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## R-701

# STUDY DIRECTED AT DEVELOPMENT OF AN IMPLANTABLE BIOTELEMETRY ION DETECTOR

by

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November 1971

THE CHARLES STARK DRAPER LABORATORY A DIVISION OF MASSACHUSETTS INSTITUTE OF TECHNOLOGY CAMBRIDGE, MASSACHUSETTS 02139

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# STUDY DIRECTED AT DEVELOPMENT OF AN IMPLANTABLE BIOTELEMETRY ION DETECTOR

#### ABSTRACT

This study was directed at the long-range objective of developing an implantable biotelemetry ion detector.

A literature search was conducted to currently update known information in the field of ion-selective electrodes. The review attempts to identify present trends in cation and anions selective electrodes pertinent to the area of bioimplantable units.

An electronic circuit was designed to provide the high impedance interface between the ion-selective sensors and signal-processing equipment. The resulting design emphasized the need for low power and miniaturization. Many of the circuits were constructed and used to evaluate the ion-selective electrodes.

A cuvette capable of holding the ion-selective and the reference electrodes was designed and constructed. This equipment was used to evaluate commercially available ion-selective electrodes and the electrodes designed and constructed under this contract. The results of the electrode tests are included in this report.

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#### SECTION 1

#### INTRODUCTION

This contract has been for a level of effort study of the problems which must be solved in order to develop an implantable biotelemetry electrolyte monitor.

All areas of medical technology and research are growing at an expanding rate. The number and range of tests that should be available to aid the physician in diagnosis for both the routine and complex cases increases daily. This could constitute a virtual revolution in medical information; but these techniques are not finding their way much beyond the research environment. Hospitals are also experiencing critical problems in merely staying up with the current call for medical tests and information, especially in clinical chemistry. What is required is the development of laboratory techniques to the point where they can be routinely applied on the hospital ward or in the examining office by persons not necessarily trained to operate complex instrumentation. The cost of these new techniques, and therefore the real degree of their use, will not improve unless some new concepts in medical instrumentation begin to take hold. It is our feeling that biotelemetry and the use of ion-selective sensors and other forms of contact sensors are useful concepts which can help bring these improvements to medical instrumentation.

Telemetry systems show promise, especially for critical monitoring procedures where it is perhaps inconvenient to disturb the patient for frequent sample removal or other procedures. They also can be of great aid when continuous monitoring of a critical parameter is necessary, so that sudden changes can be detected as soon as possible. The benefits of continuous monitoring have been shown in intensive care units in several areas, mostly for vital signs. The expansion of the technique to the whole spectrum of medical measurements will improve the ease, quality and speed of diagnosis in the entire spectrum of critical situations.

The concept of a contact sensor which gives an electrical signal correlated to the parameter of interest merely by contact with the subject or with a sample without requiring any alteration of the subject is clearly one which can benefit the development of simpler medical instrumentation. One very useful type of contact sensor recently developed is the ion-selective electrode. Sensors have been

developed which can measure several biologically important ions; this set of sensors, along with the blood gas sensors for  $P_{CO_2}$  and  $P_{O_2}$ , can cover approximately 75% of the clinical chemistry procedures routinely called. In other words, this is an instrumentation technique which can be useful today and help relieve the load on the hospital laboratory. The sensors still require some development to meet this need, but the technique is there. The future of the sensor, of course, is in long-term monitoring procedures, most probably in conjunction with telemetry: because of their noncontaminating and nondepleting nature.

The first step of the study for the development of an implantable biotelemetry ion system was a literature search to currently update known information in the field of ion-selective electrodes. The results of this search can be found in Appendix A. A great deal is currently written about ion-selective electrodes on a theoretical basis, but very little information is actually written on the problem associated with these electrodes in actual use. Since the electrodes are an essential part of the biotelemetry system, most of the effort exerted for this study was concentrated on sensor test and development.

In order to test the sensors a cuvette with a temperature-controlled water jacket was needed. Pictures of the cuvette and water jacket can be seen in Fig. 1 and 4. In addition to the cuvettes, interface circuitry which accepts the high impedance output from the sensors was required to perform the test. An electronic interface circuit which was capable of being miniaturized and capable of operating off batteries was developed. Many of these circuits were built and utilized to perform sensor tests.

Further electronic development was not investigated in order to concentrate maximum effort in the sensor area. Many of the recent technical developments in solid-state technology are directly applicable toward microminiaturized implantable electronics. Complimentary MOS operating at extremely low power and capable of operating at one volt is now a reality. The real limitation at this time is the cost of customizing monolithic silicon structures in order to get the components for a microminiaturized biotelemetry system. Once developed, these same components would be applicable to all types of implantable systems and would find wide applications. The initial cost is high and the technology is available, so that no further effort was expended in the electronic area.

Commercially available sensors were procured and some sensors were designed and developed for this study. The following sections report on the results.



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Fig. 2 Reference Sensor Developed from Machined Parts



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Fig. 3 Glass Sodium Sensors: Upper Modified Sensor, Lower Beckman 39046





#### SECTION 2

#### THE ION-SELECTIVE SENSOR

The concept of an implanted biotelemetry system performing long-term monitoring of body chemistry parameters is dependent on having adequate ionselective sensors. The type of sensor required for this concept is one which does not require or consume any sample material, does not require active interface with the experimeter, has an excellent long-term stability without calibration, and does not affect or is not affected by the subject under study. A unique and potentially suitable approach is the use of electrochemical contact sensors, and specifically for electrolyte studies, the family of ion-selective sensors. Simple contact of the ion-selective sensor and reference electrode pair with the solution of interest will give a voltage which can be correlated to the ionic concentration. This technique does not require or consume samples, and can be implanted in contact with tissue fluid and left by the experimenter. These two advantages alone put this technique far ahead of any other and have led us to do a study of the problems of stability and the biological interface in order to assess the feasibility of this measurement technique.

This sensor technique is obviously applicable in a wide variety of other biological measurement situations. The first is the simple method of dipping the electrode pair into a solution of interest, in other words, discrete sample measurement. A second application is the use of the sensors in a continuous-flow system by inserting them into a fluid line. The third general application is insertion of the sensor into a tissue area or fluid volume of interest for continuous monitoring. The three applications all have advantages and disadvantages, and it was felt that, even though the third would be our ultimate application, a great deal concerning sensor performance could be learned by studies in all three areas.

The dipping technique is the easiest to implement operationally. All that is needed is a sample of sufficient size to cover the ends of the two electrodes. One generally inserts the pair into two standard solutions to establish a response curve, and then into the unknown and notes the position on the curve. It is difficult to achieve high accuracy and reliability with this technique. Simply moving the electrodes disturbs the fragile surfaces and, in fact, simply removing the electrodes from a standard solution and immediately replacing in the same solution can give a potential shift, i.e., an error. Also, removing the electrodes serves to open the

electrical circuit which had been completed by the electrolyte solution. The high impedance of the sensors coupled with variable capacitance effects will also cause errors. This situation also inevitably invites mechanical damage to the sensors in terms of hairline fractures, electrical leakages, and other failures which lead to erratic response. In short, this is a traumatic situation for the sensors. This problem, coupled with the difficulty of maintaining the necessary temperature control, means that the sensors cannot be relied on to give routine accuracy to better than 5%. With considerable care exercised by an experienced investigator and with frequent restandardization, accuracies approaching 1% can be achieved if necessary. The dipping method can also be used for a gross test of sensor performance. But it should be emphasized that the major failure of this technique centers around the continual removal and replacement of the sensors in the fluid.

The flow-through system of measurement has been found to give much better results. It can be used either for continuous, on-line monitoring of a flowing sample stream or for processing of discrete samples injected into the line serially. Since the sensors are solidly connected to the fluid line and not moved, they are not mechanically abused. Also, excepting errors in procedure, the sensors are continuously wetted, thereby maintaining a constant environment for the sensitive surface and also preserving the electrical circuit. Exact temperature control is also far easier to implement, simply by controlling a large block containing the sensors. In this situation, accuracy on the order of 1 or 2% is relatively easy to achieve if all other precautions are taken. The only potential problem associated with continuous-flow systems is that of the so-called streaming potential, which can cause redistribution of the ion flux in the region of the reference electrode, but, it is totally a flow phenomena and disappears upon flow stoppage. Since we were concerned with static performance, this effect was not studied, but a theoretical analysis has been published.<sup>1</sup> For our work, two temperature-controlled jackets each containing two flow-through electrode chambers were constructed for electrode studies. A considerable amount of information concerning sensor performance has been gained from these chambers.

