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Evaluation of a clinical turbidometric method for the determination of serum potassium

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EVALUATION OF A CLINICAL TURBIDOMETRIC METHOD FOR THE DETERMINATION OF SERUM POTASSIUM

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INTRODUCTION

During the past fifteen years, rapid advancement in the knowledge and understanding of fluid and electrolyte changes occuring in various pathological conditions has led to a need for rapid, accurate, simple methods for the determination of the various electrolytes concerned. The role of potassium in health and disease has only recently been brought to the attention of the clinician. Thus, only recently, has the need for rapid potassium determinations been felt. The chemical methods available for the analysis of potassium in biological materials are, by necessity, complicated and time consuming. Colorimetric methods, while requiring somewhat less time than gravimetric and titrimetric methods, still require a relatively large expenditure of time. Electrocardiographic changes associated with altered potassium levels in the serum, while rapid and diagnostic, do not give absolute answers. Such conditions as the dying heart, slow nodal rhythm with auricular standstill associated with myocarditis, anoxia or severe arteriosclerotic heart disease and organic bundle branch block may alter the electrocardiograph changes found with altered potassium levels (41). With the application of flame photometry to the determination of

sodium and potassium, a rapid and accurate means for these determinations is available. Due to the expense of the flame photometer, however, and only the larger medical centers are able to afford them. This paper concerns a rapid simple method for the estimation of serum potassium which is applicable to even the smallest clinical laboratories.

Methods For Serum Potassium Analysis

MacCallum, A. (1903), (1) was one of the first investigators to attempt to determine the potassium content of biological materials. In his investigations of the effects of the various salts of sea water on the Medusoe, he found it necessary to first determine the amount of the salts present in the animals studied. He suspended the animals in a muslin bag and applied pressure to the bag. This served not only to remove the fluid from the external surface, but also the fluid from the gastro-vascular compartments. He then converted the material to be analyzed in such a way that the total bases were converted to sulphates. Hydrochloric acid and a known excess of platinic chloride were added which precipitated out the potassium as the insoluble potassium chloraplatinate. This precipitate was then heated to 300°C. and dry hydrogen gas passed over it. This served to reduce the platinum united as potassium chloraplatinate to metallic platinum. From the amount of platinum recovered the amount of potassium present could be calculated. From his determinationshe concluded that there was a remarkable similarity between the potassium content of the Medusoe and mammal blood, as determined by him.

The first attempt to determine potassium in blood

by the cobaltinitrite precipitation was apparently made by Hamburger in 1915 (3). The precipitation was accomplished in a graduated capillary tube and the precipitate measured. Each gradation on the tube was equal to 0.0001 mgm. of potassium. This method required at least sixteen hours for completion. No results for plasma, serum or blood were reported.

In 1918, S. W. Clausen (2) developed a titrimetric method for the estimation of potassium in blood. This method consisted of three steps: (1) the precipitation of potassium as potassium sodium cobaltinitrite, $K_2NaCo(NO_2)_6$, (2) the oxidation of the precipitate in an acid solution at a boiling temperature with an excess of potassium permanganate, and (3) the titration of the excess potassium permanganate with oxalic acid. The author found that calcium, magnesium and iron, present in quantities found in blood, did not affect the accuracy of the determinations. The organic material in the blood was removed by a "wet ashing" process.

An improvement over the above described method was reported by Benjamin Kramer (3) in 1920. He substituted a dry ashing procedure for the wet ashing used by S. W. Clauson. He found that ammonia and phosphates, unless present in small quantities would

interfere with the determinations. Dry ashing served to remove any ammonia which was present. The precipitate was oxidized with potassium permanganate, oxalic acid added until the solution was clear, then the solution was titrated back to a permanent pink with potassium permanganate. The amount of potassium in the sample was then calculated.

The error of this method using three cc. of serum was found not to be in excess of ±5%. The potassium in normal human serum was found to be between 16 and 22 mgm. per 100 cc. The author also demonstrated that calcium, magnesium, sodium, barium, strontium, zinc, iron, sulphate, nitrate and chloride would not interfere with the determination.

Qualitative tests performed by Kramer and Tischall (4) in 1921 showed that sodium cobaltinitrite does not precipitate creatine, creatinine or urea. It does form a precipitate with ammonia; which, however, is present in serum in only small amounts. These observations suggested the possibility of precipitating potassium directly from serum without preliminary ashing, if the ph were adjusted so that serum proteins would remain in solution. They employed a solution of sodium cobaltinitrite, adjusted to pH5.7, which is more alkaline than the isoelectric point of serum

proteins, thus resulting in the precipitation of potassium while leaving the serum proteins in solution. The precipitate is then titrated as in the original method described by Kramer (3). The authors stressed the precautions necessary in obtaining the sample of serum to be analyzed. Syringe and needle must be absolutely dry so as to preclude hemolysis and the serum must not be left in contact with the red cells longer than necessary. If serum is left in contact with erythrocytes for an appreciable length of time, potassium migrates from the cells to the serum. The serum sample should be analyzed within 48 hours, during which time it should be kept in the refrigerator.

Potassium determinations on serum obtained from ten normal adults gave an average value of 19.6 mgm. per 100 ml. The values ranged from 19.0-20.0 mgm per 100 ml. Potassium determinations on sixteen normal sera after ashing resulted in an average value of 19.8 mgm. per 100 ml, with values ranging from 17.9-21.3 mgm. per 100 ml. He concluded that for normal human adults and children the serum potassium levels were constant. The maximum variation being 18-21 mgm. per 100 ml.

In 1928, Morgulis and Perley (5) found that the factor used by Kramer (4) for the conversion of cc.'s

of potassium permanganate, used in the titration, to mgm. of potassium was derived emperically rather than stoichometrically. They found that this factor varied with the amount of potassium in the sample analyzed. The titration results, when plotted as ordinates against potassium in milligrams as the abscissae, gave a straight line. Thus a curve can be constructed from which the amount of potassium present in the unknown can be read directly. The authors also found that phosphorus in equivalent concentrations at least, did not interfere with this method.

