1962

The effect of x-rays on the membrane permeability of human erythrocytes

Garver, Richard M.; Saylor, William L.,

THE EFFECT OF X-RAYS ON THE MEMBRANE PERMEABILITY OF HUMAN ERYTHROCYTES

RICHARD M. GARVER
and
WILLIAM L. SAYLOR

THE EFFECT OF X-RAYS ON THE MEMBRANE PERMEABILITY OF HUMAN ERYTHROCYTES

* * * *

Richard M. Garver

and

William L. Saylor

THE EFFECT OF X RAYS ON THE MEMBRANE PERMEABILITY OF HUMAN ERYTHROCYTES

by

Richard M. Garver
Lieutenant Commander, United States Navy

and

William L. Saylor
Captain, United States Army Reserve

Submitted in partial fulfillment of the
requirements for the degree of

MASTER OF SCIENCE
IN
PHYSICS

United States Naval Postgraduate School
Monterey, California

1962

THE EFFECT OF X-RAYS ON THE MEMBRANE PERMEABILITY OF HUMAN ERYTHROCYTES

by

Richard M. Garver

and

William L. Saylor

This work is accepted as fulfilling the thesis requirements for the degree of

MASTER OF SCIENCE
IN
PHYSICS

from the

United States Naval Postgraduate School

ABSTRACT

Human erythrocytes were irradiated in order to study the effect of x-rays on the cell membrane. The red blood cells were exposed to various dosages of 145KVP x-rays up to 10,000 r. The effect of the x-rays on the physical characteristics of the cell membrane was evaluated by use of an alternating current wheatstone bridge. Changes in the rates of ion transport through the membrane were evaluated by K^{42} tracer studies. The x-irradiation was found to have no detectable effect on the physical characteristics of the membrane, or upon the rate of uptake of potassium; however, x-irradiation of 10,000 r was found to cause a substantial increase in the rate of potassium loss by the cells. These results are attributed to disturbance by the radiation of some intracellular process which is involved in the maintenance of the observed ionic concentration gradients.

The authors especially wish to express their appreciation for advice and assistance of Professor George H. Marmont of the Department of Electronics.

TABLE OF CONTENTS

LIST OF ILLUSTRATIONS

1 Introduction

The mammalian erythrocyte is a non-nucleated cell with a diameter of 7-8 microns and an external form of a biconcave disc. In the human, the red cells comprise about 45% of the total blood volume, with about five billion red cells per cc in the average adult. The external surface of the red cell is a semi-permeable membrane about 100 Å thick. The membrane appears to consist of three layers, a center layer of lipid molecules bounded both on the inside and on the outside by a layer of protein molecules (2).

Red cells, like other living cells, require a closely regulated internal environment for life and normal functioning. This may require the cell to maintain internal concentrations of certain substances several times higher or lower than the concentrations of these substances immediately outside the cell. An example of this type of concentration gradient is the high potassium-low sodium ratio maintained internally by the red cell. Solomon (6, 14) reports the internal potassium and sodium concentrations to be 143 mM/lt and 13.9 mM/lt respectively, while the extracellular (plasma) levels are 5 mM/lt and 154 mM/lt. The means by which the red cells maintain these gradients has been and is being studied by a number of investigators (4, 5, 24, 25, 26). The relatively simple structure of the erythrocyte (no nucleus or extensive internal substructure) makes it a suitable choice as a subject for the investigation of the possible role of the cell membrane in the maintenance of these gradients.

There are basically two methods by which these gradients might be established; 1) the membrane may be semipermeable to cations, and an active transport, by the membrane, of potassium and/or sodium out of the cell would maintain the gradient; or 2) the potassium might be selectively accumulated inside the cell, thus relieving the membrane of any active part in ion transport. Evidence has been presented by various investigators favoring one or another of these possibilities, but the problem is presently the subject of considerable effort by several groups.

The enveloping membrane of the cell was first thought to selectively pass various ions as a result of electrically charged "pores" in the membrane. The charges in the pores, being positive, would exclude cations, and the size of the pores would determine which anions could pass. However, largely through the work of Conway, Boyle and Kane (25, 26) the cell membranes were found to pass anions freely (specifically the lactate and the chloride ions). As a result, Boyle and Conway (26) proposed a "sieve" theory in which it was the critically important pore size alone, and not the electrical characteristics, that determined which ions could pass through the membrane. They predicted that the membrane would be permeable to the smaller ions, such as hydrated potassium and chloride, but impermeable to the larger ions such as the hydrated sodium ion, calcium and others. Solomon (3, 6, 10), supporting a pore theory, has postulated the existance of pores in the cell membrane to be 3.5A in radius, as compared to the radii of the hydrated sodium and potassium

ions which are 2.5A and 1.9A respectively. With the development of radioactive tracer techniques, however, it became apparent that although the sodium ion is maintained at a relatively low concentration inside the cell, it engages in an active and rapid exchange with the extracellular sodium. Thus it appeared that the permeability of the membrane to various ions and the maintenance of concentration gradients across the membrane might be independent processes.

In an attempt to overcome the difficulties encountered with the "sieve" theory, a "sodium pump" was postulated. (35, 36, 37). According to this theory, the intracellular sodium level is maintained by a membrane mechanism which continually pumps sodium out of the cell. However, some investigators maintain that sufficient energy is not available from cellular metabolism to operate such a pump (21, 27, 28). Ussing and Levi (27) have proposed an "exchange diffusion" or "ferry" process by which ion transfer across the membrane was accomplished without requiring expenditure of energy from cellular metabolism. By this process ions would be ferried back and forth by an enzymatic carrier, the impetus for movement being thermal agitation. To avoid a net energy requirement the ferry would have to carry an ionic "passenger" for every crossing in either direction. Solomon (3, 6) has also given recent evidence for potassium transport by carrier enzyme in human erythrocytes.

According to a "fixed charge" hypothesis proposed by Ling (21, 29) the concentrations of ions within muscle cells are the result of selective ionic accumulation by negatively charged side chains on the polypeptide

chains of the intracellular protein. He suggests that the selectivity is a function of the size of the ion, for example, the hydrated potassium ion would be absorbed preferentially over the larger hydrated sodium ion. The smaller ion, being able to approach more closely to the fixed charge, would represent a lower energy state than the larger ion. The potassium ions are not regarded as bound in the sense that they have lost chemical and electrical identity, but rather the ion and its fixed charge counterpart together act as an oscillating dipole. The net effect of this "selective accumulation" within the cell is an increased potassium concentration and, since electrical neutrality must be maintained, a decreased sodium concentration. Regarding transport of ions across the membrane, he concludes " ... that cations enter by association with, and dissociation from fixed negative charges on the cell surface ... "

Figure 1. Schematic Representation of Mechanisms for Transport of Ions Across Cell Membranes

Investigations into the effects of ionizing radiation on living cells have provided evidence in support of active sodium and potassium transport mechanisms (12, 13, 15, 17). Bruce and Stannard (16) have shown, using yeast cells, that radiation increased the rate of potassium leakage but decreases the rate of uptake of potassium. Sheppard and Martin (18)

studying human red cell in vitro, found the cation exchange rate to be insensitive to 1200r of x-rays, and also to the oxygen or hemoglobin concentrations. Sheppard and Beyl (19) found a definite increase in the rate of loss of potassium from human red cells after irradiation greater than 6750r. A change in the rate of uptake of potassium was not observed for any dosage up to 54,000r, suggesting either that there may be separate mechanisms in the membrane for influx and efflux of potassium or that the ionic concentration gradients may be maintained internally by the cell, and independent of the external cellular membrane.

These investigations of the ion transport mechanisms involved the use of radioactive tracers (K^{42}, Na^{24}) to determine the influx and efflux of Na and K across the cell membrane. Changes in the transport rates as influenced by changes in environment, and chemical or radiation damage to the cell have formed the basis for most of the work to date (12, 17, 38).

In addition to the observation of ion transfer rates, the membrane of the red blood cell may also be studied by measurements of the electrical impedance of cells in suspension. The cells and the suspending medium can be represented by a simple electrical circuit such as that shown in Figure 2. The measured impedance of the suspended cells are then interpreted in terms of the various components of the equivalent circuit. Fricke and Morse (11) have applied this technique to study the red blood cells of the calf. They showed that, over the frequency range from 78 KC to 4.5 MC, the red cell suspension could be represented by the circuit of Figure 2(a), where R_2

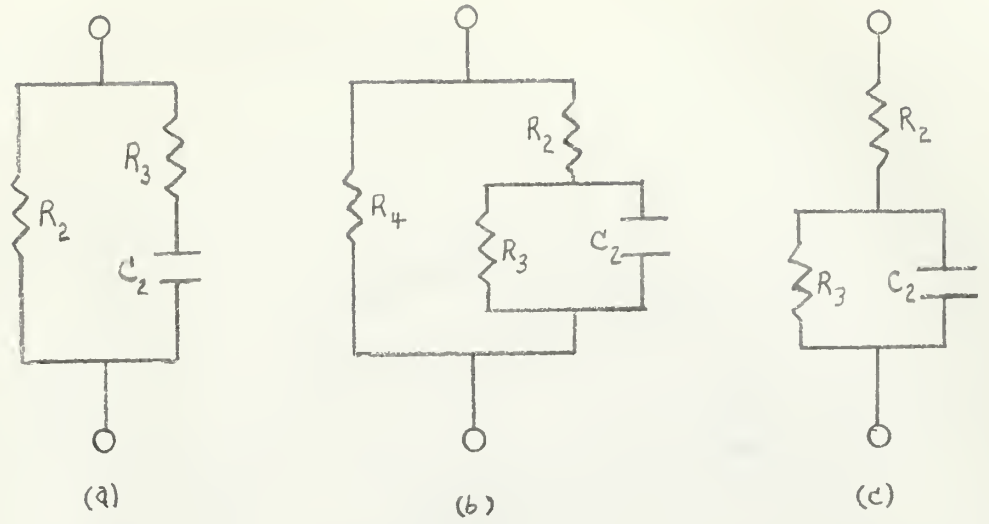


Figure 2. Simple Circuit Equivalents of Cellular Membranes. (a) Circuit Assumed by Fricke and Morse (11) for Calf Blood; (b) Circuit Assumed by Phillipson for Various Tissues (22), and by the Authors for Human Erythrocytes; (c) "Dummy" Circuit Used to Calibrate Experimental Apparatus.