The third technique is similar to the flow-through system in that a continuous sample contact is maintained. The sample is monitored continuously for concentration changes. The applications are for continuous <u>in vivo</u> measurements or continuous monitoring of industrial batch processes. The technique has the advantages of the flow-through system but has one major disadvantage: direct calibration is not achievable. The flow-through system allows occasional injection of a standard, but this technique of continuous monitoring is defeated if the electrodes are removed or if a small sample is removed for an independent measurement. This technique, therefore, is the most demanding in terms of electrode stability and reliability. None of this type of work was done but was simulated by simply retaining samples for long periods in the flow-through system.

#### SECTION 3

#### ION-SELECTIVE SENSOR THEORY

In this section we will give a brief outline of the theory of the ion-selective electrode. The complete, detailed theory as presently understood is best described in the articles by Eisenman and Ross<sup>2</sup> in the monograph published by the National Bureau of Standards.

The total system of ion-selective sensor and reference electrode is shown in Fig. 5.. The reference electrodes will be dealt with in a later section. The measured potential, E, is the sum of the potentials developed at each material interface. A potential is developed between the Ag-AgC1 internal electrodes and the respective internal solutions, another is developed from the internal filling solution to the internal side of the sensor, there is a potential across the liquid junction, and there is a potential across the ion-selective surface. In a properly operating pair, as the concentration of the ion being measured is changed, all of these potentials will remain constant except for the one developed at the ionselective surface, which will follow the concentration change in a complex but predictable fashion. Making this assumption, we can make a short derivation of the general ion-selective surface response.

Let us consider a situation as shown in Fig. 6. An ion-selective surface is interposed between two solutions of different concentrations of a salt  $A^+ B^-$ . Let us assume that the surface will pass only the positive ions,  $A^+$ . If the left side concentration,  $A_1^+$ , is greater than the right side concentration,  $A_2^+$ , the diffusion law states that ions will move from left to right.  $B^-$  ions would also flow by diffusion until the concentrations were equal, but are stopped by the membrane. After a very short period of diffusion flow, the migration of  $A^+$  will leave a net negative charge on the left side and build up a net positive charge on the right side. A charge separation such as this will automatically have an associated electric field vectored right to left. This field will oppose further migration of positive ions, resulting in an equilibrium position. Since the diffusion forces driving positive ions is clearly a direct function of the concentration ratios, so will be the resulting potential. The actual form of this dependence is derived by Eisenman in his work on glass electrodes; the simple form is as follows:









$$V_{1-2} = \frac{RT}{nF} \ln \left\{ \frac{a_{A_2}}{a_{A_1}} \right\}$$

 $V_{1-2}$  = potential across membrane from left to right.

n = ion charge number.

R = gas constant.

T = absolute temperature.

F = Faraday's constant.

a = activity of ion.

The first important point is that the electrodes respond to the activity of the ion, rather than the concentration in that they are equivalent at low concentrations (less than  $10^{-2}$  molar total solution ionic strength), but the activity becomes a smaller and smaller fraction of the concentration as it increases. The functional dependence is complex and depends not only on the concentration of that ion but also on all other species in the solution. But since it is the activity rather than the total concentration of the ion which determines the effect the ion has on various physiologic functions, this sensor actually measures a more important quantity. For measurements of parameters in normal physiologic fluids, the activity coefficients are sufficiently well known so that correlations can be made to yield the concentration data.

There are several modifications which must be made to the above equation. First, the electrodes do not respond exactly according to the slope factor RT/nF, so an efficiency factor,  $\eta$ , must be included. Another consideration when measuring biological fluids is that they are multiple ion systems, and the electrodes are not totally specific to a given ion. They are normally very selective for cations over anions or vice versa, but may not necessarily be selective between two cations or anions. Eisenman<sup>4</sup> has shown that this can be accounted for by a simple linear summation with coefficients. Also, in a real measurement system, one cannot measure the isolated potential at the membrane surface, but must measure the total potential of the electrode pair. This leads to the following functional relationship:

$$V = E^{O} + \frac{\eta RT}{F} \ln \left\{ \sum_{i=1}^{j} K_{li} a_{i}^{n_{i}} \right\}$$

- $E^{O}$  = sum of constant potentials.
- $\eta$  = efficiency factor.
- $K_{1i}$  = specificity coefficient of species i.
- $n_i = charge no. of species i.$

a<sub>i</sub> = activity of species i.

All of the ion-selective sensors respond in the manner of the above equation. Since the response is logarithmic, the sensors are reasonably accurate over a very wide range, typically 3 to 5 orders of magnitude. The limitation on the range at the low end is usually near  $10^{-5}$  or  $10^{-6}$  molar, and generally resulting from this being the solubility of the sensor material in water. The limitation at the high end is normally at 0.1 to 1 molar, often because the activity coefficients vary widely in this range, making correlation difficult.

The sensors can be classified into four types, each with their own particular set of operational characteristics. The first type is the glass electrode, which consists of a glass tip of special formulation to respond to the ion of interest. These were the first sensor type invented, but they are limited to measuring pH, sodium, and potassium or ammonium. They are characterized by very high electrical resistance, reasonable stability, fragility, but very long lifetime if properly handled, and good selectivity in the pH and sodium types. It is not likely that any new sensors of this type will appear. The next type is the solid-state crystal membrane. It consists of a solid crystal of very low solubility made up of the ion of interest and an ion not generally of measurement interest; for example, an AgCl crystal will measure chloride, silver not normally being present. These are available for fluoride, chloride, bromide, iodide, sulfide, copper, lead, cadmium, and a few others. They are characterized by relatively low electrical impedance, good stability, ruggedness, very long lifetime, and variable selectivity. They are easily the best performing sensor type. However, it is not likely that many new sensor types beyond those already discovered will be found since it is a matter of finding proper crystalline compounds, and the list has been thoroughly searched. The third type discovered is the liquid membrane system first described by Ross in 1967<sup>5</sup>. A thin hydrophobic membrane is placed between the reference inner solution and the test solution. An organic solvent containing a specific complex organic carrier is applied at the edge of the membrane and soaks it. The organic carrier compound is selected to be specific for carrying only one ionic species. All other ions are repelled since the membrane is hydrophobic and therefore rejects polarized materials. Thus the ion-selective and potential-producing character is achieved. They are available for a very wide range of ions, notably calcium,

potassium (more selective than glass), magnesium, copper, lead and nitrate. They are characterized by moderate electrical impedance, poor stability, difficulty of use, short lifetime, and good selectivity. They seem to show the most promise for discovery of sensors to measure new parameters because of the very large number of potential organic carrier systems. A fourth type which recently appeared is the heterogeneous membrane type. It includes a very broad class of sensors presently in the research phase. In general, they consist of a support mechanism of some sort such as a silicone rubber, paraffin or epoxy which suspends the ion-selective material and perhaps a silver salt or other system to maintain a low electrical resistance. Systems of this sort have been developed for calcium, potassium, sulfate, iodine, and a few others. Commercially available models of these sensors have not gained wide acceptance, but we have not studied any. The research, which is discussed by Covington in the NBS publication, would indicate that there are both stability and lifetime problems with these sensors. However, Covington concludes that there is good potential for both improved models of current sensors and also new sensor types.

#### SECTION 4

#### STUDIES ON ION-SELECTIVE SENSORS

The investigation of ion-selective electrodes has been conducted with the aim of finding suitable sensors for several biomedical applications, including discrete sample analysis and on-line monitoring of flowing fluids, notably blood. We therefore have adopted several sets of criteria in evaluating sensor performance, and our experience reflects this.

Before we could begin real evaluation, we discovered problem areas initially in electronics and then in the reference electrodes. The problems are described in other sections. We are now proceeding with some confidence that these problems are under control. Furthermore, we have in this initial overview determined that the problems of the sensors themselves are far more difficult than those so far encountered and seemingly conquered, but these problems are nevertheless also soluble.

