A METHOD FOR THE ESTIMATION OF LEUKOCYTE POTASSIUM

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ABSTRACT

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by Gary Hammerberg

In an attempt to eliminate the technical difficulty of many of the current procedures for the evaluation of intracellular potassium as an index of total body potassium a reliable procedure was sought. Leukocytes were chosen for study because they are nucleated and have greater metabolic activity than erythrocytes, and therefore might more accurately reflect the status of the body stores of intracellular potassium.

This project resulted in the development of a reliable method for the analysis of leukocyte intracellular potassium. Analyses for leukocyte potassium can be carried out in duplicate with as little as 20 ml. of heparinized whole blood from an individual with a normal leukocyte count.

The potassium concentration in leukocytes obtained from normal subjects with normal leukocyte counts was 86.4 ± 9.6 mEq of potassium per liter of packed leukocytes.

The isolation of leukocytes was accomplished with a 3% dextran solution for rapid sedimentation of the erythrocytes; this provided an ample specimen of viable cells.

The method of cytolysis for removing the intracellular potassium yielded results comparable to ultrasonic disruption.

The concept of volume measurement, that is measuring the volume of the cells used in the test procedure in milliliters, was introduced and it proved to be an efficient technique for cell quantitation.

A METHOD FOR THE ESTIMATION OF LEUKOCYTE POTASSIUM

By

Gary Hammerberg

A THESIS

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For Carol

and the kids

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INTRODUCTION

The purpose of this study was to devise a rapid, practical method for estimating potassium concentrations in leukocytes. In clinical disturbances of water and electrolyte balance profound alterations of intracellular potassium may take place which are not reflected in changes in serum potassium. The largest proportion of body tissue mass is composed of skeletal muscle and, ideally, direct measurements of muscle potassium content would be the most useful. While measurements of muscle potassium are possible with experimental animals, the bases of objections to multiple assays on human patients are obvious. Although intracellular potassium is known to vary between different tissues and cell types, changes in various tissues occur more or less in parallel (Corsa, et al., 1950). Thus, theoretically, almost any cell type available for analysis should be preferable to serum potassium determinations. Erythrocyte potassium levels have been studied, and the results indicate that their response to changes in total body potassium are too slow to be reliable. When tissue potassium changes occur slowly over prolonged time intervals, erythrocytes may be expected to yield fairly accurate reflections of total tissue potassium. However, for following acute changes or responses to therapy, the results of Corsa, et al., indicate that the equilibration lag is too long when erythrocytes

are used as indicator.

The essential notion involved in the investigations to be reported here are that leukocytes, being nucleated and actively metabolizing cells, could be expected to respond to potassium changes more closely than erythrocytes. The technical difficulties are related to the relatively small number of leukocytes than erythrocytes in the circulation. Although procedures have been devised for measuring leukocyte potassium (Baron and Roberts, 1963), these procedures are too time consuming to be of much practical use in clinical studies.

The procedure developed in these studies involved sedimentation and differential centrifugation of cells suspended in dextran. This permitted separation of leukocytes from erythrocytes and other blood components.

REVIEW OF LITERATURE

The concentration of intracellular potassium averages about twenty times that of plasma potassium, and there is a 3:2 ratio of extracellular sodium to intracellular potassium (Hoffman, 1964). The steady state that exists between a high intracellular and low extracellular potassium concentration is maintained by metabolic energies of the cell and slow permeability to sodium and potassium ions (White, Handler and Smith, 1968). Changes in intracellular potassium concentration were reported by Follis (1958) to follow (1) decreased intake as in experimental restriction and starvation, (2) excessive loss via the kidneys, as in diuresis, acidosis, renal disease and hormonal effects, (3) loss from the gastrointestinal tract as in vomiting and diarrhea and (4) uptake or loss from cells corresponding to synthesis or breakdown of glycogen.

Total Body Potassium.

