1955

Studies on the Use of Differential Centrifugation Procedures for the Measurement of the Intracellular Distribution of Potassium in Dog Heart Muscle

Rene Richard Kempen
Loyola University Chicago

STUDIES ON THE USE OF DIFFERENTIAL CENTRIFUGATION PROCEDURES FOR THE MEASUREMENT OF THE INTRACELLULAR DISTRIBUTION OF POTASSIUM IN DOG HEART MUSCLE

by

Rene Richard Kempen

A Thesis Submitted to the Faculty of the Graduate School of Loyola University in Partial Fulfillment of the Requirements for the Degree of Master of Science

February

1955

LIFE

Rene Richard Kempen was born in Kankakee, Illinois, March 24, 1928

He was graduated from St. Anne Community High School of St. Anne, Illinois in June, 1946. In September of the same year he entered St. Joseph's College, Collegeville, Indiana, from which he received the Degree of Bachelor of Science in June, 1950.

He entered the Graduate School of Loyola University of Chicago in September, 1950.

ACKNOWLEDGMENTS

I wish to express my deepest thanks to Doctor Akira Omachi of the Department of Physiology and Doctor Norton Melchior of the Department of Biochemistry for counsel, guidance, and effort in the development of the research and writing of this thesis.

TABLE OF CONTENTS

LIST OF TABLES

CHAPTER I

INTRODUCTION

Potassium is one of the most vital substances in biological processes and may be considered a basic stuff of life. Being the ion of greatest abundance inside the cell it is one of the important substances in regulating the osmotic pressure in the cell. It plays an important part in the development of the membrane potential and is believed to be concerned with at least part of the permeability of cell membranes. It is thought to play a vital role in the conduction of nerve impulses and the contraction of muscles. It is important in many enzyme systems, pyruvic phosphoferase, phosphofructokinase, fructokinase, choline acetylase, malic enzyme, phosphotransacetylase, aldehyde dehydrogenase and the lactase reaction of adenosine triphosphate. The reaction, acetate and coenzyme A to form acetyl-coenzyme A, also requires potassium. The optimum concentration of potassium for these enzyme activities varies from 0.04 to 0.15 M. Higher and lower concentrations tend to inhibit many of these enzyme systems.

One of the interesting facts with regard to potassium

is its distribution in the body. In the average human body 175 grams are found in the intracellular compartment while only 3 grams are found in the extracellular spaces. This presents a fascinating and difficult problem which has resulted with the formulation of many theories on the mechanisms of potassium accumulation in the cell.

Since potassium plays such a vital role in cell physiology, it was considered important to describe the distribution of this ion within the cell. The advent of cell fractionation techniques by differential centrifugation in relatively recent times has allowed a more precise determination of the localization of some of the chemical constituents in certain cell structures. For example, many enzymes have been localized in the mitochondrial fraction and for this reason it may be expected that this cell fraction would contain potassium. The reason for potassium being localized in other parts of the cell may be difficult to assess at this moment.

The general significance of the present study may lie not only in the description of cellular localization of potassium but also in providing information which may bear on unanswered problems, such as the cellular accumulation of potassium. The subject of this thesis is, then, a determination of the distri-

bution of potassium ion in certain cell fractions of dog heart muscle.

Qualitative Intracellular Localization of Potassium by Histochemical Techniques

Measurements of the localization of potassium inside the cell have been done directly by the histochemical technique. The histochemical method is essentially that of Macallum (1905), with modifications by Gersh (1938) and Scott & Packer (1939). Macallum applied a cobalti-nitrate reagent directly to living muscle fibers. The potassium appeared as crystals of sodium potassium cobalti-nitrate. Under oil immersion, those crystals are just visible as brilliant yellow short rods with rounded ends against a pale yellow diffuse background. The crystals are quite soluble at room temperatures so precautions are needed to maintain the reagents, slides, microscope, etc. at 0° C where the crystals are exceedingly insoluble. Macallum used the muscles of a variety of animals, including insects which have widely spaced bands, and concluded that potassium was located in the A bands.

Gersh (1938), who used frozen dried frog muscles (which have closely spaced bands), termed the results of Macallum as

artifacts. He showed that if a slight amount of water was added to the dried preparation the potassium was differentially concentrated from an evenly dispersed condition.

Bureau (1934) using fresh fibers from the frog, confirmed Macallum's results. He also showed that even the slightest contraction caused an even distribution of potassium and that during normal resting conditions the potassium is located in the A bands. It seems probable that the even distribution of potassium observed by Gersh may represent a contracted state of the muscle. This is particularly plausible in the light of the work of Scott & Packer (1939) who demonstrated that during quick freezing the fibers of a block of muscle are in a contracted state or are starting to contract.

Dubuisson (1942) reviewed the whole question of potassium distribution and felt strongly that the localization as shown by Macallum, Bureau, and others to bear a definite relationship to the chemical makeup of the fibers and is probably related to the normal distribution. It is rather difficult to criticize this type of work because the personal element is very important in such delicate work. Histochemical methods have been criticized (Scott, 1933; Policard, 1932; Gersh, 1941; Steinbach, 1947) because: 1) they alter the intracellular substances, 2) the color and crystals lack character which makes positive identification

difficult, 3) the possibility of adsorption of reagents on the complex colloidal surfaces which may cause abnormal localisation of substances, and 4) the need of succession of treatments (especially during fixation procedures as quick freezing and drying) which may actually cause shifts or morphological alterations.

Qualitative Intracellular Localisation of Potassium by Microincineration Techniques

The microincineration technique is a procedure developed essentially by Policard and his co-workers (1923). This method simply consists of mounting sections of tissues and heating them in a red hot furnace so as to burn away all the organic compounds. Only the entire mineral part of the tissues remains, arranged presumably as it occurs in the living cells. This reproduces completely the outlines and general features of the cells. Policard (1923) used free-hand sections of unfixed tissue or paraffin sections. Later he (1929) and Oddels (1930) showed additional advantages with frozen sections.

The most satisfactory cytologic and fine histologic details are obtained by using paraffin sections fixed with a mixture of nine parts of absolute alcohol and one part of neutral formalin. The incinerated tissue ash can be qualitatively examined for potassium by the microcrystallographic test. Small

droplets of the reagents can be applied on limited ash particles by means of micropipettes attached to micromanipulators. Another method of qualitative analysis is the determination of the water soluble fraction of the ashes. The water soluble mineral matter can be extracted by cautiously dipping the slide in distilled water. When the slide has dried, it is again examined and the rarified areas as compared to the controls shows the removal of the water soluble mineral, which for the most part inside the cell is potassium. The ash remaining consists only of calcium and magnesium oxides and phosphates, together with certain amounts of iron sesqui-oxide derived from hemoglobin.

Several criticisms of Policard microincineration method for the determination of potassium distribution are recognized. Most of the fixation procedures used before incineration require dehydrating agents and seem to remove the potassium (Policard & Oddels, 1930; Policard & Pillet, 1928; Draper & Hodge, 1950). Deucher noted that electronmicrographs similarily fixed showed faint A bands as compared with unfixed preparations. He concluded that the alcohol-formalin fixation removed the potassium from the A bands of these fixed slides.