A colorimetric method for serum potassium determinations was described in 1928 by Shahl and Bennett (6). Serum or plasma was dry ashed in a platinum crucible. The ash is redissolved and the potassium precipitated as the chloraplatinate. The potassium chloraplatinate is then converted into the iodoplatinate by the addition of potassium iodide. This results in the formation of a deep wine color due to the iodoplatinate. The solution is then compared and the colorimetric with known standards. Amounts as small as 0.1 mgm. of potassium may be determined by this method.

The potassium chloraplatinate may be titrated with sodium thiosulfate; if in neutral solution, the

color of the iodide formed being used as the end point.

The diazotization process of Ilosnay (8) was adapted to serum potassium determinations by Taylor (7) in 1930. Taylor (8) also introduced tungstic acid as a more efficient means of precipitating serum proteins, and the use of 30% alcohol for washing the sodium potassium cobaltinitrite precipitate. The precipitate is less soluble in alcohol than in the saline solution originally used. In a series of seven determinations, the values ranged from 19.9-24.0 mgm. per 100 ml. The author also stressed the fact that hemolysis invariably resulted in high values.

Difficulties in applying the method of Kramer and Tisdall (4) were encountered by Breh and Goebler (9) in 1930. These difficulties consisted of interference of serum protein and to some extent, of the lipemia which existed in the serum. Thus, these authors investigated the possibility of using silver cobaltinitrite to precipitate the potassium. They employed tungstic acid filtrate from which all the chlorides had been removed. For the determination of the potassium in the potassium silver cobaltinitrite precipitate, they utilized the reaction between salts of cobalt and ammonium thiocyanate in alcoholic solution. This produces a blue color, the intensity of which,

has a significant deviation from proportionality. Thus an emperical curve must be constructed using solution of known potassium concentrations. It was shown also that potassium silver cobaltinitrite was much less soluble than the sodium salt.

The results by this method, as compared with the ashing process, showed an average answer which was 0.7 mgm. higher for the ashing process.

Jacobs and Hoffman (11) in 1931 applied a hitherto unmentioned color reaction to the determination of serum potassium. The determination depends upon the formation of an emerald-green color when a solution of sodium ferrocyanide is added to a mixture of a cobaltous salt and choline hydrochloride in water. Preliminary experiments showed that in the presence of a given excess of ferrocyanide and choline, the reaction could be used colorimetrically to determine small amounts of cobalt. The color develops within a few minutes and is stable for hours. The solution may be diluted without losing accuracy. The potassium is precipitated from serum by the Kramer-Tisdall (4) method and the cobalt in the precipitate determined by the ferrocyanide-cobalt-choline color reaction. Ammonium salts were found to interfere with this method.

Another method for the determination of potassium colorimetrically was described by Sobel and Kramer (12) in 1932. This method depends upon the formation of a cobalt-cysteine-hydrogen peroxide complex. In an alkaline phosphate solution cobalt forms an olive-green complex with cysteine. Upon the addition of hydrogen peroxide a light yellow complex forms, the intensity of which is directly proportional to the amount of cobalt present.

In 1937, Harrison and Darrow (13) developed a volumetric method for potassium analysis in biological materials. The potassium was precipitated by the chloraplatinic acid method and the chlorine of the precipitate determined by titration. This method proved to be more accurate, in the authors hands, than previously described titrimetric procedures. The method is applicable to the determination of 0.004-0.04 mM. of potassium and is accurate within 1% on theoretical solutions.

The spectrograph was introduced into the analysis of potassium in biologic materials by Thomson and Lee (14) in 1937. They employed a rotating jet, through which the solution to be analyzed was passed and passed a spark through the resulting spray. They found that the rate of flow through the jets influenced the

results, a low rate of flow giving the most accurate results for the determination of potassium. This method was faster than any of the existing methods and almost eliminated the human factor as a source of error.

The Kramer-Tisdall (4) method was further modified by Hubbard and Gorbutt (16) in 1939. They carried out two precipitations of the potassium and introduced an improved method for washing the precipitate. The amount of potassium present is determined by titration with potassium permanganate as in the original procedure. This method was applicable to solutions containing from 5-80 mgm. of potassium per 100 ml.

In a study of the potassium content of human blood, Weichselbaum, Somogyi, and Rusk (17) in 1940 developed a manometric method for potassium analysis which was sufficiently accurate and less time consuming than the currently available methods. They precipitated the potassium as potassium silver cobaltinitrite, which they found to be less soluble than the sodium cobaltinitrite salt. They then added sodium hydroxide to the precipitate which caused the formation and precipitation of silver oxide and cobaltous hydroxide. The remaining solution was transferred quantitatively to the Von-Slyke apparatus, sulfuric acid added and the nitrous acid was measured. From the volume of gas,

they calculated the amount of potassium in the precipitate.

They also introduced cupric sulphate and silver nitrate to precipitate out the chlorides and to reduce the error of silver tungstate formation which removes some of the silver and, due to the acidity, adversely affects the solubility of the potassium silver cobaltinitrite precipitate. The largest variation in a comparison of titrimetric and manometric methods on a series of five samples was 0.4 mgm.%. In a series of forty potassium determinations on normal adult subjects they found 4.93 mE/L. to be the average. The largest value found was 5.26 mEq/L. and the lowest was 4.60 mEq/L.

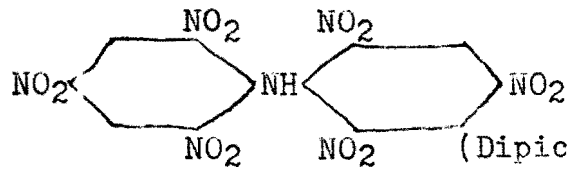
The photoelectric colorimeter was applied to potassium determination in 1940 by Tenery and Anderson (18). The potassium was precipitated as the chloroplatinate as in the method of Shahl and Bennett (6). The potassium chloroplatinate was then converted to the iodoplatinate and the reddish-orange color measured by photoelectric colorimeter. Using known solutions, a smooth curve was obtained with the Evelyn Photoelectric Colorimeter. Differences in concentration of less than 1% could be detected.