represents the suspending medium, R_3 the interiors of the red cells, and C_2 the capacitance of the cell surface. Using conversion formulas (see appendix I(c)) the assumed circuits were evaluated in terms of the parallel resistance-capacitance networks of the measurement apparatus. In his work on the impedance of tissues, Phillipson (22) assumed the circuit of Fig. 2(b), where R_3 and C_2 represented the resistances and capacities of the cell membranes, R_2 the resistance of the cell protoplasm, and R_4 the suspending medium. In extension of this work, and based upon a derivation by Maxwell of the equation for the resistance of a suspension of homogeneous spheres, Cole (1, 7, 8, 9) showed that the measured electrical characteristics of biological samples could be represented by a "circle diagram" such as Fig. 3, which is the locus of the series

impedance vector on a plot of the equivalent reactance vs. the equivalent series resistance of the

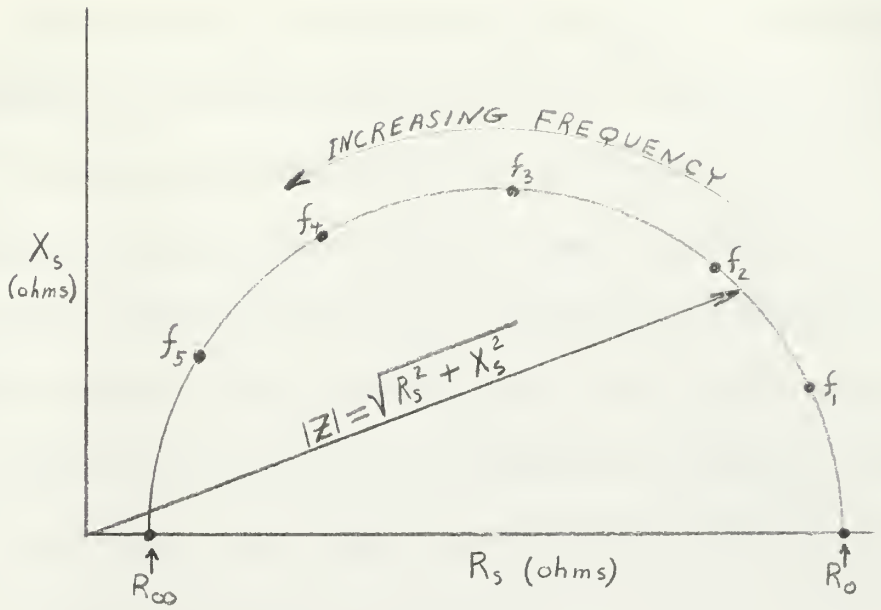


Figure 3 Plot of Equivalent Series Impedances of Biological Samples

measured values. By use of an alternating current "wheatstone Bridge, Cole has applied this analysis to a number of his own studies of biological specimens, as well as to those of Fricke and Morse (11) and others. Lessler (23), using these techniques to evaluate the effects of x-rays on amphibian erythrocytes, reported the surprising results of increased capacitance and increased uptake of potassium after in vitro radiation with only 100 r.

2. Experimental Apparatus.

Impedance measurements of suspensions of red blood cells were made using the experimental set-up as shown in Figure 4. A Hewlett-

Packard Model 650A Test Oscillator was used to provide input signal at frequencies ranging from 23 kilocycles to 2 megacycles. Isolation of the bridge was accomplished by use of a General Radio Bridge Transformer. The ratio arms of the bridge (R_2 and R_3) consisted of matched composition resistors with values of 150.25 ± 0.05 ohms. The balance arm of the bridge consisted of a parallel resistance-capacitance network. The resistance consisted of a number of fixed composition resistors in series with a calibrated carbon strip potentiometer. Wire-wound potentiometers and decade resistors were found to be unsatisfactory at the higher frequencies due to their inductive reactance. The capacitance measurements were made using a decade condenser in parallel with a calibrated variable air condenser.

In order to achieve the required sensitivity, it was necessary to feed the output of the bridge through two Hewlett-Packard Model 400-D VTVM/amplifiers in cascade, each amplifier providing a gain of approximately 100. It was also found necessary to incorporate into the circuit a parallel resonant filter network. The filter was constructed to provide pass bands at the frequencies of interest between 50 and 2000 kilocycles. The filtered and amplified signal from the bridge was finally put into the vertical input of an oscilloscope, the horizontal input receiving the signal directly from the oscillator. The resulting Lissajous figure then provided a visual indication of the bridge balance.

The performance to the bridge was evaluated by measurement of a "dummy circuit" over all frequencies. The dummy, similar to that of

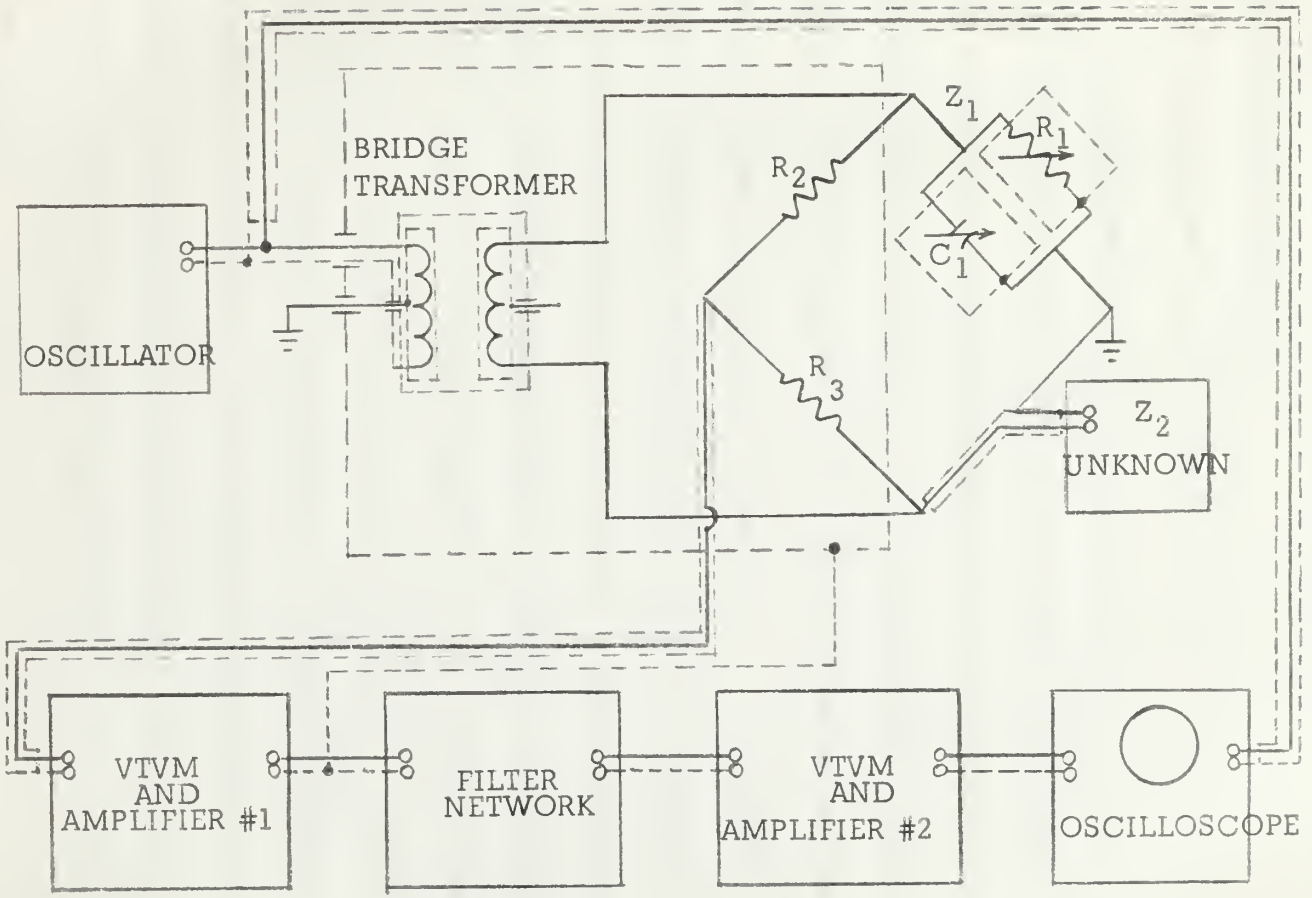
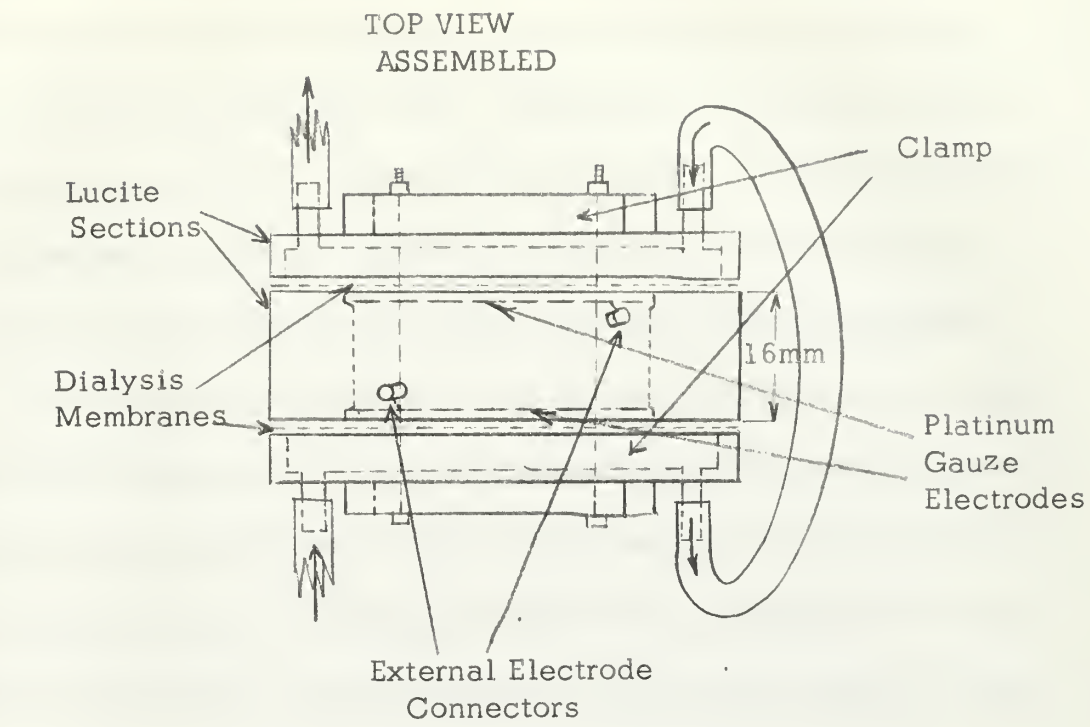