Policard & Oddels (1930 observed a loss of 10-14% of the total ash during the incineration and suggested a method

which would retain sodium and potassium in the ash during incineration. They suggested that the tissue be subjected to sulfuric anhydride to convert all the chlorides of potassium and sodium to the sulphates which are much more stable and resistant to volatilization.

In tissues which are rich in potassium, the potassium salts tend to fuse and cause serious structural disarrangement of the ashes (Policard & Oldels, 1930).

Electron-Induced Microincineration Method

Draper & Hodge (1950) examined the myofibrils of rabbit skeletal muscle with the electron microscope before and after electron-induced microincineration. This microincineration technique consists of passing a stream of electrons, much more intense than that used in electron micrography, through the tissue to be incinerated, assuming that the process is one of oxidation brought about as a result of the high temperatures attained under intense electron bombardment. There is a possibility that water plays some part in the reaction also.

By comparison of electronmicrographs of non-incinerated slides with micrographs of incinerated slides they were able to show that electron-induced incineration completely evaporates all of the potassium from the slide. Also, the effect of for-

malin-fixation on the potassium content of small pieces of whole muscles was determined and it was found that this fixation removes practically all of the potassium.

In electron-induced microincineration the Z band does not contain any and the A band has little ash. However, since Scott (1932) found a high concentration of ash in the A and Z bands after thermal microincineration of unfixed material, the electron-induced microincineration must cause complete evaporation of the potassium in the A and Z bands.

In summary, we find that very little meaningful work has been done in the field of potassium distribution inside the cell. The microincineration and histochemical procedures have provided very limited information so that no firm conclusions can be made.

Cell Fractionation by Differential Centrifugation

The method consists of subjecting a broken cell suspension to increasing centrifugal forces thereby isolating particles of various sizes. The cell components in the various tissue fractions have been verified histologically. The amount of separation is sufficient so that quantitative biochemical methods may be directly applied.

The method of differential centrifugation of broken

cell suspensions was introduced in 1934 by Bensley and Hoerr, who described the isolation of mitochondria from guinea pig liver. Since then, many papers have appeared dealing with the isolation of various individual components, while others have described the procedure for a more complete fractionation of a tissue into nuclei, mitochondria, submicroscopic particles, and soluble material (Hogeboom, Schneider, & Pallade, 1948; Schneider, 1946; Claude, 1946; Schneider, 1948; Schneider & Hogeboom, 1950).

The procedure most commonly employed at the present time is that of Hogeboom, Schneider, & Pallade (1948). Hereafter in this thesis this method will be referred to as the "Procedure of Hogeboom" for the sake of brevity.

The first step in this procedure is to prepare a broken cell suspension by first passing the tissue through a tissue press and then homogenizing the tissue mash in the Potter-Elvehjem homogenizer (Potter & Elvehjem, 1936). This homogenizer breaks the cell membranes and suspends the cell components without damage to an appreciable amount of nuclei or other components. The use of the Waring "Blendor" is entirely unsatisfactory for the preparation of homogenates (Hogeboom, 1951) because in liver, for example, its action for periods of time sufficient to disrupt most of the cells results in the breakage of many of the nuclei

and even some of the mitochondria.

The broken cells are suspended in isotonic sucrose (0.25 M) before homogenization. Use of water or other hypotonic solutions causes prohibitive morphological alteration and the use of isotonic solutions of electrolytes produces aggregation of the particles to such an extent that they cannot be adequately separated.

The fractionation procedure is usually carried out entirely in the cold, the temperature being maintained between 1° and 5° C. The first fraction is obtained after the homogenate is subjected to a centrifugal force of 600 x gravity (G) for 10 minutes. This fraction, the cell debris fraction, contains the nuclei, myofibrils, and unbroken cells as well as the formed elements of the blood. The second centrifugation is carried out on the supernatant from the first centrifugation at 18,000 x G with the removal of the large granule fraction. A third fractionation is accomplished on the second supernatant at 57,000 x G with the separation of the microsomes. The remaining supernate is called the supernate fraction which contains all the cell components which resist the gravitational forces applied.

Two other types of differential centrifugation have been used. Chantrenne (1947) emphasized the fact that the isolation

of two cytoplasmic fractions (mitochondria and microsomes) might be arbitrary and on the basis of his studies, suggested that there might actually exist a more or less continuous spectrum of sizes and chemical composition of particles. There have been other evidence of this biochemical heterogeneity (Barman & Huseby, 1948; Keller, 1951; Hygaard & Ris, 1952; Paigen, 1954), both among the isolated microsomes and to a lesser extent among the isolated mitochondria. In order to determine if a mixture of heterogenic mitochondria exist, Kuff & Schneider (1954) developed two methods: 1) packing under high centrifugal force and 2) by sedimentation in a specific gravity gradient. The packing technique consist of suspending mitochondria in isotonic sucrose and packing the mitochondria with a centrifugal force of 108,000 x G for 30 minutes. They obtained four distinct layers of mitochondria. The sedimentation technique consist of a discontinuous sucrose density gradient obtained by layering 0.4 ml of 2.22 M, 0.8 ml each of 1.59 M, 1.51 M, 1.42 M, 1.34 M and 1.26 M and 0.5 ml of 0.636 M sucrose in a celluloid centrifuge tube of approximately 5.5 ml capacity. Each layer of sucrose solution mixes with adjacent layers only minimally during the experiment. Six mitochondria zones were separated.

No literature is available for the measurement of the

distribution of potassium in the cell component fractions separated by the methods of Hogeboom or Kuff and Schneider. As far as can be ascertained no literature is available for the direct centrifugal separation of the cell fractions without the use of diluting fluid. If such a separation is possible, measurement of the concentration of potassium in each cell fraction may give more accurate information as to the true distribution of potassium inside the cell.

CHAPTER II

METHODS AND MATERIALS

Procedure The hearts from normal adult mongrel dogs were used. The dogs were anesthetized with a 6.5% nembutal, 6.5% urethane, 10% ethyl alcohol mixture. The dose for both nembutal and urethane was 30 mg per kilogram of body weight. After allowing about 30 minutes for the anesthetic to take effect, the dogs were prepared for the removal of the heart. The heart was removed quickly through an incision between the third and fourth ribs and cooled as quickly as possible in the deep-freeze (-25° C) without freezing. If the ventricle continued to beat, a notch of tissue in the approximate area of the A-V node was taken from the septum which usually caused cessation of all contractions.

The tissue was then prepared for homogenization by forcing small portions of the left ventricle through a perforated steel disk in a tissue press. The procedure removed most of the blood vessels and gross connective tissue. This procedure and all of the remaining procedures were done in the cold.

In the first group of experiments, the tissue was treated and fractionated according to the Hogeboom procedure. Usually

one half gram of tissue mash was placed in the Potter-Elvehjem homogenizer and isotonic sucrose added to make a 1:20 dilution. The cell debris, large granule, and the supernate from the large granule separation (hereinafter referred to merely as the supernate fraction) were separated by forces of 600 x G, and 18,000 x G.