A review of the objections to the sodium and silver cobaltinitrite methods for the precipitation of potassium and modified version of the Shahl-Bennett (6) method was presented in 1940 by Salit (19). He pointed out that the exact composition of sodium cobaltinitrite and silver cobaltinitrite precipitates was uncertain and that the ratio of potassium to sodium or silver varied with the concentration of the sample and reagents, the temperature at which the precipitation occurred and the time involved in the precipitation. The composition of potassium chloroplatinate, however, always has a definite composition. Iron, aluminum, magnesium, barium, calcium, manganese and copper interfere with the cobaltinitrite precipitation while only ammonia, alcohol, iron, copper and ferrocyanide are said to interfere chloroplatinate precipitation. The methods of ashing plasma or serum have been tedious and time-consuming. The author ashes the sample in specially made nickel tubes in the presence of an oxidizing agent, mercuric oxide. The potassium in the ash is determined colorimetrically by the Shahl-Bennett (6) method. Details are given for potassium determinations on blood, feces and urine.

Harris (20) in 1940 found the usual methods of potassium analysis by sodium or silver cobaltinitrite

precipitations to have insufficient accuracy and to entail an unaccounted number of variables. He modified the method of Breh and Goebler (9) by precipitating the potassium silver cobaltinitrite from a 14% alcohol solution in a water bath at 20°C. This method was less time consuming and was found to be accurate to within ±2%.

Since the determination of potassium in biological materials is most troublesome and time-consuming by standard methods, Harington (21), in 1941, adapted a new method which was comparable in accuracy to existing methods. Dipicrylamine (di(2:4:6 trinitrophenyl)amine) forms a deep orange-red potassium salt.



(Dipicrylamine)

The solubility of this salt is comparable to sodium potassium cobaltinitrite. The reagent is specific for potassium in the presence of other metals of the fourth and fifth groups except rubidium and caesium and in the absence of ammonium salts. Potassium dipicrylamine is soluble in alcohol giving a strong yellow color.

A simple photoelectric colorimeter and a Zeiss step-photometer were employed in the determination of

the color developed. With the step-photometer, for ranges of 0-0.1 mgm. potassium per 100 ml. The observations were in strict accordance with Beer's Law, but at higher concentrations, the deviation became considerable. With the simple photoelectric colorimeter, however, no linear relationship existed. This was attributed to incomplete elimination of infa-red and the wide transmission band of the filter employed. The range of normal serum potassium by this method was 17.5-24 mgm. per 100 ml.

Wood (22) in 1942 described a method of adapting the procedure devised by Breh and Goebler (9) to the photoelectric colorimeter. The potassium silver cobaltinitrite precipitate goes into solution rapidly when exposed to hot acid or alkali. The amount of silver, cobalt or nitrite in the resulting solution serves as an index to the amount of potassium present. The cobalt ion in a relatively non-aqueous solution, such as 95% alcohol, reacts with ammonium thiocyanate to form a blue compound, $(NH_4)_2Co(CNS)_4$. This is then determined by photometry. A calibration curve, using known concentrations of potassium, must be constructed, by plotting the log of the galvanometric deflection a-gainst the potassium concentration. The probable error of this method for a single determination was

reported to be 2.3 micrograms of potassium per cc. of plasma of 1.2%.

In 1942, Sobel, Hanak and Kramer (23) introduced the procedure of electrodialysis to the determination of serum potassium levels. Electrodialysis of the reagents eliminated the need for a reagent blank. Electrodialysis of the serum to be analyzed removed the organic constituents which interfere with potassium determination. Wet ashing of serum cannot be used because ammonia must be completely removed. Dry ashing involves high temperatures with the resultant loss of some potassium by valitalization. The whole process of electrodialysis takes place at low temperatures and the loss of potassium is prevented. Hydrochloric acid was used, (chloride ion), for trapping the cations of the serum, thus further reducing errors due to the presence of sulfates, phosphates and carbonates. The potassium was precipitated as the sodium cobaltinitrite salt, which was then oxidized with an excess of ceric sulfate was determined by the sensitive iodometric titration.

The color produced by sulfanilamide and N-1 naphthylethylenediamine in the presence of nitrite was used by Looney and Dyer (24) in 1943 to estimate the amount of potassium in human serum. They precipitated

potassium as the silver cobaltinitrite salt and determined the nitrite present in the precipitate by photoelectric measurement of the color produced by the reaction described above. They found that a stable color was produced which was sensitive to 0.002 mgm. of potassium in 100 ml. of solution. The color produced follows Beer's Law up to 0.01 mgm. potassium in the final solution.

Pereira (25), in 1945, employed a color reaction first described by Chiorattino (26) in 1933, to the quantitative determination of potassium. Potassium is precipitated as the silver cobaltinitrite complex. The color is produced by adding dimethylglyoxime and benzidine. This reaction is highly specific for cobalt. The color system follows Beer's Law and an error of less than 3% can be expected from this method.

The application of flame photometry to the analysis of sodium and potassium in biologic materials was described by Overman and Davis in 1947. The principle of flame photometry was first used by Lundegordh (28) in 1929. Overman and Davis (27) used the Model 18 Perkin-Elmer flame photometer. The filter for the potassium cell covered a range of 7000-9240 A° with a peak transmission of 7716 A°. The accuracy obtained was ±2% for potassium. They also noted that the

presence of high concentrations of sodium in solution during analysis of potassium increased the intensity of the light emitted by the potassium ions. Thus they stated that the potassium standards used in calibration of the instrument should contain a ration of sodium:potassium approaching that found in blood serum. They also suggested that the application of an internal standard may reduce the error due to the presence of large concentrations of sodium.

The flame photometer was devised by Barnes, Richardson, Berry and Hood (30) in 1945 to measure the concentrations of sodium and potassium in solutions for industrial use. Hold (29) in 1947 adapted the flame photometer for use with biologic materials. The solution to be analyzed is discharged through an atomizer and drawn into a flame. The light produced by the combustion of the elements in the vaporized solution is drawn into an optical system, with appropriate filters, and is focused on a photoelectric cell, which is connected, through an amplifier, to a galvanometer.

The variable factors in this method are: (1) the atomizer, (2) the flame, (3) the air pressure, (4) the preparation of the material and (5) dilution of the material and (6) interfering substances. No ashing is

required. The atomizer, flame and air pressure must remain constant for each analysis. The serum is diluted with 0.9% saline 1:25, because of the low concentration of potassium in serum. The presence of iron in the analysis of whole blood interferes with the determination of sodium but has no appreciable effect upon potassium determinations. The accuracy of this method was found to compare favorably with standard chemical methods of analysis. The elimination of preparatory procedures, especially ashing, is most time saving.