Figure 4. Schmetic Diagram of Impedance Bridge and Associated Equipment.

figure 2(c), was constructed of fixed resistors and capacitors whose values were precisely known. The equivalent series resistance and reactance of the dummy circuit was compared at each frequency with that of the measured bridge values by use of appropriate conversion formulas (see Appendix I (b)).

The measurement cells were machined from 1 3/4 inch lucite round stock. The center portion of the cells were bored to give a blood sample capacity of about eight ml. Platinum electrodes were constructed from 52 mesh platinum gauze and were mounted on the center portion. Prior to use the center and end sections of the measurement cells were then assembled as shown in Figure 5 with a dialysis membrane mounted between the center portion and each end section. The joints were made leaktight by the use of four pliable plastic gaskets (Parafilm) for each cell, one being inserted on either side of each dialysis membrane. The measurement cells were then mounted on a wooden form between two ground glass joints and the entire apparatus was rotated constantly during irradiation in order to insure proper mixing of the sample suspensions and uniformity of the radiation. To reduce polarization effects, which were significant at the lower frequencies of interest, it was found necessary to coat the platinum gauze electrodes with a layer of platinum black. This was accomplished by electrolysis in a 0.11 M platinum chloride solution.

The x-rays were obtained by use of 150 KVP industrial radiography machine, manufactured by the North American Philips Company.



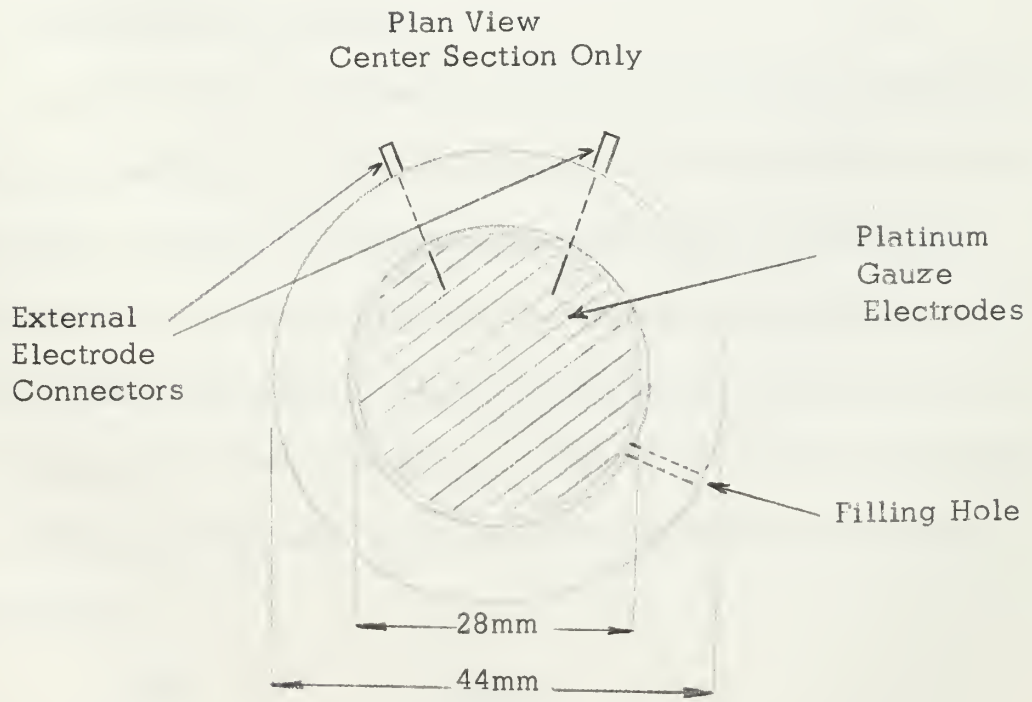


Figure 5. Diagram of Measurement Cell

Filtration consisted solely of the inherent filtration of the tube. The half value layer was not determined. Dosage rates were determined with a Victoreen condenser-r meter. Attenuation of the x-ray beam by the measurement cell was accounted for by placing the Victoreen thimble in a lucite case similar in dimensions to the measurement cells, and mounting it on the rotating rig in place of a cell during calibrations.

The relative radioactivities of the packed red blood cells or of the buffer were determined by use of a conventional beta counting apparatus, consisting of a shielded end window G-M tube and a Technical Associates Model 100 Scaler. Measured samples were evaporated on copper planchets for counting. Total potassium concentrations within the red cells were determined with a Coleman Flame Spectrophotometer.

3. Experimental procedure.

From 70 to 120 ml. of venous blood was collected by syringe and immediately washed several times until the cell suspension was nearly plasma free. Heparin was used as an anticoagulant in preliminary experiments. In all the experiments from which the data was obtained, the blood was defibrinated by stirring with sterile wooden applicators. The buffer used for washing and for circulating around the measurement cells during the experiment was a Tyrode's buffer with the following composition:

NaCl	8.00 g
KCl	0.20 g
$CaCl_2$	0.20 g
$MgCl_2.6H_20$	0.10 g
$NaH_2PO_4.H_20$	0.05 g
$NaHCO_3$	1.00 g
glucose	1.00 g
Distilled Water	1000 cc

The buffer was prepared immediately prior to use. pH was adjusted to 7.2 to 7.4 During the centrifugation, the measurement cells (Figure 5) were assembled and clamped. After assembly, the interior of the measurement cells were wet down with Tyrode's and buffer was circulated through the system past the exterior of the dialysis membranes prior to adding the red cell suspension. The measurement cells, with a capacity of 8-9 cc. were filled by needle and syringe through small holes drilled in the lucite rims. The holes were then sealed with paraffin. After preparation, the measurement cells were secured to the rotating mechanisms for a few minutes to insure equilibrium of the samples and the circulating buffer prior to the first measurement. There were two of these rotating mechanisms, one for the samples to be irradiated and one for the control sample. Usually, three cells were attached to each apparatus, so that there were three irradiated samples and three controls.

The circulating Tyrode's solution, through which pure oxygen was bubbled, was fed through the system by gravity flow, and the tubing was passed through a water bath to bring the temperature to about 28° C. prior to its circulation about the blood samples. The flow rate was controlled by insertion of short pieces of glass capillary tubing into the tubing just upstream from each cell. The length and bore size of these capillaries were adjusted to permit a flow rate of about 3 ml. per minute around each cell. During the later experiments, the solution was not recirculated after having once passed through the system. This was done in order to

avoid the possible deleterious effects of the substances formed in the radiation of the Tyrode's solution.

Irradiation of the blood samples was carried out at a dose rate of approximately 30 r/min. During this time, the control and the samples being irradiated (all cells) were rotated to insure proper mixing and, in the case of the irradiated sample, uniform dose. The test samples were rotated in a vertical plane in the center of the x-ray beam. The average target-to-sample distance was approximately 25 cm.

Just prior to irradiation, and after irradiation dosages of 1000, 3000, and 5000r the electrical impedance of the red cell suspensions were measured on the bridge. Each of the six measurement cells was shaken, connected to the bridge, and measured at five different frequencies (approximately 50, 100, 500, 1000, and 2000 kilocycles.) Since the impedance is dependent upon the extent of settling of the red cells, the measurement cells were shaken immediately before each measurement and the values of the parallel resistance and capacitance were recorded from the bridge after exactly 30 seconds of settling. Each measurement cell was fitted with flexible plastice tubing (Tygon) of sufficient length to permit this mixing without requiring interruption of the circulating buffer solution. At the end of each experiment, hematocrits (i.e. the volume fraction of packed red cells) were determined for each of the irradiated and control samples.