A rapid, accurate estimation of total body intracellular potassium would reveal the degree of potassium depletion of a patient and serve as a guide to potassium replacement therapy.

Measurements of plasma potassium concentration reflect the concentration in extracellular fluid, but do not reflect intracellular concentrations. Black (1957) has summarized the evidence that normal plasma potassium levels may occur with decreased

intracellular levels. With diabetic acidosis several factors contribute to depletion of intracellular potassium. Breakdown of glycogen stores, particularly in the liver, liberate potassium which is now free to diffuse out of the cell. As part of the effect produced by acidosis, the dynamic exchange of potassium and hydrogen ions for sodium ions across cell membranes promotes movement of potassium out of the cells into the extravascular fluids. The increase in potassium concentration in the plasma is rapidly corrected by renal losses accompanying the osmotic diuresis produced by hyperglycemia. Furthermore, even in the absence of such diuresis, it has been shown (Elkinton and Danowski, 1955) that renal clearance of potassium is unrelated to intracellular potassium content, and therefore any increase in plasma potassium concentration is inevitably accompanied by increased excretion in the urine. Vomiting which frequently occurs during diabetic acidosis serves to further aggravate losses of potassium from the body.

Potassium Assay by Isotope Dilution.

Many attempts have been made to evaluate intracellular potassium levels. These include erythrocyte and muscle analysis and isotope dilution techniques. Isotope dilution studies reported by Corsa, et al. (1950) require expensive instrumentation, and the 12.4 hour half-life of the isotope of potassium makes it impractical to maintain a supply in smaller institutions. The procedure itself is technically difficult, although it does yield good results.

Uniform distribution of isotype is essential to estimating total body potassium by isotope dilution, but equilibration is slow, being no more than 85% complete in 24 hours. Equilibration is even slower in the presence of edema or circulatory disturbances. Contamination of the potassium isotope by radio-sodium is another source of error (Kerman, 1965).

Muscle Biopsy Analysis.

Barnes, Gordon, and Cope (1957) described an improved procedure for skeletal muscle potassium analysis. Intracellular levels of muscle potassium would probably provide the most useful information regarding a patient's potassium status, as muscle mass makes up approximately 40% of body tissue and operates vital functions such as respiration and cardiac activity. However, muscle biopsy will never become a popular routine procedure. The procedure is long, and the specimens are contaminated with fat and blood which is difficult to remove before analysis. Biopsies are associated with prolonged discomfort, and since muscle tissue does not regenerate, the material available is limited.

A question that arises about muscle biopsy is how representative is the sample to the mean muscle potassium concentration. Flear (1962) reported good statistical correlation between random muscle specimens in rats, but in a later report Flear and Florence (1963) found this is not necessarily true for man because the weight-to-weight relationship of muscle biopsy to total tissue is entirely different than that of the rat. He concluded that the

fat tissue and interstitial fluid must be taken into consideration when analyzing muscle specimens. The coefficient of variation for a single potassium analysis could be reduced from 8 to 5 percent if the simultaneous variations of sodium and potassium are considered in the samples analyzed.

Erythrocyte Analysis.

Kerr (1937) reported erythrocyte potassium values of 108 mM of potassium per liter of red blood cells for pigs and 90 to 100 mM of potassium per liter of red blood cells for rats and rabbits. Sheppard, Martin and Beyl (1951) measured potassium levels in human erythrocytes and found 95 mM per liter of red blood cells. But Corsa, et al. (1950) showed with isotope studies that erythrocytes lag behind muscle and other tissues in the loss of potassium in the process of a developing hypokalemia, thus making the erythrocyte a poor specimen for analysis in evaluation of total body intracellular potassium.

Leukocyte Analysis.