In a second group of experiments, no diluting fluid was added to 30 grams of dog cardiac tissue mash which was homogenized in the Potter-Elvehjem homogenizer. The material was placed in plastic centrifuge tubes and spun either at (1) 24,000 x G in the International Refrigerated Centrifuge (for 30 minutes or 3 hours) or at (2) 108,000 x G in the Spinco Model E Ultracentrifuge (for 30 minutes or 1 hour). The later instrument was made available to us through the courtesy of Dr. L. Abood at the Neuro-psychiatric Institute of the University of Illinois College of Medicine.

Upon removal of the tubes from the centrifuge a visible separation was apparent, a supernate and precipitate. The supernate was carefully poured off and because no visible separations of the precipitate were discernible, three arbitrary fractions were chosen, samples from the upper, middle, and bottom portions of the precipitate.

Each of these portions of the precipitate was homogenized again in isotonic sucrose. The suspensions were fractionated according to the Hogeboom procedure in order to determine whether a differential separation of cell constituents might have taken place in the high speed packing procedures.

Aliquots were taken at each step for potassium, analysed by the internal standard procedure using the Perkin Elmer Flame Photometer (Overman & Davis, 1947), and nitrogen, analysed by the Micro-Kjeldahl or Nesslerization procedures (Hawk, Oser, and Summerson, 1951). In one experiment analysis of the nucleic acids (Volkin & Cohn, 1954) was carried out.

In general most of the experiments were carried out in duplicate except for Experiments #13 and #14 which were done in triplicate and Experiments #6 and #7 which were done in quadruplicate. Only one potassium determination was done for each fraction except for Experiments #13 and #14 in which duplicates were done, nevertheless, several readings were taken for the single determination. In general the nitrogen determinations were done in duplicate except for Experiments #15 and #16 which were carried out in triplicate.

Preparation of Tissue for Nitrogen Determinations

Samples of tissue or tissue fractions containing approx-

imately 10 micro-moles are placed into previously weighed 100 ml pyrex test tubes. The tubes are again weighed and the weight of the samples determined by difference. The following procedure completely oxidizes all of the organic material. Two mls of 1:4 dilution of H_2SO_4 is added to the tubes. The tubes are heated gently until the tissue becomes charred. The heat is then increased until the white smoke in the tube disappears. The tubes are removed from the heat and allowed to cool. Two drops of 30% H_2O_2 are added and the tubes reheated until the fluid becomes clear. If the fluid is not completely clear, the H_2O_2 procedure is repeated.

Nessalerization Procedure

After digestion the samples are diluted with 15 ml of 0.5 N NaOH and distilled water to a final volume of 25 ml. It was pointed out to us that good precision in the Nessalerization procedure was obtained only if the acidity was controlled. We are indebted to Dr. J. Melchior and Mr. Dan Ziegler for aid given in this procedure. The following procedures were employed to assure this consistency. Three to five ml aliquots are titrated with 0.5 N NaOH and the amount of alkali needed to completely neutralize the aliquot is thus determined. This amount less 0.3 ml is added to each aliquot to be analysed for nitrogen. The

volume is made to 9.7 ml with water. Three tenths of a ml of Nessler's reagent is rapidly added and the tubes shaken immediately. After ten minutes readings are taken on the Coleman Spectrophotometer at a wave length of 550 μ.

In Experiments #8 to #12 inclusive the Micro-Kjeldahl method for nitrogen was employed. A change from this method to the Nessler's procedure was made because of the small amount of material available for analysis in the mitochondrial and supernate fractions. The Micro-Kjeldahl method necessitates that a certain minimal amount of nitrogen, 100 to 200 micro-moles, be present in each sample for good precision. The samples obtained from the mitochondria and supernate fractions contain only about 15 to 25 micro-moles which is considerably less than this minimal amount.

With respect to other fractions containing adequate of nitrogen the analytical results obtained by the Micro-Kjeldahl procedure may be comparable with those obtained by the Nesslerization procedure. However, the authors feel that only limited comparison is warranted since sufficient control studies were not performed which would indicate that the two methods could give essentially the same results.

Preparation of Tissue for Potassium Determinations

Samples of tissue containing from 5 to 150 micro-equiv-

alents of potassium are placed into previously weighed 100 ml pyrex test tubes. The tubes are again weighed and the weight of each sample determined by difference. Approximately three ml of concentrated HNO_3 is added per gram of tissue to each tube. The tubes are heated until the tissue completely dissolves, and then the heat is increased until the volume is reduced to about 0.25 ml. Dilutions of 10, 25, 50, and 100 ml are made depending on how much potassium is present and appropriate amounts of $LiNO_3$ for the internal standard are added. The readings are taken on the Perkin-Elmer Flame Photometer which quantitately measures the emission spectra of potassium heated to incandescence.

Preparation of Tissue for Determination of Deoxyribonucleic Acid and Ribonucleic Acid

To one ml of a water homogenate (20%) is added 2.5 ml of cold trichloroacetic acid (TCA) and the mixture is centrifuged. The procedure is repeated with 2.5 ml of TCA and the supernates which contain the acid soluble fraction are discarded. The following solvents are mixed successively with the acid insoluble fraction of the tissue and the suspensions centrifuged in order to remove the phospholipids: 1) one ml water plus 4 ml of 95% ethyl alcohol, 2) 5 ml ethyl alcohol, 3) 3:1 alcohol ether mixture which is repeated three times. The residue is then treated for 16 hours at 37° C with 1 N KOH in order to hydrolyse the ribose

from the ribonucleic acid. The mixture is then neutralized with 6 N HCl, one volume of 5% TCA is added, and centrifuged. The precipitate is washed once with 5 ml of 5% TCA and the supernates are combined for the ribose determination of ribonucleic acid. The residue is resuspended in 5 ml of 5% TCA and heated at 90° C for 15 minutes to hydrolyse the desoxyribose from the desoxyribonucleic acid. The mixture is centrifuged, the precipitate washed once with 2.5 ml of 5% TCA, and the supernates are combined for the desoxyribose determination of desoxyribonucleic acid. The orcinol reaction for ribose and the diphenylamine reaction for desoxyribose were done according to Volkin & Cohn (1954).

CHAPTER III

RESULTS

Potassium Content in Cell Fractions of Heart Tissue Homogenized in Isotonic Sucrose and Separated by the Hogeboom Procedure

In the first series of experiments, cell fractions were separated from tissue homogenized in isotonic sucrose according to the differential centrifugation method of Hogeboom, et al (1948). The distribution of potassium, nitrogen, as well as the potassium/nitrogen ratios in these cell fractions are shown in Table I. Potassium concentration is expressed in terms of milliequivalents (meq) per kilogram of wet tissue weight. Nitrogen concentration is expressed in units of moles (M) per kilogram of wet tissue weight. The potassium/nitrogen ratio is also an expression of concentration and may be a better unit since the actual weight of each fraction may be difficult to assess, especially when diluting fluid is used.

In order to ascertain the recovery of material at each step of the cell fractionation, samples of tissue from the same heart were determined directly without homogenization for potassium and nitrogen. These values are indicated in the top hori-

zontal column of Table I under the heading "Non-homogenized Samples". The values compare favorably with results reported by others for the concentration of potassium (Manery, 1954) in normal dog myocardium.

After the tissue was homogenized in isotonic sucrose with the Potter-Elvehjem homogenizer, aliquots were taken for analysis. These results are reported in the second horizontal column of Table I under the heading "Homogenate". The average potassium and nitrogen values of three samples agree very well with the non-homogenized values within a range which is not particularly large. The nitrogen determinations were done with the Nesslerization procedure in this series of experiments.

