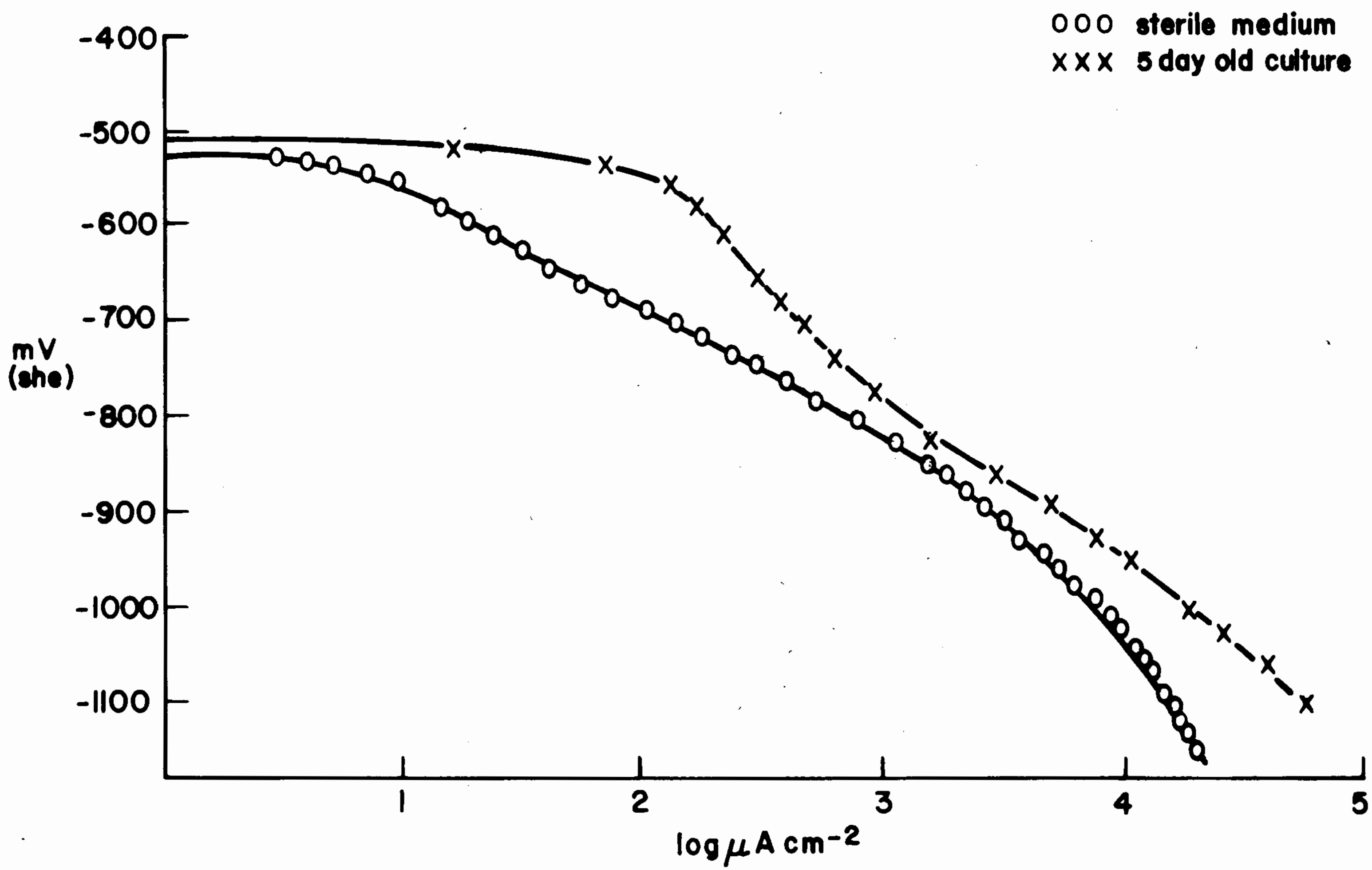


FIGURE. 56.

Semilogarithmic plot of cathodic polarization curve for WVB culture

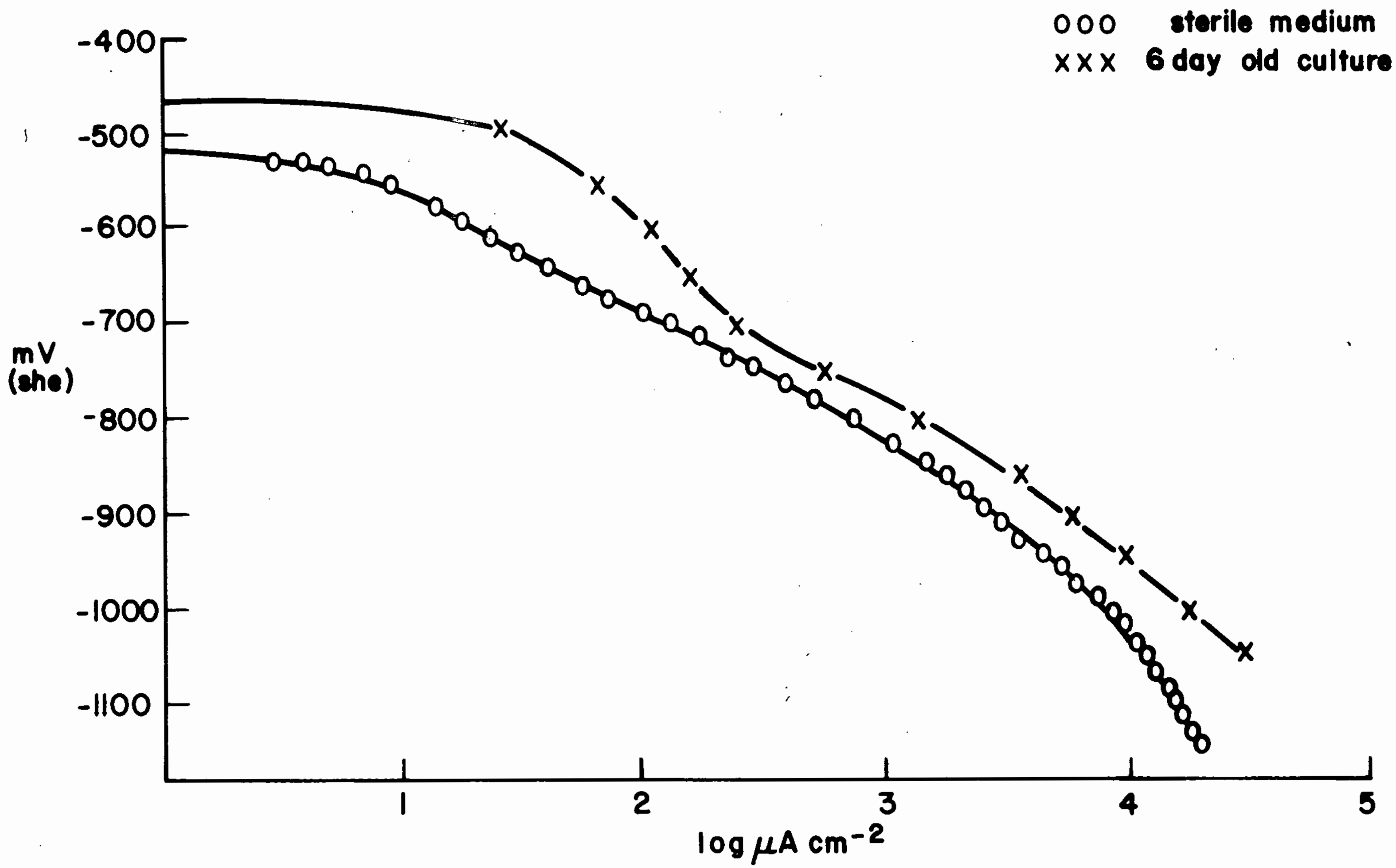


FIGURE .57.

Semilogarithmic plot of cathodic polarization curve for culture BR

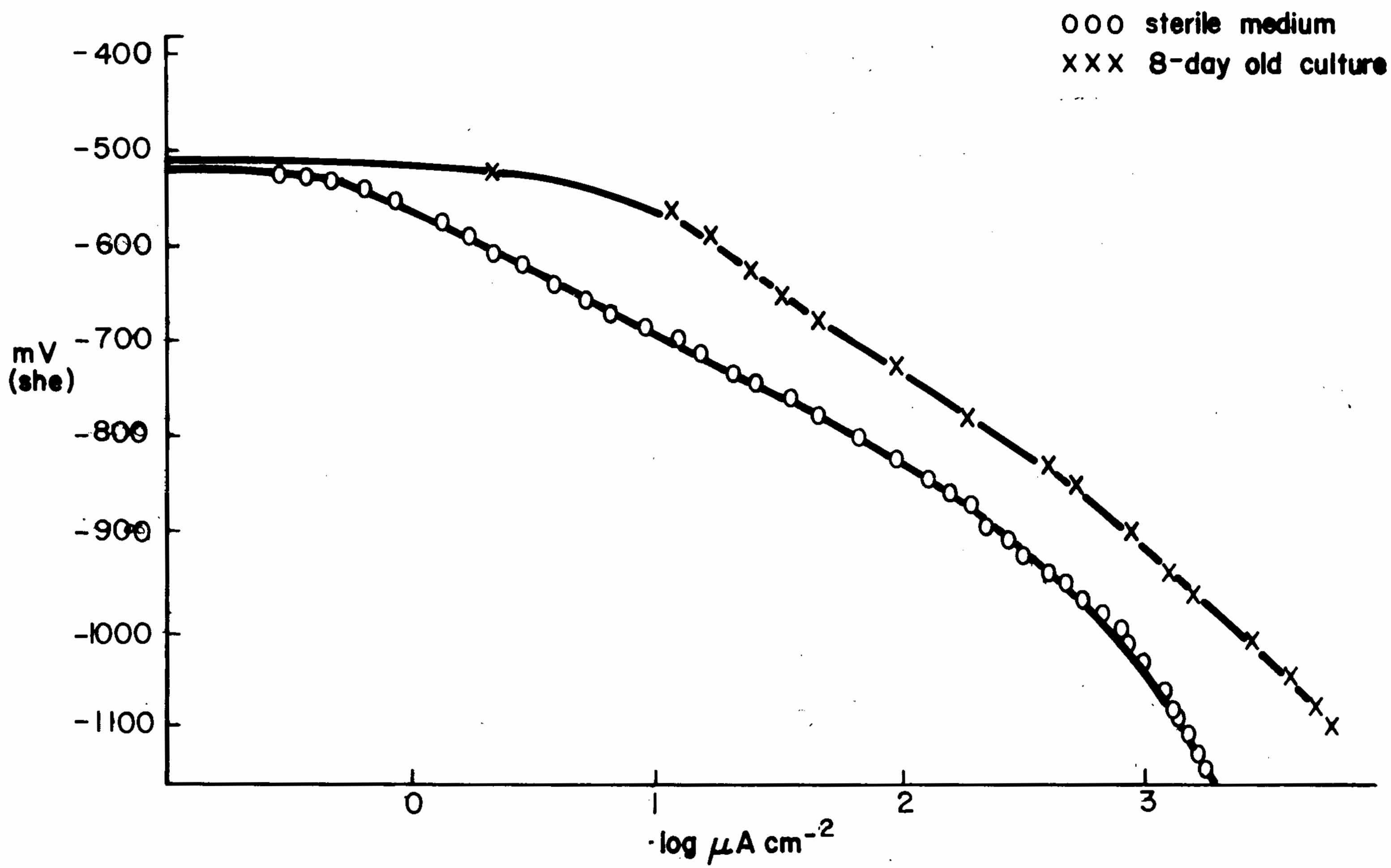


FIGURE 58

Semilogarithmic plot of cathodic polarization curve for culture DB

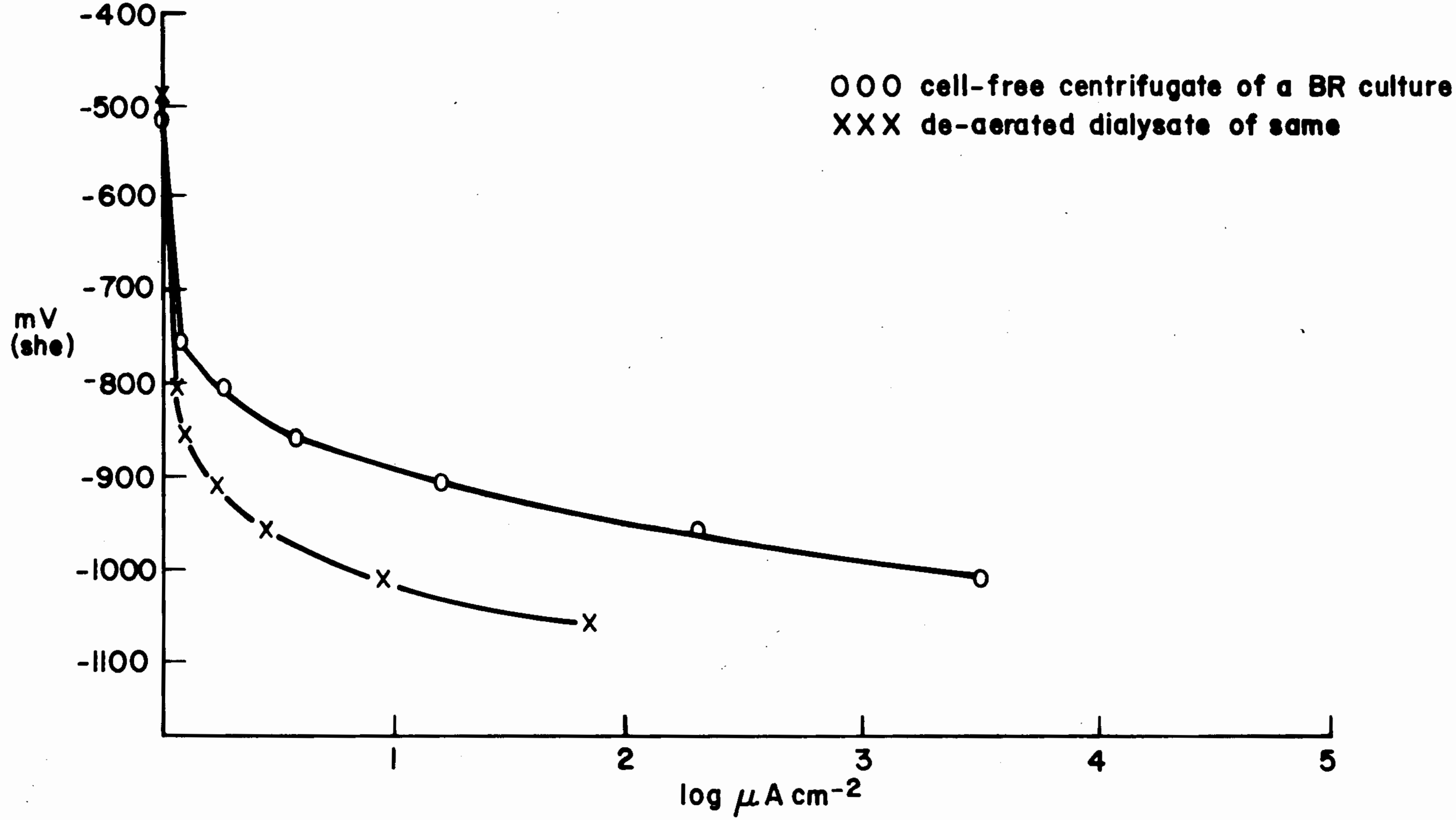


FIGURE.59.

Cathodic polarization curves recorded for mild steel in centrifugate of BR culture and its dialysate

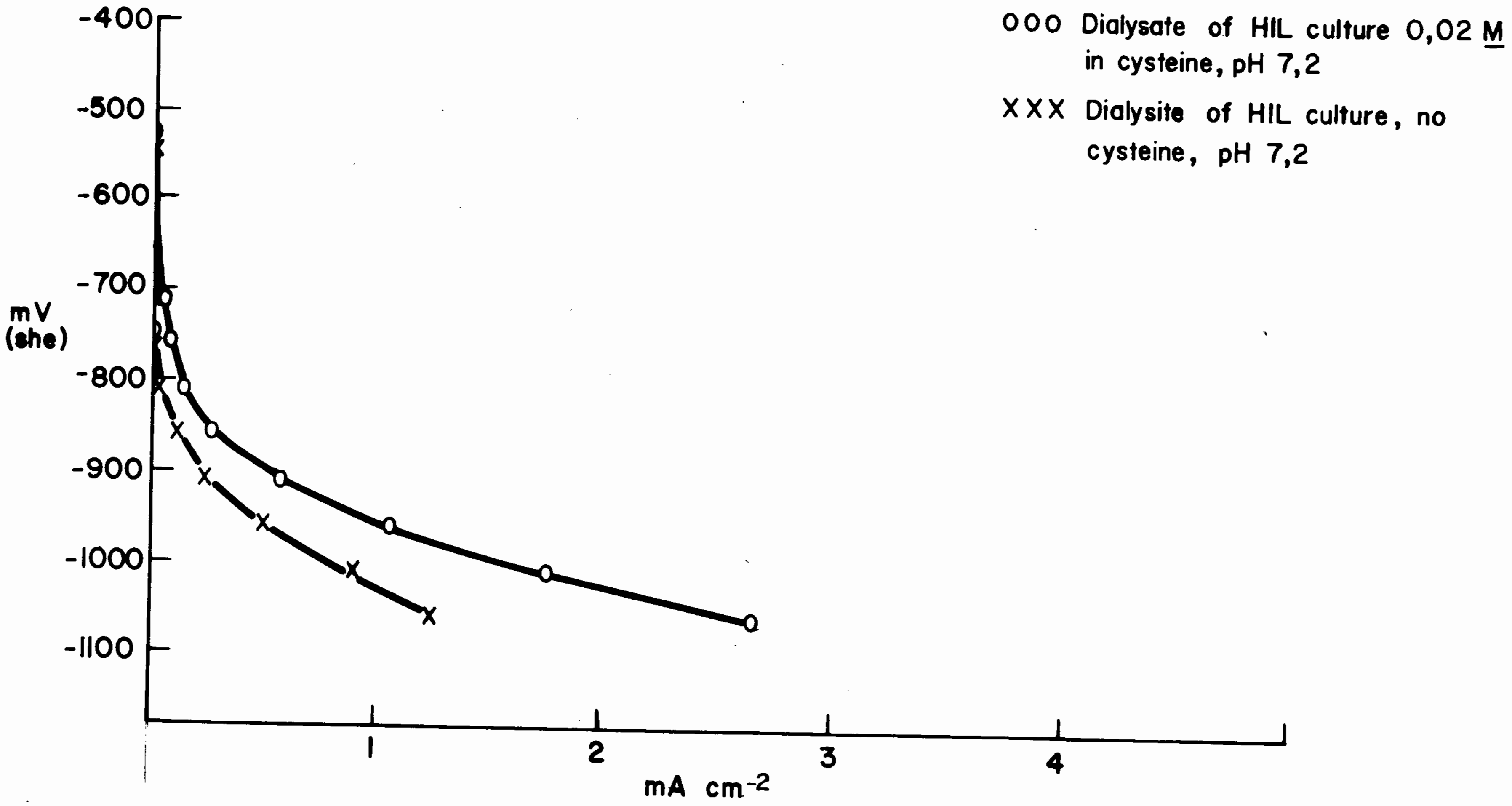


FIGURE 60

Cathodic polarization curves recorded in HIL culture dialysates with and without cysteine additions