The next problem encountered is that the electrodes require very tight temperature control of the sensor, the reference, and the sample to achieve accuracy and reliability. The slope factor,  $\eta RT/nF$ , changes directly with absolute temperature, an effect which varies depending on the ratio between the internal reference solution and the test solution, having no effect at unity ratio. One normally fills the sensor with a solution near those to be encountered to minimize this effect. More important is the fact that the  $E^{O}$  term in the sensor equation will change enough with temperature to give significant errors for changes over + 0.5<sup>O</sup>C. This problem varies from unit to unit and is not well understood. Thermal cycling over 5<sup>O</sup>C can cause irreversible changes in the glass structure which leads to a hysteresis in readings. We were therefore compelled to construct a heated jacket system for all electrode test chambers for control to  $\pm 0.1^{O}C$ .

The initial experience has been with the small glass sensors marketed by Beckman, the clinical sodium sensor, type 39046, and the clinical cation sensor, type 39047. The cation sensor is appropriately named in that it is nearly equally sensitive to potassium and ammonia, and about ten or twenty times less sensitive to sodium. The sodium sensor is at least 1000 times more sensitive to sodium than any other ion except silver, which may be neglected in biological systems since it

precipitates as AgCl and is therefore not ionized and not measurable. These glass sensors, as well as the normal glass pH sensor have thin hydrated layers on the internal and external surfaces of the sensitive glass tip. Because of the nature of the glass structure, these hydrated layers act as ion exchange surfaces with interstitial sites which can accommodate ions of the proper size, thus giving rise to their measurement potential.

The hydrated layers are supported by the bulk of the glass membrane, which does not participate in the measurement. However, this glass bulk acts as a very high resistance (glass being an insulator) in series with the potentials developed at the surfaces, thereby giving rise to the characteristic very high source resistance of the sensor. The manufacturer can make the glass very thin to reduce this resistance, but at the expense of producing a very fragile sensor tip. Also the hydrated glass very slowly dissolves into the test solution, so that, even not considering fragility, a very thin sensor would have reduced lifetime. As a result, it is difficult to make reliable bulk sensors with a thickness less than 0.2 mm. The resistance of the sodium sensors at room temperature varies from unit to unit in a range of  $2 \times 10^8$  to  $10^9$  ohms, but at body temperature of  $37^{\circ}$ C, they are more manageable 50 to 200 megohms. The cation sensors are better, 50 to 100 megohms at 23<sup>°</sup>C, and 5 to 10 megohms at 37<sup>°</sup>C. This required special instrumentation to measure the sensor potential. This instrumentation has been developed, so that the high impedance is now a problem only in that the response is slow. However, most of the present problems with glass sensors are materials problems associated with the hydrated surface layers.

There is an entire set of problems which could be described as operational and which must be overcome before the sensor can either be studied or used in a measurement. Some of these have already been discussed: temperature, sample handling, reference electrodes, and electronic interface. The sensors also require an initial stabilization period to bring the surfaces to the proper level of hydration. The pH sensor stabilizes in about two days, a sodium sensor in two to five days, but the potassium sensor can require two to four weeks. During this stabilization period, there is a random drift in sensor potential which requires restandardization with known solutions so often as to render the sensor virtually useless. Also, any time even a well-stabilized electrode is inserted into a new measurement situation or if the electronics interface is turned off and then on, there is an initial drift period of perhaps thirty minutes. Much care must be taken to be certain that a stabilized sensor is maintained wet in electrolyte solution; dehydration of the surface will require another complete stabilization period.

After overcoming the above difficulties, one can begin to study the accuracy and stability performance of the sensors. The first observation, which is somewhat

misleading, is that gained when a sensor is left continually in the same fluid at constant concentration. After perhaps 12 hours, the sensor drift settles down to less than 1% per day on some units, even the cation sensors. This gives the false impression that the sensor is very stable. However, if the concentration is changed and then returned to the original value even after a few days of stable operation, there is a small shift. (This is all done in a flow-through situation so that the sensors are always wet). The magnitude of this shift is somewhat correlated with the concentration ratio.

In a normal measurement situation, where we first use a standard solution, then test solution, we have consistently been able to get 1% accuracy in routine situations with well-stabilized sensors, both sodium and cation. However, this is only a stability requirement of five minutes. We have had a limited amount of experience with the sensors in an on-line measurement of guinea pig blood during a perfusion experiment. The sensor readings are immediately inconsistent but after an hour begin to give good readings. It is felt that the change from simple electrolyte standard solutions to the complex blood chemistry situation causes a potential shift which recovers slowly. However, the sensor surfaces were not seriously impaired after eight hours in the blood stream.

At this point, we have decided that the glass pH and sodium sensors are valuable in biological situations and deserve further development. However, the glass cation electrode is unsuitable except in well-controlled situations of discrete sampling where both the ammonium and sodium are either known constant or negligible. The selectivity ratios of the cation sensor are not only small and thus require compensating but they exhibit a slow drift in time, thereby rendering the sensor ineffective.

The pH and sodium sensors are already readily applicable in discrete sampling situations where frequent standardization can be accomplished. We feel that we will soon be able to apply them to relatively short-term on-line monitoring situations of less than four hours. However, considerable development is still necessary to reduce the drift problems now encountered before they can be applied in long-term monitoring situations where calibration is difficult, i.e., an implant.

It is very likely that the drift observed is a combination of two effects, both a result of materials handling. The first problem is that during the cooling of the glass, strains are set up which effect the surface states in the hydrated layers. Since glass has a very slight fluid character, and since the hydrated surfaces are slowly but continually dissolving into the solutions, these strains slowly shift and alter the surface states. Sudden changes in the electrode environment also induce

new strain on the surface. The other problem is that the sensor properties are highly sensitive to composition changes. If the glass is inhomogeneous, as the surface slowly dissolves, a different composition will appear at the surface, thereby giving a different potential. There may also be some merit to considering surface treatments, either to reduce strain or to retard the leaching process. In any case, it is our feeling that the materials technology is available which can bring these sensors to the point where they can be implanted with confidence that they will exhibit long-term accuracy. Biological compatibility is another matter which will be considered later.

Because of the failure of the glass cation sensor to reliably measure potassium, we searched for a replacement. In the Fall of 1970, Orion Research announced a liquid membrane sensor with a rejection ratio of over  $10^{+4}$  with respect to sodium and approximately 100 with respect to ammonium ion. The organic carrier is a form of valinomycin. We purchased one of these sensors and have given it a brief trial to determine the feasibility of its use in various applications.

A schematic of the construction is shown in Fig. 7. The acetate filter disc is given a hydrophobic treatment, which prevents direct exchange between the test solution and internal reference solution. To use the sensor one must assemble the sensor by squeezing the small filter disc between the internal body and the external sleeve, introducing the two filling solutions and then inserting the central AgC1 electrode. Sensor performance seems to revolve around the quality of the filter disc. Initially, with a good disc, the sensor will give as much as a 56-mv potential difference for a decade activity change (theoretical is 60 mv). The distribution runs from about 45 mv to 56, 53 being normal. In use, this response slope degrades to virtually zero in anywhere from one to three weeks. The internal materials slowly leach into the test solutions, but do not run out unless the sensor is assembled improperly and allows a slow leak. We therefore concluded that the membrane condition is critical. This is preliminary, and we are conducting further tests.

The electrical resistance of the sensor is on the order of 50 megohms at  $23^{\circ}$ C and goes down to about 10 megohms at  $37^{\circ}$ C, so that they are less fragile electrically than the glass sodium. The response time is reasonably rapid, approximately 15 seconds for doubling or halving the concentration at a level around  $10^{-3}$  molar. The speed is a direct but not linear function of concentration being faster at high concentration. The short-term drift rate is far worse than that of a sodium sensor, on the order of a few percent in ten minutes. One advantage of this sensor is a much shorter initial stabilization time; it will achieve its maximum performance in at most four hours, and can be easily used within an hour after assembly.