The detailed construction and operation of a flame photometer was described by Domingo and Kline (31) in 1949. They found that the effect of sodium upon potassium determinations could be almost completely eliminated by use of a butane-air flame in place of an acetylene flame. They also found that calcium and ammonia did not interfere with the analysis of potassium. The presence of acid, in concentrations greater than 0.2N, significantly lowered potassium readings.

Abul-Fodl (32) in 1949 devised a colorimetric method for potassium determination based on the estimation of cobalt in a potassium silver cobaltinitrite precipitate. The reaction depends on the reduction of the phosphomalybdic-phosphotungstic acid phenol reagent by cobalt in the presence of amino acids (glycine

or alanine), which results in a blue color which is directly proportional to the amount of cobalt present. The conversion of cobalt to cobaltinitrite does not affect the reaction.

Theoretically, this method is more accurate than that of Looney and Dyer (24) because the reaction depends upon the cobalt which is more stable than the nitrite employed by Looney and Dyer (24).

Bernstein (34), (1950), stated "Flame photometric analysis of cations in biological fluids and tissues is, because of its simplicity, rapidity and greater accuracy, replacing laborous chemical procedures". The author went on to describe the application of the internal standard to flame photometry.

Absolute light intensity measurements are subject to errors due to variable viscosity, surface tension and composition of samples. However, the addition of lithium to all samples analyzed provides an internal standard by which light intensity ratios are measured. This materially reduces the errors from the above sources. A notable exception to this, however, is potassium, which in the presence of a relatively large concentration of sodium, results in higher reading. This is due to the phenomenon of mutual excitation by which potassium, in the presence of large concentrations

of sodium, emit more luminous energy per mEq. Hence reference to a potassium calibration curve is necessary to compensate for the error introduced by the presence of sodium.

The physical basis of flame photometry was described by Spencer (33) in 1950. When an atom is heated, one of its electrons absorbs the energy and jumps to the next outer orbit, where it rotates at a higher energy level. The electron is now in an unstable position and tends to return to its original orbit. As it returns to the original orbit, it releases as light the difference in energy required for rotation in the orbits concerned. The intensity and wave length of the light emitted is dependent on the position and energy level of the electron. Hence, each element when heated in a flame under standard conditions emits a characteristic light spectrum not emitted by any other element. The intensity of the light emitted is proportional to the concentration of the element present.

The method of Abul-Fodl (32) was modified by Lockhead and Powell (35) in 1951 to make the procedure less time consuming. They found the accuracy of this method to compare favorably with that obtained by the flame photometer. They suggest that since this method is less expensive than flame photometry, it is more

suitable for the small laboratory.

Elliott and Howell (36), (1951), reported on the results of four hundred serum potassium determinations on normal human subjects using the Beckman flame photometer. They obtained 4.18 mEq/L. as an average value with a range of 3.1-5.5 mEq/L.

A review of the methods available for the determination of serum potassium and their objections were presented by Shukers (56) in 1952. The chemical methods for potassium determination depend upon the precipitation of potassium as an insoluble salt, followed by the analysis of some component of the precipitate which bears a constant relation to the amount of potassium present. The chief criticism of the cobaltinitrite method is that sodium potassium cobaltinitrite is not of constant compostition. It has also been stated that the cobaltinitrite is too soluble for accurate analytic procedures. In spite of these objections, the cobaltinitrite method is capable of giving satisfactory results. The silver cobaltinitrite, while being less soluble than the sodium cobaltinitrite precipitate, has the disadvantages of requiring the preliminary removal of chlorides and protein. With reasonable attention to details, chemical methods are

accurate to within ±2%, but they require from three to thirty six hours for completion. With the advent of the flame photometer, a rapid and accurate method has been made available.

Review of Potassium Metabolism

Dietary potassium is derived chiefly from cereals, fruits and vegetables (37). The potassium is rapidly absorbed from the gastrointestinal tract and diffuses from the blood stream into the cells of the body. The kidneys are responsible for the excretion of more than 90% of the potassium, the remainder being lost in the perspiration and feces and via gastrointestinal juices. During the first few days of fasting, the potassium in the urine is decreased, and the concentration of potassium in the urine is much less than the serum potassium concentration indicating that the kidney tubules are able to conserve potassium to some extent. Potassium enters the urine by simple glomerular filtration, and as mentioned above, some is reabsorbed by the kidney tubule, but the rest is excreted. The daily intake of potassium is between three and four grams and the same amount is excreted.

The steroid hormones elaborated by the zona glomerulosa of the adrenal cortex seem to act as a regulator of potassium distribution in the body. These

hormones regulate the amount of potassium excreted in the urine, the amount taken up by the cells, the distribution between the cells and fluid and the relative proportions of sodium and potassium to be found in the perspiration. The hormone favors the excretion of potassium and the retention of sodium.

The concentration of potassium in human serum varies normally between 4.09 to 5.63 mEq/L. The interstitial fluid, of course has the same concentration. Body cells, however, contain about twenty times as much potassium as the serum. Red cells contain about 420 mgm./100mL. of potassium and three-fourths of the body potassium is to be found in the muscle cells. The cellular potassium is not completely ionized, most of it being in combination with protein and glycogen. It is alterations in the extracellular potassium which cause the clinical symptoms of hypo and hyper potassemia. The concentration of potassium in the extracellular compartment depends on the amount of potassium entering by way of the gastrointestinal tract and the cells, and by losses in the urine, perspiration and gastrointestinal secretions and that amount of potassium entering the cell. Dehydration and rehydration alter the extracellular potassium by shrinking and expanding this compartment without changes in the total amount of potassium

present. The potassium level of the extracellular fluid is accurately reflected in plasma potassium according to the Gibles-Donnan equilibrium. This is exceedingly valuable because it provides an accurate means of determining the amount of potassium bathing the body cells. Homeostatic mechanisms are so delicately adjusted, however, that only major disturbances result in altered potassium levels. When these alterations occur sericus consequences result.