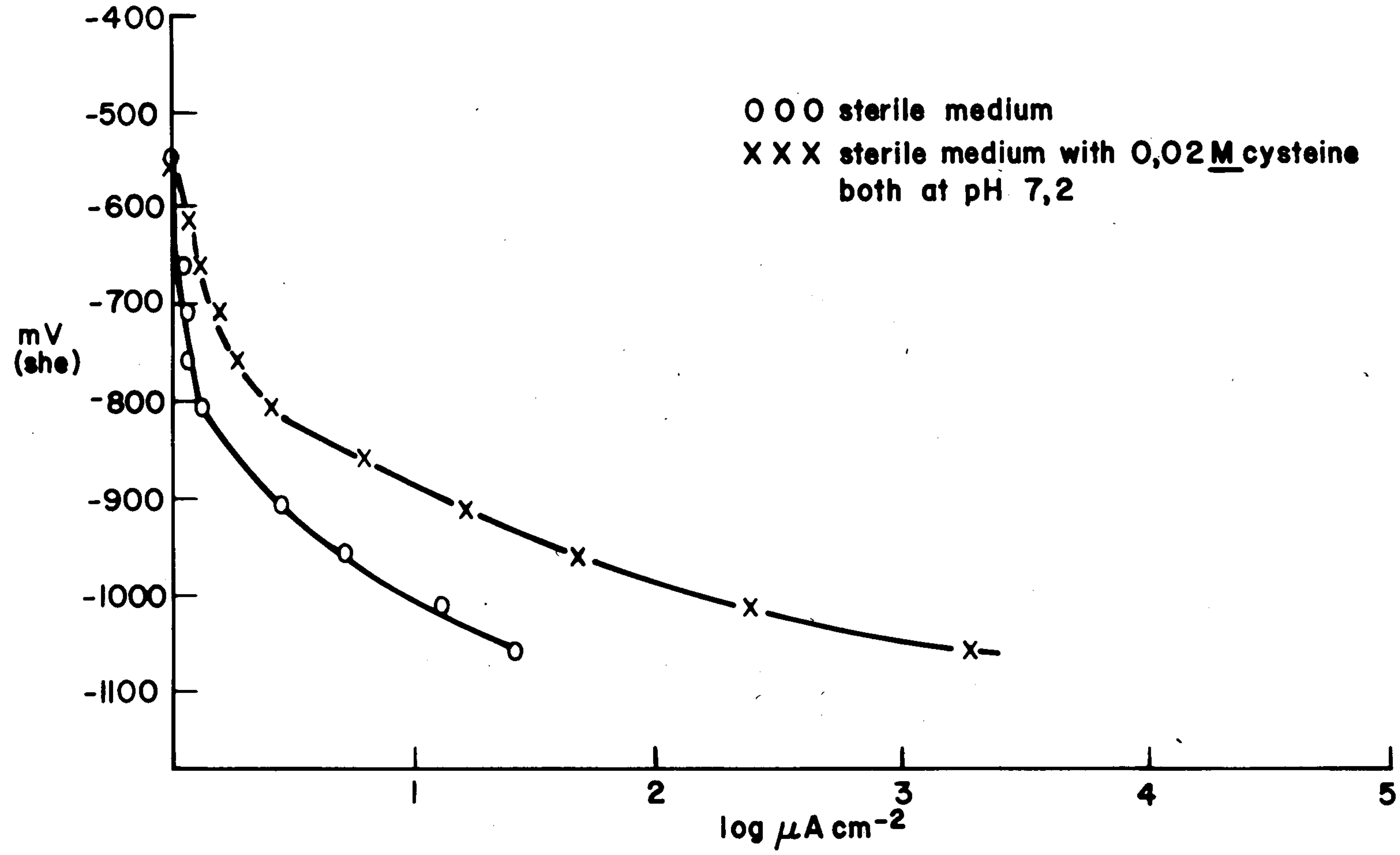


FIGURE. 61.

Cathodic polarization curves recorded in growth medium with cysteine additions

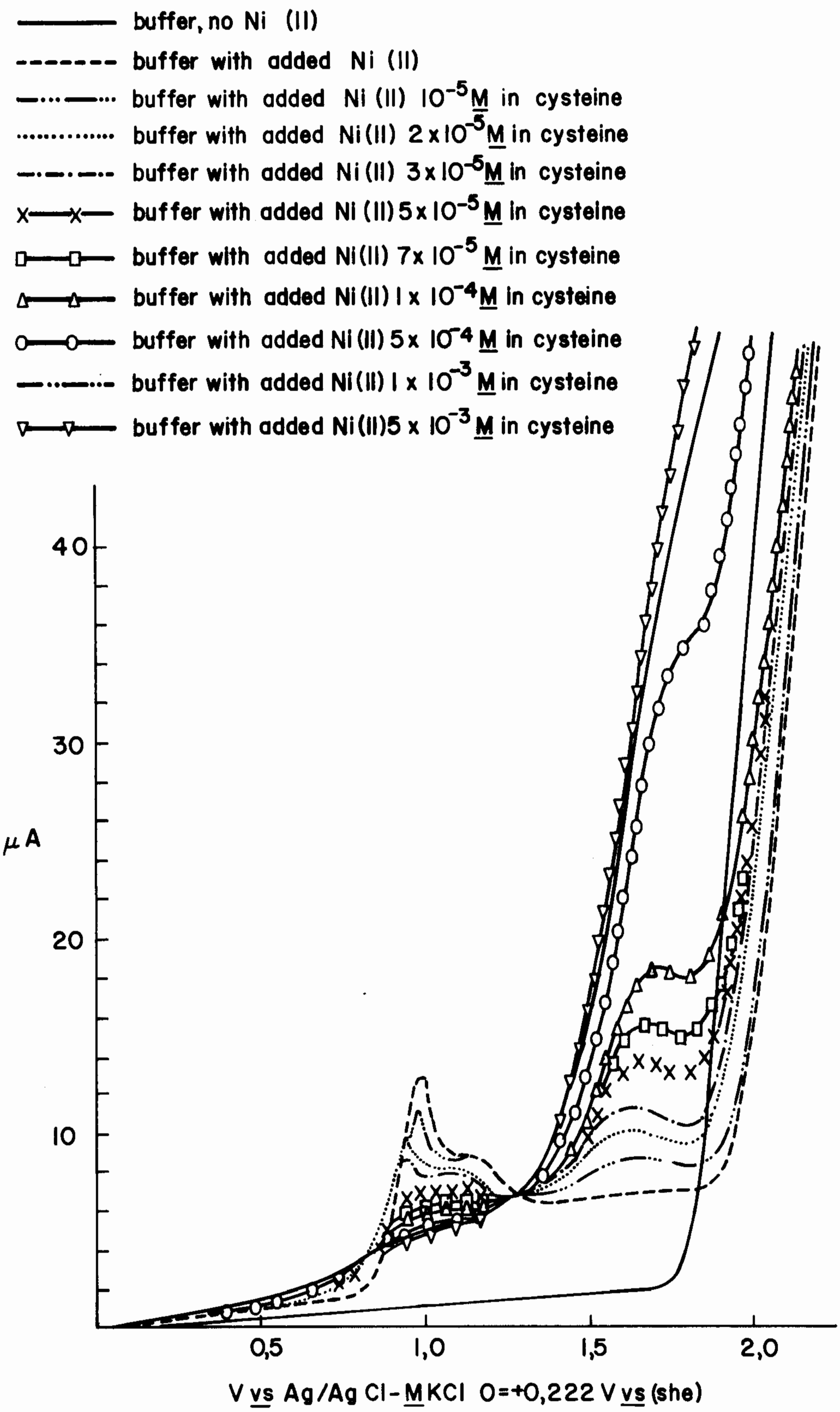


FIGURE 62.

Polarograms recorded in Brdička's buffer 0,002 M in Ni (II), containing various cysteine additions. Typical catalytic waves are visible at voltages between 1,5 V and 2,0 V

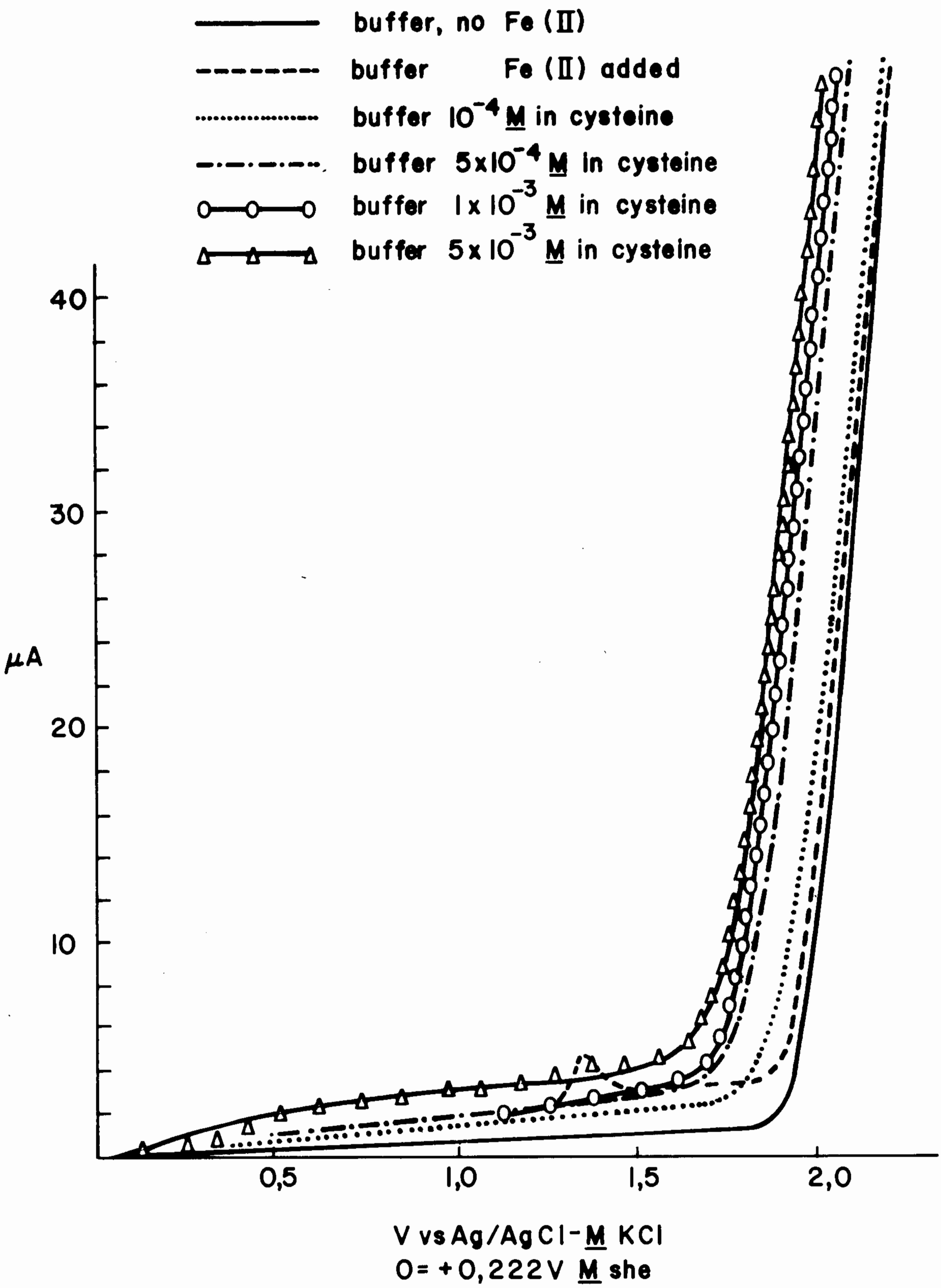


FIGURE. 63.

Polarograms recorded in Brdička's buffer 0,002 M in Fe (II) containing various cysteine additions. No catalytic waves are present.

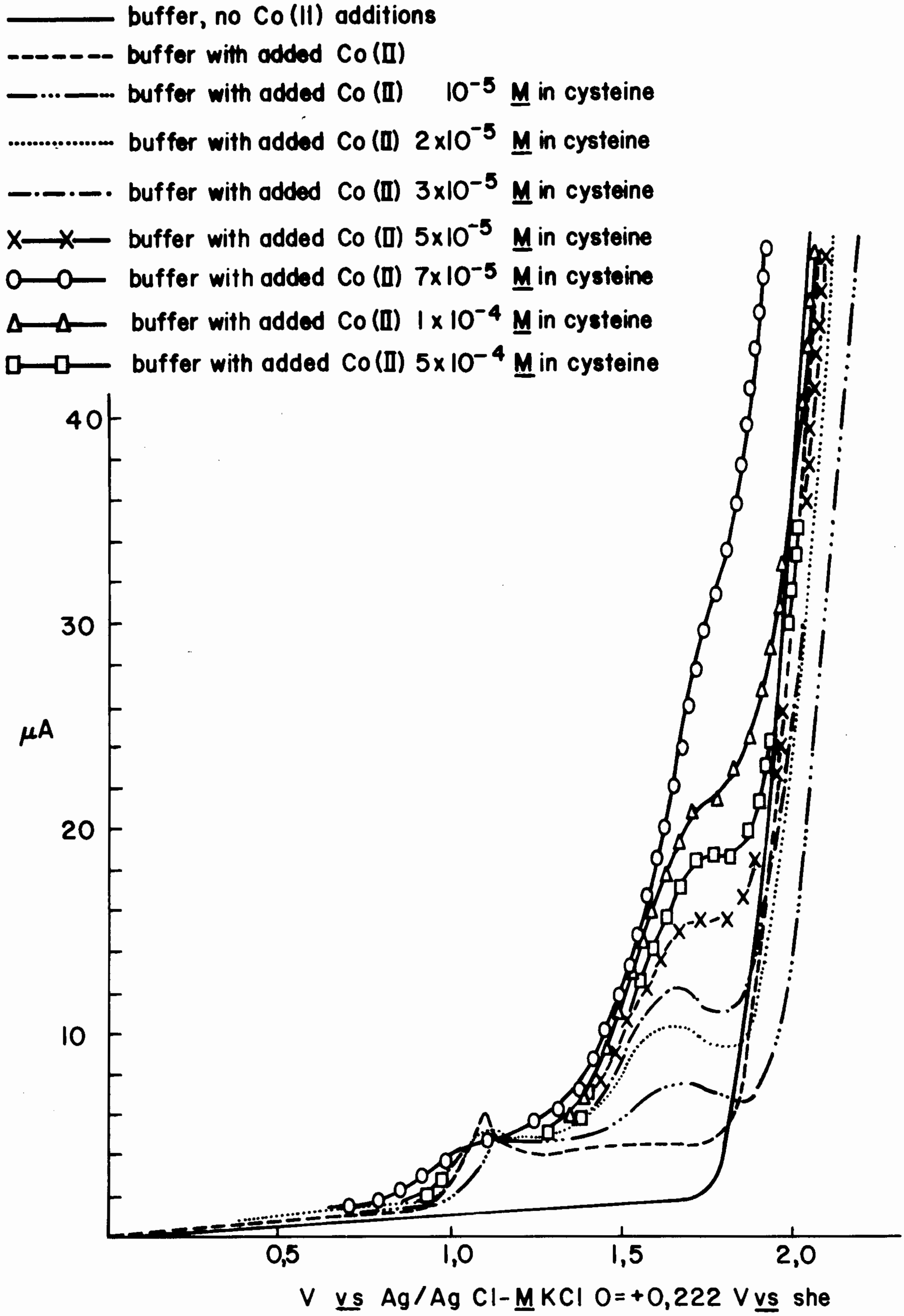


FIGURE 64

Polarograms recorded in Brdička's buffer, 0,002 M in Co (II), containing various cysteine additions. Typical catalytic waves are visible at voltages between 1,5 and 2,0 V

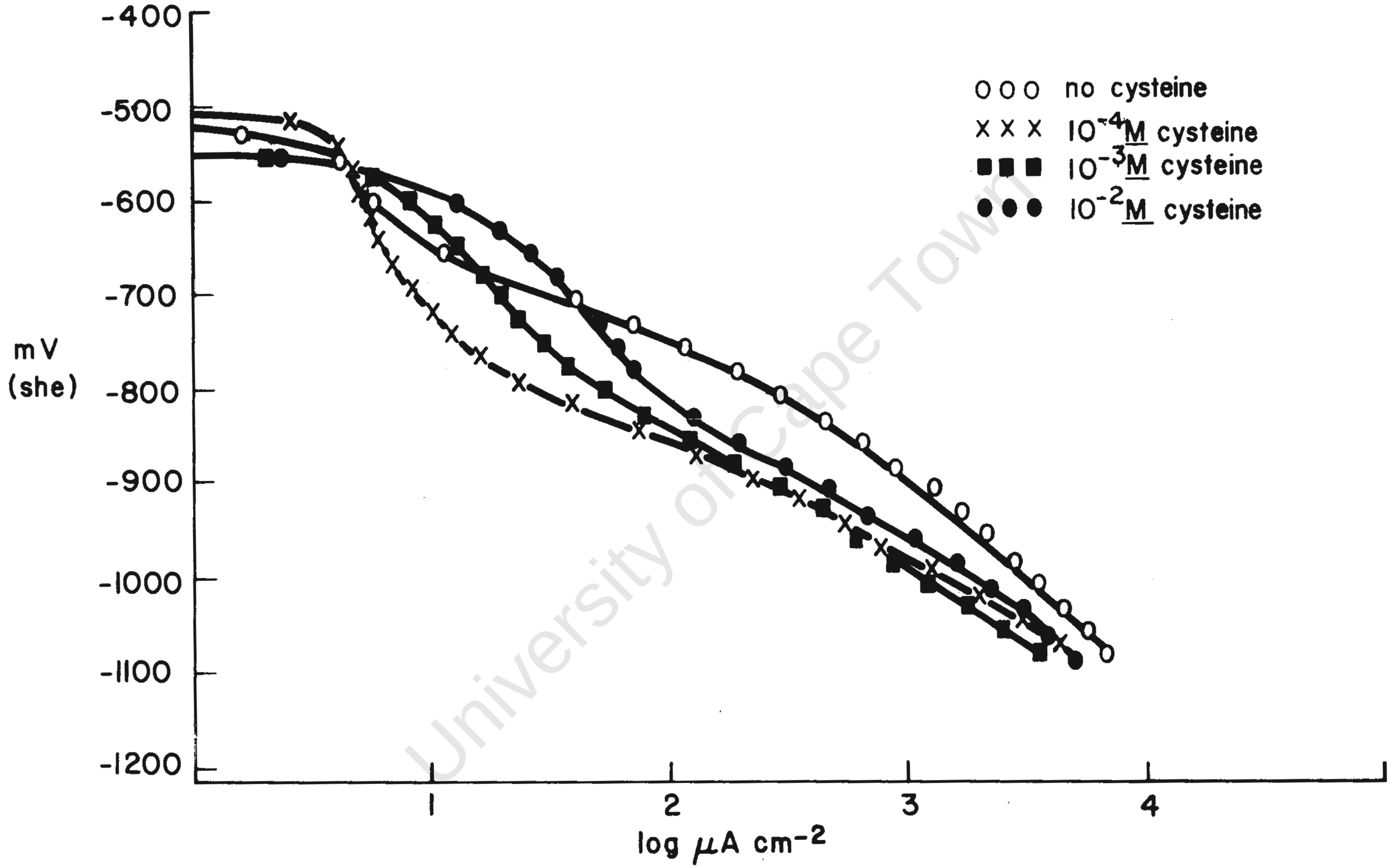


FIGURE 65

Semilogarithmic plots of cathodic polarization curves for mild steel in Brdicka's Buffer, 0,002 M in Fe^{++}, containing cysteine additions.