Fig. 7 Liquid Membrane Sensor

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We have concluded that this sensor is suitable for discrete sample analysis and some forms of short-term, on-line monitoring. The nature of its present form is not suitable for an implant, however. There are three reasons for this. First, the stability of this sensor is at least two orders of magnitude away from that required for a long-term noncalibrated situation. However, this is a new sensor whose performance can undoubtedly be improved; the glass sodium sensors are much better now than when first reported by Eisenman in 1957. Second, the short lifetime of at most three weeks must be improved considerably but is very likely a matter of design. Third, the presence of a very slow leaching of the valinomycin and its strong organic solvent would very likely cause considerable irritation at the implant site, thus possibly causing large shifts in the local chemistry — and perhaps of the entire organism. The most fruitful direction to take for solution of all of these problems will undoubtedly involve a search for a sensor tip which retards the leaching of the internal solution or in fact stabilizes it in some matrix. Beckman has a sensor of this form, but it has a very short lifetime. We will also try forms of dialysis membrane external to the sensor which will allow electrolyte exchange but blockage of larger organic compounds. Several tradeoffs are involved, the major ones being sensor accuracy and response rate. However, again we feel that this is a materials problem which can be overcome, although this is probably a more difficult problem than the glass sodium sensor.

We have also done a small amount of work with the solid-state crystal sensors. We have used an <u>In Vivo</u> Metrics Ag-AgCl bulb sensor for measuring chloride and an Orion Model 94-09 with a lanthanum fluoride crystal for fluoride. Initial studies have shown that both types are very stable and excellent for this application. The electrical resistances are under one megohm, mainly because the crystals are ionic lattices capable of supporting conduction. The crystals dissolve very slowly into an electrolyte solution. This does not affect sensor lifetime, which is apparently indefinite, but differing inhomogeneities in the crystal structure are revealed to the test solution, thereby causing a slow drift. Process control to reduce material impurity and to reduce crystal defects will undoubtedly bring sensor performance to the quality required for an implant. The problems of the biological interface must still be considered.

#### SECTION 5

#### REFERENCE ELECTRODES

A necessary element in all ion-selective measurement systems is the reference electrode. Since the measured parameter is the electrode voltage, it is clear that two electrodes are required to complete the electrical circuit. The second electrode, the reference, should maintain an absolutely stable potential in the sample fluid, independent of fluid changes, flow rates, or temperature changes. At first glance this may seem simple; all that need be done is to insert a wire into the solution. However, complex physical phenomena occur at metal surfaces in electrolyte solutions which generate reduction-oxidation potentials. These potentials vary considerably with time as the surface state of the metal changes under reaction with the solution. In fact, the only surfaces which give reliable, relatively stable potentials in electrolyte solutions are in fact forms of ion-selective electrodes. Furthermore, if one could rely on a given ionic species as having an absolutely constant concentration even though others vary, the best reference would be an electrode selective to that species. However, that situation seldom exists, especially in biological materials.

As a result of all of the above constraints, virtually all measurements employ as a reference the liquid junction system, as shown in Fig. 5. In this technique, a highly stable ion-selective electrode (usually AgCl or  $HgCl_2$ ) is placed in a compartment which is absolutely stable in the species it measures (usually a saturated solution): this compartment is then connected to the sample solution by some form of restricted liquid connection, the concept being to have a merely resistive and nonpotential generating connection between the two liquids. With proper design, stable potentials can be achieved with the above technique. The major difficulties arise in trying to achieve the proper form of liquid junction, because, unfortunately, there must be a finite fluid flow with associated ionic flow (if not, there is poor electrical connection), and net ionic flow will generate a potential. This potential is predictable from the integrated Nernst-Planck equation:

$$E = -\frac{RT}{F} \ln \left[ \frac{\sum_{i=1}^{n} K_{is} U_{is} a_{is}''}{\sum_{i=1}^{n} K_{is} U_{is} a_{is}'} \right]$$

R = gas constant

T = absolute temperature

F = Faraday constant

K<sub>is</sub> = partition coefficient of charged species i

U<sub>is</sub> = mobility in flow region of species i

a'<sub>is</sub> = activity of species i in external phase

a," = activity of species i in internal phase

The use of the equation is not possible in any normal situation because many of the ionic flow rates cannot be determined. But years of experimentation have shown that the best reference electrodes employ an absolute minimum flow rate in a symmetrical flow pattern. It is also helpful to use an electrolyte filling solution whose positive and negative ion carriers have similar diffusion coefficients.

The two major types of internal electrodes are Ag-AgCl (silver chloride deposited on silver wire) and the calomel (an amalgam of mercury and mercurous chloride). The calomel has historically been more popular for wide-range general chemistry use in pH measurement probably because it is cheaper. But the calomel is unstable and subject to hysteresis with temperature changes, and if a sensor were to fail in an implanted situation, the mercury would prove highly poisonous. We have, therefore, considered only the silver chloride internal reference.

The next problem to consider is that of the liquid junction. Our first response was to investigate the various commercially available types. Two major firms in this area for several years are Beckman and Corning; their reference junctions are somewhat the same in that most employ some mechanical means to produce the restricted flow in a short tube. The general practice is the use of a small hole about 1 mm in diameter, 3 to 4 mm long, which is filled with a semiporous material, such as a packed fiber, a glass frit or a porous ceramic plug. Almost all of these tend to clog in time by crystallization in the plug; although some units can be recovered by boiling in nitric acid. The flow in the junction is not well-controlled so that erratic potentials result and the filling solution needs to be replenished rather often. All of these solutions were rejected.

A relative newcomer in the field is Orion Corporation. Their reference electrodes use two concentric pieces closely machined to produce a tight-fitting cone at the bottom, as shown in Fig. 8. The reference fluid flows by capillary action to establish the liquid junction. This type is far superior in that it is easier to use, more durable, and easily recovered even if allowed to dry out completely. The electrode typically given has potentials reproducible to  $\pm 0.2$  mv. The major



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Fig. 8 Orion Reference Electrode

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problems with them are that they are large (1/2") diameter by 5" long) and can have a high rate of electrolyte flow, making them unsuitable for implant work. However, they are among the best available for general laboratory work.

#### Development Work on Reference Electrodes

Upon discovering that there are really no commercially available reference electrodes which can be directly applied to an implanted ion sensor system, we began a search for other techniques. It was soon discovered that very good potential liquid junction would be dialysis tubing used for artificial kidney equipment. This type of material readily passes small ions and water while restricting bulk flow as well as large molecules. In use, it takes up water and swells to create a structure of small pores. A few configurations were tried, with the one shown in Fig. 9 and 3 being a final version. The inner sleeve has a 1-mm hole drilled through the center in the end. The dialysis membrane is stretched over the end of the sleeve and is pulled tight and sealed with the outer sleeve, which is drilled for a tight fit. Assembly is performed in a bath of electrolyte to be certain that the small end hole is wetted and free of bubbles. An Ag-AgCl electrode is then inserted in the center. The electrode must then be soaked for about two days in an electrolyte solution to allow the membrane to swell properly and establish proper flow. The membranes can be presoaked but are more difficult to handle and likely to tear during assembly. References of this type have been used reliably in measurements with  $\pm 0.1$ -mv reproducibility. The membranes have lifetimes approaching six months, and the electrolyte loss is such that the sensors need be refilled only once a month at most. A quantitative study of the sensors investigating parameters of the membranes, hole geometry, or filling solutions has not been undertaken, mainly because the first model was satisfactory.

Although we have attempted no theoretical study of the sensor performance, it is felt that the high degree of symmetry as well as the very abrupt nature of the junction, combined with very low flow rate, are the major factors which contribute to the superior performance. We feel that it can be readily adapted to an implant situation. The surface material, dialysis membrane, has already been proven in biological applications. Also, the sensor is easily miniaturized and can operate for long periods without maintenance. Continued study will be required to be certain of long-term tissue reaction as well as the above-mentioned parameters in order to optimize performance, but it is felt that this sensor type will be the proper one to employ.



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Fig. 9 Dialysis Membrane Reference Electrode

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#### SECTION 6

#### THE BIOLOGICAL INTERFACE

There are several problem areas relating to the biological interface which must be studied in order to achieve a successful implant situation. Our literature search has revealed an outline of these areas, as well as suggesting research directions. We are concerned with this interface in two respects: first, the biological fluids and rejection mechanisms may coat the sensor or otherwise render it ineffective; second, the foreign surfaces or fluids of the sensors or even their mere presence may alter the local or total chemistry of the organism to a point that any measurements are invalid.