Aliquots of the homogenate were then separated into cell fractions with the results appearing in the lower portion of Table I. The total nitrogen distribution in each fraction is similar to the distribution of the total protein calculated from the data in the literature (Ludwig & Chamutin, 1950; Schneider & Hogeboom, 1950). The nitrogen values, in other words, gives a quantitative estimation of the amount of each component separated by the Hogeboom procedure. No literature was found for the distribution of nitrogen in the components of heart tissue.

The cell debris fraction, which presumably is made up

mainly of nuclei, myofibrils and unbroken cells, contains about 71% of the total tissue nitrogen with only 20% of the total potassium. Since the amount of potassium is not proportionately as large as the nitrogen, the potassium/nitrogen ratio is only 25% of that of the whole tissue. There appears to be fairly good agreement between the three samples. Since the myofibrils constitute 40-60% of the whole tissue (Cantarow & Schaedharty, 1954), the high nitrogen concentration may be due largely to this cell component.

The large granule fraction, which presumably is predominately mitochondria, contains roughly 7% of the total tissue nitrogen. Since the potassium concentration is relatively lower here also, the potassium/nitrogen ratio is lower than that of the total tissue, although greater than the cell debris fraction. As the cell debris fraction is rather heterogeneous, further comparison seems unwarranted.

The supernate from the mitochondria separation, which presumably contains most of the microsomes and other cell components which resist centrifugal forces of 18,000 x G, appears to have about 25% of the total tissue nitrogen. The amount of potassium present is relatively much greater so that the ratio of potassium/nitrogen is some 5.6 times that of whole tissue. Since

the microsomes are probably only a small part of this fraction, it seems more justified to ascribe these results to the "cytoplasm proper".

In the last two horizontal columns of Table I, the totals obtained by summing the potassium and nitrogen values of the component fractions are given. The totals agree very well with the whole tissue samples and show a recovery of about 97 to 105 percent for both potassium and nitrogen. This appears to be good confirmation of the precision of the separation and analytical procedures.

It appeared to us at this point, that homogenization in a sucrose solution, although isotonic, may be such an unphysiological procedure for the cell constituents that potassium may easily move from one compartment to another during the separation. The measurement of the potassium in these cell fractions, then, may not give a true account of the distribution inside the cell.

Potassium Content in Cell Fractions of Heart Tissue Homogenized without Addition of Diluting Fluid and Separated by Forces of 108,000 x G for 30 Minutes

In order to obtain a more nearly correct sample of each cell fraction, it was considered that a centrifugation procedure without the addition of a diluent might be feasible. In this attempt, forces of 108,000 x G were applied for 30 minutes with

the aid of the Spinco Model E Ultracentrifuge. Two fractions were obtained, a supernate and a precipitate. This supernate will be hereinafter be referred to as "Initial Supernate" in order to distinguish it from the supernate obtained from the Hogeboom separation.

The analysis of the initial supernate is given in the second horizontal column of Table II. Its potassium concentration is slightly higher than that of the non-homogenized tissue samples. Since the nitrogen content is only about one half of the control tissue sample, the potassium/nitrogen ratio is roughly twice that of the non-homogenized tissue. In comparison with the supernate from the Hogeboom separation in Table I, it appears that relatively more nitrogen is extruded than potassium. This may be related to the absence of microsomes in the "Initial Supernate".

The precipitate had the appearance of being homogeneous throughout so that an artificial separation was done. The precipitate was carefully divided into upper, middle, and bottom portions. The concentration of potassium and nitrogen was determined and found to be practically the same in each portion of the precipitate indicating that no large separation of the components occurred. However, it appears that the upper portion may have a lower nitrogen value and a higher potassium/nitrogen ratio.

Otherwise, these values agree well with the results obtained for the homogenate in Table I.

In order to ascertain if a partial separation might have occurred, the Hogeboom fractionation procedure was employed on each portion of the precipitate. The value for nitrogen in each portion of the precipitate were found to be practically identical with the respective values in the other portions of the precipitate indicating that there was essentially no separation. The one exception is the low value for the upper portion in the cell debris fraction. Compared to the nitrogen values in Table I, the mitochondria value here is higher and the supernate and cell debris fractions appears to be about the same with the exception noted.

Potassium Content in Cell Fractions of Heart Tissue Homogenized without Addition of Diluting Fluid and Separated by Forces of 100,000 X G for 1 hour

In Experiments #16 and #17 shown in Table III, the duration of centrifugation in the Spinco centrifuge was extended to 1 hour. The results of this series are somewhat similar to the results of the previous experiment. A larger volume of the initial supernate fraction was obtained which was a little different in gross appearance from the previous experiment (Table II). A semi-clear layer was observed at the top which is presumably

made up of phospholipids (Strittmatter & Ball, 1954). The dilution of this fraction by the phospholipids may account for the slight decrease in the potassium concentration relative to the non-homogenized tissue samples observed in this fraction.

The potassium and nitrogen values in the three arbitrarily spooned layers of the precipitate were in the same order of magnitude as Experiment #15. In Experiment #16, two widely varying figures were noted for the nitrogen value in the upper portion of the precipitate. The lower figure is believed to be closer to the true value since the total of the fractionated values shown in the Hogeboom separations gives a value which is much closer to this figure. This indicates that in all three experiments with the high speed centrifugation, the upper portion of the precipitate had a lower value than the middle and bottom portions.

Low nitrogen values were found in the cell debris fraction of the upper portion of the precipitate in Experiment #16 (Table III) as was observed in Experiment #15 (Table II). This is believed to be due to contamination by supernate. The supernate nitrogen was higher in the upper portion of the precipitate in Experiment #16 but appears to be the same in Experiment #15.

In the last experiment of this series, an analysis of the nucleic acids was performed. The results show that there

seems to be a slightly lower concentration of the nucleic acids in the upper portion of the precipitate. However, since only one experiment was carried out it is felt that this result needs to be interpreted cautiously.

The normal biological variation of the potassium/nitrogen ratios in the non-homogenized tissue samples occurring between the different hearts seems remarkably constant in the experiments employing the Nesslerization procedure for nitrogen (Experiments #13 to #17). This is rather interesting in light of the fact that the potassium/nitrogen ratios are dependent on two variables.

Potassium Content in Cell Fractions of Heart Tissue Homogenized without Addition of Diluting Fluid and Separated by Forces of 24,000 x G

In preliminary experiments, tissue homogenates were fractionated with a multispeed attachment of the International refrigerated Centrifuge Model PR-1 at forces of 24,000 x G for 30 minutes and 3 h ours. In these experiments the nitrogen values were determined according to the Micro-Kjeldahl method. Due to variations in the results these data are considered to be less credible and are therefore incorporated in the Appendix.