The inorganic salts of potassium in the body are highly ionized, thus the principle effects of potassium are assumed to be due to the ion. There are three known isotopes of potassium having atomic weights of 39, 40 and 41. About 93% of body potassium is K^{39}, the remainder being made up of K^{41}. The radioactivity of potassium is due entirely to K^{40}. (38)

Potassium reduces the contractility of the heart and favors relaxation. In the presence of high enough concentrations of potassium, the heart will stop in diastole. Calcium has the opposite effect, increasing contractility and causing the heart to stop in systole. The presence of calcium and potassium in proper proportions ensures the rhythmicity of the heart beat (37). Darrow and Miller (39) observing the occurence of cardiac failure in some patients with Addison's disease

being treated with DOCA, were led to investigate the reasons behind the cardiac failure. Some cases were explained on the basis of pre-existing cardiac disease, the return of blood volume and retention of water and sodium increasing the cardiac burden, and low serum potassium as a result of therapy with DOCA. Using rats and cats, the authors, by repeated injections of DOCA were able to produce cardiac lesions in some of the animals. These lesions were characterized by small or large areas of necrosis which were replaced by fibrous connective tissue. Polymorphonuclear infiltration was absent. The lesions in these animals could be prevented by the addition of potassium to the diets.

Nerve fibers are exceptionally rich in potassium. Ratios as high as 65:1 on the inner and outer surfaces of vertebrate nerves have been reported. Polarization of the nerve is believed to be due to this difference. When a nerve is stimulated, potassium diffuses rapidly into the surrounding fluid. It is restored during rest (repolarization). The excitability of a nerve is markedly decreased by perfusing it with a solution high in potassium. Potassium is also necessary for transmission of nerve impulses across synapses and across the myoneural junction (37). It has also been found that potassium is essential for muscular contraction,

leaving the muscle cell on contraction and re-entering it on rest.

The role of potassium in acid base balance has not as yet been adequately explained. Clinically, hypokalemia and hyperkalemia frequently occur with alkalosis and acidosis respectively. Other important roles of potassium concern protein and carbohydrate metabolism. During the polymerization of glucose to glycogen in the liver, potassium and phosphorus are laid down with the glycogen in a definite weight-to-weight ratio. Conversly, during the process of glycolysis and liberation of glucose into the blood stream, potassium is liberated. In presence of a positive nitrogen balance, and when protein is being laid down in the cells, potassium is deposited with it in a ratio of 1 gm. N. to 2.38 mEq K. In the presence of protein catabolism, potassium is liberated into the extracellular fluid.

Hypopotassemia

In contrast to hyperkalemia, hypokalemia has been described under a great variety of clinical conditions and occurs more frequently than hyperkalemia. The mechanisms producing a low serum potassium are: (1) dilution of the extracellular fluids by potassium free solutions, (2) loss of potassium by excretion in the urine, gastrointestinal juices and perspiration, (3) transfers

of potassium into the cells (45). The signs and symptoms of hypokalemia involve the cardiovascular and muscular systems. They occur when serum potassium reaches 2.5-3.0 mEq/L. The most prominent feature is generalized muscular weakness involving all the striated muscle of the body, though characteristically, not involving muscles above the neck. The rapid, shallow, gasping respirations seen are due to paresis of the voluntary muscles of respiration. Flaccid paralysis occurs when the serum potassium reaches a level of about 2.5 mEq/L. At about this level, alterations in cardiac physiology occur resulting in an increased pulse pressure, Corrigan type pulse, cardiac dilitation, systolic murmurs and arrythmias. Characteristic EKG changes are most evident in the precordial leads, especially V_3. They consist of negative T waves, increase in QT interval and depression of ST segment (40).

Martin and Wertman (46), (1947), in a study of fourteen cases of diabetic acidosis reported the occurence of low serum potassium levels. They found the lowest levels to occur within the first twenty four hours of therapy and usually between twelve and twenty four hours. The serum potassium returned to normal within two to three days. Two of the patients who showed low serum potassium complained of muscle weakness

after therapy had been instituted. On admission most of the patients showed high potassium levels. This was explained on the basis of hemoconcentration secondary to dehydration and to decreased renal excretion of potassium secondary to shock associated with acidosis. Tuynman and Wilhelm (47) in 1948 reviewed the pathogenesis of hypokalemia occuring during therapy in diabetic acidosis. The factors involved are: (1) the excessive loss of potassium in the urine due to the polyuria induced by the excretion of large amounts of sugar in the urine, (2) acidosis results in increased excretion of water and electrolytes, (3) loss in urine by diuresis caused by the large amounts of fluid administered during therapy, (4) the process of glycogenesis, induced by insulin, removes potassium from the serum and deposits it in the body cells, and (5) the relative decrease in serum potassium invoked by the hemodilution incurred by fluid therapy. Greenman (48), (1949), found that patients being treated for diabetic acidosis continued to secrete measurable amounts of potassium in the urine. Also, if no potassium is given during this time, the over-all balance of cell potassium remains consistently negative.

The normal kidney while being able to conserve sodium, is unable to conserve potassium. Thus, even in the presence of hypokalemia, some potassium is lost in

the urine. Normally, potassium is excreted in the glomerular filtrate, some being reabsorbed by the tubules, where ammonia is substituted as part of the base conserving mechanism of the body. In some forms of nephritis with decreased tubular function, thetubules are unable to replace potassium with ammonia. This results in increased loss of potassium in the urine. With tubular damage greater than glomerular damage, hypokalemia states are more likely to be seen. When glomerular damage is great enough to cause an increased NPN, hyperkalemia states may occur depending upon the character of the renal damage (40). Brown, et. al. (48), (1944), reported two cases of nephritis who developed flaccid paralysis and EKG changes characteristic of hypokalemia. Both of these patients had tubular damage far greater than glomerular damage as evidenced by a normal NPN in one patient and only a slight elevation in the other.

Familial periodic paralysis has been shown to be associated with low serum potassium levels. The disease is characterized by episodic transient periods of paralysis of skeletal musculature without the presence of other neuralgic abnormalities. This disease has been recognized for over 100 years, but the disturbance in serum potassium was discovered only about six years ago.