Several different investigators have found that bare glass surfaces of any nature will immediately absorb protein from solution so that a nonporous coating is built up. It has also been found that these coatings will build up on the surfaces of the sodium and pH-type glass sensors and render them ineffective after perhaps 24 hours. There is a variety of possible approaches to these problems. Some investigators have found that heparin binding or silicone coatings have reduced the coating problems considerably, but this has not been tried with the sensors. Such coatings may alter the response considerably. Others have shown that vacuum degassing of glass surfaces reduces their biological interference. Another possible solution is the use of dialysis membrane as an interface which passes the ions to be measured but does not pass the larger offending protein systems. Ross has reported that this is absolutely necessary for the liquid membrane potassium sensor since the serum proteins render the sensor ineffective in a short time (see Appendix A). The use of a membrane of a material similar to dialysis membrane would seem to be the solution with the greatest potential, but considerable study is still required. Perhaps a combination of solutions will be the optimum, but a considerable amount of research is still needed to resolve the surfaces problem. Solving these can improve the areas of discrete sample testing and on-line monitoring, considerably.

The other part of the problem is that of minimizing the trauma and tissue reaction associated with foreign body rejection mechanisms. Studies we have found show that disc-shaped objects placed in areas of low movement show the greatest promise, but there is still a need for investigation. Very little research has been

done on local effects on the interstitial chemistry. It is our feeling that the most important development in this area will be to minimize the size of the sensor as much as absolutely possible and to maintain uniform surfaces. But we also feel that the major portion of the research in this area will involve implantation of devices into normal, healthy subjects and observing the results, both locally and systemically.

#### SECTION 7

#### ELECTRICAL INTERFACE CIRCUITRY

One of the first problems encountered in the development of an implantable electrochemical sensor system was the stringent requirement imposed on the electronics interfacing with the sensor. The sensor is essentially a dc source with a source resistance up to 100 megohms, which must be measured to an absolute accuracy of better than 50  $\mu$ v on a long-term basis. This means that the circuit must have a low offset voltage and a very low input bias current, less than 0.5 pa. For the implantable situation, it is also desirable to have a minimum power requirement to allow battery lifetime of at least a few weeks. Considerable work has been done in this area, and a circuit capable of performing this function has been developed.

The initial design for this amplifier was undertaken by Mr. David Kress and reported in an unpublished Master's thesis. The latest version of the design was developed by Mr. Kress for this project.

A block diagram of the circuit is shown in Fig. 10. It is chopper-stabilized to insure very low offset voltage drift. Present direct-coupled FET input amplifiers exhibit drifts of 50 to  $100 \mu v/{}^{0}C$ , and long-term drifts in hundreds of microvolts. The drawback, of course, with most chopper systems is high input bias current and a requirement to operate inverting, which severely limits input impedance. In order to achieve very high input impedance, typically  $2 \times 10^{12}$  ohms, a noninverting chopper system was used, whereby a unity-gain feedback system signal is compared with the input signal to drive the system to a null at the chopper. This clearly gives very high input resistance, since at null there is no voltage drop across the MOSFET switch pair and thus no current. The input terminal sees only a capacitor and the MOS gates as leakage paths, both of which are over  $10^{14}$  ohms for properly selected units.

The major leakage components in this situation are actually the transients associated with the switching of the MOSFET pair. If the transients are not matched, there is a net charge transfer on each cycle which then leads to a net leakage current. This is definitely an ac effect since the leakage increases linearly with frequency. This leakage can be nulled out by using a trimming capacitor. However, this does



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Fig. 10 Block Diagram

not help input impedance; even though the leakage can be trimmed to less than 0.1 pa at a given input voltage, it will vary as the input changes. The reason for this, as discovered, is that the MOS transistors have capacitances which vary with the voltage applied across them. Since the level of the switch must vary with the input, the voltage across the gate will vary if the drive voltage is referenced to ground. Therefore, the capacitances which were balanced at one input level will be unbalanced at another level, therefore giving rise to a net transient again. The solution for this problem was to float the oscillator voltage supply referenced to the unity gain output so that the relative gate voltage is constant. Implementation of this system immediately led to an input impedance well over  $10^{12}$  ohms, the design goal. This technique also led to a great improvement in common-mode rejection ratio of 110 dB. It is felt that this model will not perform well over a wide temperature range as designed, partly because the oscillator frequency is not well stabilized, but this is not critical since the device will be used either in a laboratory environment or in vivo at the well-controlled 37<sup>°</sup>C body temperature. However, such stabilization could very likely be designed in. Long-term drift tests of a few weeks have shown offset voltage drift of 1 to  $2 \mu v$ , and offset current drift of  $\pm 0.5$  pa. Preliminary temperature drift studies show a typical drift of  $\pm 0.25 \,\mu v/^{\circ}C$  and  $\pm 0.3 \,pa/^{\circ}C$  with no hysteresis.

The circuit operation can be followed from the block diagram of Fig. 6. By alternately switching on the MOSFETs in the chopper, an error signal is formed, e(t), which is a square wave between the input and the output. It is fed into the source follower which has an ac input impedance of  $10^{12}$  ohms over a wide range of input. This signal then goes to a very high gain, (about 30,000) ac-coupled amplifier. This amplified square wave is then demodulated by going through a comparator in a synchronous connection. According to the phase of the chopping cycle, the square wave and ground potential are alternately switched from the positive to the negative input terminal so that a finite error signal can be detected, resulting from the output being high or low. The output of the comparator drives an integrator which is essentially a very low pass filter. At null the output of the integrator matches the input, and all signals will theoretically go to zero; noise voltages keep the comparator section oscillating at null, but the integrator removes this noise. One note, however, is that most circuit functions are handled by the Solitron UC4250C type amplifier which will operate on  $\pm 1.35$  volt supplies at 5  $\mu$ a supply current. The present system uses  $\pm 1.35$  and -2.7 volt supplies, the increased low voltage necessary to ensure turn on of the MOSFET devices. With lower threshold devices, the system should be able to operate with  $\pm 1.35$  volt supplies. Supply current in the present system is  $25 \,\mu$ a, but it is felt that improved design will bring this under  $10 \mu a$ .

#### **SECTION 8**

#### CONCLUSION

The state-of-the-art technology required to develop an implantable biotelemetry ion detector can be roughly divided into the electronic and sensor areas. The electronic area is primarily concerned with the high impedance interface, data encoding data transmission and data storage. Most of the electronic effort performed for this contract was concentrated in developing a high impedance interface circuit which required low power and was capable of being miniaturized. The circuit described in the report satisfied these requirements and could be hybridized into a suitable package.

The vast advancements made in solid-state technology over the past few years enable the electronic problems to be solvable with existing state-of-the-art knowledge. After a suitable electronic system is developed, miniaturization can be accomplished by existing techniques. The techniques chosen will depend on the application of the biotelemetry ion detectors and the number of systems to be built. As an example, if a large number of systems were to be built, maximum use would be made of monolithic silicon circuits developed especially for this application. If only a few systems were to be built, a hybridized structure using existing semiconductor chips would be the best approach. The electronic area is an engineering problem related to cost and application. The sensors required for a biotelemetry system are not off-the-shelf available, so that a greater portion of the contract was devoted to an evaluation of sensors.

This report has discussed the state-of-the-art and potential applications of a new class of sensor, the ion-selective electrode. At this point they show promise for use in a variety of biomedical applications, from the use in an implant for continuous monitoring without the problems of sampling collection, to routine highvolume testing in the hospital chemistry lab. The sensors, which cover at least 75% of the tests routinely performed in both research and clinical situations, have not yet been extensively applied because of a group of problems we have outlined. They have been applied successfully in many research situations where highly qualified technicians with considerable experience in the use of the sensors did the measurements. However, it is felt that the inherent potential of the concept of a

contact sensor requiring no moving parts, complex dilution and mixing apparatus, or very accurate photocell systems more than warrants continued development of these sensors to bring them into the realm of routine application.