TABLE I

POTASSIUM CONTENT IN CELL FRACTIONS OF HEART TISSUE HOMOGENIZED AND SEPARATED BY THE HOGEBOOM PROCEDURE IN ISOTONIC SUCROSE

	Exp #13			Exp #14		
	K	N	K/N	K	N	K/N
NON-HOMOGENIZED SAMPLES	78.5	2.04	38.5	79.5	1.94	40.9
HOMOGENATE						
Tube 1	80.6	1.99	39.3	79.6	2.04	39.1
Tube 2	80.2	2.03	39.5	79.1	2.00	39.5
Tube 3	75.1	2.13	35.3	77.0	2.00	38.5
Average	78.6	2.05	38.0	78.6	2.01	39.0
	K	N	K/N	K	N	K/N
Cell Debris						
Tube 1	15.7	1.53	10.3	16.6	1.61	10.4
Tube 2	15.3	1.39	10.4	16.0	1.61	13.0
Tube 3	13.1	1.39	9.7	17.5	1.43	12.1
Average	14.8	1.46	10.1	16.7	1.43	11.8
Mitochondria						
Tube 1	2.6	0.14	18.8	1.9	0.10	19.3
Tube 2	3.0	0.17	18.0	2.2	0.12	18.5
Tube 3	2.9	0.17	16.8	1.9	0.10	20.1
Average	2.8	0.16	17.9	2.0	0.11	19.3
Supernate						
Tube 1	58.5	0.44	135	61.9	0.57	109
Tube 2	61.0	0.48	127	64.4	0.58	112
Tube 3	56.2	0.46	121	62.2	0.56	111
Average	58.6	0.46	128	62.8	0.57	111
TOTALS	76.2	2.08		81.5	2.11	
% Recovery	97	101		103	105	

K = Meq/Kilo N = Moles/Kilo K/N = Meq K/Eq N

TABLE XI

POTASSIUM CONTENT IN CELL FRACTIONS OF HEART TISSUE HOMOGENIZED WITHOUT ADDITION OF DILUTING FLUID AND SEPARATED BY FORCES OF 108,000 x G FOR 30 MINUTES

Exp #15

	K	N	K/N
NON-HOMOGENIZED SAMPLES	78.6	1.98	39.6
INITIAL SUPERNATE	84.6	0.96	87.2
PRECIPITATE			
Upper Portion	78.4	1.97	39.7
Middle "	81.1	2.24	36.2
Bottom "	80.5	2.24	36.0

HOGEBOOM SEPARATIONS OF UPPER, MIDDLE & BOTTOM PORTIONS

	Cell Debris N	Mitochondria N	Supernate N
Exp #15			
Upper Portion	1.20	0.29	0.58
Middle "	1.45	0.31	0.53
Bottom "	1.43	0.33	0.56

K = Meq/Kilo N = Moles/Kilo K/N = Meq K/Mole N

TABLE IIa

RECOVERY OF MATERIAL IN HOGEBOOM SEPARATION OF THE PRECIPITATE

	Total N	% Recovery
Exp #15		
Upper Portion	2.07	105
Middle "	2.29	102
Bottom "	2.32	103

N = Moles/Kilo

TABLE III

POTASSIUM CONTENT IN CELL FRACTIONS OF HEART TISSUE HOMOGENIZED WITHOUT ADDITION OF DILUTING FLUID AND SEPARATED BY FORCES OF 108,000 x G FOR ONE HOUR

	Exp #16			Exp #17		
	K	N	K/N	K	N	K/N
NON-HOMOGENIZED SAMPLES	72.7	1.94	37.4	84.1	2.09	40.3
INITAL SUPERNATE	72.4	0.84	86.6	83.9	1.03	81.0
PRECIPITATE						
Upper portion	63.1 65.0	2.81 2.09	22.4 31.1	75.2	2.06	36.4
Middle "	64.9	2.31	28.1	83.3	2.37	35.4
Bottom "	65.0	2.31	28.1	84.2	2.32	36.3

HOGEBOOM SEPARATIONS OF UPPER, MIDDLE, & BOTTOM PORTIONS

	Cell Debris	Mitochondria	Supernate
	N	N	N
Exp #16			
Upper portion	1.29	0.28	0.66
Middle "	1.60	0.27	0.49
Bottom	1.60	0.25	0.50

NUCLEIC ACID DISTRIBUTION
Exp #17

	RNA	DNA
PRECIPITATE		
Upper portion	90	90
Middle "	106	100
Bottom "	104	109

K = Meq/Kilo
N = Eq/Kilo
K/N = Meq K/Eq N
RNA = % of Average
DNA = % of Average

TABLE IIIa

RECOVERY OF MATERIAL IN HOGEBOOM SEPARATION OF THE PRECIPITATE

	Total N	% Recovery
Exp #16		
Upper Portion	2.23	99
Middle "	2.36	102
Bottom "	2.35	102

N = Moles/Kilo

CHAPTER IV

DISCUSSION

The aim of this investigation was to study the distribution of potassium inside the cell. The results of this study indicate that differential centrifugation procedures which separate certain cellular components may not be an adequate means for attacking this problem. As indicated previously in this thesis, the major difficulty appeared to be the uncertainty that potassium shifts may take place during the course of the fractionation procedure, especially with the use of large volumes of diluting fluid.

Despite this difficulty, a rough separation appeared to be effected by the procedure of Hogeboom since the potassium/nitrogen ratios were essentially different in the fractions obtained. The mitochondria had a lower potassium/nitrogen ratio than the whole tissue and this figure might be valid as a minimal value since it is a relatively homogeneous fraction. This would be based on the assumption that movement of potassium into the mitochondrial fraction did not take place at a rapid rate. A similar description probably holds for the cell debris fraction

since its potassium/nitrogen ratio is lower than that of the whole tissue but the histological heterogeneity of this fraction renders difficult any far reaching conclusions to be made. The contamination by the supernate fraction, for example, may be as high as 100% since the wet weight of this fraction is about twice that of the original tissue. The correct potassium/nitrogen ratio obtained for the supernate may also be subject to considerable error. One important factor would be the gain of potassium and nitrogen lost from the other components. It is more likely for potassium to be extracted than nitrogen since the protein molecule would be less mobile and less apt to penetrate a restricting membrane.

In order to overcome some of these difficulties, it seemed possible that a partial separation might be effected by centrifugation in the absence of diluting fluid. This procedure was not successful. Perhaps one reason is that for an adequate separation, a certain amount of fluid volume is necessary for differential movement of the components to occur under the present condition. If the centrifugation time was extended, the viscosity fact or of tissue homogenized without the use of diluting fluid might have been overcome. A second reason may be that aggregation of the cell components occurred. It has been reported

that diluting fluids which contain electrolyte tend to aggregate certain cell components (Hogeboom, Schneider, & Pallade, 1948) and it may be possible that the contact of extracellular fluid with intracellular material may have been sufficient cause to promote aggregation in the present system.

Although the present data is subject to considerable potential error, it may be possible that some of the data obtained with the Hogeboom method or the differential packing method are actually close to the correct values. The amount of potassium found in the mitochondrial fraction could be a true estimate of the amount associated with this component if it could be assumed that there was little movement of potassium during the fractionation. The cell debris fraction has a considerable amount of contamination but the value adjusted for some of this contamination (i.e. the supernate) may be close to the true figure. The supernate has a high potassium/nitrogen ratio but it may be perfectly reasonable to find most of the cell potassium in the cytoplasm proper. However, it is undoubtedly more judicious to ascribe less than more meaning to the data at the present stage of our knowledge.