For the potassium uptake experiment, using K^{42} as a tracer (see Figure 6), the red cell suspensions were prepared and irradiated

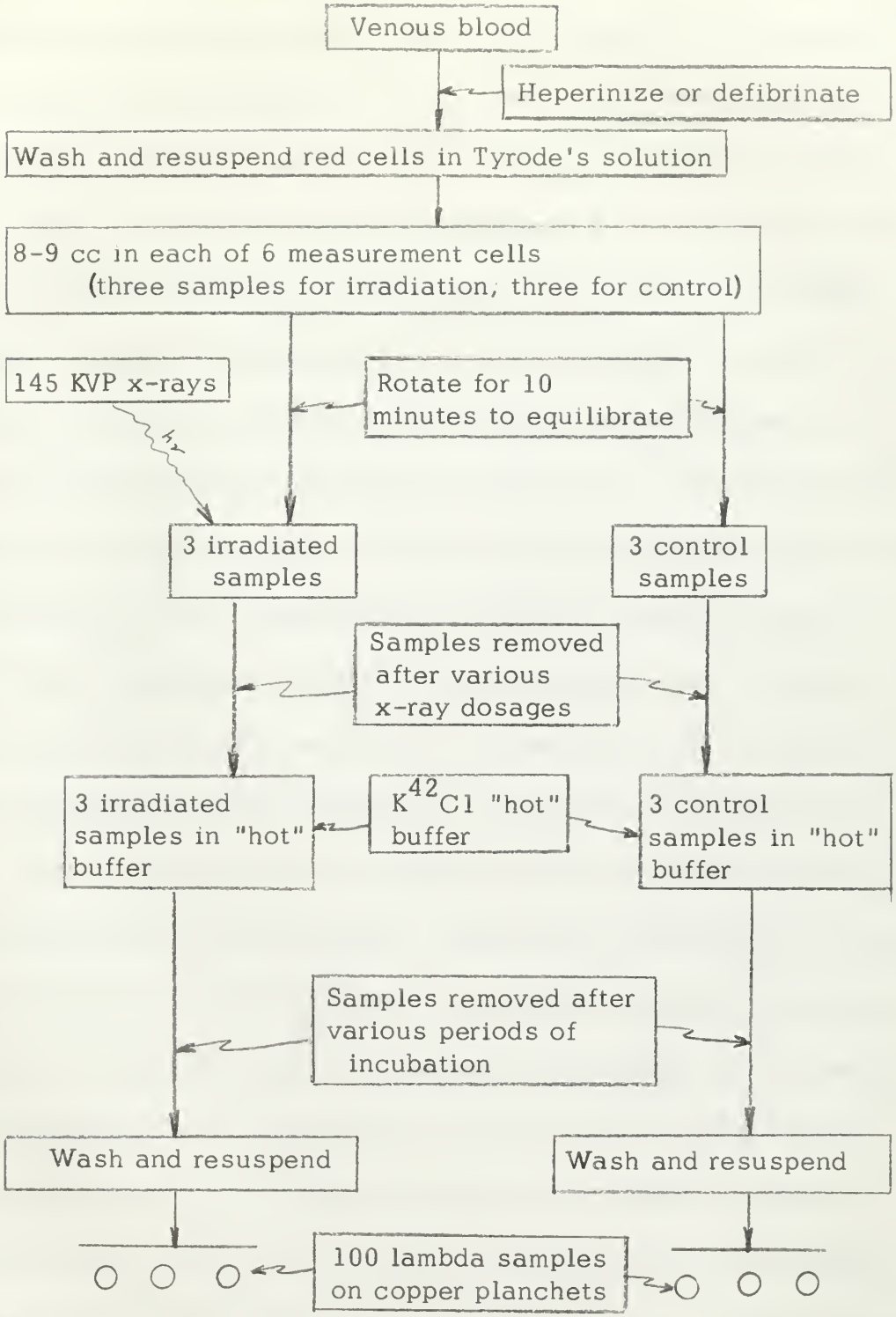


Figure 6. Flow Chart of Experimental Procedure

essentially as described above. After the test samples had received a dose of 2000r, approximately 0.5 ml was taken from each irradiated and control cell. Additional samples were taken after 5,000r and 10,000r. Two hundred lambda of each sample was pipetted into a small test tube with 1.5 ml of Tyrode's buffer made with $K^{42}Cl$ instead of the stable isotope. The radioactive potassium was obtained from Oak Ridge National Laboratory in the form of KCl in HCl, and had a specific activity of approximately 80 mc/gm K at time of use. Hematocrits were determined on the samples from each of the cells so that the exact volume of packed cells finally deposited on the counting planchets could be calculated. Agitation and aeration during incubation was sufficient to prevent the red cells from settling in the tubes. At several intervals during incubation, ranging from less than four to nine and one-half hours, approximately 0.15 ml of each sample was placed in capillary tubes of about 2mm inside diameter. These were centrifuged at 10,000 g for 10 minutes in a hematocrit centrifuge modified to handle these large bore tubes. The supernatant was removed and discarded and the cells were resuspended in non-radioactive Tyrode's and centrifuged again. The washed red cells were then resuspended to a known volume in distilled water, and a portion from each irradiated and control sample was set aside for later determination of total potassium. 100 λ aliquots were pipetted onto copper planchets. After evaporation, these were counted at a rate of about 100 cps for a total of 10^4 counts. Each sample was counted several times for consistancy. The counting rates were

corrected for coincidence losses, background (0.43 cps in shield) and efficiency, and were back decayed to an arbitrary zero time, taken for convenience as the time of counting of the first sample.

The last experiment was designed to observe the effects of 10,000 r of radiation on the efflux of potassium from erythrocytes. Because of the long incubation periods involved in this experiment, it was necessary to take special measures to maintain the samples. For this reason centrifugation and resuspension in Tyrode's prior to irradiation was omitted. Thirty ml. of blood was drawn from the donor, defibrinated, and placed directly into two lucite cells. (These were the same cells used in the impedance experiments, but with the platinum electrodes removed.) The samples were irradiated with 145 KVP x-rays as before for a total dose of 10,000 roentgen. However, for this experiment, the apparatus was modified to permit the samples to be rotated at a mean distance of about 25 cm. from the anode of the x-ray tube, giving a dose rate of approximately 160 r/min. As before, continuous rotation of the samples and circulation of oxygenated Tyrode's was carried on during the irradiation. After the irradiation, 5 2 ml. specimens were withdrawn from the x-rayed and control samples, and each was resuspended in 60.0 ml. of Tyrode's solution made up with K^{42} replacing the stable potassium. These suspensions in 300 ml. paraffin coated ehrlenmeyer flasks, were incubated in a covered water bath at 37°C. for a period of ten hours. Mixing and aeration was assured by attaching the flasks to an apparatus which provided a continuous gentle shaking. At the fifth and tenth hours of

incubation, specimens were drawn for determination of K^{42} uptake in the manner previously described. After ten hours of incubation, the samples were washed five times in non-radioactive Tyrode's and re-suspended in a packed cell to buffer ratio of 1-5. The samples were then incubated as before in the 37°C. water bath. At two hour intervals during this elution period, 0.1 ml. samples were taken from both the irradiated and control suspensions and centrifuged. Counting samples were prepared by placing 100 lambda of the supernatant on copper planchets. The water was then slowly evaporated to dryness under infra-red lamps. Counting rates were determined and corrected as for the uptake experiment.

4. Results and Discussion.

The measured parallel resistance and capacitance were converted to the equivalent series values using appropriate formulae (Appendix I (a)). Figures 7 and 8 are plots of this series impedance after various x-ray doses for an irradiated sample and the corresponding control. These are typical of the eighteen samples measured. The slight translation of the curves to the left would occur if there were a decrease in the total packed cell concentration. This would occur due to hemolysis, caused by cell shrinkage due to osmotic effects or an increase on liquid volume. Since the same translation is apparent in the controls as in the irradiated samples, this cannot be attributed to radiation damage. Figure 9 shows the results of plotting the mean values of the three irradiated and three controls of an experiment in which a single dose of 10,000 r was given. The same translation of the curves to the left with time is apparent here.

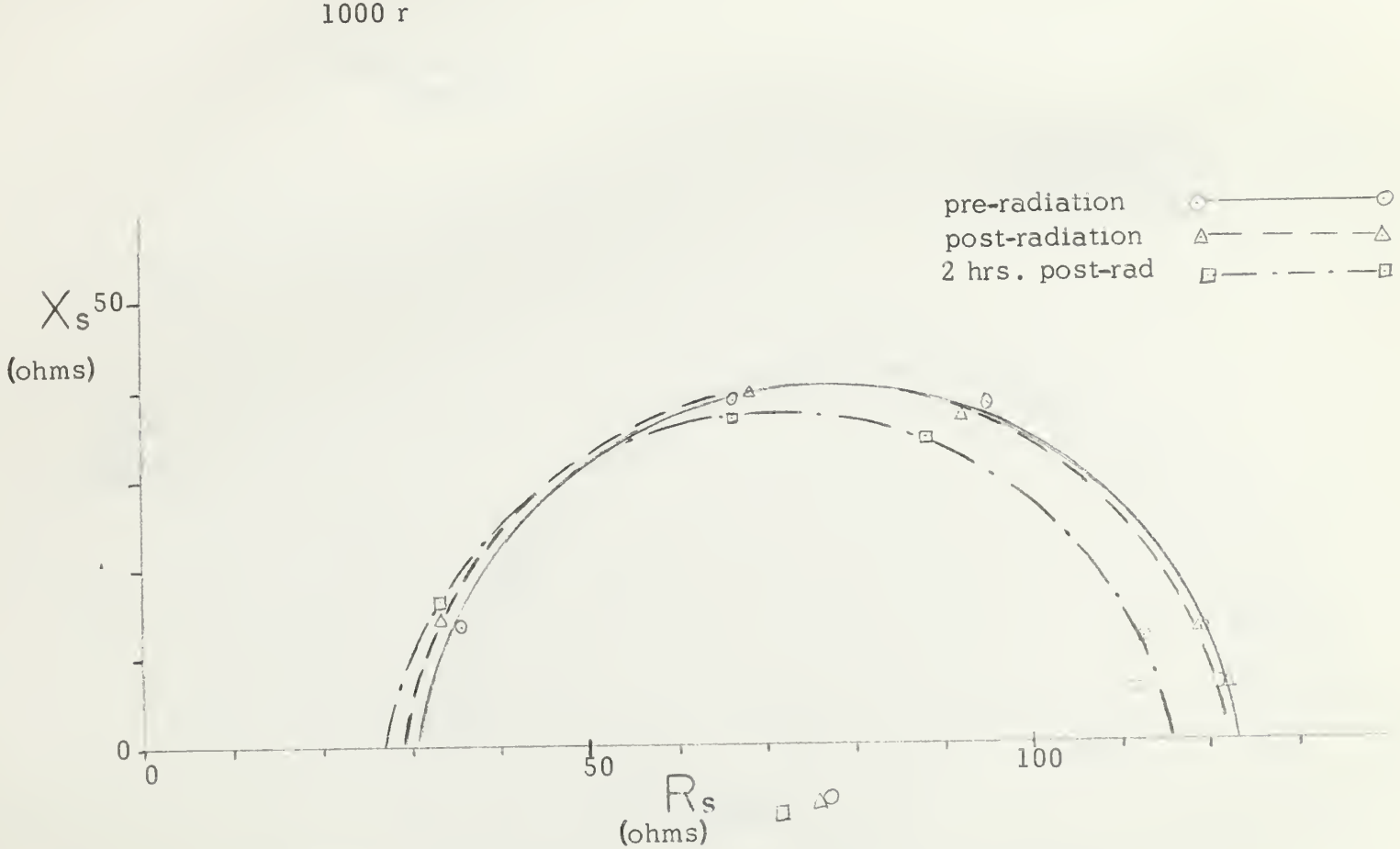


Figure 7. Series Impedance Locus of a Typical Irradiated Before and After 10,000 r of X-Irradiation