In their present form, the sensors can be used in both discrete sampling and on-line monitoring systems which are properly designed to overcome the shortcomings of the sensors. The major problem is that of drift rates, which means that frequent calibration is required, either automatically or manually. The second problem is that a replacement cycle is necessary, especially for the liquid membrane sensors. With the present sensors, on-line equipment can be designed which can give up to one-half hour of monitoring over a small concentration range of perhaps 20%. Initial improvement in the sensors would allow design simplifications as well as performance improvements of any type of this equipment.

The sensors in their present form cannot be applied to implant situations. However, with development in the areas discussed, we feel that they can be applied in implants. The commercial suppliers of these sensors have not done this development and are not likely to because it would not appear too economically attractive. The sensors have sold but not in volume, so that they are not convinced that increased investment will be economically attractive. This we conclude since all of their advertisements and descriptive brochures point out the potential of the sensors, as we have, without pointing out the difficulties. They seem to be concentrating their efforts in marketing the sensors as they are in applications where they are useful in their present form. There is also a going research effort to expand the range of ions which can be measured. Therefore, the development efforts we see as necessary are in improving the present sensors to the point where they can be more easily applied for both routine and demanding situations.

The ion-selective sensor system breaks down into four areas: the electronics interface, the sensor, the reference, and the biological interface. The electronics interface problems are virtually eliminated in comparison with other areas. For an implant, considerable miniaturization will be required along with reliability tests, but this technology is readily available. For telemetry applications additional circuit development will be necessary, but again, this is currently available technology.

The major development area will be in the improvement of the ion-selective sensors. The glass electrodes are at a level where they have a satisfactory lifetime but a drift rate such that recalibration is necessary at least every eight hours even for the best units. The problem is basically one of materials, first of producing a material not subject to minor stresses which will change with time, and also of producing an absolutely uniform structure whose properties will not vary as the

hydrated layer slowly leaches into solution. This is not a trivial materials problem, but is one that can be solved. The liquid membrane sensors for potassium and calcium will be more difficult since more components of a more complex nature are involved. It is felt that the first area of concern will be the disc or whatever saturable membrane is used in the tip of the sensor. This material is very important in determining the lifetime of the sensor, its drift properties, and also the biological contamination. Undoubtedly, this research program will be conducted in conjunction with optimizing the solvent type and organic carrier. It is anticipated that the liquid sensors will be a more difficult problem, but are worth studying since they seem to offer the greatest promise for covering a wide range of measurement situations. The third sensor type, the solid-state crystal, is perhaps closest to being applicable in long-term implants since it already has the best drift performance and sufficient lifetime. Some development will still be necessary to achieve the reliability in the miniaturized form of the sensor. All sensor development should proceed along the lines of attempting to achieve optimum performance in a sensor of manageable size and then embarking on a program of miniaturization. Large versions of an optimized sensor can be immediately applied in other pressing important situations where size is not nearly so critical.

The present state of the reference electrode is at nearly the same quality as the electronics in that we have developed a reliable long-life probe suitable for use in large-scale systems. Some development is still necessary to achieve the desired miniaturization while preserving the reliability, but this should not prove to be a major problem area.

The last problem area, the biological interface, will require considerable investigation. Previous research has involved mostly observation of local histological effects and some study of the coating of various materials <u>in vivo</u> by various subjects. Very little has been done on local chemistry changes, but this is clearly very important to the application of implanted sensors. Much of this will be conducted in conjunction with the sensor studies.

In short, we feel that the ion-selective sensor approach to measurement is a very valuable one which deserves continued research and development. The technique can be applied in several important research and clinical situations with considerable improvements and proper equipment design, many of these applications are close to implementation. The implantable sensor is still some distance from application but the ion-selective electrode method is the only way this can be accomplished. The sensors show sufficient promise so that with the proper development, an implant can be done.

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#### APPENDIX A

#### CATION AND ANION-SELECTIVE ELECTRODES

by

#### Peter P. Bradley

The literature in the fields of chemistry, biology, and medicine was reviewed in the period from January 1969 to July 1970, in terms of relevant publications to the present problem of developing electrodes for measurement of cations and anions; in particular, sodium, potassium, and chloride, in biological systems. The review attempts to elucidate the present trends in cation and anionselective electrodes of interest to us immediately (Na<sup>+</sup>, K<sup>+</sup>, and Cl<sup>-</sup>), the appearance of electrodes with new sensitivities, new techniques which are being applied to electrode use, and applications of electrode technology in chemical, biological, and medical experiments.

#### A.1 Publications on Sodium, Potassium and Chloride-Sensitive Electrodes

The Orion Newsletter, August, 1969-Vol. 1, No. 3, reports an evaluation of the potassium-selective electrode with valinomycin as the ion-exchange membrane. It exhibits close to Nernstian slope, from 1 M to  $10^{-5}$  M potassium, the limit of detection being between  $10^{-6}$  and  $10^{-7}$  M. The temperature range is  $0-50^{\circ}$ C. Electrode resistance is less than 30 megohms. It is used with a double junction reference electrode, Model 90-02. The selectivity constants are presented:

Monovalent Cation	Selectivity Constant
$Cs^+$	1.0
NH4 <sup>+</sup>	0.03
H <sup>+</sup>	0.01
Ag <sup>+</sup>	0.001
Na <sup>+</sup>	0.0002
$\mathrm{Li}^+$	0.0001

Wise, et al. (1970) studied the Corning Model 476132 K ion-exchange electrode with a selectivity of about 80:1 for  $K^+$  over Na<sup>+</sup> at 10<sup>-3</sup> mol/kg. A cellophane dialysis membrane covered the outside porous membrane of the electrode to prevent poisoning of the electrode. Reference electrodes used with it included a saturated calomel electrode with a slow-leak fiber junction, and with serum, an

Ag-AgCl electrode covered with cellophane to keep the electrolyte filler from diffusing into the medium. They found that this electrode is suitable for measuring potassium concentrations in serum; it gave results which agree with those obtained with flame photometry, without correction for sodium present in the normal concentrations.

Pearson and Elstob (1970) using a sodium ion-responsive glass electrode, Electronic Instruments, Ltd. (E.I.L.), type GEA 33, with a calomel reference electrode equipped with a saturated KCl salt bridge (E.I.L. type RJ23) obtained satisfactory results with a variety of clinical solutions, excepting solutions of sodium salts of weak acids and mixed solutions of electrolytes and dextrose, especially after autoclaving which reduces the pH due to decomposition. In this case, it was necessary to calibrate using a buffer system (0.5 M triethanolamine + HCl to pH 7) and dilute samples with the buffer before measurement.

Butler and Huston (1970) tested the Orion 92-19  $K^+$  selective electrode for activity responses. Systematic deviations from the Nernst equation were observed in a direction opposite to the observed potential difference. These results indicate that the ion exchanger is somewhat permeable to C1<sup>-</sup> as well as to  $K^+$ . Deviations were found to be smaller if the ion exchanger was fresh.

Frant and Ross (1970) found that the Orion 92-19  $K^+$  sensitive electrode suitable for use with serum samples, since the values obtained agreed closely with those obtained by flame photometry. The electrode, they reported, has a selectivity over Na<sup>+</sup> of 10,000 to 1. The electrode is made from a 5 to 10% solution of valinomycin in an aromatic solvent, such as nitrobenzene, diphenylether, chlorobenzene, or bromobenzene. The reference electrode was a double-junction with 5 M lithium trichloroacetate filling solution.

Nutbourne (1969) tested the clinical applicability of sodium and potassiumsensitive glasses BH 104 and BH 115, respectively, with a saturated KCl calomel electrode as reference. With the sodium electrode he obtained linear response, and the effect of  $K^+$ ,  $Ca^{++}$ , and  $Mg^{++}$  was negligible, as was changing the pH from 5 to 9. Values at least as good as those from the flame photometer were obtained. He concluded that the sodium BH 104 electrode is suitable for work with serum and urine.

The potassium glass electrode, BH 115, was tested with a saturated calomel electrode, equipped with a salt bridge between the saturated KCl of the electrode and the sample. The salt bridge was as close to the composition of the sample as possible. In constant Na<sup>+</sup> solutions, he observed accurate response to changes in  $K^+$  concentrations, but with changing Na<sup>+</sup>, he observed a response in error up to 10 to 35%. The electrode was observed to be sensitive to Na<sup>+</sup> and NH<sub>4</sub><sup>+</sup>. Thus, if

the concentrations of these ions are known or are zero, then the electrode may adequately be used; but it is unsuitable for use in serum due to the Na<sup>+</sup> and NH<sub>4</sub><sup>+</sup> present, as it is in urine where these two ions lead to instability.