It is usually not associated with any other serious disease although some cases have been reported to occur with thyrotoxicosis. During the typical attack there is flaccid paralysis of all the skeletal musculature with loss of deep tendon reflexes. No sensory changes occur and the sensorium remains intact. The attack may last from a few hours to a few days and disappears without any residual effects. During the attack serum potassium levels are low but return to normal with recovery. Oral administration of potassium chloride hastens recovery. Balance studies have shown that potassium is not lost in the urine but enters the intracellular compartment. Whether the paralysis is caused by low serum potassium, chronic potassium defecit in muscle or by a disorder of hepatic or muscle glycogenesis has not been determined. (50).

A syndrome of postoperative potassium defecit and alkalosis was described by Eliel, et. al, (51) in 1950. The syndrome is characterized by apathy, lithargy, nervousness and irritability, muscular weakness, metabolic alkalosis hypochloremia and hypokalemia. Prompt relief occured after administration of potassium salts. All patients with this syndrome were on a low intake of potassium preoperatively, due to vomiting and intravenous feedings. Loss of gastrointestinal secretions, removal

of potassium and chloride from stomach leads to alkalosis and hypokalemia. Hyperadrenal cortical states, secondary to stress of surgery, has been postulated as a factor in the development of hypokalemia in this syndrome by increasing the urinary loss of potassium. Prevention of this syndrome is by early replacement of potassium in the postoperative period (51).

Diarrheal states, if severe may lead to excessive loss of potassium and the development of hypokalemia. Several cases of cholera, treated with potassium free fluids, have been reported to develop low serum potassium levels. The high mortality of infantile diarrhea has been shown to be due to hypokalemia. The addition of potassium to fluid therapy in this condition has markedly reduced the mortality rate. Several cases of sprue have been reported to develop hypokalemia. The development of hypokalemia in diarrheal states is due to the excessive loss of potassium in the intestinal secretions and vomiting, if present, and to the decreased dietary intake of potassium. The use of large quantities of potassium free fluid in therapy augments the low serum potassium level (40,52).

Hyperadrenal cortical states such as Cushing's Disease and Addison's Disease overtreated with DOCA are associated with hypokalemia. The hypokalemia is due to

the increased excretion of potassium by the kidneys under the influence of increased levels of cortical steroids. Patients under therapy with adrenal cortical hormones or ACTH may develop hypokalemia as a result of overdosage.

In a study of twenty seven cases of intestinal obstruction in man, Polsoner, et. al. (53), (1939), found sixteen patients with serum potassium levels below 4 mEq/L., the lowest value being 2.37 mEq/L. From these results it is obvious that hypokalemia does not result in all cases of intestinal obstruction. Two secondary factors seem to be of importance in the pathogenesis of hypokalemia in these cases. The first and most important factor is the loss of potassium in the gastrointestinal fluids. Vomiting and gastric suction are the means by which these losses occur. The authors found that gastric juice in patients with bowel obstruction contained from two to four times as much potassium as did the serum. The remainder ofthe gastrointestinal fluids contained lower amounts. The second factor is the expansion of the extracellular compartment due to therapy with potassium free fluids. This results in a lowering of serum potassium level without an absolute decrease in the amount of extracellular potassium.

The introduction of carbo-resins in the therapy of

congestive heart failure to decrease the amount of sodium absorbed has, in some cases, also decreased the amount of potassium absorbed by the gastrointestinal tract resulting in hypokalemia. The addition of potassium to these resins has overcome this effect.

The use of mercurial diuretics and ammonium chloride lead to large transient increases in the urinary excretion of potassium. With intermittent use of mercurials, however, potassium depletion is not likely to occur. The continual use of either mercurials or ammonium chloride may induce a slowly developing hypokalemia, which may be well tolerated. Clinically, hypokalemia in treated congestive heart failure is most likely to be seen when food intake is poor (54).

Hyperkalemia

The clinical condition of hyperkalemia is not seen as frequently as hypokalemia, and symptoms of hyperkalemia do not become manifest until serum potassium levels reach extremely high values. Serum levels of 10-10.5 mEq/L. are uniformly lethal, death being due to toxicity of potassium upon the myocardium, cessation of heart action in diastole. The symptoms of hyperkalemia consist of paralysis of striated muscle all over the body, cardiac toxicity, EKG changes, and unknown alterations in cellular metabolism. The EKG changes, however,

become manifest at lower levels of hyperkalemia. Be-tween 7-9 mEq/L., T wave alterations appear, between 9-9.5 mEq/L. the P wave disappears and between 9.5-10.5 mEq/L. spreading and complete disintegration of the QRS complex occurs. The sequence of EKG changes in hyper-kalemia are: (1) elevation of T wave, (2) decrease in size of R wave and increase in S component, (3) disap-pearance of P wave. (4) depression of ST segment and (5) spreading of QRS and T wave until a smooth biphasic wave is seen (40,41).

The excessive administration of potassium as a cause of hyperkalemia has only recently been recognized. It has been shown that the administration of five grams of a potassium salt orally to normal persons will re-sult in hyperkalemia with characteristic EKG changes. Potassium has been used in very large doses in the past in the treatment of myasthenia gravis and Meniere's syndrome. With the advent of sulfa drugs, potassium carbonate was used as an alkalizing agent in patients with congestive heart failure to avoid the administration of sodium. How many cases of potassium intoxication occur-red as a result of this practice can only be surmised. (40)

Wener, et. al. (42), (1949), described the effect upon rabbits of acute intravascular hemolysis produced by the intravenous injection of saponin. They showed in. They showed

that death was due to potassium intoxication produced by the rapid liberation of potassium from the erythrocyte into the blood stream. This points to the possibility of potassium intoxication occuring in erythroblastosis fetalis, transfusion reactions and other hemolytic diseases (42).

In 1947, Fox, et. al. (43), showed that conditions which cause shock, such as burns and trauma cause an elevation of extracellular potassium. The reason for this is that the damaged cells lose their specific permeabilities and allow sodium and water to enter and potassium to leave. The action of histamine like substances on uninjured cells allow these cells to take up water and sodium and to release potassium. Serum potassium has been observed to rise to twice normal levels in some instances of shock.