There appeared to be some evidence that microsomes were layered on to the upper portion of the precipitate during high

speed (100,000 x G) centrifugation in the absence of diluting fluid. The upper portion of the precipitate had a low nitrogen content indicating that following the early separation of heavy materials or low speeds, there is an overlay of small particles from the supernate which is deposited at the higher centrifugal field. If this result were to be ascribed to the presence of larger amounts of supernate, it can be calculated that the potassium content should also have been altered significantly. Since this was not the case, this result appears to signify that microsomal separation may be possible in the future without the addition of a diluting fluid. Although the nucleic acid data appears to contradict effective microsomal separation, this data was obtained from one heart only and appears less significant at this point.

It may be of some value to note some possible approaches in the future investigations to this apparently unsolvable problem. One procedure which may be employed is to vary tissue concentrations by altering the volume of diluting fluid. If potassium concentration is unaltered by dilution, this would indicate that potassium shifts are not important in these studies. If these shifts do take place, an extrapolation of the data to zero dilution may allow a calculation of the distribution of

potassium. Another procedure may be to centrifuge for longer intervals of time. A third procedure that may be followed is to isolate certain cell components such as mitochondria and nuclei and determine the rates of exchange of potassium with the aid of radioactive potassium. If the rates of exchange are low, it may be possible to consider the data in this thesis as relatively unaffected by movement of potassium during the fractionation procedures.

CHAPTER V

SUMMARY

1. The distribution of potassium and nitrogen was measured in cell fractions of the dog myocardium obtained by using the cell fractionation technique of Hogeboom, et al. Most of the potassium was found in the supernate fraction. The cell debris, mitochondria, and supernate fractions had potassium/nitrogen ratios of 10.8, 17.1, and 122, respectively.

2. High gravitational forces, 24,000 x G and 100,000 x G, were employed also in order to effect a separation of components from tissue homogenized without the addition of a diluting fluid. A supernate and precipitate were obtained but no further separation of cell components was obtained as determined by fractionation by the Hogeboom procedure of precipitate samples taken from various areas of the precipitate. The data does, however, indicate that a possible separation of the microsomes occurred in the upper portion of the precipitate.

3. In one experiment the distribution of nucleic acids in the various areas of the precipitate were determined. Desoxyribonucleic acid was distributed evenly throughout the precipitate

indicating no segregation of the nuclei occurred. Ribonucleic acid was distributed evenly throughout the precipitate indicating that no segregation of the microsomes occurred in the precipitate. These are the results of only one experiment.

4. Interpretation of the data is difficult since the movement of potassium between fractions during the fractionation procedure is unknown. Further study is necessary before a thorough evaluation of the present data is possible.

BIBLIOGRAPHY

Barnum, C. P., & Huseby, R. A., 1948. Some Quantitative Analysis of the Particulate Fractions from Mouse Liver Cytoplasm. Arch. Biochem. 19:17.

Barnum, C. P., & Huseby, R. A., 1950. The Intracellular Heterogeneity of Pentose Nucleic Acid as Evidenced by the Incorporation of Radiophosphorus. Arch. Biochem. 29:7.

Bensley, R. R., & Hoerr, N., 1934. The Preparation and Properties of Mitochondria. Anat. Rec., 60:449.

Boyer, P. D., 1953. The Role of Potassium and Related Cations in the Action of Pyruvic Phosphoforase and Other Enzymes. J. Lancet 73:195.

Brink, F. Jr., 1953. The Role of Potassium in the Activity of Nerve Cells. J. Lancet 73:171.

Bureau, F., 1934. Recherches sur la Répartition du potassium dans les cellules et sur les déplacements qu'il subit au cours des phénomenes d'excetation. Arch. Int. Physiol. 39:311.

Cantarow, A., & Schoparty, B., 1954. Biochemistry. Saunders, Philadelphia.

Chantrenne, H., 1947. Hétérogenéité des granules cytoplasmiques du foie de souris. Biochem et Biophys. 1:437.

Claude, H., 1946. Fractionation of Mammalian Liver Cells by Differential Centrifugation. I. Problems, Method, and Preparation of Extract. II. Experimental Procedures and Results. J. Exper. Med. 80:19.

Claude, H., 1947-48. Studies on Cells: Morphology, Chemical Constitution, and Distribution of Biochemical Functions. Harvey Lectures 43:121.

Deucher, F., 1941. Topochemische Untersuchungen über Glykogen, Kalium und Aschegehalt im Warmblüterherzen. Z. Mikr. Anat. 49:401.

Draper, M. H., & Hodge, A. J., 1949. Studies on Muscle with the Electron Microscope. II. The Ultrastructure of Toad Striated Muscle. Australian J. Exper. Biol. & M. Sc. 27:465.

Draper, M. H., & Hodge, A. J., 1950. Electron-induced Microincineration with the Electron Microscope. I. Distribution of Residual Mineral Content in Vertebrate Striated Muscle. Australian J. Exper. Biol. & M. Sc. 28:549.

Dubuisson, M., 1942. Sur la répartition des ions dans le muscle strié. Arch. Int. Physiol. 111:439.

Fenn, W. O., 1953. Introduction to a Symposium on the Metabolism of Potassium. J. Lancet 73:163.

Gersh, I., 1938. Improved Histochemical Methods for Chloride, Phosphate-Carbonate and Potassium Applied to Skeletal Muscle. Anat. Rec. 70:311.

Gersh, I., 1941. Recent Developments in Histochemistry. Physiol. Rev. 21:242.

Hawk, P. H., Oser, B. L., & Summerson, W. H., 1951. Practical Physiological Chemistry. p 818.

Hogeboom, G. H., Schneider, W. C., & Pallade, G. E., 1948. Cytochemical Studies of Mammalian Tissue; I. Isolation of Intact Mitochondria from Rat Liver; Some Biochemical Properties of Mitochondria and Submicroscopic Particulate Material. J. Biol. Chem. 172:619.

Hogeboom, G. H., 1951. Separation and Properties of Cell Components. Fed. Proc. 10:640.

Keller, E. B., 1951. Turnover of Proteins of Cell Fractions of Adult Rat Liver in Vivo. Fed. Proc. 10:206.

Kuff, E. L., Schneider, W. C., 1954. Intracellular Distribution of Enzymes XII. Biochemical Heterogeneity of Mitochondria. J. Biol. Chem. 206:677.

Laird, A. K., Nygaard, O., & Nis, H., 1952. Separation of Rat Liver Mitochondria into Two Morphologically and Biolchemically distinct Subfractions. Cancer Res. 12:276.

Logan, J. E., Maxwell, M. A., & Rossiter, R. J., 1952. Estimation of Nucleic Acids in Tissue from the Nervous System. Biochem. J. 51:470.

Macallum, A. B., 1905. On the Distribution of Potassium in Animal and Vegetable Cells. J. Physiol. 32:95.

Maley, G. F., & Plaut, G. W. E., 1953. Yields of Oxidative Phosphorylation by Heart Mitochondria. J. Biol. Chem. 205:297.

Mamory, J. F., 1954. Water and Electrolyte Metabolism. Physiol. Rev. 34:334.

Mudge, G. H., 1953. Cellular Mechanism of Potassium Metabolism. J. Lancet 73:166.