Shapiro and Parpart (1937) were among the first workers to measure the ionic concentrations in leukocytes, from peritoneal washing in rabbits. Deane and Smith (1951) reported intracellular potassium values of 112 mEq per liter of intracellular water and 49 mEq per kg. of cell mass with a range of 40 to 56 mEq. Manery (1949) determined potassium, sodium, and chloride contents of rabbit leukocytes. Hempling (1954) and Wilson (1949) studied the potassium and sodium mobility in rabbit leukocytes. They

concluded that leukocytes, like erythrocytes, will lose potassium and gain sodium at 2 C and regain the loss in a reciprocal fashion at 36 C.

It was also noted that glucose in high concentrations slowed the rate of potassium loss after isolation. All this previous work on leukocytes had been done on cells isolated from intraperitoneal washings. Baron and Roberts (1963) introduced a technique of obtaining leukocytes from peripheral blood. Their procedure required 50 ml. of blood and approximately 48 hours to complete.

Isolation of Leukocytes.

Vallee, Hughes and Gibson (1947) and Tullis (1952) reported the early work done on isolation of leukocytes from whole blood. There are basically three techniques of removing leukocytes from peripheral blood: by flotation, sedimentation, and centrifugation (Skoog and Beck, 1956).

Valentine (1958), in an excellent compilation of many aspects of leukocyte isolation for biochemical studies, suggested selective erythrocyte sedimentation with either fibrinogen, dextran, or phytohemagglutinin. After sedimentation the leukocyte and platelet rich supernatant was removed and centrifuged at 800 rpm for 3 minutes. The plasma with most of the platelets is removed, and the remaining leukocytes resuspended in saline. Valentine also listed some of the methods used for disruption of the leukocyte wall. These include homogenization, surface acting agents, and osmotic forces. In conclusion, he mentioned that results of chemical analysis of cells

have been expressed by wet weight, dry weight, cell numbers and cell nitrogen. Humphrey and White (1960) claimed 100% lysis of leukocytes by repeated freezing and thawing.

Flotation Technique.

This method makes use of the different densities of erythrocytes (sp. gr. 1.092) and leukocytes (sp. gr. 1.065) in various chemical solutions. Vallee, Hughes, and Gibson (1947) used a serum albumin solution. After the albumin-blood mixture had been centrifuged the leukocytes were found at the plasma-red cell interface. The specific gravity of the solution of albumin is critical for good separation. The separated cells tend to clump and are difficult to work with. Spear (1948) used a gum acacia solution. The pH of this solution is hard to control and separation of leukocytes from it is difficult. Tullis (1952) described an elaborate setup in which blood from the patient is passed directly through a vessel containing an exchange resin which removes platelets and calcium, and then through a siliconized glass bowl containing a high-density solution which traps the plasma and leukocytes and allows the red blood cells to pass. This method requires a larger amount of blood and is expensive.

Sedimentation Method.

The principle of this procedure is to increase the erythrocyte sedimentation rate by adding substances to increase erythrocyte agglutination. Dextran and fibrinogen solutions and phytohemagglutinin are widely used presently in sedimentation techniques.

Minor and Burnett (1948) used fibrinogen solutions added to anticoagulated blood and allowed it to sediment, leaving the leukocytes in the fibrinogen-plasma supernatant. Li and Osgood (1949) separated leukocytes using phytohemagglutinin to increase the sedimentation rate of erythrocytes. Leukocyte yields by this method are extremely variable. Baron and Roberts (1963) described a procedure for dextran sedimentation in which the blood is first defibrinated with glass beads. Dextran, an inert chemical that does not alter the physiological environment of the cells, is the most popular of the sedimentation agents.

Centrifugation Technique.

These procedures involve centrifugation of an anticoagulated whole blood specimen, using various special glassware and apparatus. Butler and Cushman (1940) used a specially designed constricted centrifuge tube. Ottesen (1955) described a method using a tube containing a specially designed plastic body with a capillary tube in its axis. The specific gravity of the specimen was adjusted so that after centrifugation the leukocytes were within the capillary tube. Jago (1956) centrifuged heparinized blood immediately in a 15 ml. centrifuge tube and pipetted off the white cell layer from under the plasma. This procedure was used for the isolation of lymphocytes only.