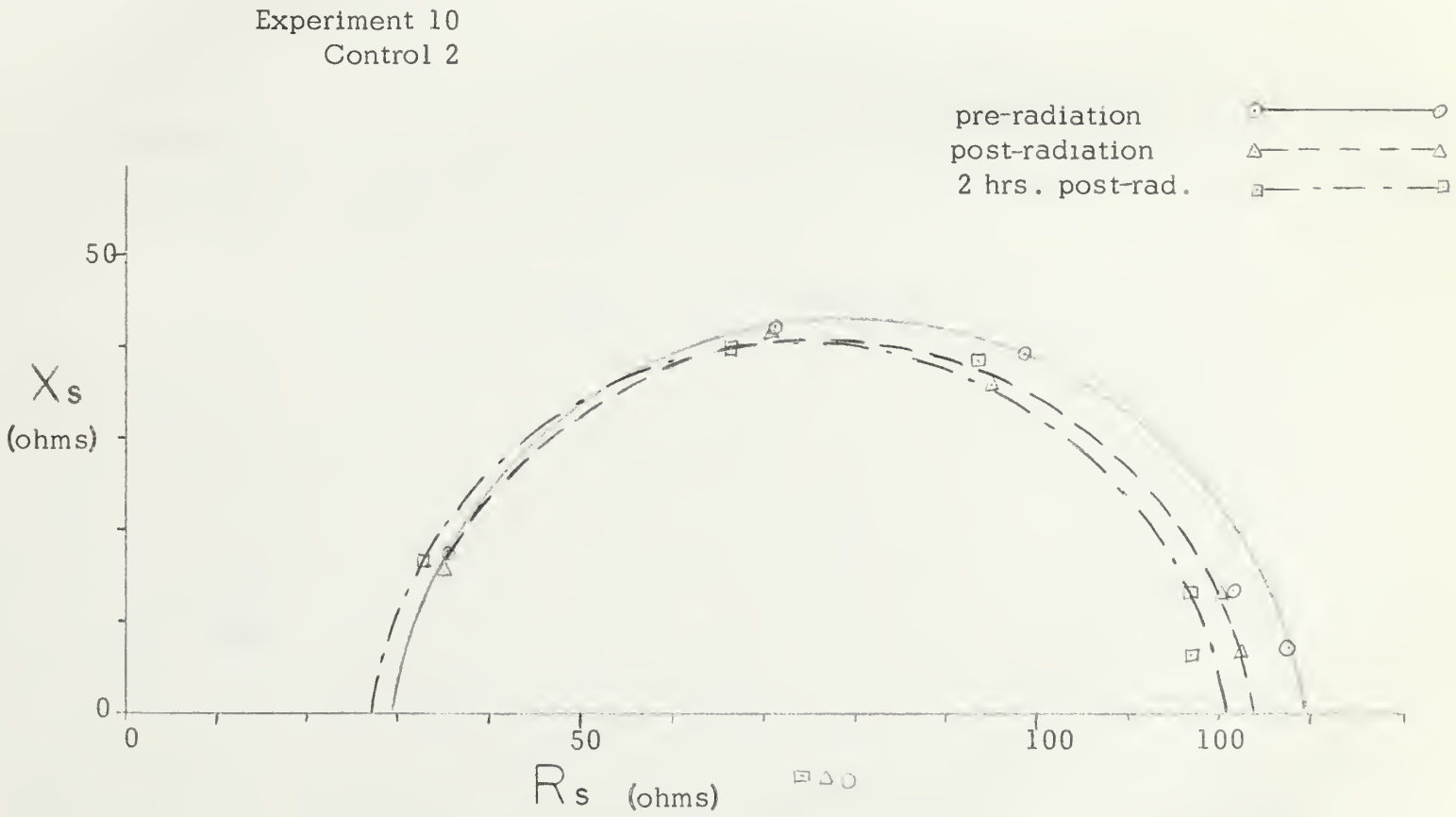


Figure 8. Series Impedance Locus of a Typical Unirradiated Control Sample Measured at Times Corresponding to Those of the X-Irradiated Sample of Figure 7.

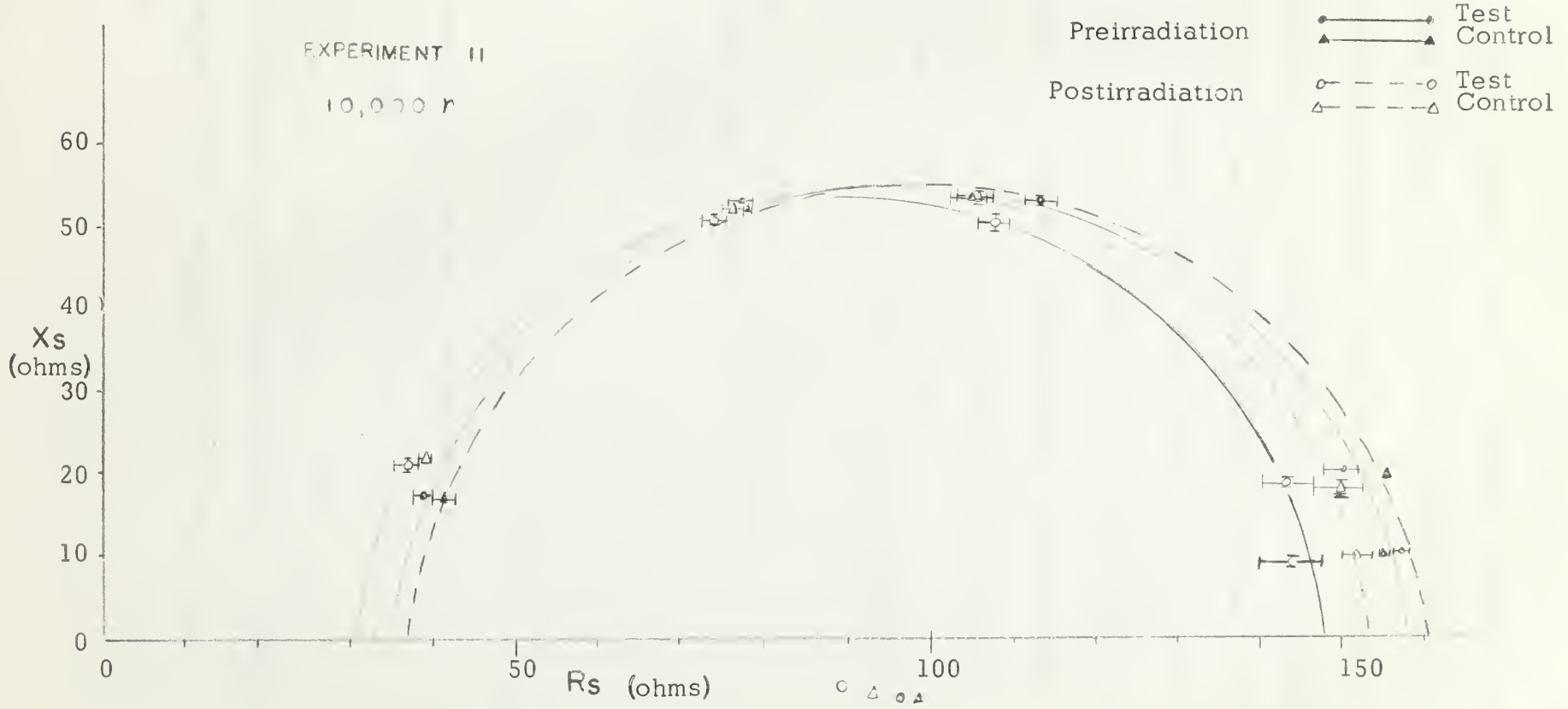


Figure 9. Series Impedance Locus on Irradiated Sample and Irradiated Control for One X-Irradiation of 10,000 r. (Mean Values of Six Samples).