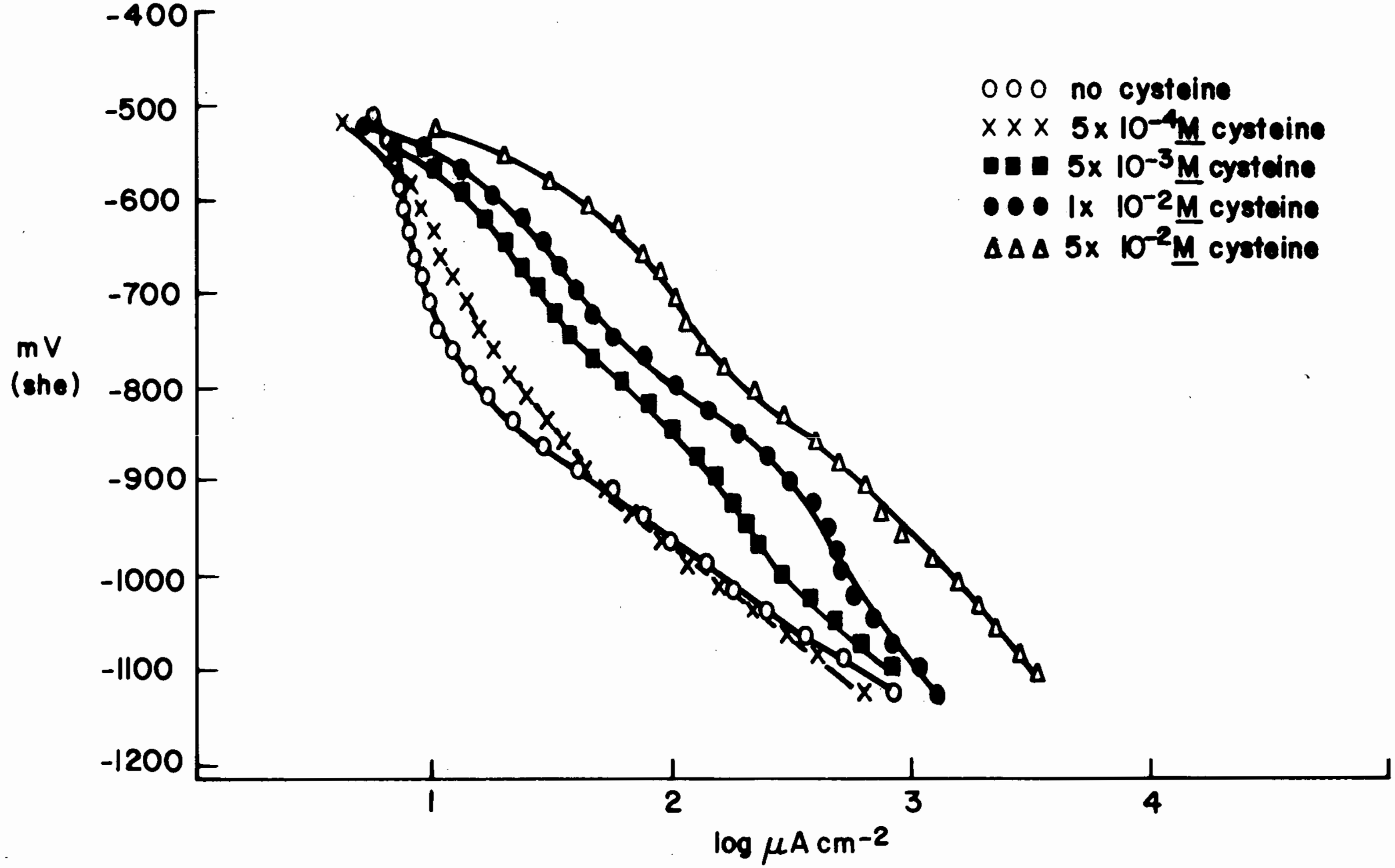


FIGURE.66.

Semilogarithmic plots of cathodic polarization curves for mild steel in 0,02 M NaCl containing 0,000025 M Fe^{++} with cysteine additions, pH, 7,1

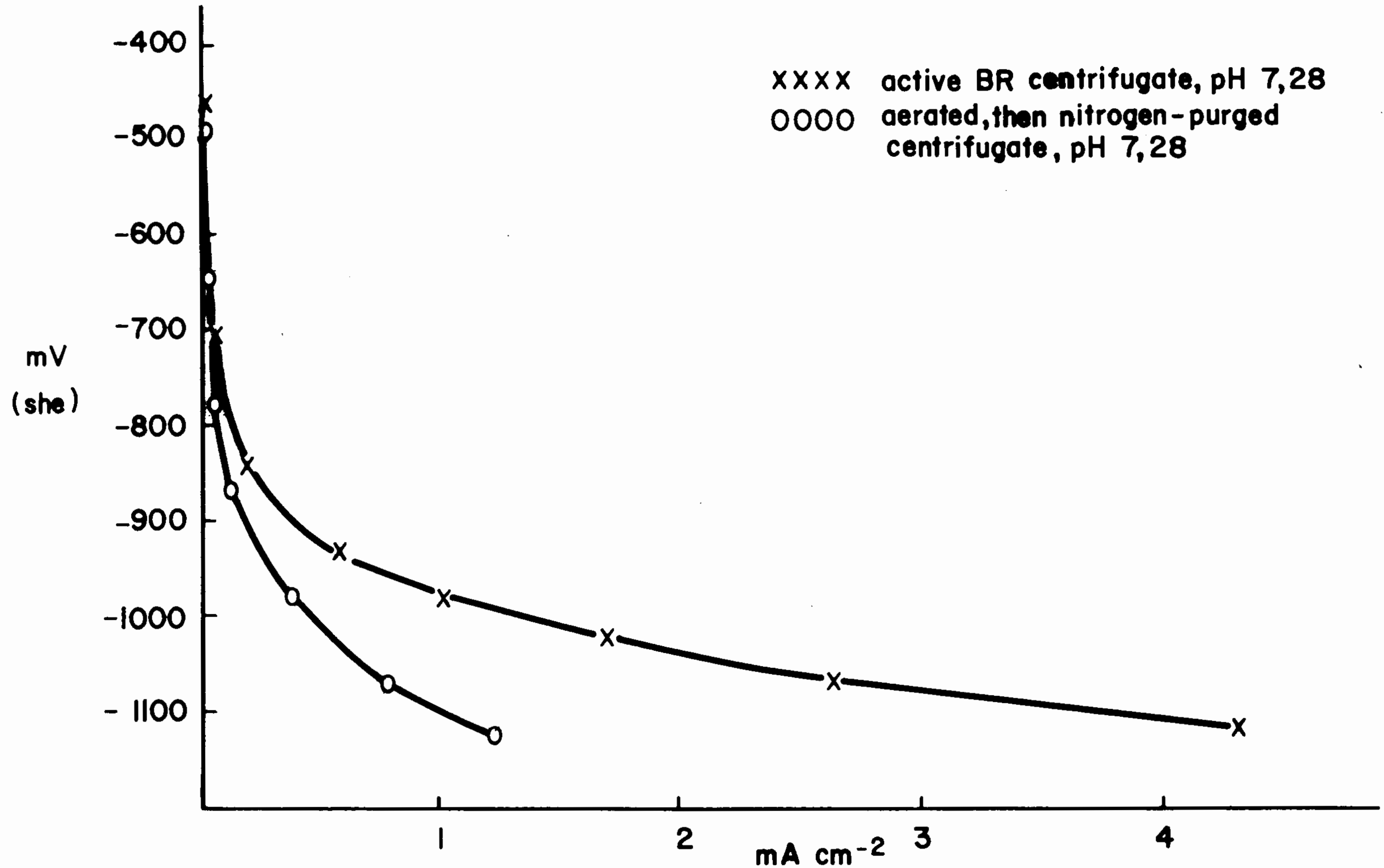


FIGURE 67(a)

Cathodic polarization curves for mild steel in centrifugates of cultures

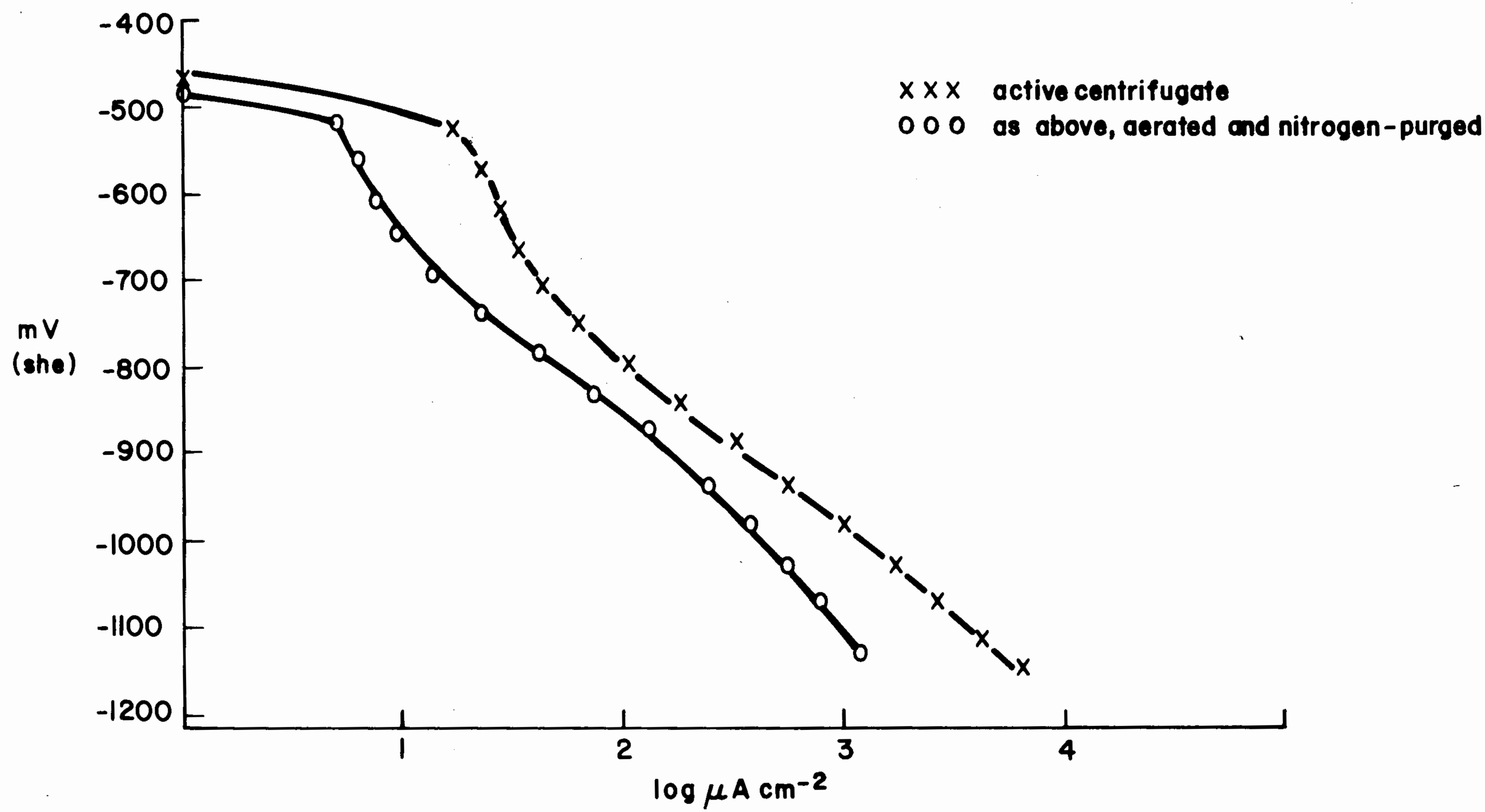


FIGURE. 67 (b)

Semilogarithmic plot of data of 67(a)

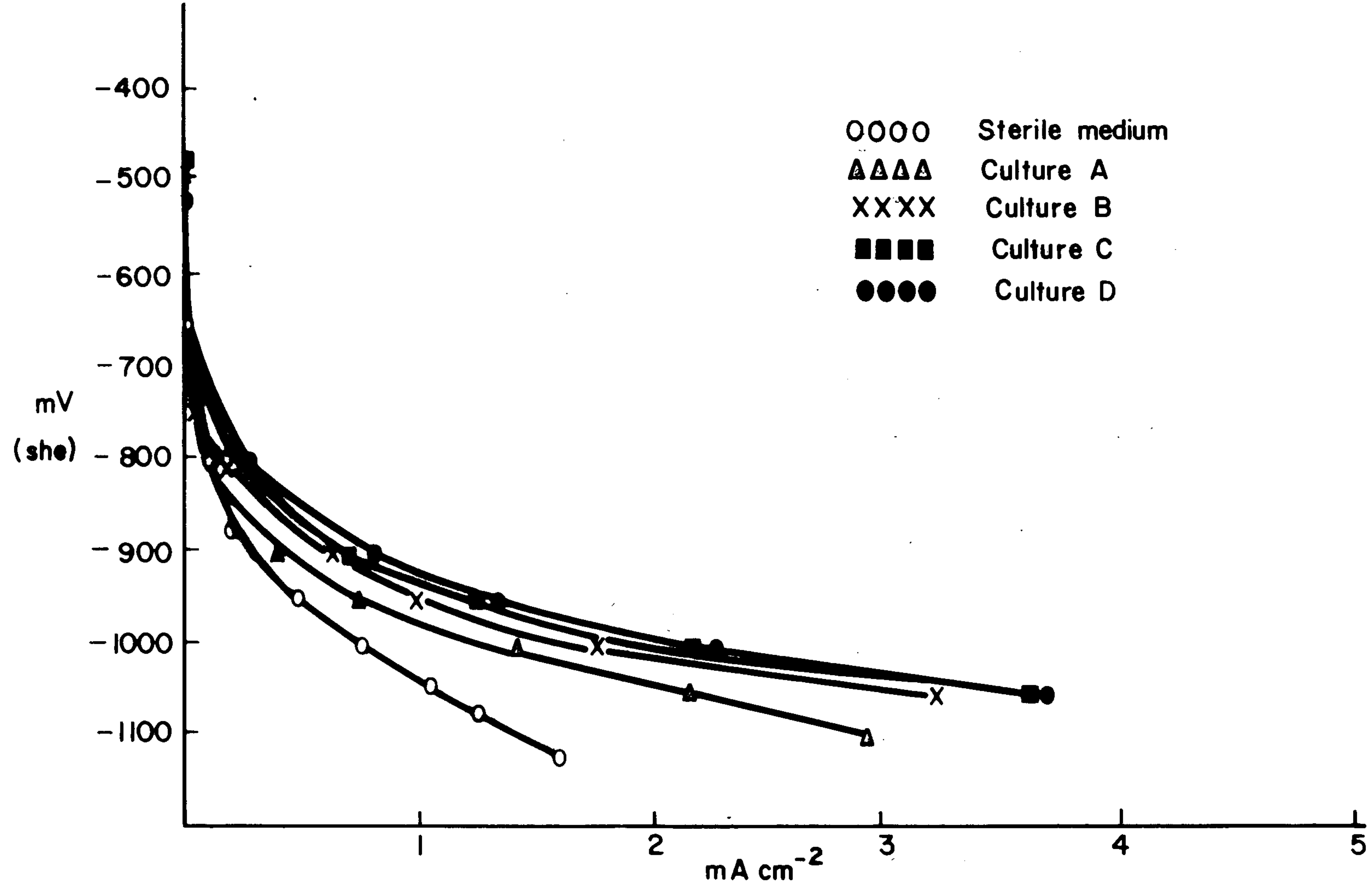


FIGURE 68

Cathodic polarization curves recorded in cultures of BR of varying pH and sulphide content, data for which are recorded in Table 10

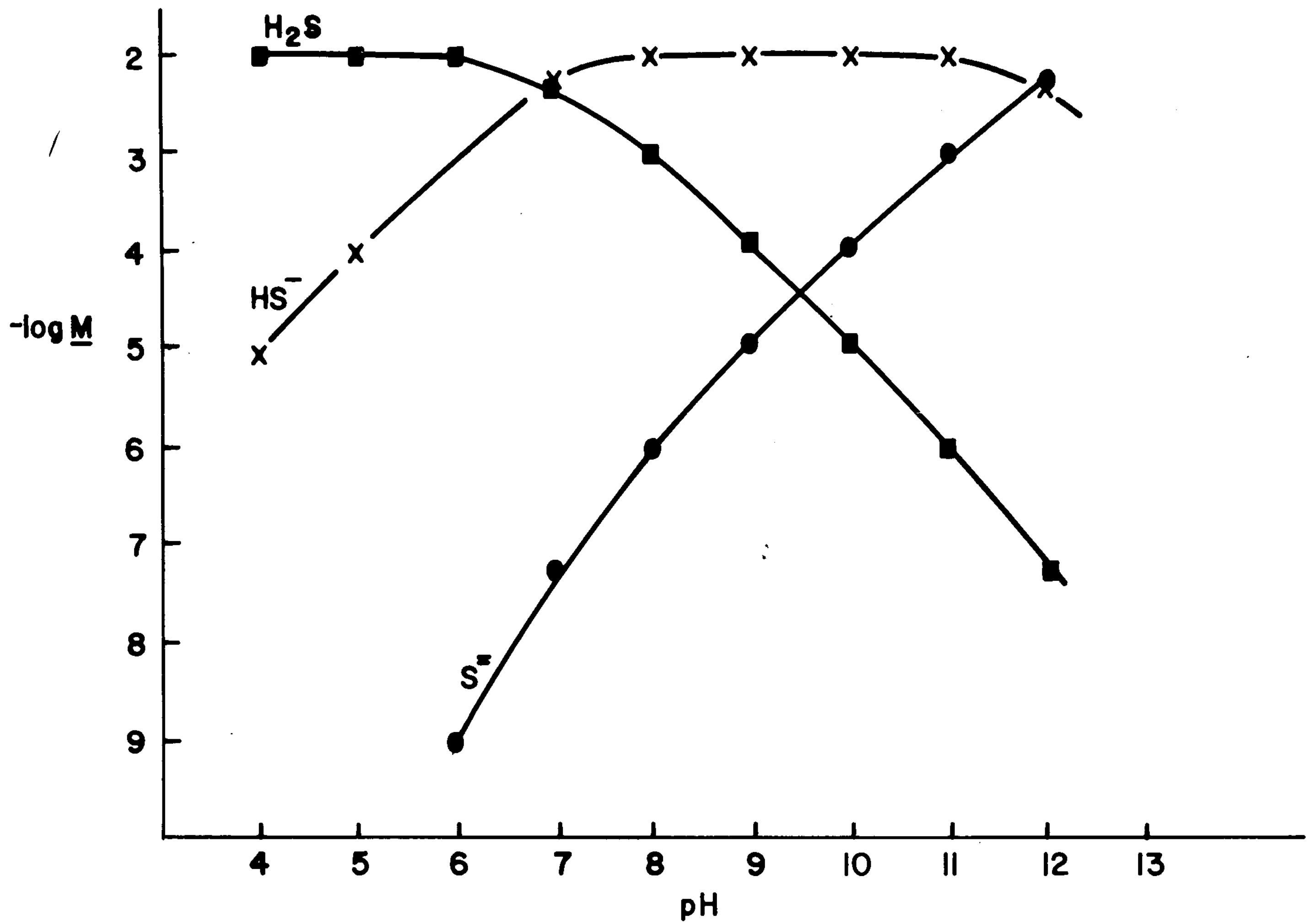


FIGURE 69.