Centrifugation methods are the easiest. There are no reagents except the anticoagulant. The speed and time of centrifugation are the only critical conditions of the procedure. The yields are poor

and the erythrocyte contamination is high when compared to the sedimentation procedures.

MATERIALS AND METHODS

Devising a procedure for the analysis of intracellular leukocyte potassium involved the solving of three separate problems which are reported as three experiments.

Experiment A.

The initial problem that had to be overcome in this project was the isolation of enough leukocytes for analysis. In the first attempt to isolate enough leukocytes the leukocyte layer from 10 ml. of whole blood was centrifuged in calibrated Wintrobe tubes. The leukocytes form a superficial layer (buffy coat). The volume of white blood cells was recorded and this layer with a small amount of the underlying red cells was removed and analyzed for potassium. The potassium from the red blood cells was taken into consideration by determining the amount of potassium per gram of hemoglobin, and then measuring hemoglobin concentrations on the sample containing the leukocytes, it was then possible to calculate the net amount of potassium from leukocytes. This method was discarded, because the volume of leukocytes isolated was insufficient for accurate potassium analysis.

A sample large enough to be analyzed was obtained from 20 ml. of blood by rapid sedimentation of erythrocytes with dextran.

To prevent loss or gain of potassium by the cells during isolation, the pH and electrolyte concentration of the suspending medium was maintained as similar to plasma as possible (see Appendix I). In addition, a high glucose concentration (200 mg./ 100 ml.) was maintained in order to minimize the exchange of ions across the cell membrane. It was not possible to prevent the exchange of ions completely, but since the exchange rate was approximately 2.0 (mM.) per hour and the isolation of the leukocytes required no more than 30 minutes, the maximum error was less than 2%.

The second problem was to analyze the isolated leukocytes.

Experiment B.

The direct aspiration of the leukocyte suspension for potassium analysis was tried, but the cells clumped and plugged the aspirating tube on the flame photometer. Lysis and liberation of potassium from the isolated leukocytes then became another problem to overcome. Nitric acid digestion was tried and found to be too time-consuming. The method of acid precipitation with trichloroacetic acid was also performed and the results were lower than those obtained with ultrasonic treatment or cytolysis. It was believed that the potassium was being complexed and trapped in the precipitate. Ultrasonic disruption provided results similar to those obtained with cytolysis. The method of cytolysis was chosen because it was less involved than preparing the sample for ultrasonic treatment.

Experiment C.

Relating mean leukocyte potassium concentration to intracellular potassium per liter of cell water was the last problem and it was accomplished by calculation. Work previously done has shown that the water content of leukocytes is very close to 80%. Considering this the results obtained from this study compared favorably with other data reported in the literature. For example, a specimen of 0.04 ml. cells was analyzed and the result found to be 86 mEq potassium/liter of cells, of that 0.04 ml. packed cells only 80% is cell water. This then became 0.032 ml. of cell water. When this new volume was considered in the calculations the resulting answer is 106.6 mEq of potassium/liter of cell water. Baron and Roberts (1963) reported 100.3 ± 6 mEq/liter of cell water.

Leukocyte Isolation

Potassium was analyzed in leukocytes isolated from peripheral blood from healthy young adults to determine normal values. Paired observations were performed for statistical analysis of the procedure.