Disturbances in potassium metabolism occuring in renal disease in man was described in 1943 by Keith, et. al., (44). Balance studies indicated that in most cases of severe renal dysfunction in man, potassium clearance was adequate to excrete the potassium derived from tissue catabolism. However, when an extra amount of potassium occured in the extracellular fluid, the kidneys excreted it at a much slower rate, and if the increased load of potassium continues, retention will occur. An

abnormally high serum potassium level in patients with chronic renal disease usually indicated an acute exacerbation. Patients with oliguria or anuria showed consistent elevations of serum potassium.

An elevation of serum potassium occurs in patients with Addison's Disease, which isdue to a deficiency of adrenal cortical steroids. The deficiency of these steroids results in loss of sodium and water and a decrease in the excretion of potassium. High potassium levels seen are due to lowered excretion of potassium, contraction of extracellular fluid volume and the inability of the cells to take up potassium (40).

The advent of blood banking has created another source of inducing hyperkalemia. Since potassium begins to shift from the erythrocytes to the plasma soon after blood is withdrawn from the vein, no doubt exists that stored blood contains relatively higher serum potassium levels than normal, especially after long storage. In patients with good renal function, little or no danger exists. However, multiple transfusions or patients with extensive burns, decreased renal function or hyperkalemia from any other source, may raise serum potassium to dangerous levels. Serum potassium determinations on stored blood are not recommended however for reasons of sterility, but the possibility of hyperkalemia resulting from multiple transfusions of stored blood should be kept in mind (57).

Turbidometric Method of Serum Potassium Estimation

The method evaluated in this paper was described by H. G. and N. B. Keitel (55) in 1953. It is based upon the estimation of the degree of turbidity produced by the precipitation of potassium as sodium potassium cobaltinitrite. The turbidity of the unknown is compared with that of two known standards.

Only a minimum of equipment is required for this procedure. The materials needed are: (1) two Sahli hemoglobin pipets, (2) two microscopic culture slides, (3) one rubber tipped stirring rod, the tip measuring 2½ mm. thick, 7 mm. wide at the base and 2 mm. wide at the tip and (4) one 15 by 15 cm. piece of black paper or overexposed xray film. Two standard potassium solutions are needed. One containing 3 mEq of potassium per liter (0.224 gm. potassium chloride per liter) and 140 mEq of sodium per liter (8.12 gm. of sodium chloride per liter). The other standard contains 5 mEq of potassium per liter (0.373 gm. of potassium chloride per liter) with 140 mEq of sodium (8.12 gm. of sodium chloride per liter). A 15% solution of sodium cobaltinitrite is used to precipitate the potassium.

Serum for the determination is obtained by venipuncture with precautions taken to avoid hemolysis.

The blood is centrifuged until cell free plasma or serum is obtained. When serum remains in contact with the erythrocytes, potassium tends to migrate to the serum. Thus the serum must be removed after centrifugation has been completed. With a Sahli hemoglobin pipet, 0.02cc. portions are transferred to two of the depressions on the microscope slide. With the same pipet, 0.02cc. portions of the 3mEq and 5mEq standards are transferred to the other two depressions on the microscope slides. The other Sahli hemoglobin pipet, marked at 0.01 cc., is then used to transfer 0.01 cc. of the 15% sodium cobaltinitrite reagent to the depressions. The sodium cobaltinitrite is added in the following order; first to the unknown, then to the 3mEq and 5mEq standards, then to the other unknown. This eliminates time as a factor in the development of the precipitates. Each sample is stirred with a rapid circular motion for five seconds with the rubber tipped stirring rod. The stirring rod is wiped dry between stirrings. The slides are then placed on a black background and a light directed across the slide. If the temperature is above 80°F. a clear distinction between the 3mEq and 5mEq standards cannot be made. The readings can usually be made within 20-30 seconds and must be completed within five minutes.

Classification of the unknowns, depending on the degree of turbidity as compared to the standards, is as follows: (1) if the precipitate was less than the 3mEq standard, (2) the same as the 3mEq standard, (3) between the 3 and 5mEq standards, (4) the same as the 5 mEq standard and (5) greater than the 5mEq standard.

When the unknown contains more than 5mEq/L. of potassium, it can be diluted with an equal amount of normal saline. This will serve to dilute the potassium present and make a closer estimation possible.

The precautions described by the authors (54) are common to all methods of potassium determinations. The test slide must be very clean and free from scratches in order to compare equally the precipitates. If either the standards of the unknown contain localized areas of precipitation, errors are likely to result. Both standard potassium solutions should be run with each test. Cigarette ash, due to its potassium, will interfere with the test. Grossly lipemic serum should not be used unless it is first cleared with ethyl ether. During collection of the sample, extreme care must be utilized to prevent hemolysis.

The authors ran a total of 145 serum samples with potassium concentrations between 2 to 10 mEq/L., as determined by the flame photometer. They found agreement

adequate for clinical purposes. Serums classified as 1 and 5 always contained abnormal amounts of potassium.

The method evaluated in this paper was set up according to the above description. The standards were prepared by weighing dried potassium chloride on a chain balance. The "3"mEq standard analyzed by the flame photometer at this laboratory contained 3.2mEq/L. and the "5"mEq standard contained 5.1mEq/L. The sodium cobaltinitrite solution was prepared from a commercial chemically pure preparation. The sodium cobaltinitrite was kept in the refrigerator.

Several difficulties were encountered in carrying out the determinations. Extreme care must be used in pipeting the serum, because bubbles are apt to occur in the serum on the slide. Usually these bubbles can be obliterated with the stirring rod, but if they persist, an accurate comparison cannot be made. The time factor was found to be very important and to obtain accurate results, the cobaltinitrite reagent must be added in the described sequence. Extreme cleanliness of the slide is re-emphasized. Minute foreign particles tend to result in localization of the precipitate and make comparisons difficult. Lint introduced on the stirring rod by wiping between stirrings has the same effect, and preferably, a lint free towel should be used. An

ordinary microscope substage lamp was used as a source of light in making the comparisons. The light was directed across the slides. This proved to be the simplest and most satisfactory method of comparison. If the environamental temperature is above 27°C., the difference between the two standards cannot be accurately determined, as precipitation is dependent upon temperature. To obtain accurate results, both standards must be run with each determination. Several determinations must be carried out before accurate and consistent estimations of turbidity can be made.