Mascini and Liberti (1969) report the fabrication of a halide-sensitive electrode, the membrane of which is composed of Ag-halide and thermoplastic polymer, fused together. Choice of halide confers specificity upon the electrode. Response is reported to be good for solutions of single halides; deviation occurs in mixed halide solutions. It was not evaluated in biological solutions. The reference electrode used was a saturated calomel, fiber type (Beckman 39170).

Lal and Christian (1970) in evaluating the Orion 92-19 K<sup>+</sup> selective electrode found a slightly less than Nernstian response and a linear calibration curve which was obtained from  $10^{-4}$  to  $10^{-1}$  M K<sup>+</sup>. Slight anion effects are observed with I<sup>-</sup>, OH<sup>-</sup>, CrO<sub>4</sub><sup>-</sup>, and oxalate. The reference electrode used was a saturated KCl calomel with a 3% agar-saturated ammonium nitrate salt bridge.

Krull, et al. (1970) is reported to have developed a solid membrane  $K^{\dagger}$  selective electrode. This publication is not available to us at present.

#### A.2 Miscellaneous Publications on Patented Electrodes and Evaluation Studies

Fleet and Rechnitz (1970) utilized a rapid-mixing continuous-flow system with liquid membrane ion-selective electrodes to measure reaction times as short as 10 msec under turbulent flow conditions of complex formation of  $Ca^{++}$ ,  $Mg^{++}$ and  $Be^{++}$  with the biological ligands: lactate, gluconate, maleate, and tartrate. Electrodes used included: Calcium Orion 92-20, divalent ( $Mg^{++}$ ) metal ion Orion 92-32-02 (with  $Be^{++}$ , equipped with an internal reference solution consisting of  $10^{-3}$  M Be<sup>2+</sup> in 1 M KCl). Reference electrodes were Orion flow-through 98-20 or Beckman saturated calomel, type 39410, with a quartz fiber junction.

Whitfield and Leyendekkers (1970) studied the selectivity isotherms for the Orion calcium-selective ion exchange electrode with the following system: Ca(II)-Na(I)-Cl(I)-water. Variation in response was reported in the range 0.03-6 M as a function of solution composition as well as of ionic strength.

Settko and Wise (1969) report the fabrication of a liquid organic phase with ion-exchanging properties which can detect  $C1^{\pm}$  or  $Ca^{2+}$ .

Ross (1969-1970) patented a cation-sensitive electrode which responds to  ${\rm Cu}^{++}$ .

Ross (1969-1970) patented an anion-sensitive electrode which responds to  $ClO_4^-$ , Br<sup>-</sup>, I<sup>-</sup>, NO<sub>3</sub><sup>-</sup>,  $ClO_3^-$ , and which is reproducible to 1%.

Ross (1969) patented several polymeric membranes (e.g., polyethylene) with preferentially liquid transport paths between the organic phase (solid ion-exchanger in organic solvent) and the test solution.

Rechnitz and Hseu (1969) found reduced interference by  $H^+$  and univalent and divalent cations in analytical and biochemical measurements with a Ca<sup>++</sup> selective solid membrane electrode.

Pain and Mukherjee (1969) used bentonite clays to prepare electrodes sensitive to  $Ca^{++}$  in the presence of high  $Na^{+}$  concentrations.

Frant and Ross (1969) patented electrodes for the determination of  $Cu^{++}$ ,  $Cd^{++}$ , or  $Pb^{++}$ .

The following are the reference electrodes used in the first two parts of this review:

With the Orion valinomycin 92-19, double junction, model 90-02.

With Corning 476132 potassium electrode:

 Saturated calomel with a slow-leak fiber junction for "pure" solution work.

(2) Ag-AgCl covered with cellophane for serum work.

With E.I.L. sodium glass, type GEA 33, a calomel with saturated KCl salt bridge (E.I.L. type RJ23).

With Orion 92-19, Ag-AgCl filled with 0.01 M KCl saturated with AgCl. With Orion 92-20, 92-32-02 (for  $Mg^{++}$ ):

(1) Orion 98-20.

(2) Beckman saturated calomel, type 39410 quartz fiber junction.

Whitfield and Leyendekkers used a Janaer thalamide reference electrode (cat. 3183) with Orion 92-20 Ca and Orion 92-17 Cl.

Frant and Rose (1970) used a double junction reference electrode with 5 M lithium trichloroacetate filler with the Orion 92-19  $K^+$  selective electrode.

Scibona, <u>et al</u>. (1970) used a calomel electrode with saturated KCl filler with their alkylammonium salt electrode.

Nutbourne (1969) used a saturated calomel electrode as the reference electrode (with a salt bridge in the case of the potassium electrode) in studying the E. I. L. electrodes BH 104 (sodium) and the BH 115 (potassium).

With a halide-sensitive electrode, Mascini and Liberti (1969) used a saturated calomel fiber type Beckman 39170 electrode.

Suga, <u>et al</u>. (1970) used an Ag-AgCl reference electrode filled with 1.5 M RbCl, with the microelectrode of NAS 11-18 (sodium-sensitive glass). With NAS 27-4 glass microelectrodes (potassium selective), they used the above NAS 11-18 glass microelectrode as the reference.

Pioda, <u>et al</u>. (1969) used a saturated KCl calomel electrode with 0.1 M  $\rm NH_4 NO_3$  as the second solution of a double junction reference electrode with a valinomycin system.

Lal and Christian (1970) used a saturated KCl calomel electrode with a 3% agar saturated  $\rm NH_4NO_3$  salt bridge with the Orion 92-19 K<sup>+</sup> selective electrode.

#### A.3 Publications on In Vivo Monitoring of Cation Concentrations

Suga, <u>et al</u>. (1970) measured the sodium and potassium concentrations <u>in</u> <u>vivo</u> in the cochlear endolymph of the guinea pig. Endolymph, unlike most interstitial fluid, exhibits high  $K^+$  concentration and low Na<sup>+</sup> concentration. They used microelectrodes to measure cation concentrations; for sodium, Corning NAS 11-18 glass capillary tubing; for potassium, Corning NAS 27-4 glass capillary tubing. NaCl solution (0.1 M) and 0.1 M KCl, each buffered to pH 7.6 by use of trisbuffer, were used as filling solutions into which was suspended an Ag-AgCl wire. In measuring activities, the NAS 11-18 glass microelectrode was used as the reference electrode for potassium, since the NAS 27-4 responds to both potassium and sodium. In the case of sodium, the reference electrode was a micropipette filled with 1.5 M RbCl, connected to an Ag-AgCl wire.

Friedman, et al. (1969) used the  $K^+$  glass electrode to monitor ion transfers during the rewarming of the single rat tail artery.

Dick and McLaughlin (1969) measured Na and K activities and concentrations in toad oocytes using Na and K sensitive microelectrodes and a flame photometer.

Glass Microelectrodes, edited by Lavallee, Marc, Schanne, Otto F., and Hebert, Normand C., John Wiley and Sons, New York, 1969. Techniques are described from which Suga derived his procedures. Discussed are "Single and Coaxial Microelectrodes in the Study of the Retina", by Tsumeo Tomita, "The Difference of Electric Potentials and the Partition of Ions Between the Medium and the Vacuole of the Alga Nitella", by Gregor A. Kurella, "Cation and Hydrogen Microelectrode in Single Neurons", by Raja H. Khuri, "Measurement of Activity of Hydrogen, Potassium and Sodium Ions in Striated Muscle Fibers and Nerve Cells", by P. G. Kostyuk, et al. It should be noted that the above research was completed before 1968.

#### A.4 Publications on Applications of Electrodes to Biomedical Fields

Webber and Wilson (1969) measured sodium concentration in high-purity water, and obtained a Nernst-response down to 1  $\mu$ g/liter Na<sup>+</sup>, by controlling pH and solution flow around the electrode.