Total leukocyte counts and differential counts were done on all samples. Twenty milliliters of blood were drawn in plastic syringes. The sample was immediately transferred to 50-ml. heparinized plastic centrifuge tubes and inverted gently to mix. A 3% dextran solution was added to the blood in a 2:1 dextran: blood ratio and the erythrocytes were allowed to sediment for 10 minutes. The supernatant plasma-dextran mixture, containing

leukocytes, platelets, and a few erythrocytes, was transferred to 50-ml. plastic centrifuge tubes and spun at 150 x g. for 5 minutes. The platelet-rich supernatant was discarded and the cell mass resuspended in a few drops of a balanced electrolyte solution with a high concentration of glucose and a pH of 7.40. The remaining erythrocytes were lysed by suspension in 1.8 ml. deionized water for 15 seconds. The ionic concentration was restored with a salt solution of 4 times the concentration of the balanced electrolyte solution. The specimen was then centrifuged at 500 x g. for 1 minute and the supernatant was discarded and the pure leukocyte button washed once in a 10 x 75-mm. siliconized tubes with the balanced electrolyte solution. The volume of packed cells was determined by centrifugation in a microhematocrit centrifuge in disposable micropipettes. The cells were resuspended in 1 ml. of distilled deionized water and a dilution factor calculated. The cell suspension was shaken periodically during a 30-minute period to remove the potassium from the cells. The cell suspension was then diluted with 0.02% sterox for potassium analysis with the Coleman Flame Photometer, Model 21. The flame photometer was adapted for microsampling by the method described by Warwick (1968). The direct reading scale was used and the instrument was standardized with potassium standards (see Appendix I). The mEq read from the scale were multiplied by the dilution factor for each sample and the results expressed in mEq of potassium per liter of packed leukocytes.

All glassware was siliconized or of unwettable plastic to increase the yield of leukocytes by preventing their sticking to untreated glass. All nonplastic material was siliconized (see Appendix I).

RESULTS

The results of Experiment A on the isolation of leukocytes are recorded in Table 1. It was found that dextran sedimentation provided a more efficient method for the isolation of leukocytes. The results of Experiment B on the comparison of the techniques for lysis and liberation of potassium from leukocytes are recorded in Table 2. Cytolysis was chosen as the easiest and most effective technique for releasing the intracellular potassium for analysis.

The results of the intracellular potassium analysis on leukocytes from normal human adults are recorded in Table 3. The mean leukocyte potassium was found to be 86.4 mEq potassium per liter of packed cells. Paired observations were made to determine the variation of the method and it was found that two standard deviations equaled 7.2 mEq potassium per liter of packed cells. The results of the paired data are recorded in Table 4.

Microscopic examination of the final preparation of the leukocytes prior to lysis showed that there were very few platelets and only a few erythrocyte ghosts present. The white blood cells were intact, and the ratio of polymorphonuclear to mononuclear leukocytes was the same as in the whole blood specimen prior to sedimentation.

Table 1.--Comparison of methods of isolating leukocytes from peripheral blood.

Trials	Centrifugation of the buffy coat from 10 ml. blood in Wintrobe tubes (ml. packed leukocytes)	Sedimentation of 20 ml. blood with 3% dextran solution (ml. packed leukocytes)
1	0.001 ml.	0.016
2	0.012	0.024
3	0.006	0.014
4	- *	0.019
5	-	0.073
6	-	0.056
7	0.005	0.058
8	0.002	0.047

* less than 0.001 ml.

Table 2.--Comparison of trichloroacetic acid (TCA) precipitation, nitric acid digestion, ultrasonic treatment, and cytolysis with distilled deionized water, for the lysis of leukocytes and liberation of potassium from them.

Trials	TCA ppt.	HNO_3 digestion	Ultrasonic	Cytolysis
1	30*	32	81	80
2	20	22	82	86
3	40	24	-	-

* mEq of potassium per liter of packed leukocytes

Table 3.--Intracellular potassium values for leukocytes from normal adults.

Sample	mEq of potassium per liter of packed cells
1	84.0
2	85.6
3	78.0
4	88.8
5	90.0
6	93.0
7	87.2
8	89.8
9	86.4
10	88.8
11	86.4
12	92.8
13	80.0
14	92.4
15	76.0
16	90.0
17	82.8
18	83.9

$\bar{x}$ = 86.4 9.6 (2 S.D.)