Omachi, A., Barnum, C. P., & Glick, D., 1948. Quantitative Distribution of an Esterase among Cytoplasmic Components of Mouse Liver Cells. Proc. Soc. Exper. Biol. Med. 67:133.

Paigen, K., 1954. The Occurrence of Several Biochemically Distinct Types of Mitochondria in Rat Liver. J. Biol. Chem. 206:945.

Policard, A., 1923. Sur une méthode de microincineration applicable aux recherches histochimiques. Bull. Soc. chimique de France 33:1551.

Policard, A., & Pillet, D., 1928. Application de la technique histochimique de la microincinération à l'etude des pigments antracosiques pulmonaires. C. R. Soc. Biol. 98:985.

Policard, A., 1929. La microincinération des cellules et des tissue. Protoplasma 7:464.

Policard, A., & Oddels, H., 1930. Localising Inorganic Substances in Microscopic Sections. Anat. Rec. 44:349.

Pollicard, A., 1932. Some New Methods in Histochemistry. The Harvey Lectures 27:204.

Potter, V. R. & Elvehjem, C. A., 1936. A Modified Method for the Study of Tissue Oxidations. J. Biol. Chem. 114:495.

Schmidt, G., & Thannhauser, S. J., 1945. A Method for the Determination of Desoxyribonucleic Acid, Ribonucleic Acid, and Phosphoproteins in Animal Tissues. J. Biol. Chem. 161:83.

Schneider, W. C., 1945. Phosphorus Compounds in Animal Tissues. I. Extraction and Estimation of Desoxypentose Nucleic Acid and of Pentose Nucleic Acid. J. Biol. Chem. 176: 259.

Schneider, W. C., Hogeboom, G. H., & Ross, M. H. 1950. Intracellular Distribution of Enzymes. VII. The Distribution of Nucleic Acids and Adenosinetriphosphatase in Normal Mouse Liver and Mouse Hepatoma. J. Nat. Cancer Inst. 10:977.

Schneider, W. C., 1951. Cytochemical Studies of Mammalian Tissue; The Isolation of Cell Components by Differential Centrifugation. Cancer. Res. 11:1.

Scott, G. H., 1932. Distribution of Mineral Ash in Striated Muscle Cells. Proc. Soc. Exper. Biol. & Med. 29:349.

Scott, G. H., 1933. The Localization of Mineral Salts in Cells of Some Mammalian Tissues by Micro-incineration. Am J. Anat. 53:243.

Scott, G. H., 1933. A Critical Study and Review of the Method of Micro-incineration. Protoplasma 20:133.

Scott, G. H., 1937. The Microincineration Method of Demonstrating Mineral Elements in Tissues. Handbook of Microscopical Technique, edited by C. E. McClung, p 643.

Scott, G. H., & Packer, D. M., 1939. An Electron Microscope Study of Magnesium and Calcium in Striated Muscle. Anat. Rec. 74:31.

Shoppard, C. W., 1951. New Developments in Potassium and Cell Physiology. Science 114:65.

Stolnbach, N. D., 1947. Intracellular Inorganic Ions and Muscle Action. Ann. N. Y. Acad. Sci. 47:849.

Strittmatter, C. R., & Ball, E. G., 1954. The Intracellular Distribution of Cytochrome Components and of Oxidative Enzyme Activity in Rat Liver. J. Cell. & Com. Physiol. 43:57.

Teng, C. T., 1953. Effect of Potassium Ions and Other Electrolytes on Carbohydrate Metabolism in Liver Slices. J. Lancet 73:192.

Von Korff, R. W., 1953. The Effect of Alkali Metal Ions on Acetate Activation by an Enzyme from Heart Muscle. J. Lancet 73:196.

APPENDIX I

POTASSIUM CONTENT IN CELL FRACTIONS OF HEART TISSUE HOMOGENIZED WITHOUT ADDITION OF DILUTING FLUID AND SEPARATED BY FORCES OF 24,000 x G FOR 30 MINUTES

	Exp #6 K	Exp #7 K	Exp #8 K	N	K/N
NON-HOMOGENIZED SAMPLES	73.1	76.1	-	-	-
INITIAL SUPERNATE	97.6	92.3	93.5	0.80	117
PRECIPITATE					
Upper portion	73.6	77.0	89.2	2.50	37.0
Middle "	74.0	73.3	79.6	2.03	39.2
Bottom "	75.0	78.0	80.6	2.03	39.7

HOGEBOOM SEPARATIONS OF UPPER, MIDDLE, & BOTTOM PORTIONS

	Cell Debris K	N	K/N	Mitochondria K	N	K/N	Supernate K	N	K/N
Exp #7									
Upper Portion	14.2			5.9			55.5		
Middle "	18.4			7.0			50.3		
Bottom "	20.0			5.4			46.6		
Exp #8									
Upper Portion	14.6	1.43	10.2	2.6	0.28	9.3	69.8	0.38	183
Middle "	10.8	1.13	9.6	2.8	0.25	11.2	56.3	0.34	165
Bottom "	11.7	1.32	8.9	3.2	0.25	12.7	52.1	0.32	163

K = Meq/Kilo N = Moles/Kilo K/N = Meq K/Mole N

APPENDIX Ia

RECOVERY OF MATERIAL IN HOGEBOOM SEPARATION OF THE PRECIPITATE

	Total K	% Recovered	Total N	% Recovered
Exp #7				
Upper Portion	75.6	103		
Middle "	75.7	103		
Bottom "	72.0	96		
Exp #8				
Upper Portion	67.0	98	2.09	84
Middle "	69.0	89	1.72	85
Bottom "	67.0	84	1.89	93

K = Meq/Kilo N = Moles/Kilo

APPENDIX II

POTASSIUM CONTENT IN CELL FRACTIONS OF HEART TISSUE HOMOGENIZED WITHOUT ADDITION OF DILUTING FLUID AND SEPARATED BY FORCES OF 24,000 x G FOR THREE HOURS

	Exp #9			Exp #10			Exp #11		
	K	N	K/N	K	N	K/N	K	N	K/N
NON-HOMOGENIZED SAMPLES	79.7	3.26	24.4	77.6	1.76	43.9	90.3	1.95	47.3
INITIAL SUPERNATE	98.4	1.14	85.6	84.8	0.96	88.1	82.1	1.05	78.9
PRECIPITATE									
Upper Portion	79.7	1.73	41.1	77.6	1.92	40.3	78.4	1.48	53.8
Middle "	81.9	1.94	38.4	75.0	1.99	37.7	80.9	1.27	42.1
Bottom "	90.9	1.98	42.2	74.7	1.95	38.2	80.1	1.59	50.6

HOGEBOOM SEPARATIONS OF UPPER, MIDDLE, & BOTTOM PORTIONS

	Cell Debris			Mitochondria			Supernate		
	K	N	K/N	K	N	K/N	K	N	K/N
Exp #9									
Upper Portion		1.08			0.31			0.43	
Middle "		1.13			0.37			0.48	
Bottom "		1.27			0.31			0.53	
Exp #10									
Upper Portion	11.6	1.02	11.4		0.18			0.52	
Middle "	14.2	0.87	16.2		0.28			0.54	
Bottom "	12.5	0.93	13.1		0.22			0.50	
Exp #11									
Upper Portion	16.5	1.21	13.6		0.19		63.8	0.62	92.0
Middle Portion	16.9	1.31	12.3		0.20		61.5	0.67	92.2
Bottom "	15.7	1.45	10.9		0.19		63.6	0.72	89.8