Comparison of Slide Method With Flame Spectrophotometer

One hundred samples of serum were analyzed by the method of Keitel and Keitel for their potassium content. Each sample of serum had been previously analyzed by the flame spectrophotometer. The serum potassium levels ranged between 3.2 and 16.3 mEq/L. Hemolysis occured in the two samples containing 10.0 and 16.3 mEq/L. Ten samples were graded as 5. One of these contained 5.9 mEq/L. and nine contained greater than 6 mEq/L. The number of samples grade 4 was forty five; twenty serum of these were between 5-6 mEq/L., seventeen between 4-5 mEq/L. and one between 3-4 mEq/L. Forty-three samples were graded 3. Of these, twelve contained between 5-6

mEq/L., twenty six contained between 4-5 mEq/L. and five between 3-4 mEq/L. Only two samples were graded 2 and both contained between 3-4 mEq/L. No samples were graded 1 (See Table 1).

Grade	Number of Patients				
	0-3mEq.	3-4mEq.	4-5mEq.	5-6mEq.	>6mEq.
1	0	0	0	0	0
2	0	2	0	0	0
3	0	5	26	12	0
4	0	1	17	27	0
5	0	0	0	1	9

Table 1

An accurate differentiation between grades 3 and 4 were particularly difficult to make. Two factors in this series influenced the relatively large number of samples graded 3 whose actual potassium level as determined by the flame spectrophotometer ranged between 4-5 mEq/L. The first is the fact that the 3 mEq/L. standard contained 3.2 mEq/L. as determined by the flame spectrophotometer and the second, and the most important, is the relatively large number of sera containing over 4 mEq/L. as compared with those containing less than 4 mEq/L. Those samples graded 3 which contained between 5-6 mEq/L. must be accounted for by errors in grading. These errors were probably due to the introduction of

foreign particles such as lint and dust which cause localization of the precipitate. The same factors influenced the number of samples graded 4. Only one serum was graded 5 which contained between 5-6 mEq/L. and nine sera were graded 5 which contained over 6 mEq/L. There were no sera in this series which contained less than 3.2 mEq/L., and no samples were graded 1.

A tabulation of the results listing actual potassium levels as determined by the flame spectrophotometer and the grade given the sample will be found in the appendix.

Every sample in this series graded 5 contained a greater than normal amount of potassium. Although several samples were graded 4 which contained slightly over 5 mEq/L, the elevations were not out of the accepted limits of normal except for only a few cases. With a reasonable amount of care and practice in carrying out this procedure, significant deviations in serum potassium levels can easily be determined. It is well to emphasize again that practice is needed before accuracy in grading can be accomplished. Despite the fact that this series did not contain any sera below 3.2 mEq/L., it is felt that any significant hypokalemia would be picked up by this method.

CONCLUSIONS

The results obtained by this method were found to correlate with results by flame spectrophotometric analysis with a sufficient degree of accuracy to be applicable for clinical use. Every sample graded 5 contained an amount of potassium greater than normal. Samples of sera containing 6 mEq/L. or greater of potassium were picked up without undue difficulty. This series did not contain any sera below 3.2 mEq/L; thus no conclusions can be drawn as to the accuracy obtainable in the analysis of sera containing lower concentrations of potassium.

SUMMARY

The methods available for the determination of serum potassium are reviewed. A summary of the advantages and disadvantages of these methods is also presented. A review of potassium metabolism and the various clinical conditions in which serum potassium levels are altered is described.

A series of one hundred sera were analyzed by slide method of Keitel and Keitel and the results compared with those obtained by the flame spectrophotometer. A detailed description of the method employed is included.

Finally, the author wishes to acknowledge the assistance in the technical portions of this paper rendered by Miss Helen Black.

APPENDIX A

	Keitel Method	mEq/L.-Flame Spectrophotometer
1.	4	3.82
2.	4	5.0
3.	4	3.1
4.	3	5.0
5.	3	4.0
6.	5	10.0*
7.	5	6.3
8.	3	4.5
9.	3	5.1
10.	5	5.9
11.	4	4.4
12.	4	5.6
13.	4	5.6
14.	3	3.9
15.	4	4.3
16.	4	5.6
17.	4	5.1
18.	3	4.6
19.	2	3.9
20.	4	5.0
21.	3	4.5
22.	4	4.7
23.	4	4.9
24.	3	5.6
25.	3	4.6
26.	5	16.3*
27.	3	3.9
28.	4	4.6
29.	4	5.2
30.	4	5.8
31.	3	4.0
32.	5	6.1
33.	3	6.2
34.	4	5.2
35.	3	5.8
36.	4	5.0
37.	4	4.8
38.	4	5.2
39.	3	4.0
40.	4	4.0
41.	3	4.75
42.	5	7.1
43.	4	5.3
44.	3	4.7
45.	4	4.0

(*Hemolyzed)

	Keitel Method	mEq/L.-Flame Spectrophotometer
46.	3	4.0
47.	3	4.5
48.	4	5.2
49.	3	4.2
50.	3	4.5
51.	2	3.2
52.	5	6.0
53.	4	5.6
54.	3	5.6
55.	4	5.6
56.	4	4.9
57.	5	6.5
58.	3	5.1
59.	4	5.7
60.	3	4.9
61.	3	4.3
62.	4	5.0
63.	5	6.8
64.	4	4.9
65.	3	5.0
66.	4	5.7
67.	3	4.5
68.	3	5.0
69.	4	5.2
70.	3	5.3
71.	4	4.9
72.	4	5.2
73.	4	5.6
74.	4	5.0
75.	5	6.3
76.	3	4.2
77.	3	4.5
78.	3	5.1
79.	4	5.6
80.	3	4.3
81.	3	4.6
82.	3	4.8
83.	4	4.4
84.	3	4.9
85.	3	4.5
86.	3	5.2
87.	3	4.6
88.	4	4.9
89.	4	4.7
90.	3	3.8

	Keitel Method	mEq/L.-Flame Spectrophotometer
91.	3	4.6
92.	4	5.0
93.	3	4.2
94.	4	5.5
95.	4	4.6
96.	3	3.8
97.	4	5.2
98.	4	4.8
99.	3	3.7
100.	4	4.5

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