It seems reasonable to expect that, had there been a net radiation effect, it would have manifested itself by a decreased electrical resistance of the cellular membrane. Assuming the suspension of cells to be represented by the circuit of Figure 2 (b), it is seen that this effect amounts to a decrease in R_3. To compare such a decrease in membrane resistance with the experimental data as displayed in Figures 7-a, we might plot the series impedance locus of a suspension of red cells, as represented by the circuit of Figure 2(b), for a particular value of the membrane resistance (R_3) and for a 10% decrease in this resistance. To do this, the circuit of 2 (b) is first converted to the equivalent series circuit (Appendix I(d)). The results are:

$$(a)\quad \frac{R_s(\omega C_s)^2}{1+(\omega R_s C_s)^2} = \frac{(R_2+R_3+R_4)(R_2+R_3)+R_2(R_2+R_4)(\omega C_2 R_3)^2}{R_4[(R_2+R_3)^2+(\omega C_2 R_2 R_3)^2]}$$

$$(b)\quad \frac{C_s}{1+(\omega R_s C_s)^2} = \frac{C_2 R_3^2}{(R_2+R_3)^2+(\omega C_2 R_2 R_3)^2}$$

For $\omega = \infty$, these become, respectively:

$$(c)\quad R_s = \frac{R_2 R_4}{R_2+R_4}$$

$$(d)\quad C_s R_s^2 = C_2 R_2^2$$

From Figure 7, for $\omega=\infty$, $R_s = 33.7\,\Omega$. Also, taking Fricke's (11) value of 3.5 for the ratio R_2/R_4 for red blood cells, we have from (c):

$$33.7 = \frac{3.5\,R_4^2}{R_4(4.5)}$$

or, $R_4 = 43.3\,\Omega$ and $R_2 = 3.5\,R_4 = 151.6\,\Omega$.

From (b) we see the expression to be independent of R_4. Therefore, as a first approximation, we may consider the simpler expressions (Appendix I (e)) =

(e) $C_s = \frac{1+(\omega C_2 R_3)^2}{\omega^2 C_2 R_3^2}$ $\qquad R_s = \frac{R_2(1+(\omega C_2 R_3)^2)+R_3}{1+(\omega C_2 R_3)^2}$

Now, for a 10% decrease in R_3, these become:

(f) $C_s' = \frac{1+0.81(\omega C_2 R_3)^2}{0.81(\omega C_2 R_3)^2}$ $\qquad R_s' = \frac{R_2(1+0.81(\omega C_2 R_3)^2)+0.9R_3}{1+0.81(\omega C_2 R_3)^2}$

And if initially we consider only the lower frequencies, the expressions are approximately equal to unity. In this case (f) becomes:

(g) $C_s' = \frac{1}{0.81(\omega C_2 R_3)^2}$ $\qquad R_s' = R_2 + 0.9\,R_s$

Then, the ratios of the original equivalent series values to those representing a 10% decrease in the membrane resistance, R_3 are approximately:

(h) $\frac{X_s'}{X_s} = 0.81, \quad \frac{R_s'}{R_s} = \frac{R_2+0.9R_3}{R_2+R_3} \simeq 0.9$ (since $R_3 >> R_2$)

We see that a change in R_3 will show itself to a lesser degree with increasing frequency. The results of this analysis are shown in Figure 10, the curve (a) representing the pre-irradiation curve of Figure 9 and the curve (b) the effects of a 10% decrease in the membrane resistance. Comparing this to Figure 9, and taking into account the translation of the entire curve to the left, it apparent that there seems to be no significant change in the membrane characteristics for irradiations up to 10,000 r.

The results of the potassium uptake experiments are shown in Figure 11. From this it appears that there is no significant effect on the uptake of potassium after x-ray dosages up to 10,000 r. Although the 5000 r data of experiment 14 does suggest an effect after 11 hours of incubation, the accumulated errors (hematocrit, volume measurements, etc.) are such as to render this result inconclusive. These essentially

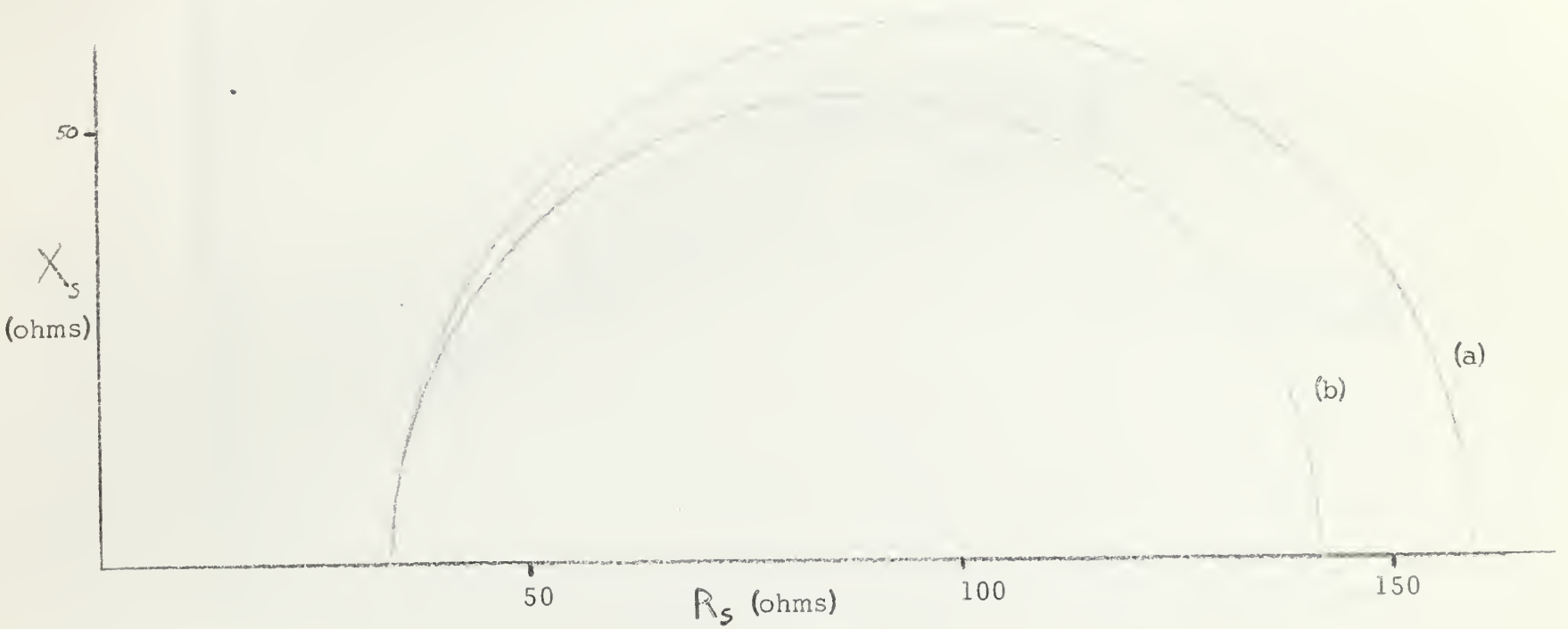


Figure 10. Series Impedance Locus of a Suspension of Red Blood Cells Comparison of Loci for (a) No Change, and (b) a 10% Decrease in Cell Membrane Resistance.

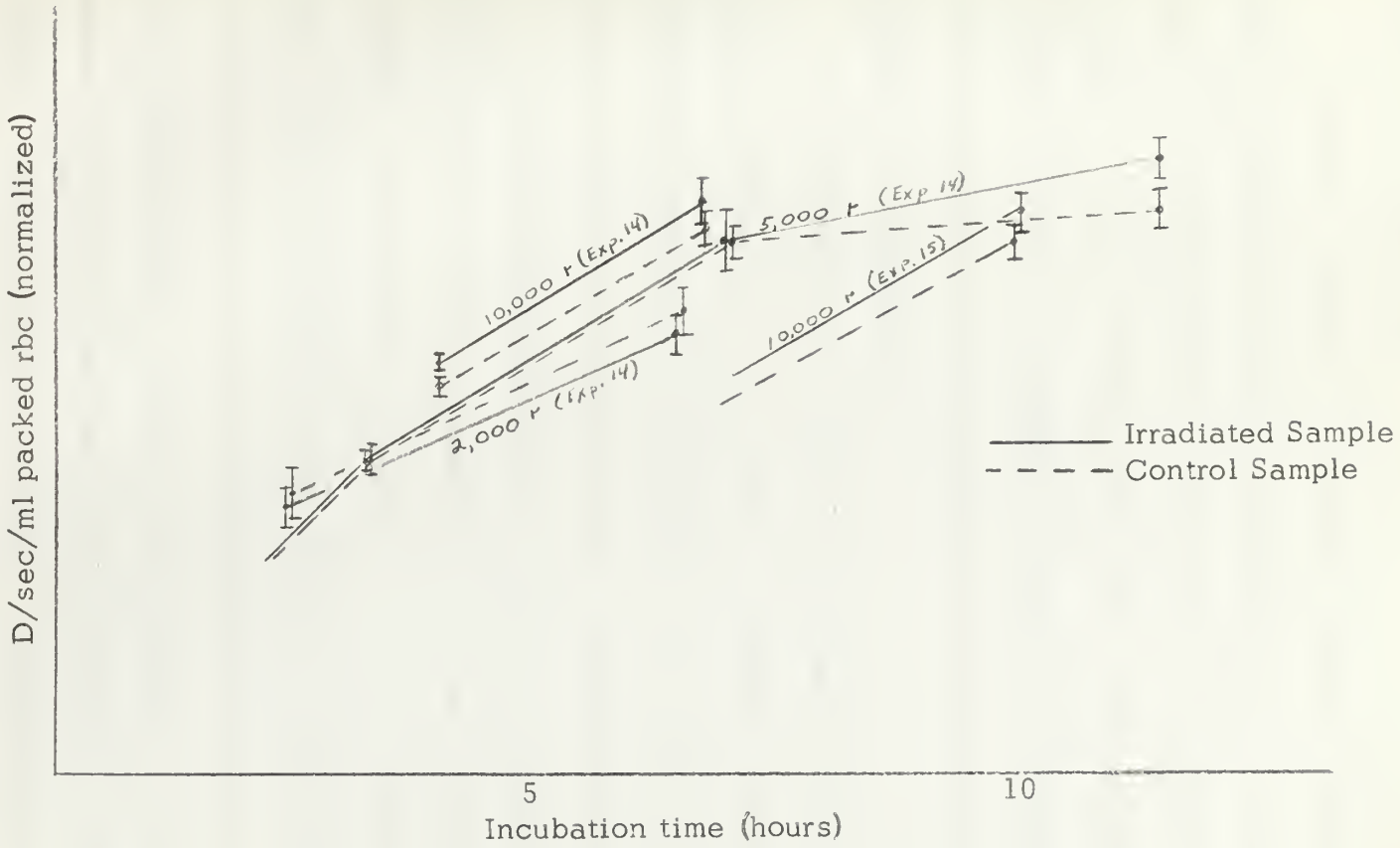


Figure 11. Potassium Uptake by Erythrocytes as a Function of Incubation Time after Various Doses of X-irradiation.

negative results for the effect of radiation on the rate of potassium uptake are in agreement with the findings of Sheppard and Beyl for human erythrocytes (19).