Log molarity *vs* pH data for the relevent sulphide species.

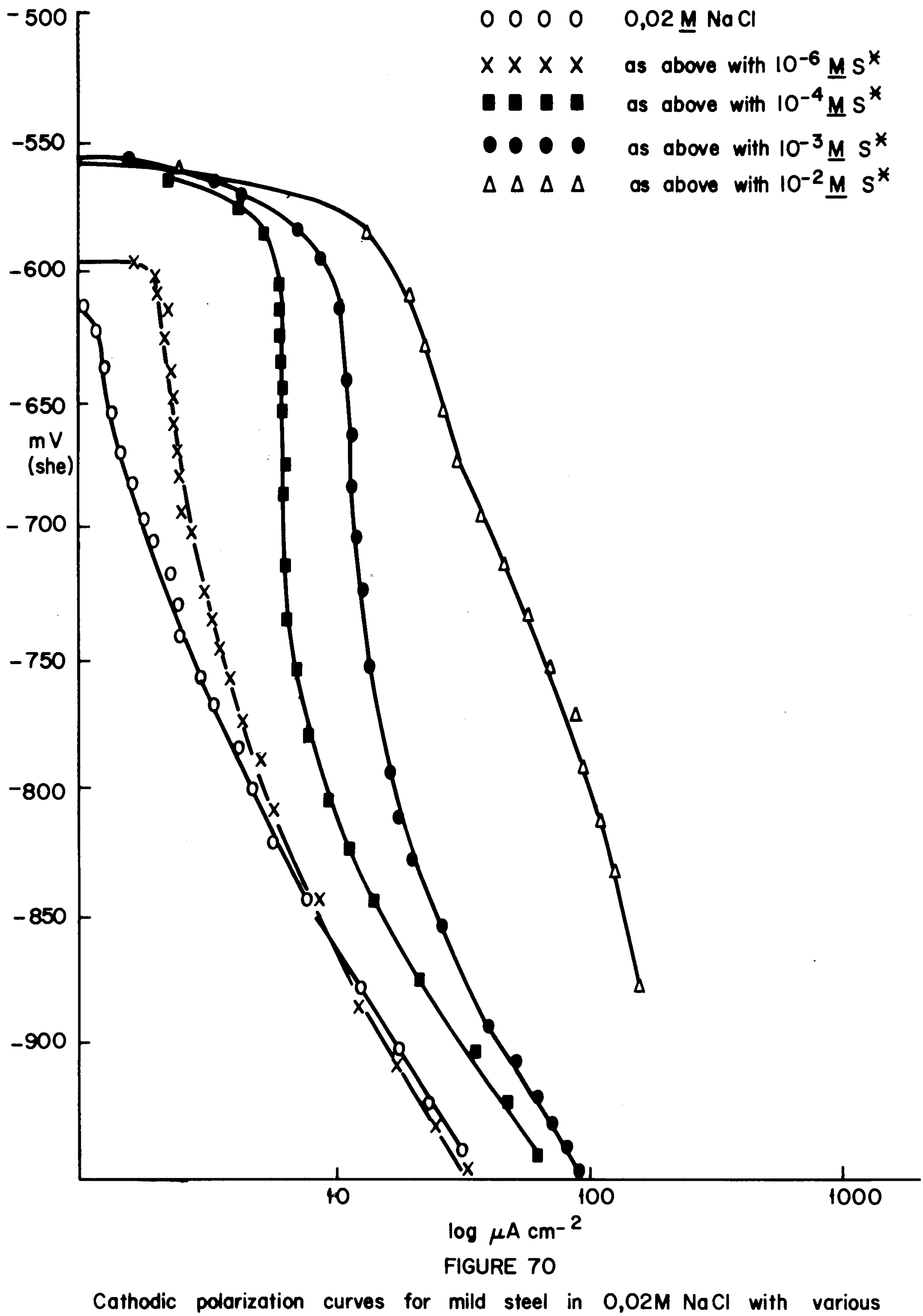


FIGURE 70

Cathodic polarization curves for mild steel in 0,02M NaCl with various additions of sulphide as $Na_2S.9H_2O$. pH of all solutions 7,40

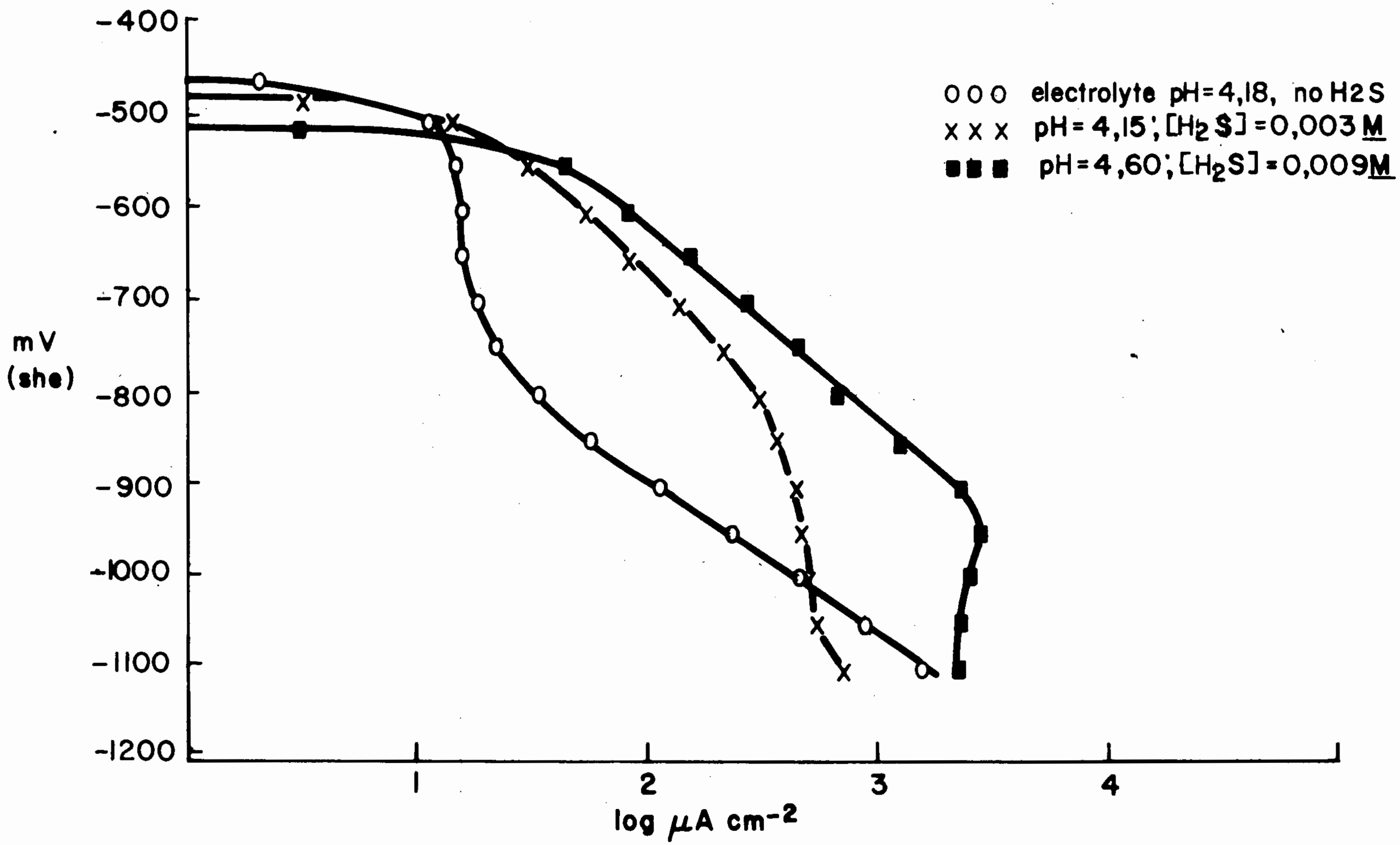


FIGURE 71

Semilog plots of cathodic polarization curves for mild steel in 0,02 M NaCl with H_2S added as gas from Kipps' generator

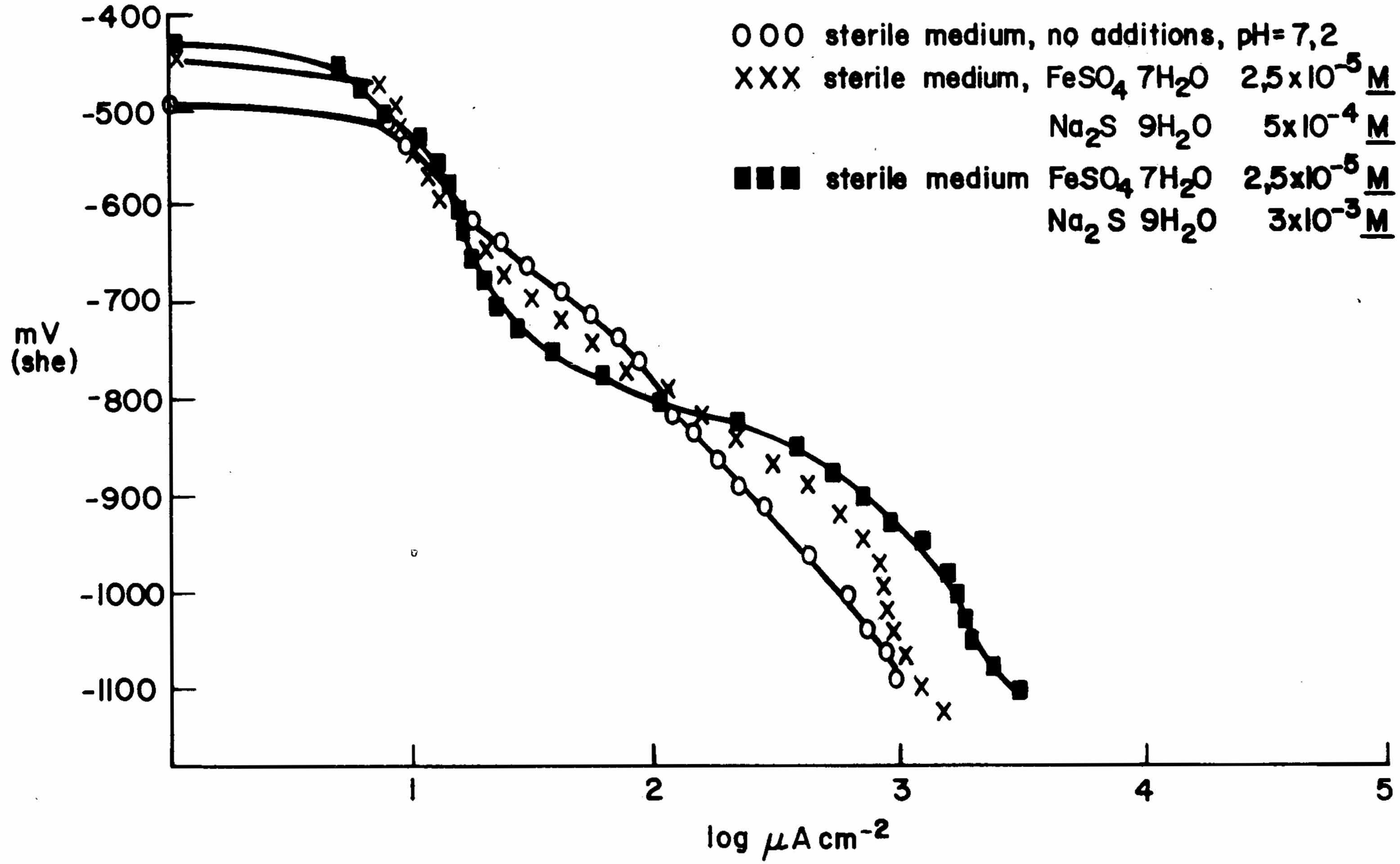


FIGURE. 72.

Semilog plots of cathodic polarization curves for mild steel in growth medium to which sulphides are added

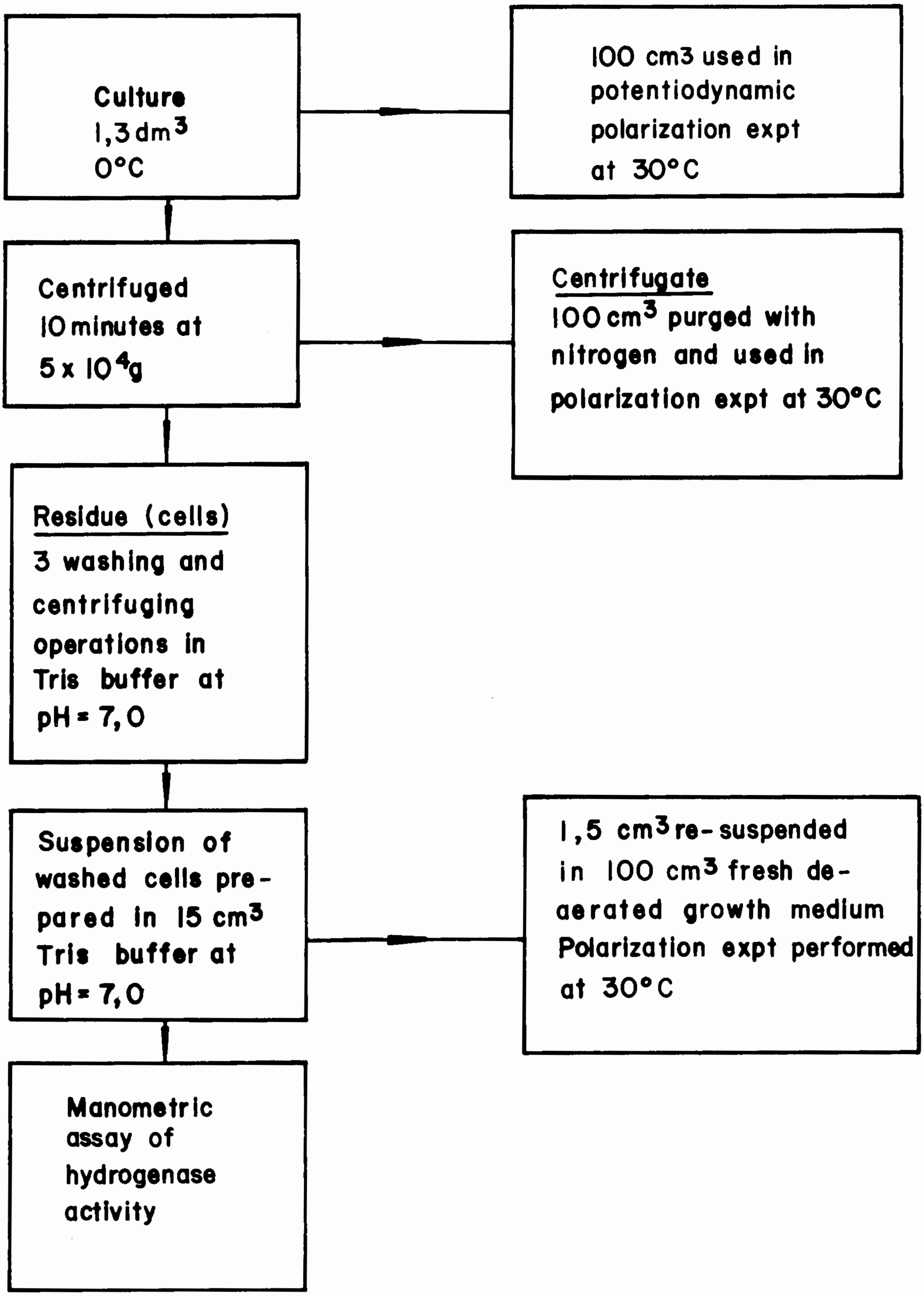

Culture
1,3 dm3
0°C
100 cm3 used in potentiodynamic polarization expt at 30°C
Centrifuged 10 minutes at 5 x 104g
Centrifugate
100 cm3 purged with nitrogen and used in polarization expt at 30°C
Residue (cells)
3 washing and centrifuging operations in Tris buffer at pH = 7,0
Suspension of washed cells prepared in 15 cm3 Tris buffer at pH = 7,0
1,5 cm3 re-suspended in 100 cm3 fresh de-aerated growth medium
Polarization expt performed at 30°C
Manometric assay of hydrogenase activity