$s_{\bar{x}}$ = 0.267

s^2 = 23.2

s = 4.8

Table 4.--Paired observations for the analysis of the variance of the method.

Sample	X	X'
1	84.2*	86.0
2	84.5	87.9
3	94.3	88.8
4	80.0	85.6
5	92.7	89.0
6	71.0	71.2
7	86.0	88.1
8	76.9	72.5
9	94.8	96.6
10	88.2	88.0

$s^2 = 13.02$

$s = 3.6$

2 S.D. = 7.2

* mEq of potassium per liter of packed leukocytes

DISCUSSION

Procedures developed to date for the estimation of intracellular potassium are either inadequate for clinical evaluations or impractical for many institutions due to expense and technical difficulty. Plasma and erythrocyte potassium levels do not accurately reflect total body potassium values. Muscle biopsy analyses are impractical for repeated analysis, and the procedure is technically difficult. Isotope and metabolic intake and excretion studies probably provide the best information on total body potassium levels, but the procedure is long and requires expensive equipment.

Work done on leukocytes in the past have used cells isolated from peritoneal washings (Hempling,1954 and Wilson, 1946). The most recent work by Baron and Roberts (1963) used peripheral blood, but the test required 50 ml. of blood and 48 hours to complete which makes it nearly useless as a clinical diagnostic tool. A practical test for the evaluation of total body potassium must be short, easily performed, and must not require instrumentation difficult to use. The procedure developed here meets these requirements.

The leukocyte isolation was accomplished by rapid sedimentation of erythrocytes with dextran. Conrat, Chew and Lee (1964), Skoog and Beck (1955), and Baron and Roberts (1963) all reported the isolation of viable cells with this procedure. Microscopic

observation of the leukocytes after isolation revealed intact well preserved cells. Wilson (1946) in his work studied the effect of centrifugation on leukocytes and found that the process of centrifugation did not damage the cells.

The author experimented with many methods of disrupting the cell to release the intracellular potassium. Among these were nitric acid digestion, trichloroacetic acid precipitation, ultrasonic treatment, and cytolysis in distilled water. The method of cytolysis, whereby the ionic contents of the cell diffused into the deionized media, was chosen for this work because it was easily performed and provided good results. Hempling (1954) had compared this technique with ashing the specimen and found no significant differences in potassium values. The quantitative step of the analysis involves determining the packed cell volume by centrifugation in a high speed microhematocrit centrifuge. From this it is then possible to calculate the volume of cells used for analysis. This type of cell quantitation is similar to that used in determining the volume of erythrocytes for various clinical analyses (Maturen 1968).

The expression of results in terms of volume is a new concept in leukocyte analysis. Until now, all the workers have reported their results in terms of cell number, cell weight or by cell nitrogen. The technique of cell quantitation in this procedure works well and is quick and accurate.

With regard to permeability and movement of potassium from the cell after isolation, it has been established by Hempling (1954), Wilson (1949) and Streeter and Solomon (1954) that there is a constant exchange of ions in living cells. It is therefore most important to maintain optimal conditions for the preservation of the leukocytes *in vitro* to insure reliable results. The medium in which the cells are suspended must contain a balanced electrolyte content similar to plasma, have a pH from 7.30 to 7.40, and contain a glucose concentration of 200 mg./100 ml. The high glucose concentration has been demonstrated to slow the ionic exchange of sodium and potassium.

The results indicate that the leukocytes isolated from an individual with a normal differential leukocyte count have 86.4 9.6 mEq of potassium per liter of leukocytes. The evaluation of the procedure with the paired observations indicates that it is a reliable and reproducible method for the analysis of intracellular leukocyte potassium (2 S.D.=7.2 mEq potassium per liter of packed cells).

The results also indicate that the method is an accurate procedure. When the mean leukocyte concentration is converted to values of potassium per liter of cell water by considering that 80% of the cellular material is water, the results obtained compare well with values from past work.