As shown in Figure 12, the rate of efflux of potassium from the red cells is significantly affected by a single dose of 10,000 r. The curve represents the total potassium efflux from the intracellular spaces as a function of time as obtained from activity measurements. The dotted extensions to the curves indicate the expected convergence of the activities as equilibrium is reached between the intracellular and extracellular K^{42} concentrations.

Summarizing then, 10,000r of x-irradiation 1) did not affect the physical characteristics of the red cell membrane as reflected by changes in electrical impedance of suspensions of the cells, and 2) resulted in an increase in potassium efflux without changing the uptake rate.

If we assume the existance of "pores" in the membrane, the absence of an observable change in impedance indicates no significant damage induced by the radiation to the pores, or change in the charges on the pores. This would appear to argue against an active transport mechanism within the membrane, since it seems reasonable that a change in resistance would accompany a change in the membrane. A sodium pump, if damaged to such an extent that the rate of potassium efflux would be effected, would be expected to "leak", and this should be reflected as a decrease in membrane resistance; however the observed increased potassium efflux, with no change in influx following irradiation is

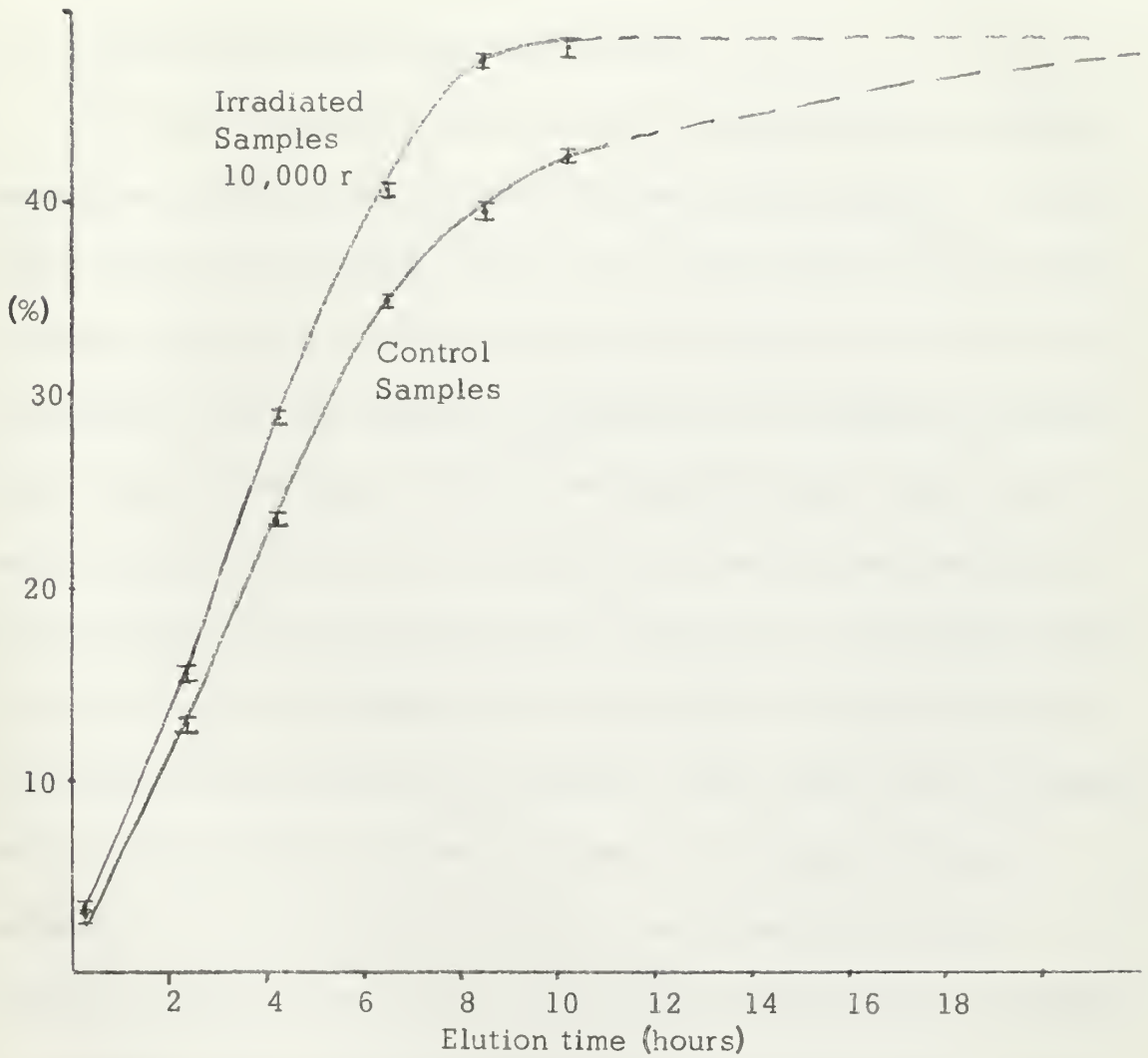


Figure 12. Percent of Intracellular Potassium Appearing in the Supernatant as a Function of Elution Time.

compatible with an active transport system involving a "sodium pump" or a method of "exchange diffusion" not involving an intact membrane, since radiation damage might be expected to decrease the activity of such a mechanism, and the net effect would be a trend toward equilibrium, i.e. a sodium influx and a potassium efflux.

Our results are also consistent with a radiosensitive intracellular mechanism for maintaining the potassium and sodium gradients. Ling's "fixed charge" hypothesis (21, 29) is such a mechanism. In this case, the cell membrane plays a passive role in ion transport and accumulation, so the lack of physical damage as indicated by our impedance measurements would be predicted. Further, any radiation effect which would compromise the accumulation of potassium by the intracellular protein would be expected to produce the results observed. This radiation effect could be due to direct damage of the intracellular "bonding" mechanism, or to disturbances of metabolic processes, which would lead to the same result. The generally observed phenomenon of increased membrane permeability as a non-specific reaction to anything which hinders metabolism (30) and the evidence that irradiation may result in inhibition of processes which depend upon ATP as an energy source (31), tends to support any hypothesis which is dependent upon an active mechanism involving the expenditure of energy derived from metabolism. Among such mechanisms may be included an active "exchange diffusion" or "ferry", a "sodium pump", or ionic accumulation by "fixed charges".

Bacq and Alexander (32) attribute similar radiation effects primarily to a release of enzymes, which in turn is considered to be a result of

the direct effect of the radiation on the external and internal cellular membranes. However, it is difficult to envisage a release of enzymes through a physically intact membrane, and it would appear that physical damage sufficient to allow such a release of enzymes would have resulted in an observable change in our impedance measurements. This argument, of course, is predicated upon the assumption that the erythrocyte membrane is representative of the internal membranes of all mammalian cells. Recent evidence, particularly electron microscope pictures (33), indicates that this a reasonable assumption.

In conclusion, up to 10,000 r of x-irradiation resulted in no physical damage to the cell membrane as reflected by changes in impedance measurements, but did show an increased potassium efflux from the erythrocytes. These findings are interesting since they may result from an ion transport mechanism which is not dependent upon the physical integrity of the membrane. If one assumes that the erythrocyte membrane is representative of all intracellular membranes of other cells, the lack of physical damage after high doses of radiation does not appear to be in agreement with an "enzyme release hypothesis" which attempts to explain cellular damage by the breaking down of cellular membranes.

BIBLIOGRAPHY

1. K. S. Cole and H. J. Curtis, "Wheatstone Bridge and Electrolytic Resistor for Impedance Measurements over a Wide Frequency Range", Rev. Sci. Instr., 8, 333, (1937).

2. J. A. Kolmer, "Clinical Diagnosis by Laboratory Examinations", D. Appleton-Century Co., Chapter 1, (1944).

3. A. K. Solomon, "Pores in the Cell Membrane", Sci. Am., 146, Dec. 1960.

4. R. B. Dean, T. R. Noonan, L. Haege, and W. O. Fenn, "Permeability of Erythrocytes to Radioactive Potassium", J. Gen. Physiol., 24, 353, (1941).

5. I.M. Glynn, "Sodium and Potassium Movements in Human Red Cells", J. Gen. Physiol., 134, 278, (1956).

6. A. K. Solomon, "Red Cell Membrane Structure and Ion Transport", J. Gen. Physiol., 43, (Supplement), 1-15, (1960).

7. K. S. Cole, "Electric Conductance of Biological Systems", Cold Spring Harbor Symposia 1, (1933).

8. K. S. Cole, "Electric Impedance of Suspensions of Spheres", J. Gen. Physiol., 12, 29, (1928).

9. K. S. Cole, "Electric Phase Angle of Cell Membranes", J. Gen. Physiol., 15, 640, (1932).

10. A. K. Solomon, "The Permeability of the Human Erythrocyte to Sodium and Potassium", J. Gen. Physiol., 36, 57, (1952).

11. H. Fricke and S. Morse, "The Electric Resistance and Capacity of Blood for Frequencies Between 800 and 4 1/2 Million Cycles", J. Gen. Physiol., 9, 153, (1925).