FIGURE 73

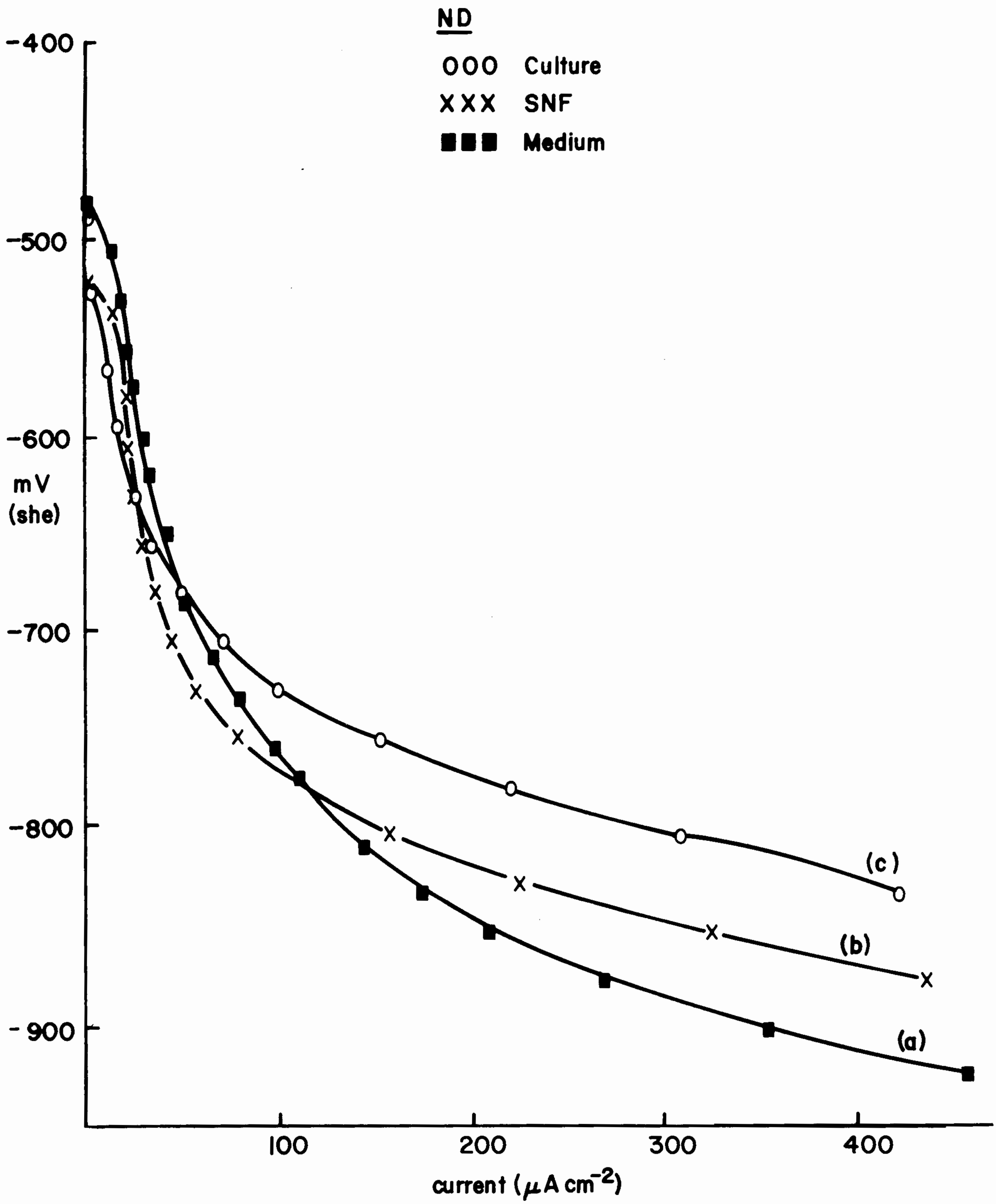


FIGURE 74

Cathodic polarization curves recorded in (a) Sterile medium. Suspension of hydrogenase-active cells from DB culture in sterile medium (common curve for the two cases). (b) cell free centrifugate of DB culture. (c) DB culture).

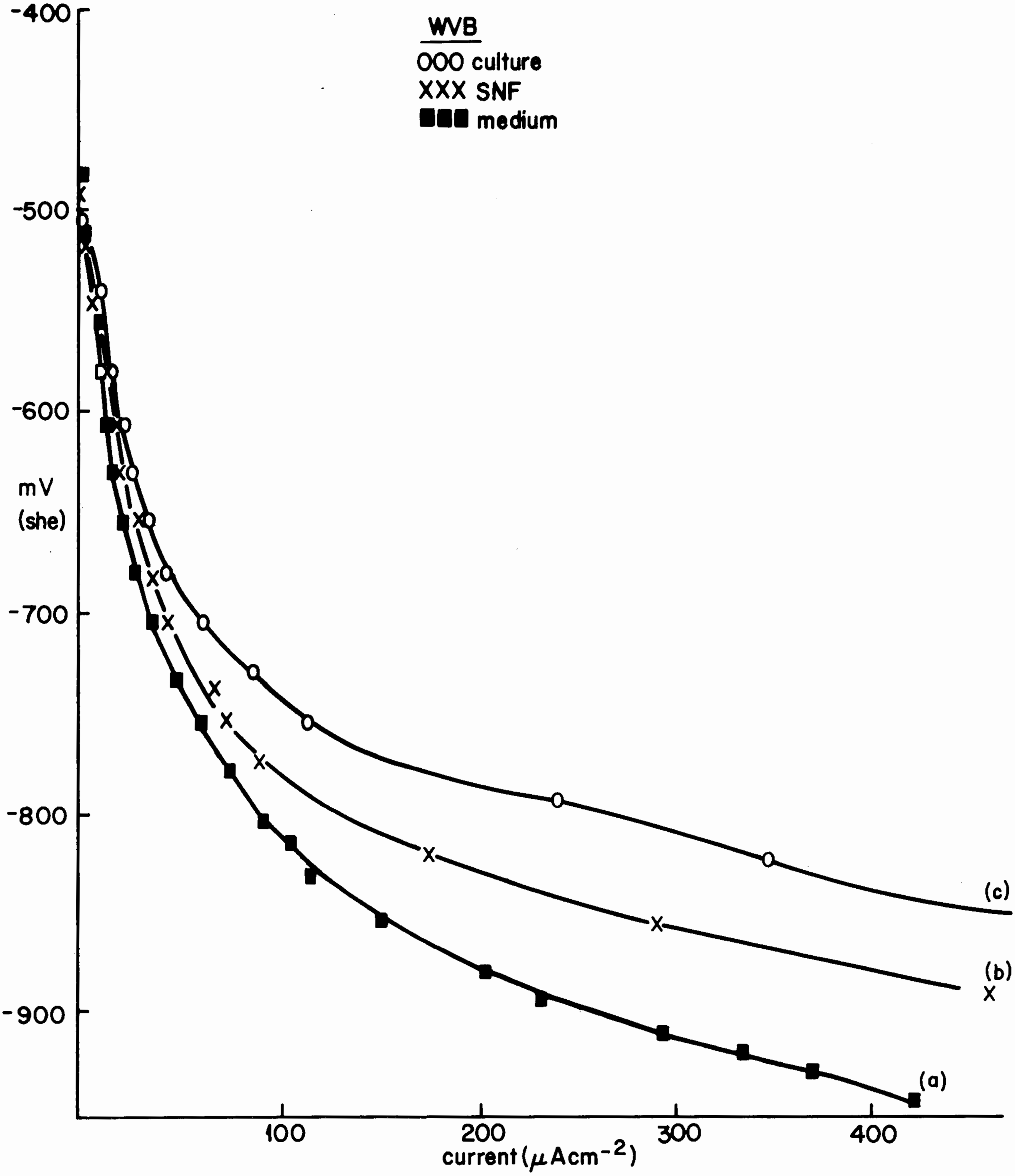


FIGURE 75

Cathodic polarization curves recorded in: a) Sterile medium. Suspension of hydrogenase-active cells from WVB culture in sterile medium (common curve for the two cases) b) Cell-free centrifugate of WVB culture. c) WVB culture.

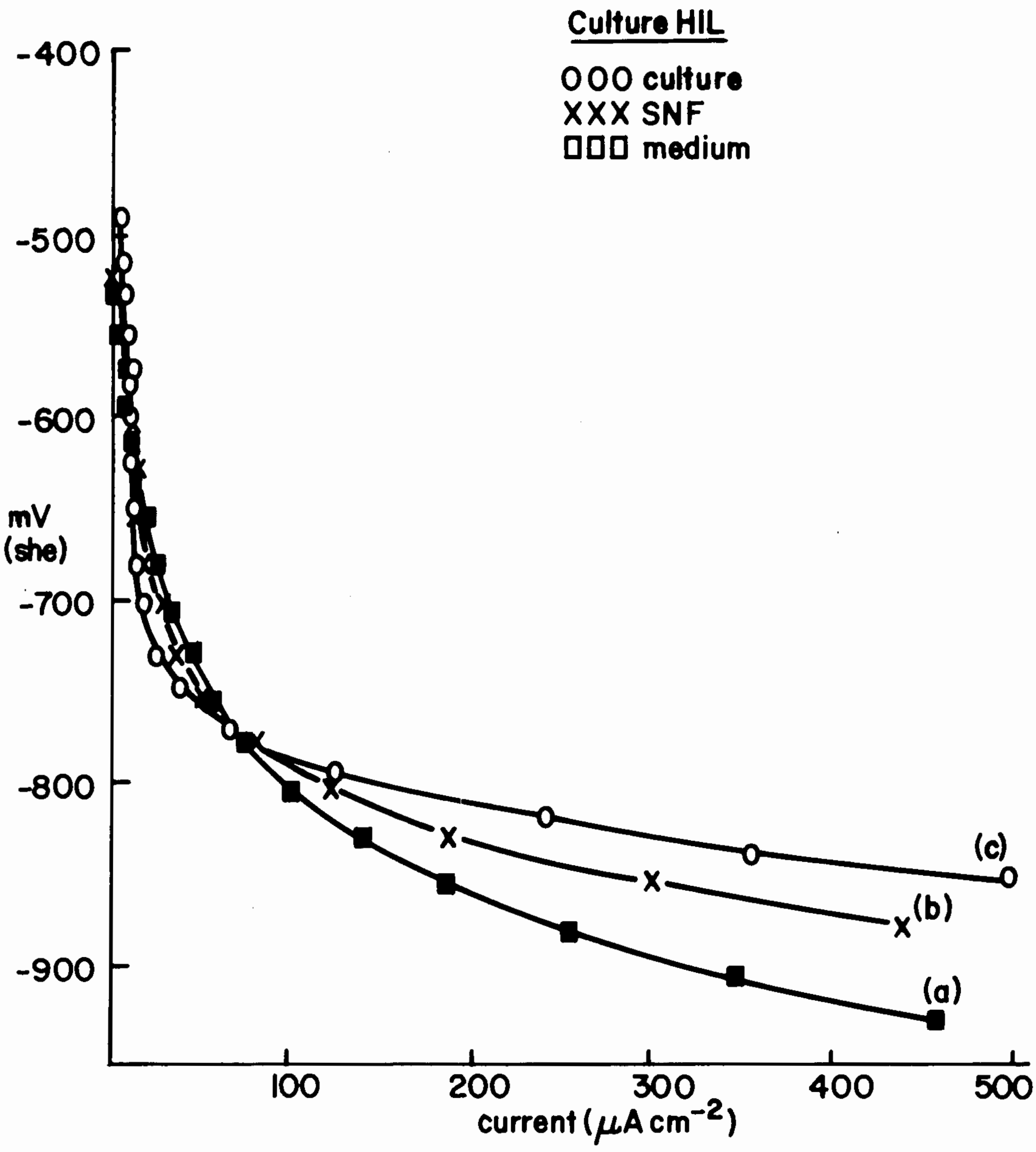


FIGURE 76.

Cathodic polarization curves recorded in (a) Sterile medium. Suspension of hydrogenase-active cells from HIL culture in sterile medium (common curve for the two cases). b) Cell-free centrifugate of HIL culture. (c) HIL culture.

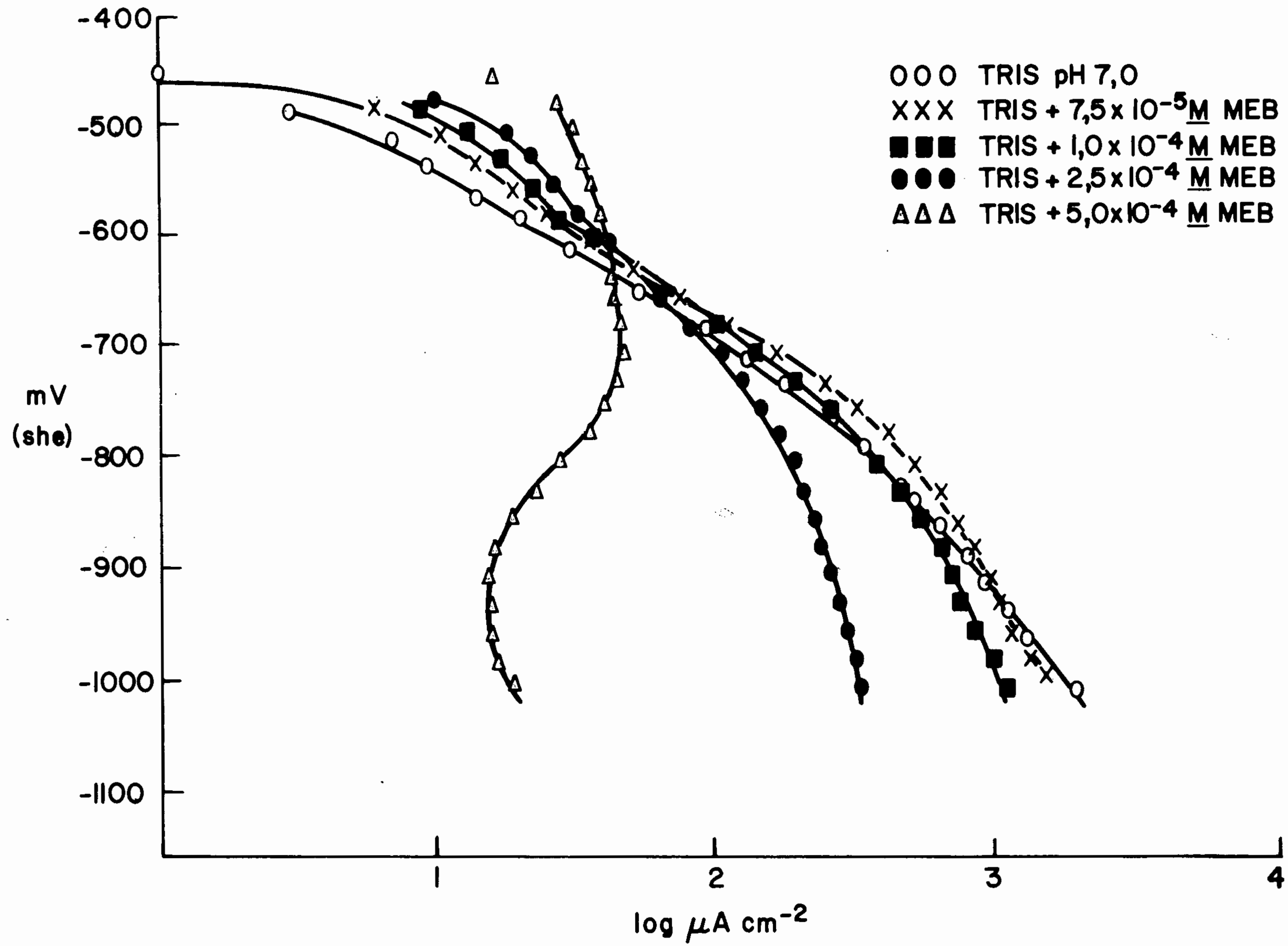


FIGURE. 77.