The next step in this study would be the evaluation of potassium levels in leukocytes in individuals with electrolyte disturbances. Comparison of leukocyte potassium levels with

those of muscle, erythrocyte or isotope dilution studies would give an indication of the sensitivity of the analysis. The comparison of potassium values in patients with lymphocytosis and granulocytosis must be done to determine if there is a significant difference in the content of potassium between lymphocytes and granulocytes.

SUMMARY AND CONCLUSIONS

In an attempt to eliminate the technical difficulty of many of the current procedures for the evaluation of intracellular potassium as an index of total body potassium a reliable procedure was sought. Leukocytes were chosen for study because they are nucleated and have greater metabolic activity than erythrocytes, and therefore might more accurately reflect the status of the body stores of intracellular potassium.

This project resulted in the development of a reliable method for the analysis of leukocyte intracellular potassium. Analyses for leukocyte potassium can be carried out in duplicate with as little as 20 ml. of heparinized whole blood from an individual with a normal leukocyte count.

The potassium concentration in leukocytes obtained from normal subjects with normal leukocyte differentials counts was 86.4 9.6 mEq of potassium per liter of packed leukocytes.

The isolation of leukocytes was accomplished with a 3% dextran solution for rapid sedimentation of the erythrocytes; this provided an ample specimen of viable cells.

The method of cytolysis for removing the intracellular potassium yielded results comparable to ultrasonic disruption.

The concept of volume measurement, that is measuring the volume of the cells used in the test procedure in milliliters,

was introduced and it proved to be an efficient technique for cell quantitation.

With further investigation, as previously outlined in the discussion section of this paper, it will be possible to determine if leukocyte potassium values do reflect the total intracellular potassium levels of the body.

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APPENDIX I

Materials used for leukocyte isolation and analysis.

Balanced electrolyte solution:

Lactic acid	2.80 ml.
Sodium hydroxide	1.17 gm.
Sodium chloride	8.00 gm.
Potassium chloride	0.35 gm.
Magnesium chloride	0.21 gm.
Calcium chloride	0.20 gm.
Glucose	2.00 gm.

Deionized water to one liter

Dextran:

Type 200 C 204,000 average molecular weight
Sigma Chemical Company
3500 Dekalb Street
St. Louis, Missouri

Dextran	3 gm.
Balanced electrolyte solution to	100 ml.

Siliconized glassware:

Siliclad (silicone concentrate)
Clay Adams
New York 10, New York

1. Dilute concentrate to a 1% solution
2. Soak glassware 5 seconds
3. Rinse in distilled deionized water
4. Dry at 212 F for 10 minutes

Sterox:

Stock Reagent #3766
1% solution
Diluted 1:50 for 0.02% working solution

Harleco
Hartman Leddon Company
Philadelphia, Pennsylvania

Sodium-Potassium:

Stock Flame Photometry Standard #29148
Na - 140 mEq/1000 ml. - K - 4 mEq/1000 ml.

Working dilution 1 to 100 for working standard.

Harleco
Hartman Leddon Company
Philadelphia, Pennsylvania

VITA

The author was born in Los Angeles, California, on May 18, 1942. He graduated from Zion Benton Township High School in June 1960. He attended Ferris State College from September 1960 to December 1962 at which time he transferred to Michigan State University. He received his B. S. degree in Medical Technology from Michigan State University in June 1964, and completed professional training in Medical Technology at Edward W. Sparrow Hospital, Lansing, in June 1965. In August 1965 he was registered as a medical technologist with the American Society of Clinical Pathologists. After working at Edward W. Sparrow Hospital and Olin Memorial Hospital, he accepted a position as instructor in the School of Medical Technology at Michigan State University and enrolled in a program of graduate study in Clinical Laboratory Science in the Department of Pathology, Michigan State University in June 1967. He is a member of the Lansing, the Michigan, and the American Societies of Medical Technologists.