12. K. Hsu, Thesis, "Radiation Effect on the Permeability of Yeast Cells to Sodium and Potassium Ions", Univ. of Calif., (1959).

13. J. S. Robertson, "Theory and Use of Tracers in Determining Transfer Rates in Biological Systems", Physiol. Rev., 37, 133, (1957).

14. A. K. Solomon and G. L. Gold, "Potassium Transport in Human Erythrocytes: Evidence for a Three Compartment System", J. Gen. Physiol., 38, 371, (1955).

15. A. P. Harrison, Jr., A. K. Bruce, and G. E. Stapleton, "Influence of X-Irradiation on Potassium Retentivity by Escherichia Coli", Pro. Soc. Exp. Biol. Med., 98, 740, (1958).

16. A. K. Bruce and J. N. Stannard, "The Effect of X-Irradiation Upon Potassium Retentivity of Yeast", J. Cell. Comp. Physiol., 51, 325, (1958).

17. R. E. Eckel, "Potassium Exchange in Human Erythrocytes. General Aspects of the Fluoride Effect", J. Cell. Comp. Physiol., 51, 81, (1958).

18. C. W. Sheppard and W. R. Martin, "Cation Exchange Between Cells and Plasma of Mammalian Blood. Methods and Applications to Potassium Exchange in Human Blood", J. Gen. Physiol., 33, 703, (1950).

19. C. E. Sheppard and G. E. Beyl, "Cation Exchange in Mammalian Erythrocytes. 3. The Prolytic Effect of X-rays on Human Cells", J. Gen. Physiol., 34, 691, (1951).

20. C. W. Sheppard and M. Stewart, "The Direct Effect of Radiations on Erythrocytes", J. Gen. Physiol., 39, Suppl. 2:179, (1952).

21. G. N. Ling, "Muscle Electrolytes", Am. J. Phys. Med., 34, 89, (1955).

22. M. Phillipson, Bull. Acad. Roy. Belgique, Cl. Sci., 7, 387, (1921).

23. M. Lessler, "Low-Level X-ray Damage to Amphibian Erythrocytes", Science, 129, 1551, (1959).

24. J. W. Raker, I. M. Taylor, J. M. Weller, and A. B. Hastings, "Rate of Potassium Exchange of the Human Erythrocyte", J. Gen. Physiol., 33, 691, (1950).

25. E. J. Conway and F. Kane, Biochem J., 28, 1769, (1934).

26. P. J. Boyle and E. J. Conway, Am. J. Physiol., 100, 1, (1941).

27. H. Levi and H. H. Ussing, Acta. Physiol. Scandinav., 16, 232, (1948).

28. E. J. Conway, Science, 113, 270, (1951).

29. G. N. Ling, "Symposium on Phosphorous Metabolism", 748, Johns Hopkins Press, Baltimore, (1952).

30. Z. M. Bacq and P. Alexander, "Fundamentals of Radiobiology", 2nd Ed., Pergamon Press, New York, (1961), p. 341.

31. Ibid., p. 315.

32. Ibid., p. 270.

33. J. David Robertson, "The Membrane of the Living Cell", Sci., Am., 65, April 1962.

34. F. H. Johnson, H. Eyring, and M. J. Polissar, "The Kinetic Basis of Molecular Biology", John Wiley & Sons., New York., (1954), Ch. 11.

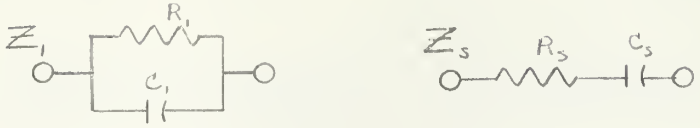
35. A. Krogh, Proc. Roy. Soc., London s. B., 133, 140, (1956).

36. R. Dean, Biol. Symp., 3, 331, (1941)

37. A. L. Hodgkin, Biol. Rev., 26, 339-409, (1951).

38. A. K. Bruce and J. N. Stannard, "The Effect of Temperature on Potassium Transfer in Irradiated Yeast", J. Cell. and Comp. Physiol., 52, 165-176, (1958).

APPENDIX I
CONVERSION FORMULAS FOR SIMPLE NETWORKS

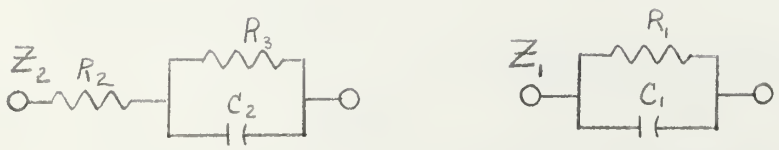
(a) 

$$Z_1 = \frac{1}{\frac{1}{R_1} - \frac{\omega C_1}{j}} = \frac{R_1}{1+(\omega C_1 R_1)^2} - \frac{j\,\omega C_1 R_1^2}{1+(\omega C_1 R_1)^2}$$

$$Z_s = R_s + X_s = R_s - \frac{j}{\omega C_s}$$

Setting $Z_1 = Z_s$:

$$R_s = \frac{R_1}{1+(\omega C_1 R_1)^2} \qquad X_s = \frac{\omega C_1 R_1^2}{1+(\omega C_1 R_1)^2}$$

(b) 

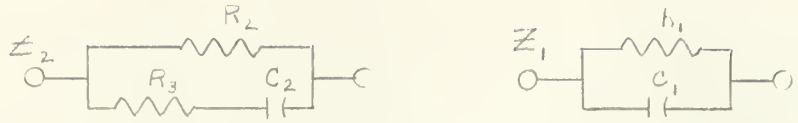
$$Z_2 = R_2 + \frac{R_3}{1+j\omega C_2 R_3} = \frac{(R_2+R_3)+j\omega C_2 R_2 R_3}{1+j\omega C_2 R_3}$$

$$\frac{1}{Z_2} = \frac{(R_2+R_3)+R_2(\omega C_2 R_3)^2}{(R_2+R_3)^2+(\omega C_2 R_2 R_3)^2} + j\,\frac{(\omega C_2 R_3(R_2+R_3)-\omega C_2 R_2 R_3)}{(R_2+R_3)^2+(\omega C_2 R_2 R_3)^2}$$

$$\frac{1}{Z_1} = \frac{1}{R_1} + j\omega C_1$$

Setting $\frac{1}{Z_2} = \frac{1}{Z_1}$:

$$R_1 = \frac{(R_2+R_3)^2+(R_2\omega C_2 R_3)^2}{(R_2+R_3)+(\omega R_2 R_3)^2 R_2}, \qquad C_1 = \frac{C_2 R_3^2}{(R_2+R_3)^2+(R_2\omega C_2 R_3)^2}$$

(c) 

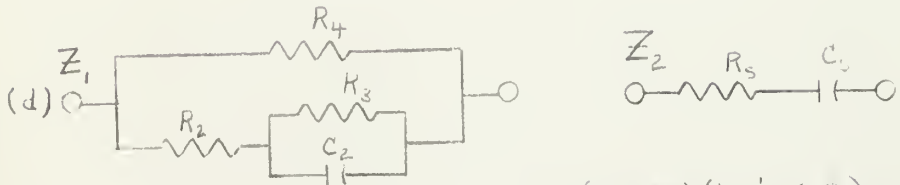
$$\frac{1}{Z_2} = \frac{1}{R_2} + \frac{1}{R_3 - \frac{j}{\omega C_2}} = \frac{1}{R_2} + \frac{R_3 + \frac{j}{\omega C_2}}{R_3^2 + \frac{1}{(\omega C_2)^2}}$$

$$= \left[\frac{1}{R_2} + \frac{1}{R_3 + \frac{1}{(\omega C_2)^2 R_2}}\right] + j\left[\frac{\omega C_2}{1 + (\omega C_2 R_3)^2}\right]$$

$$\frac{1}{Z_1} = \frac{1}{R_1} + j\omega C_1$$

Setting $\frac{1}{Z_1} = \frac{1}{Z_2}$:

$$\frac{1}{R_1} = \frac{1}{R_2} + \frac{R_2(\omega C_2)^2}{1 + (\omega C_2 R_2)^2}, \quad C_1 = \frac{C_2}{1 + (\omega C_2 R_2)^2}$$

(d) 

$$\frac{1}{Z_1} = \frac{1}{R_4} + \frac{1}{R_2 + \frac{R_3}{1 + j\omega C_2 R_3}} = \frac{(R_2 + R_4)(1 + j\omega C_2 R_3) + R_3}{R_4[R_2 + R_3 + j\omega C_2 R_2(R_3)]}$$

$$= \frac{[R_4(R_2 + R_3)(R_2 + R_3 + R_4) + R_2 R_4(R_2 + R_4)(\omega C_2 R_3)^2] + j[R_4(R_2 + R_4)(R_2 + R_3)\omega C_2 R_3 - R_2 R_4(\omega C_2 R_3)(R_2 + R_4)]}{R_4^2(R_2 + R_3)^2 + (\omega C_2 R_2 R_3 R_4)^2}$$

$$\frac{1}{Z_2} = \frac{1}{R_5 - \frac{j}{\omega C_5}} = \frac{R_5(\omega C_5)^2 + j\omega C_5}{1 + (\omega C_5 R_5)^2}$$

Setting $\frac{1}{Z_1} = \frac{1}{Z_2}$:

$$\frac{R_5(\omega C_5)^2}{1 + (\omega C_5 R_5)^2} = \frac{(R_2 + R_3)(R_2 + R_3 + R_4) + R_2(R_2 + R_4)(\omega C_2 R_3)^2}{R_4[(R_2 + R_3)^2 + (\omega C_2 R_2 R_3)^2]}$$

$$\frac{C_5}{1 + (\omega C_5 R_5)^2} = \frac{C_2 R_3^2}{(R_2 + R_3)^2 + (\omega C_2 R_2 R_3)^2}$$