Semilogarithmic plots of cathodic polarization curves for mild steel in tris buffer pH 7,0 with methylene blue additions (MEB)

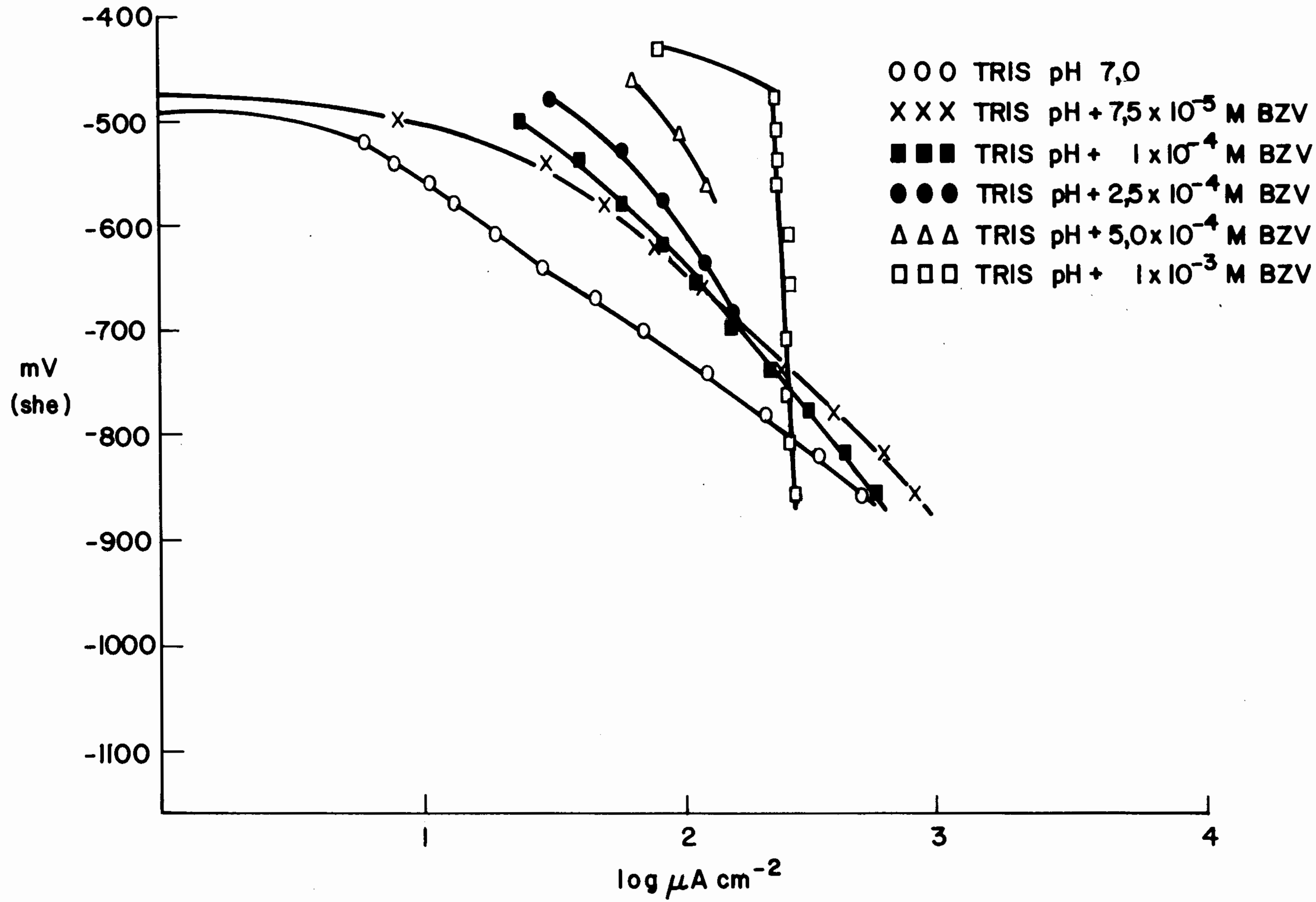


FIGURE. 78

Semilogarithmic plots of cathodic polarization curves for mild steel in tris buffer with benzyl viologen additions

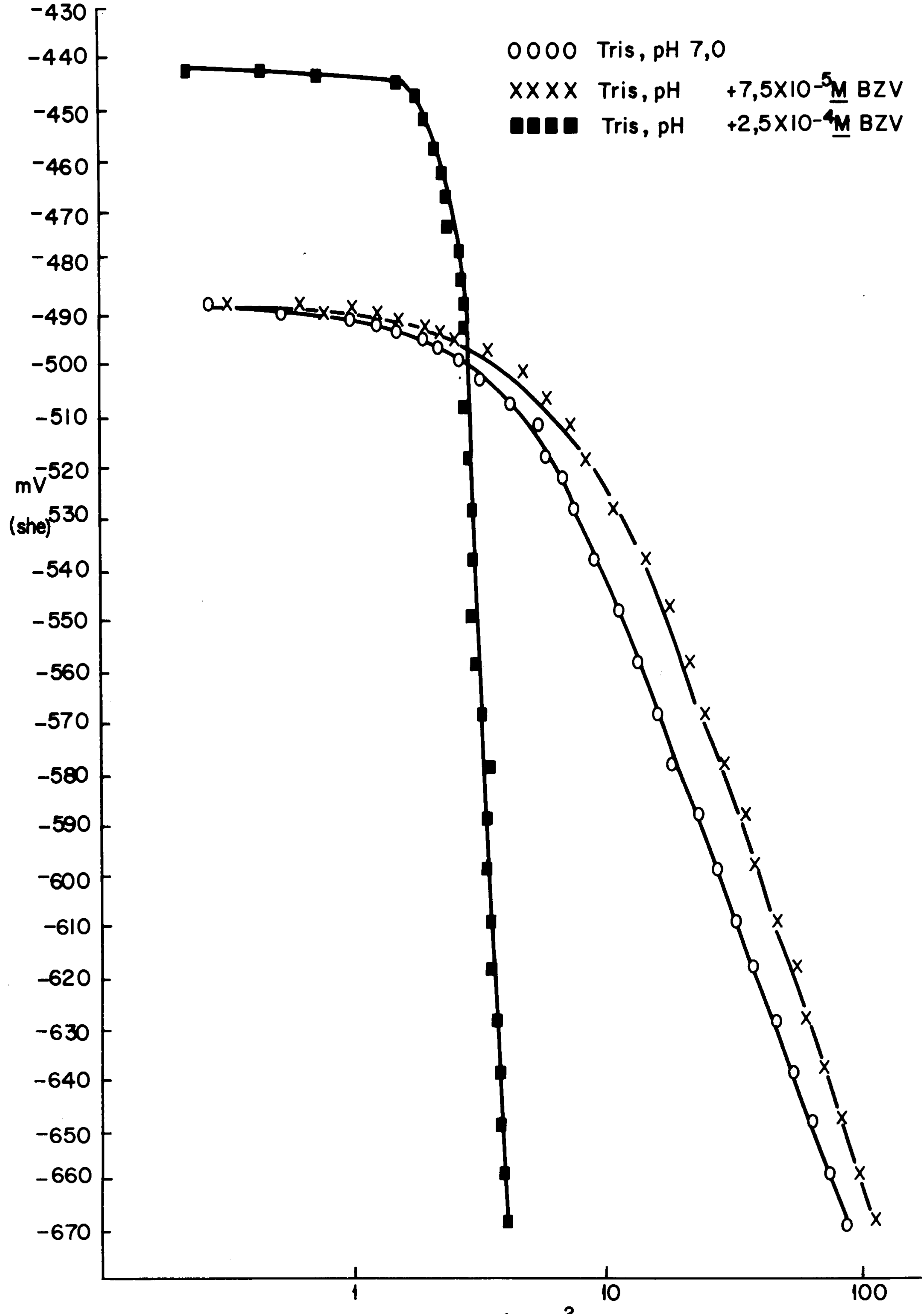


FIGURE 79

Cathodic polarization curves, determined under true potentiostatic conditions, for mild steel in Tris buffer, pH 7,0 with Benzyl viologen additions

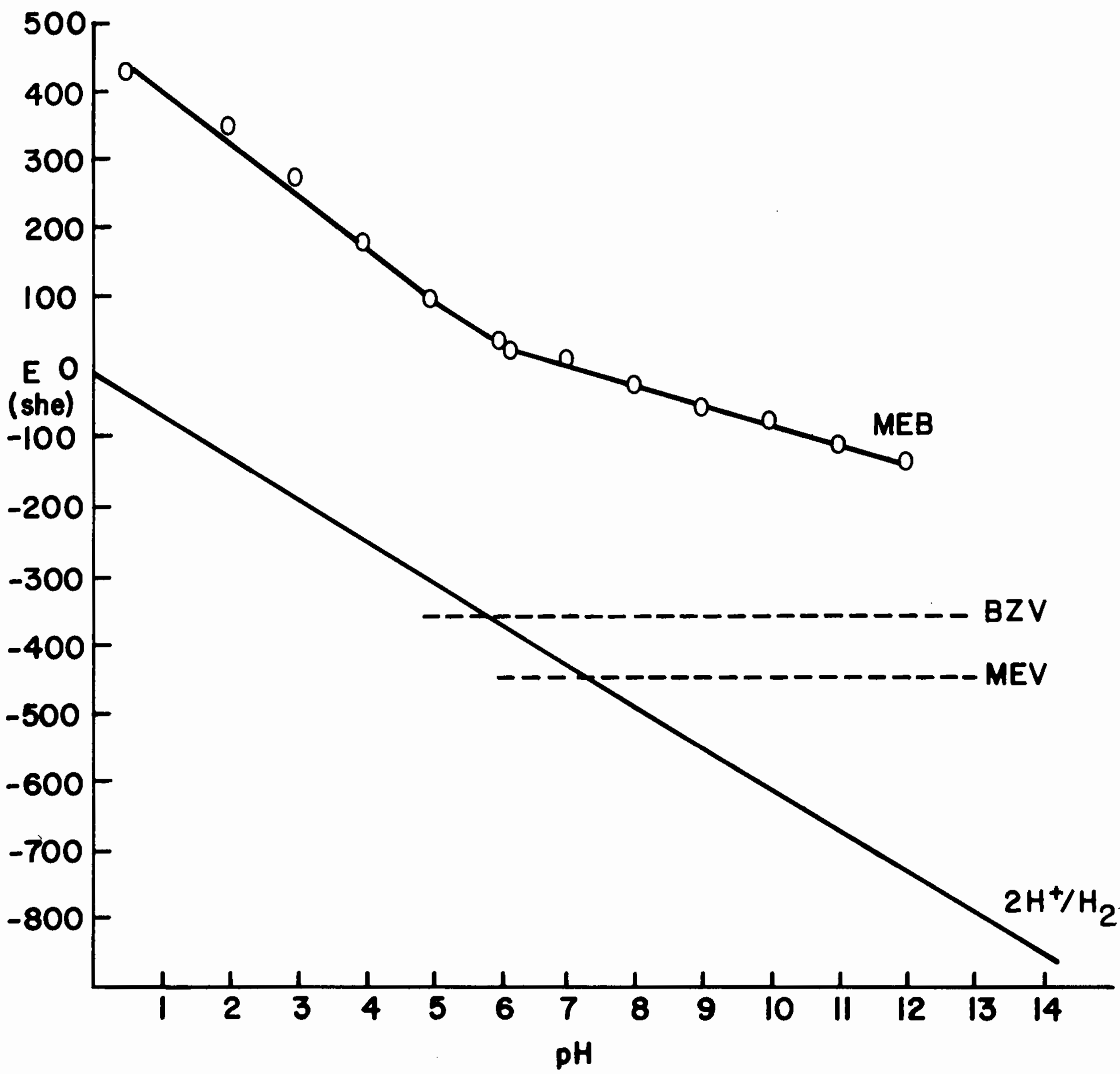


FIGURE 80

pH versus potential behaviour of redox dyes methylene blue (MEB), benzyl viologen (BZV) and methyl viologen (MEV).

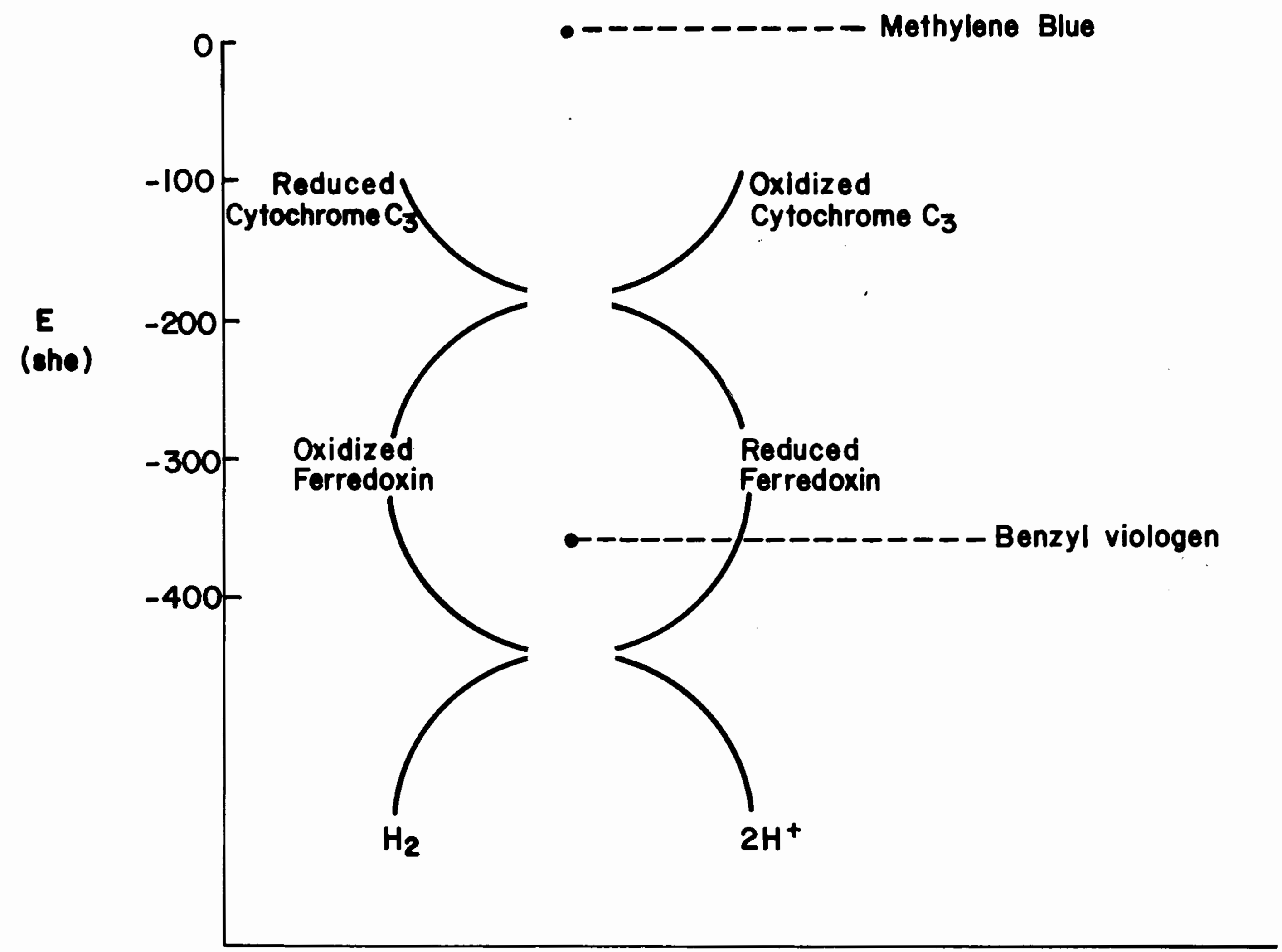


FIGURE 81.

The redox chain coupling hydrogen oxidation with sulphate reduction. Redox potential scale is superimposed together with equilibruim redox potential values for methylene blue and benzyl viologen.

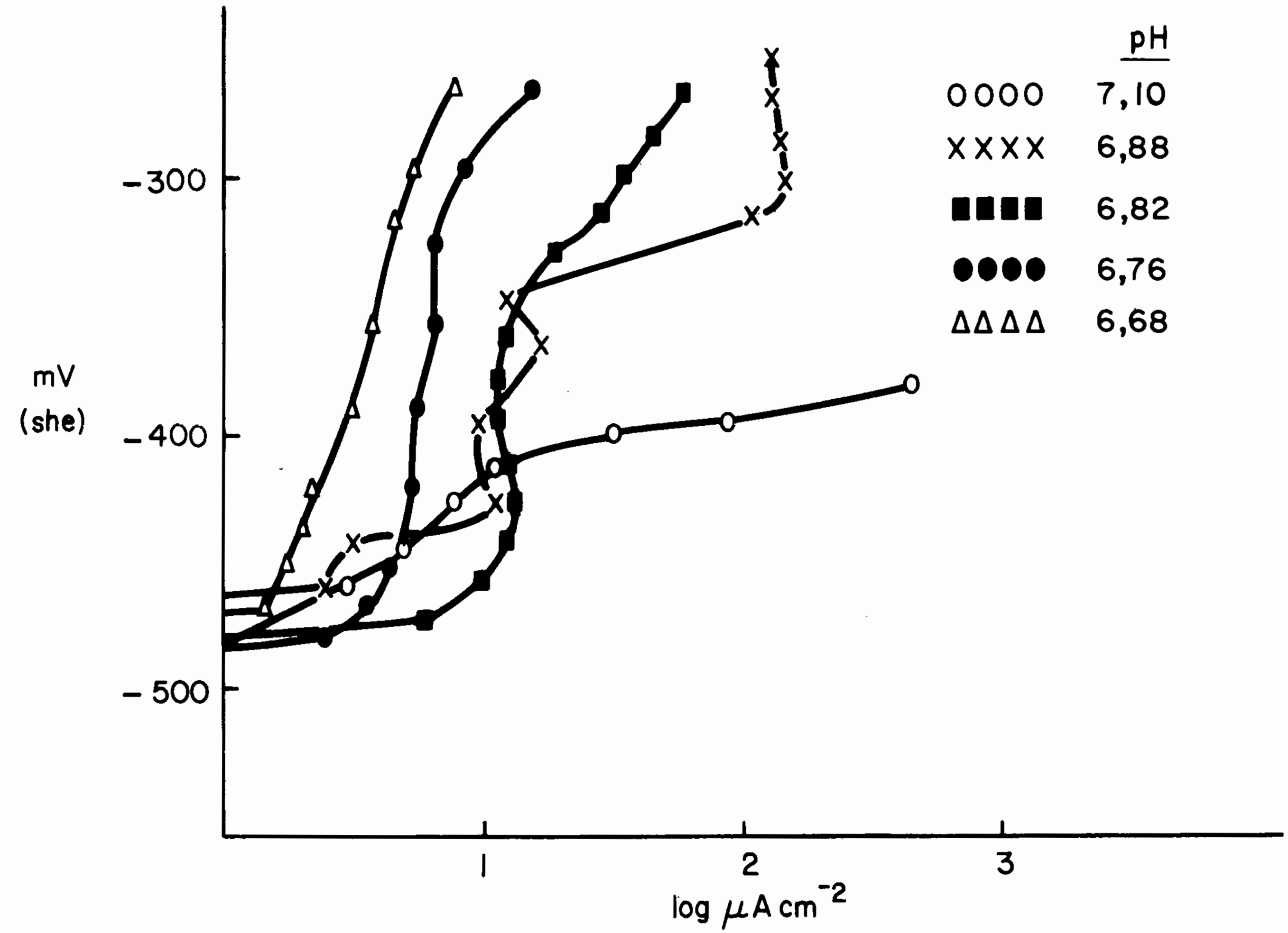


FIGURE 82

Semilogarithmic plots of anodic polarization curves for mild steel in cultures of HIL