Pioda, et al. (1969) found the valinomycin  $K^+$  sensitive electrode to have a selectivity of K over Na of over 4,000:1.

Mowbray (1969) evaluated a Corning electrode, with a greater sensitivity for  $K^{+}$  than Na<sup>+</sup> favorably for use as a biomedical tool.

Moore (1970) found that serum  $Ca^{++}$  concentration was within a narrow range, 0.94 to 1.33 moles/1, and that within an individual varied 6% over several months.

Scibona, <u>et al.</u> (1970) demonstrated that liquid membranes formed by organic solutions of long chain alkylammonium salts behave as liquid electrodes sensitive to the anion concentration. Liquid anion membranes can be used as electrodes sensitive to the aqueous concentration of metal cation though anionic complexes. Zinc and palladium were tested via their formation in the membrane of tetrachlorozinc of tetrachloropalladium (II), these two species acting as anions to the tetraalkylammonium salts. Both electrodes were Hg,  $Hg_2Cl_2$ , with saturated KCl filling solution.

#### Fluoride

Tusl (1970) used an  $F^-$  electrode to measure  $F^-$  concentration of diluted samples of human urine, after adding sodium citrate to decompose Fe and Al complexes.

Sun (1969) used  $F^{-}$  electrodes with calomel reference to determine urinary fluoride.

Manahan (1970) used the fluoride electrode as a reference in the determination of nitrate ion concentration with an error less than or equal to 0.9%.

Jones, et al. (1969) used a fluoride-sensitive electrode to measure the fluoride activity in buffered multi-vitamin preparations.

Francis (1969) determined fluoride ion concentration in products of decomposition of organic materials.

Arnaldo and Mascini (1969) by means of added fluoride determined the fluoride concentration by obtaining the equivalence point by Gram's Plot.

Anfalt and Jagner (1970) demonstrated that formate, acetate, proprionate, and butyrate interfere with the precipitation reaction of fluoride with lanthanum nitrates.

#### Calcium

LaCroix, <u>et al</u>. (1970) found that usage of the chloride ion electrode in measuring chloride ion concentration of plant extracts resulted in unreproducible and very high chloride concentration values.

#### Sodium and Chloride Ions

Kopito, <u>et al</u>. (1969) studied the use of electrodes in determining levels <u>in</u> <u>situ</u> of sweat electrolytes in cystic fibrosis diagnosis.

Friedlmander, et al. (1969) utilized sodium electrode sweat tests in the diagnosis of cystic fibrosis of the pancreas.

#### General

Kater and Leonard (1969-1970) patented the use of semipermeable membranes to cover liquid junctions when used in biological fluids to prevent fouling of electrodes.

Montalvo and Guibault (1969) and Guibault and Montalvo (1969) coated  $\text{NH}_4^+$  sensitive glass with urease and were able to measure urea concentrations due to the resulting potential of the increased  $\text{NH}_4^+$ .

Guibault, et al. (1969) used a Beckman electrode responsive to  $\mathrm{NH_4}^+$  in monitoring of a deaminase system.

Guibault and Hrabankova (1970) coated electrodes with an L<sup>-</sup> amino acid oxidase and were able to determine the concentration of L<sup>-</sup> amino acid by the potential due to increased  $NH_4^+$  which diffused into the electrode.

#### New Techniques of Determining Ion Concentration

Brand and Rechnitz (1970) developed a technique of differential potentiometry with ion-selective electrodes, which does away with the weakness of the liquid junction. Two glass (or membrane) ion-sensitive electrodes are used, one sensing a species which remains constant among all the samples tested. Manahan (1970) used this method to measure nitrate concentration.

Bergveld (1970) developed an ion-sensitive solid-state device for neurophysiological measurements. The device combines the principles of an MOS transistor and a glass electrode and can be used for measurements of ion activities in electrochemical and biological environments.

#### A.5 Publications on Biological-Glass Interfaces and Tissue Reactions To Implants

Dutton, et al. (1969) studied by reflected light microscopy and electron microscopy the thrombi formed on a variety of foreign surfaces, including soda lime silica glass, polymethylmethacrylate, and Epon epoxy resin. With histological evaluation in two dimensions, they found that the thrombi are composed of isolated cellular aggregates and an interaggregate red-cell fibrin mesh. In three dimensions, it was found that the thrombi exhibited columnar-shaped cellular aggregates extending from the foreign surface into the blood and red cell fibrin mesh surrounding the aggregates. They concluded that since platelets were not found adherent to the foreign surfaces directly, the intervening film "conditions" the surface preliminary to platelet adhesion.

Johnsson-Hegyeli and Hegyeli (1969) evaluated the compatibility of implant materials in living cells in vitro. The materials and their interaction with living human blood and tissue were observed. Blood cells-materials interfaces were prepared by incubating the materials in contact with whole blood or platelet-rich plasma. Tissue cell-materials interfaces were prepared by cultivating animal and human cells as a monolayer directly on the surface of the material to be evaluated. Implant materials included polymers, metals, glass, and carbon surfaces. Glass coverslips implanted or studied in vitro were used as controls. Tissue and cell growth were noted on the surface of the glass coverslips. Little toxicity or "abnormal" growth was anticipated (or observed) with the glass coverslips as controls.

Hersh, et al. (1969) bonded heparin ionically to glass surfaces. The heparin coating resisted fluid shear stresses as high as  $10^4$  dynes/cm<sup>2</sup> at  $30^{\circ}$ C for periods in excess of 300 hours, and 1000 W of ultrasonic cleaning at  $62^{\circ}$ C for periods of more than 45 minutes. The bonded heparin also prevents the clotting of human blood. Heparin was linked via an amino silane to the surface of a borosilicate glass. They found that physiologically significant amounts of heparin do not elute from the glass surface into the blood.

Messing, et al. (1960) found that protein solutions exposed to porous glass membranes are rapidly depleted of protein. The proteins do not migrate through the membranes but become trapped at the channel walls. The proteins, they found, became firmly bound to the surface of the porous glass. Surface deactivation of porous glass can be accomplished by treatment with methyl trimethloxysilane or x-aminopropryl triethoxysilane, silanes which react with surface silanol sites to produce a thin polysiloxane coating on the glass surface. The silane-treated tubes remained permeable to water. The methyl silane treatment results in a strongly hydrophobic coating.

Wood, et al. (1970) evaluated the significance of implant shape in experimental testing of biological materials. In adult albino rabbits, they implanted a number (304) of stainless steel discs and cylinders in the following sites: beneath the periosteum of the anterior calvarium, under the masseter muscle of the left mandibular ramus and in the body of the sacrospinalis muscle. Wood, et al. compared the tissue reaction around the disc implants with that observed around the cylinder implants through the study of histological sections. In addition, the degree of reactions observed at the various sites was contrasted with that seen at the other sites.

They found that in all cases the muscle implants showed the greatest reaction. Discs showed many "micro-areas" of tissue reaction around their periphery. In contrast, the rod shaped implants in muscle demonstrated a greater reaction towards the ends than in the mid-portion of the shaft (clubbing), but this reaction did not occur with rods implanted at other sites. They concluded that much of the histological tissue reaction observed around muscle implants is really caused by mechanical trauma which must be differentiated from a noncompatibility reaction. Thus, a site must be chosen where mechanical trauma will be minimal (submasseteric site) and an implant shape must be employed which does not result in clubbing (disc).

Rigdon (1970) demonstrated that when polyurethane was implanted subcutaneously in mice and rats, a chronic granulomatomous reaction occurs in which there is an excessive amount of hemosiderin<sup>\*</sup>; the hemosiderin apparently results from the lysis of the many erythrocytes present (some of which are partly creatinated). He suggests that the infiltration of erythrocytes and subsequent hemolysis may result from the positive change associated with this plastic, attracting the negatively charged cells. A similar reaction occurs with nylon and Teflon. It is noted, however, that there is no significant amount of erythrocytes or hemosiderin associated with the inflammatory reaction accompanying carbon black, glass beads, or Fuller's earth.

Hemosiderin is an insoluble form of storage iron in which the small particles of ferric hydroxide are so arranged as to be visible microscopically both with and without the use of specific staining methods (Dorland's Illustrated Medical Dictionary, twenty-fourth edition, p. 665).

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