K = Meq/Kilo N = Moles/Kilo K/N = Meq K/Mole N

APPENDIX IIa

RECOVERY OF MATERIAL IN ROCKBOOM SEPARATION OF THE PRECIPITATE

	Total N	% Recovery
Exp #9		
Upper Portion	1.82	105
Middle "	1.98	102
Bottom "	2.11	106
Exp #10		
Upper Portion	1.72	90
Middle "	1.59	80
Bottom "	1.65	85
Exp #11		
Upper Portion	2.02	135
Middle "	2.18	170
Bottom	2.36	148

N = Moles/Kilo

APPENDIX III

POTASSIUM CONTENT IN CELL FRACTIONS OF HEART TISSUE HOMOGENIZED WITHOUT ADDITION OF DILUTING FLUID AND SEPARATED BY FORCES OF 108,000 x G FOR 30 MINUTES

Exp #12

	K	N	K/N
NON-HOMOGENIZED SAMPLES	80.3	2.07	39.9
INITIAL SUPERNATE	95.4	0.91	103
PRECIPITATE			
Upper Portion	98.3	1.96	46.4
Middle "	89.3	2.15	42.5
Botton "	90.2	2.19	40.9

HOGEBOOM SEPARATIONS OF UPPER, MIDDLE, & BOTTOM PORTIONS

	Cell Debris			Mitochondria			Supernate		
	K	N	K/N	K	N	K/N	K	N	K/N
Exp #12									
Upper portion	15.5	1.41	10.5		0.17		71.4	0.42	159
Middle "	18.8	1.54	11.2		0.16		80.8	0.43	168
Botton "	17.7	1.73	9.3		0.24		72.0	0.46	145

K = Meq/Kilo N = Moles/Kilo K/N = Meq K/Mole N

APPENDIX IIIa

RECOVERY OF MATERIAL IN HOMOGENATION SEPARATION OF THE PRECIPITATE

	Total M	% Recovery
Exp #12		
Upper Portion	2.00	102
Middle "	2.13	99
Bottom "	2.43	111

M = Moles/Kilo

APPENDIX IV

PRECISION EXPERIMENTS ON A HOMOGENATE WITH THE POTASSIUM PROCEDURE

Sample #	Tissue gms	Total K micro-eq	K meq/kilo	S Deviation
1	0.0539	4.02	74.6	0.3
2	0.0524	3.92	74.8	0.0
3	0.0505	3.80	75.2	0.8
4	0.0497	3.74	75.2	0.8
5	0.0505	3.74	74.2	0.8
6	0.0496	3.70	74.6	0.3
7	0.0508	3.79	74.6	0.3
		Averages	74.8	0.5

RECOVERY OF ADDED MATERIAL

Sample #	Tissue gms	Average micro-eq K	K added	Recovered amount	Recovery %
8	0.0491	3.68	1.04	4.78	101
9	0.0512	3.83	1.04	4.88	100
10	0.0502	3.75	1.04	4.81	100
11	0.0523	3.91	1.04	4.91	99
12	0.0513	3.83	1.04	4.83	99
				Average	100

APPENDIX V

PRECISION EXPERIMENTS ON A HOMOGENATE FOR THE KJELDAHL METHOD

Sample #	Tissue gms	Gms N	N %	Deviation %
1	0.0496	0.0737	14.9	1.9
2	0.0501	0.0727	14.5	0.1
3	0.0510	0.0749	14.8	0.1
4	0.0517	0.0735	14.2	2.3
5	0.0523	0.0754	14.4	1.2
6	0.0506	0.0748	14.8	1.4
		Averages	14.6	1.2

APPENDIX VI

GLYCINE IN SUCROSE RECOVERY WITH NESSLER'S PROCEDURE

Sample #	Reading gamma	Average amount	% Deviation
1	37.5 38.0	37.8	0
2	37.5 37.6	37.6	0.5
3	38.0 37.3	37.7	0.3
4	37.4 37.6	37.5	0.8
5	38.2	38.2	1
6	37.5 38.0	37.8	0
7	37.8 38.0	37.9	0.3
8	38.0 37.8	37.9	0.5
	Averages	37.8	0.4
	Less Blanks	0.6	
	Actual	37.2	
	Theoretical	36.0	

A glycine standard containing 241.4 mg/50 ml was made in isotonic sucrose. Aliquots of 0.5 ml were digested in 2 ml of 1:4 H_2SO_4 and made to a final volume of 50 ml. Four ml aliquots which theoretically contain 36 gamma N were used in the Nesslerization.

APPENDIX VII

PRECISION EXPERIMENTS ON A HOMOGENATE WITH THE NESSLERISATION PROCEDURE

Sample #	Tissue gms	Total N gamma	N gm %	Deviation %
1	0.01508	444	2.93	0.3
2	0.01525	441	2.89	1.3
3	0.01530	459	2.98	1.6
4	0.01630	481	2.95	0.7
5	0.01659	475	2.87	2.3
6	0.01870	566	3.01	2.6
		Averages	2.93	1.5

RECOVERY OF ADDED MATERIAL

Sample #	Tissue gms	Average gamma N	Gamma added	Recovered amount	Recovery %
6	0.01507	442	100	543	100
7	0.01548	454	100	562	101
8	0.01510	443	100	550	101
9	0.01497	439	100	552	102
				Average	101

Aliquots (0.3 to 0.4 ml) of homogenate were weighed and digested in 2 ml 1:4 H_2SO_4. They were made to a final volume of 25 ml with two ml aliquots being used in the Nesslerisation.

APPENDIX VIII

PRECISION EXPERIMENTS ON A HOMOGENATE FOR THE RNA & DNA METHODS

Sample #	Tissue mgs	RNA %	RNA Deviation %	DNA %	DNA Deviation %
1	1.940	98.2	1.8	155	55
		98.3	1.7	96	4
2	1.920	101.8	1.8	103	3
		100.8	0.8	103	3
3	1.948	100.0	0	103	3
		99.5	0.5	103	3
4	1.964	97.3	2.7	103	3
		97.3	2.7	103	3
5	1.957	103	3.8	88	12
		107	7.5	88	12
6	1.960	100.0	0	81	19
		98.6	1.4	81	19
7	1.986	99.0	1.0	96	4
		100.0	0	96	4
8	1.955	101.1	1.1	103	3
		100.6	0.6	96	4
9	1.964	100.6	0.6	96	4
		100.5	0.5	96	4
10	1.933			96	4
				117	17
	Averages	100	1.6	100	9

APPROVAL SHEET

The thesis submitted by Rene Richard Kompen has been read and approved by three members of the faculty of the Stritch School of Medicine, Loyola University.

The final copies have been examined by the director of the thesis and the signature which appears below verifies the fact that any necessary changes have been incorporated, and that the thesis is now given final approval with reference to content, form, and mechanical accuracy.

The thesis is therefore accepted in partial fulfillment of the requirements for the Degree of Master of Science.

January 19, 1955
Date

Alvira Quadri
Signature of Adviser