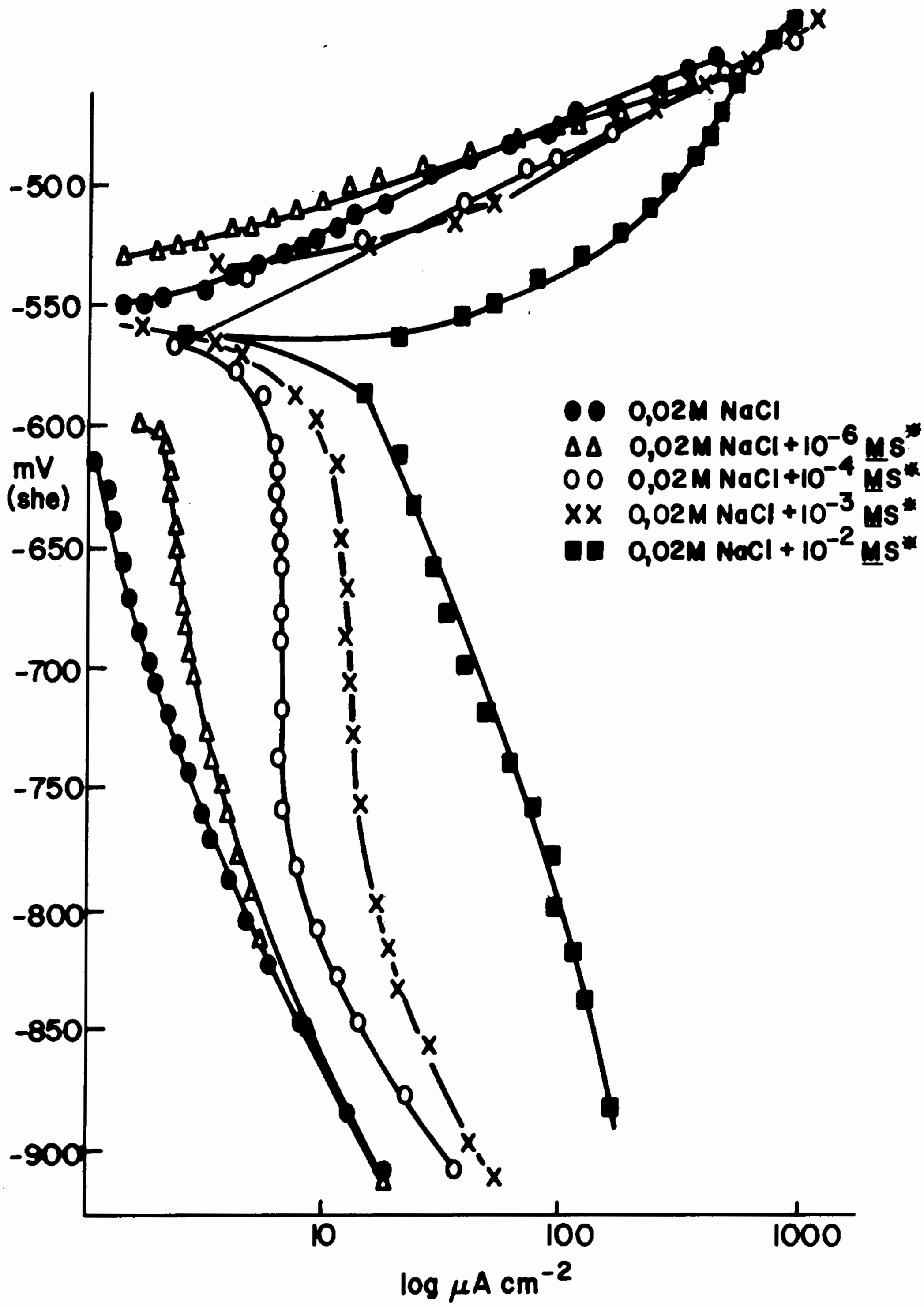


FIGURE 83

Anodic and cathodic polarization curves for mild steel in 0,02 M NaCl solutions with additions of sulphide as $Na_2S.9H_2O$

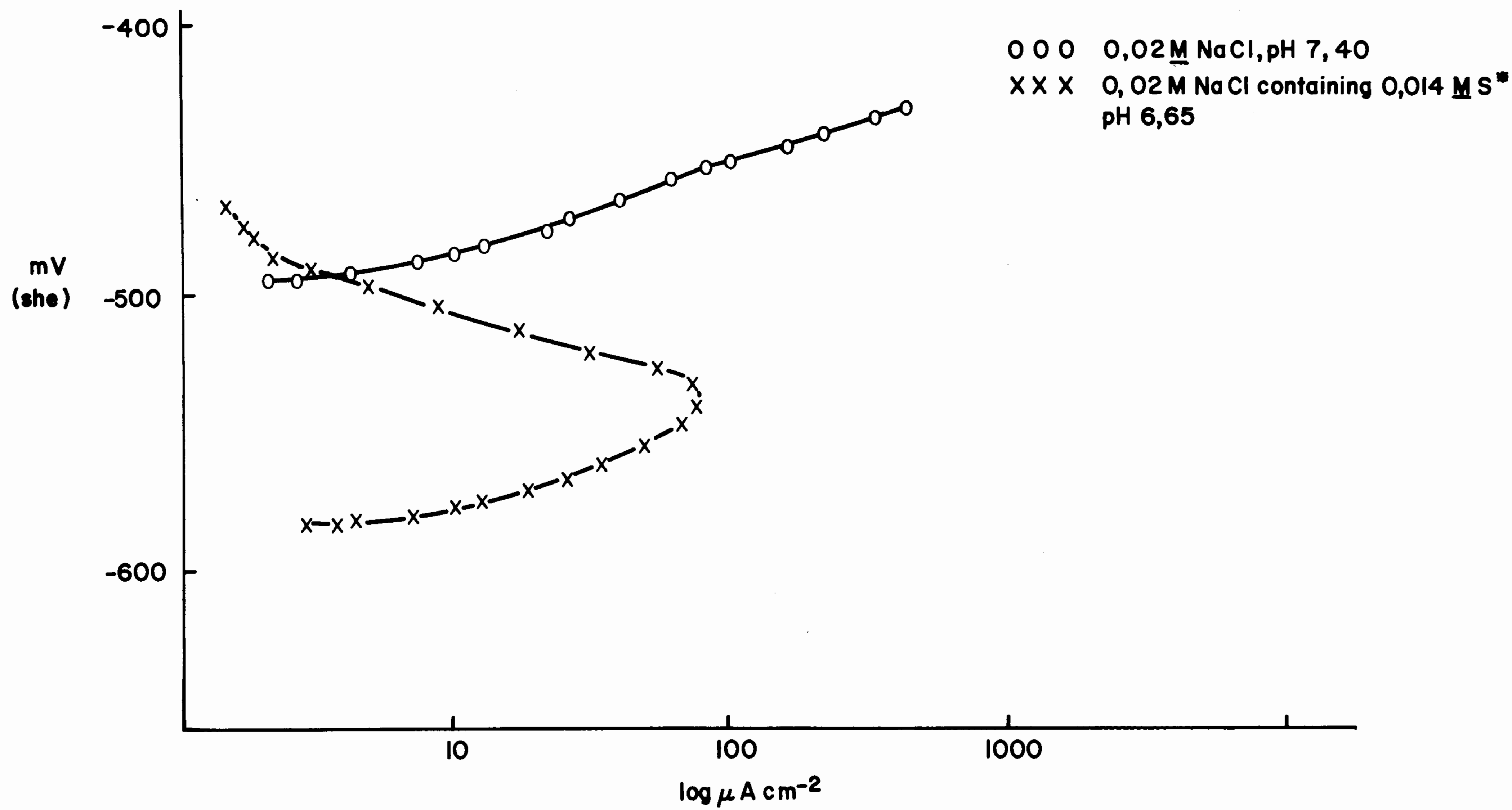


FIGURE 84

Anodic polarization curves for mild steel in 0,02 M NaCl. with sulphide additions

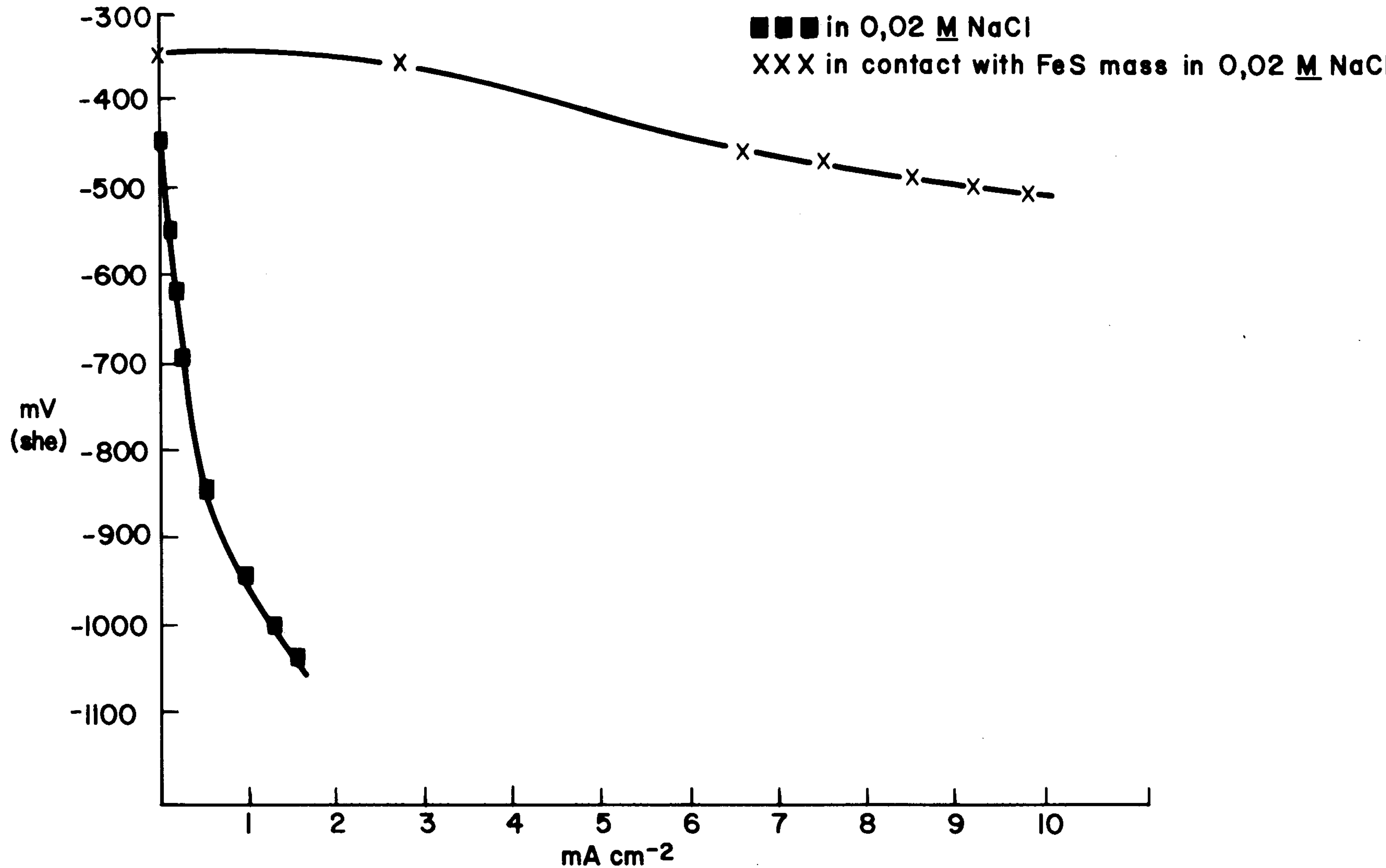


FIGURE. 85.

Cathodic polarization curves for mild steel in contact with solid FeS

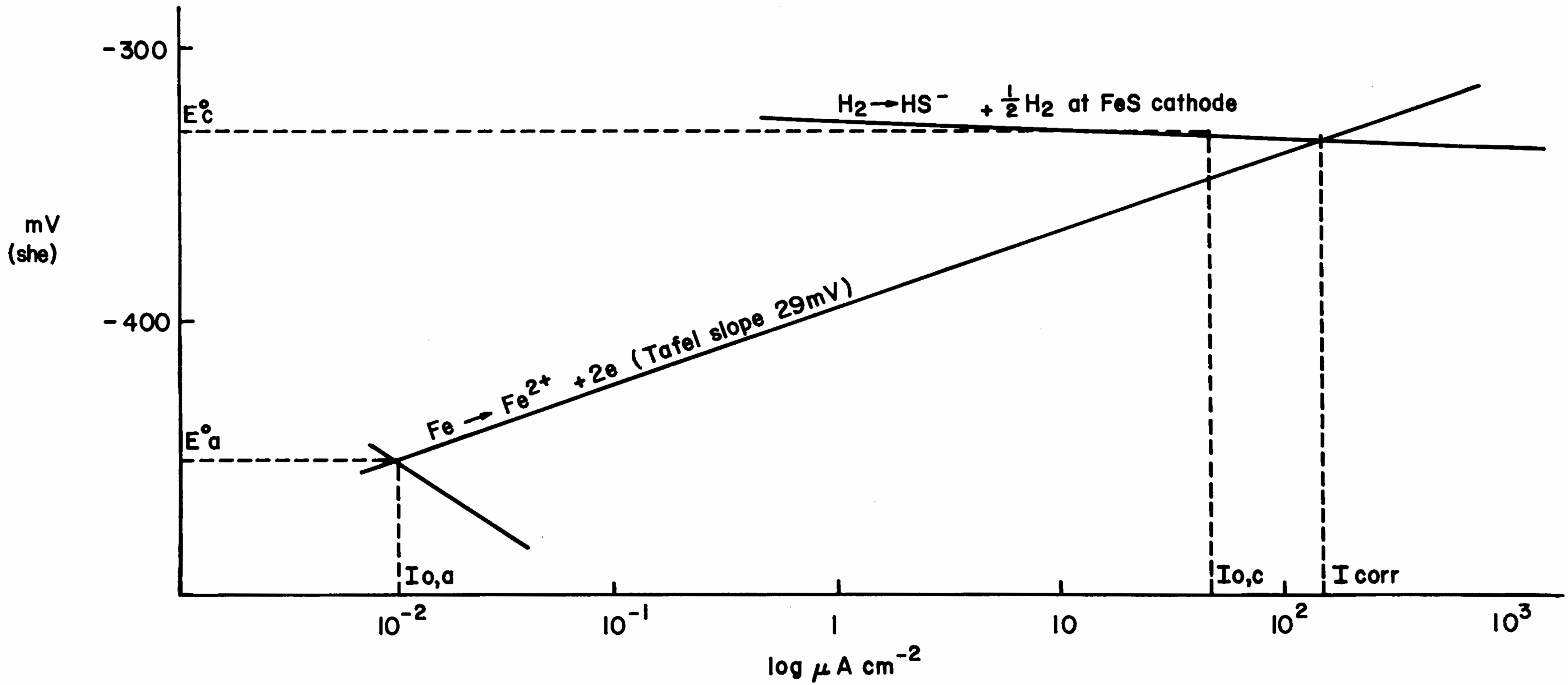


FIGURE 86(a)

Wagner Traud type diagram of proposed mechanism. The value for Io,a is the smallest of those quoted in Figure 30. Higher values would give a higher I corr. The value of I corr, greater than $10^2\ \mu A\ cm^{-2}$ is consistent with corrosion rates observed under field conditions.

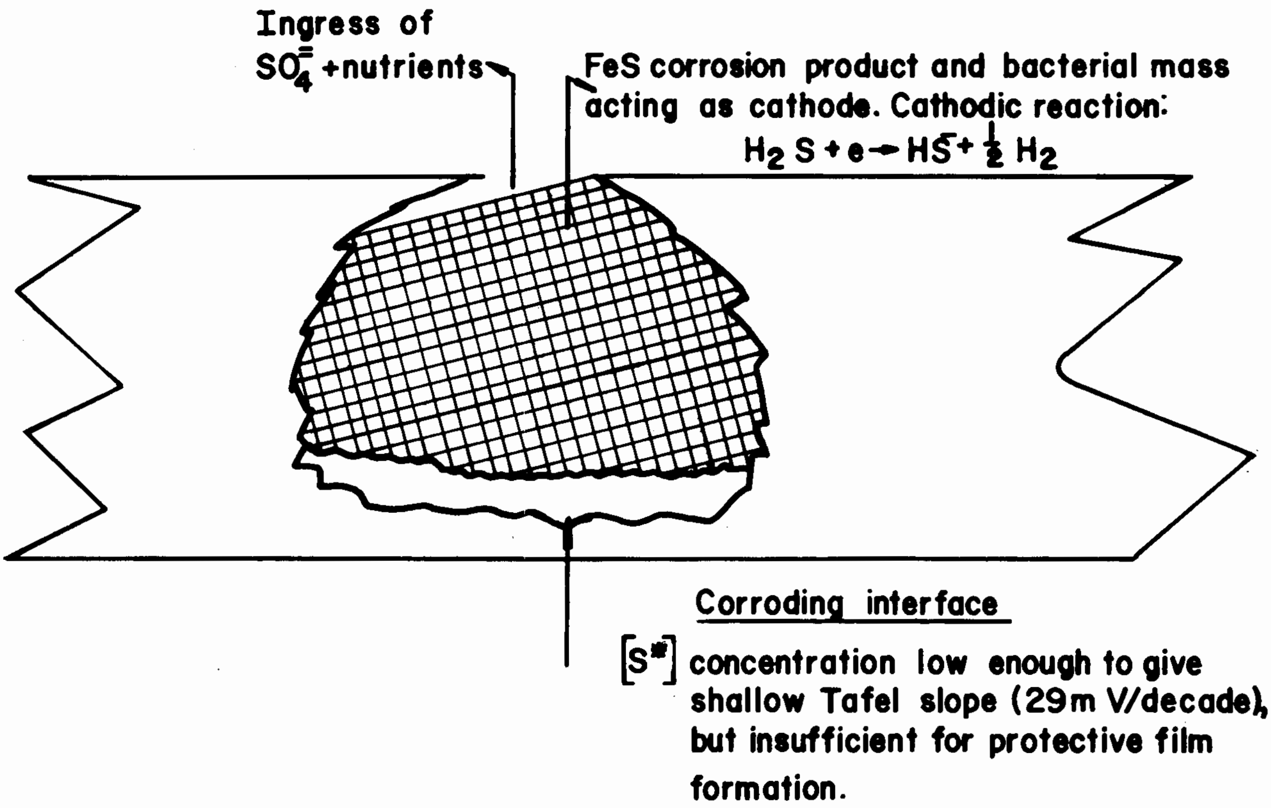


FIGURE 86(b)

Schematic diagram of actively corroding pit with electrochemical characteristics of Figure 86 (a).

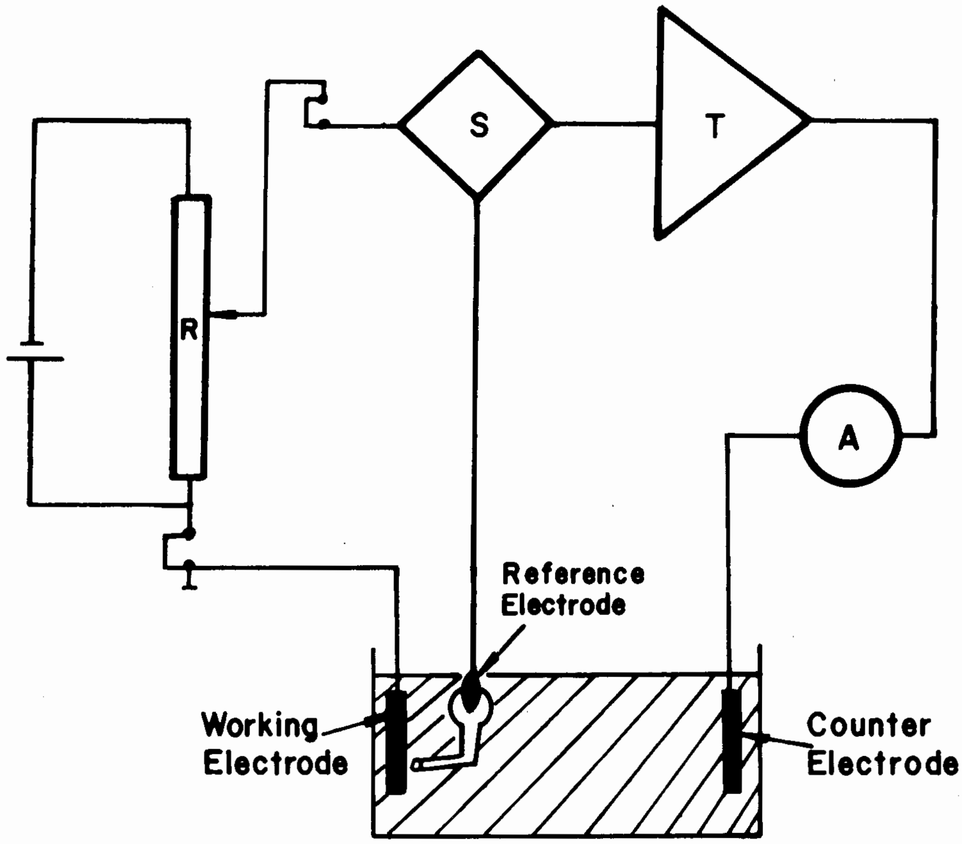


FIGURE 39

Diagram of the basic components of a potentiostatic circuit. The potential between reference and working electrodes is compared (S) with the potential tapped from a reference supply (R). The error signal is amplified (T) and generates a current measurable at (A) between counter electrode and working electrode such as to minimise that error

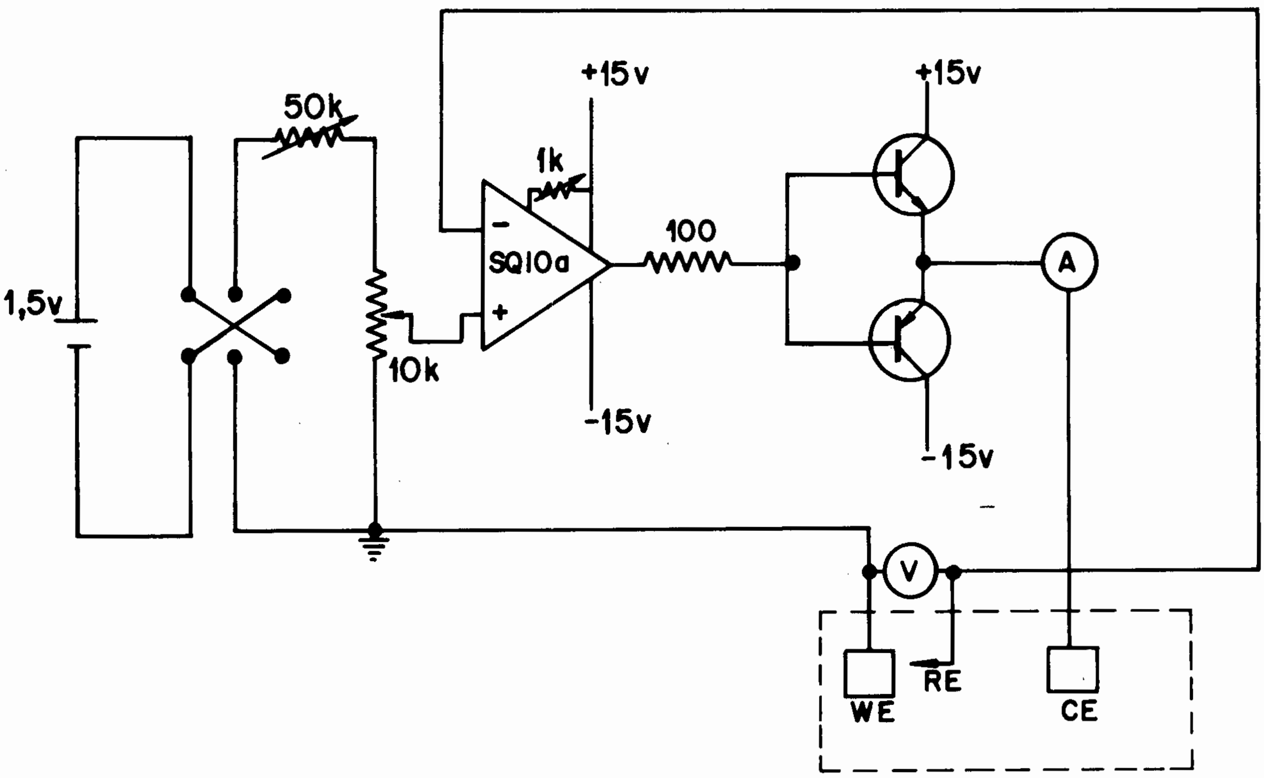


FIGURE. 40

Potentiostatic control circuit. The operational amplifier employed was a Philbrick Nexus SQ10a. Dotted area represents electrochemical cell containing working electrode (WE), reference electrode (RE), and counter electrode (CE).

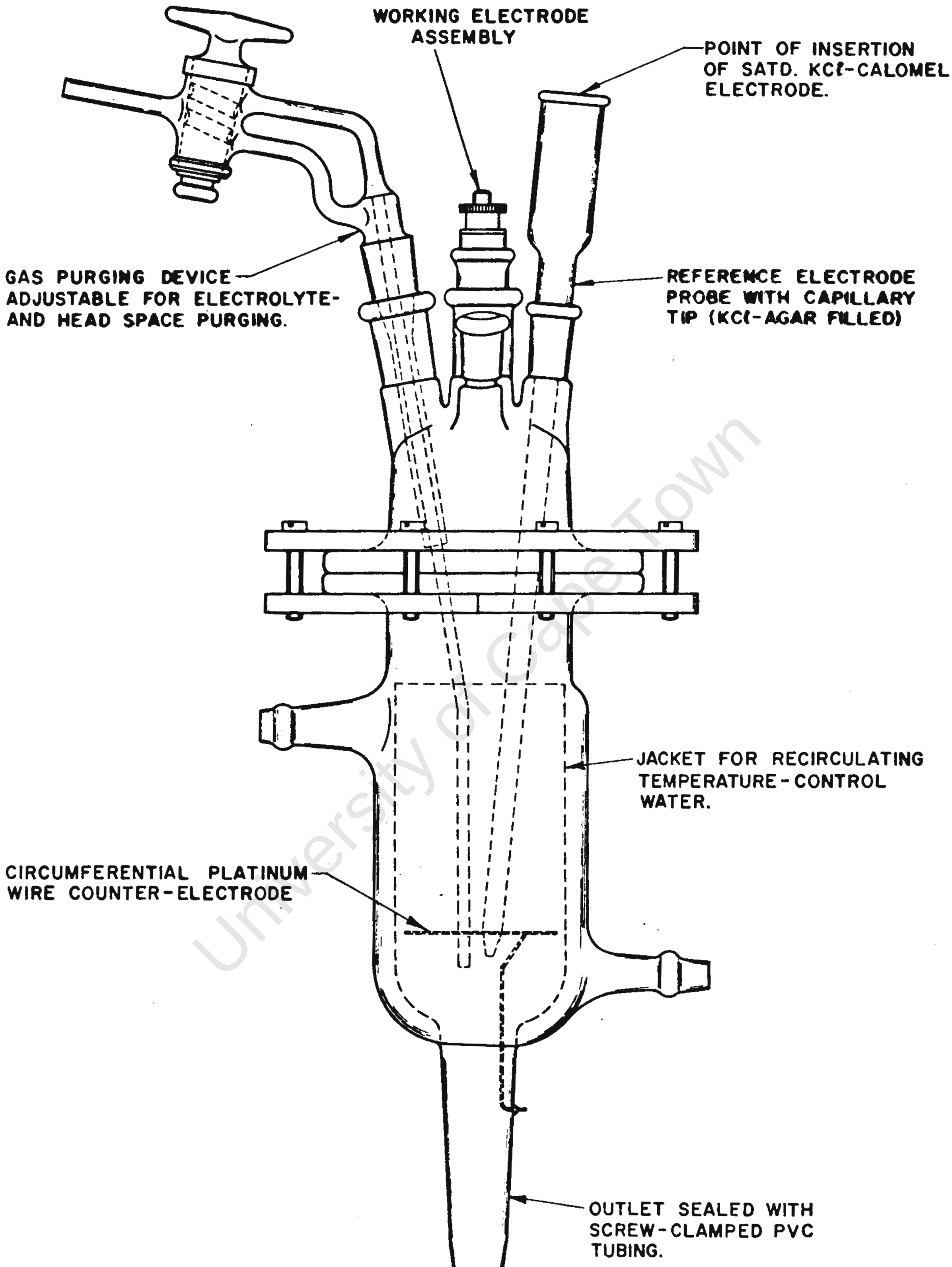


FIGURE 41 POLARIZATION CELL

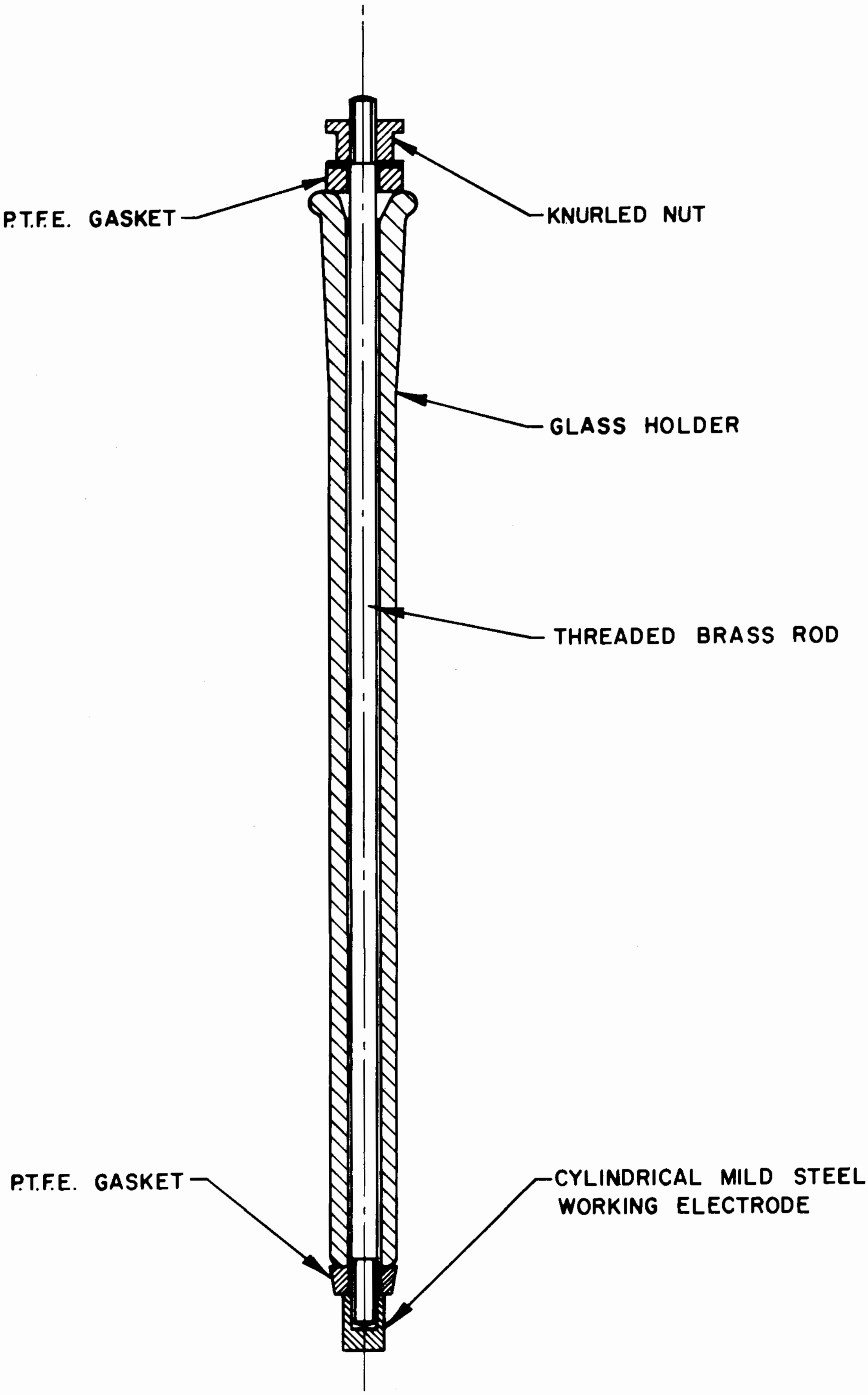


FIGURE.42 WORKING ELECTRODE ASSEMBLY

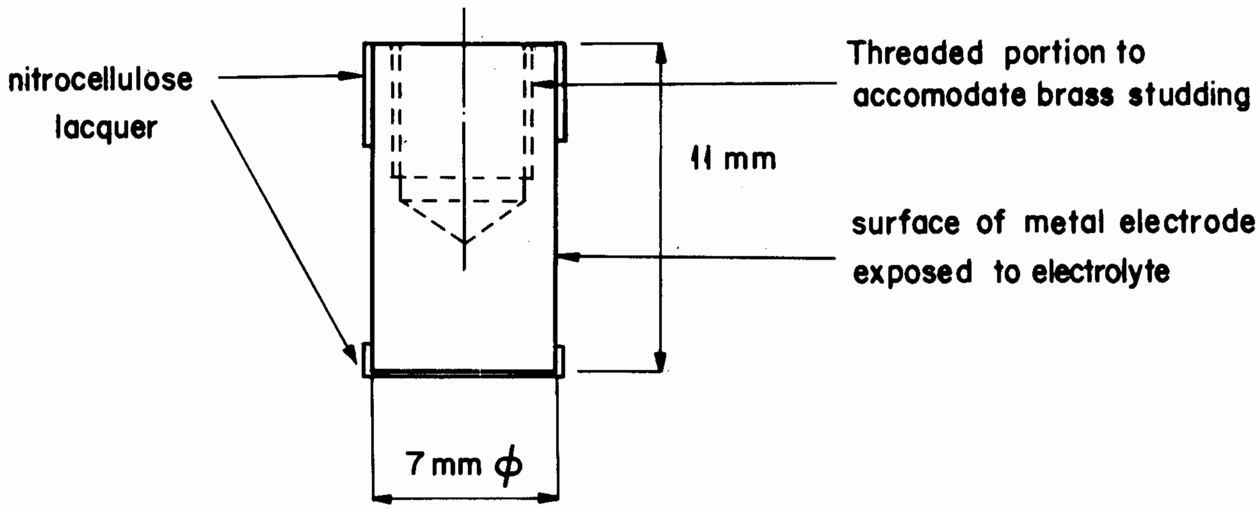


FIGURE 43
Working electrode showing application of nitrocellulose lacquer (not to scale)

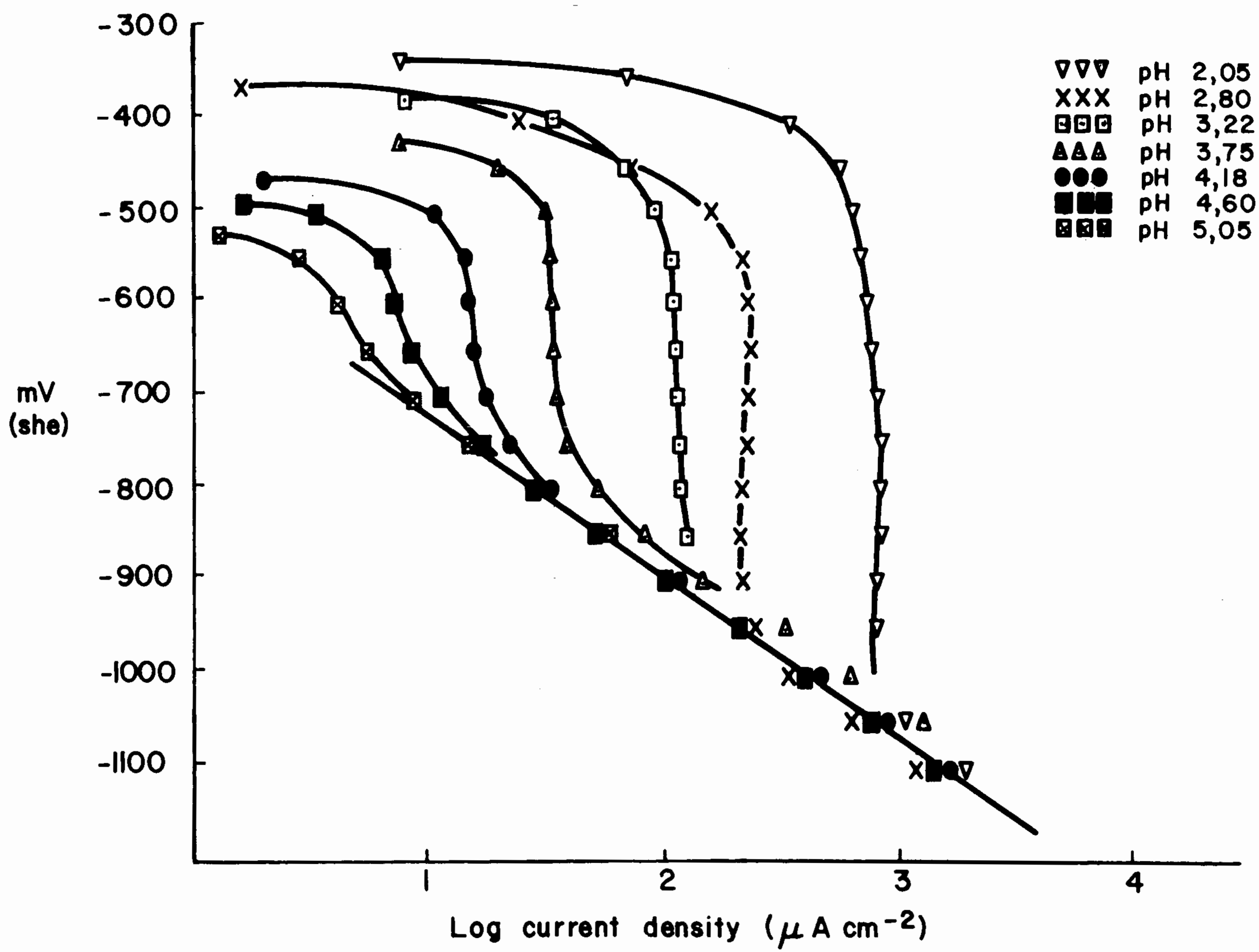


FIGURE 44

Cathodic polarization curves recorded in 0,02M Na Cl solution at various pH values