

THE DETERMINATION OF SODIUM
IN HUMAN SERUM

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SECTION I

THE PROBLEM

Within the past few years physicians and surgeons have come to appreciate more keenly the importance of maintaining or re-establishing a normal electrolyte balance in the blood of their patients. This has led, in turn, to a greater demand for determinations of the concentration of sodium which is the chief cation in the blood plasma. To meet this demand there is need for a method which is not only accurate but reasonably rapid since it would clearly be useless to calculate the amount of intravenous fluid needed on the basis of the sodium content of the plasma many hours earlier.

The problem of devising a satisfactory method for the determination of sodium in human serum is beset with at least two serious obstacles. The relative solubility of sodium and potassium salts in aqueous solution and their insolubility in many organic solvents is well known. Methods of separating the two cations from a complex mixture by ordinary analytical techniques are therefore involved and consequently require considerable time.

Another difficulty arises which is peculiar to the determination of sodium in serum. Next to the pH, the sodium concentration of normal human plasma is the most constant

feature in blood, ranging from 300 to 330 or from 325 to 350 mg. per 100 ml., depending upon the method used for the determination. The difference between extreme normal limits is therefore only about 10 per cent, and the variation from a mean value, approximately 5 per cent. Any method to be of value must therefore be accurate to within a few per cent at least.

Many of the chemical methods available are indeed sufficiently accurate, but in general, they are so time-consuming as to be of limited value when a series of determinations is essential, as in the precise diagnosis and controlled treatment of body-fluid disturbances. By means of the flame photometer the requisite rapidity and accuracy can be achieved in the hands of skilled workers when proper precautions are exercised, but the instrument is expensive and not likely to be found in most routine hospital laboratories. The need therefore still exists for a more rapid yet accurate chemical method for the determination of sodium in serum, a method moreover, in which only small quantities of serum are required for analysis: the purpose of this investigation therefore was to develop such a procedure, which would be suitable for use in the average routine hospital laboratory.

SECTION II

REVIEW OF EXISTING METHODS

Since 1850, when Schmidt estimated sodium in the serum of choleric patients, many methods have been proposed for the determination of serum sodium. They fall into two general classes, chemical methods, and physico-chemical methods, and are therefore reviewed under these headings.

CHEMICAL METHODS

Introduction

The early methods which were chiefly gravimetric are fully reviewed by Peters and Van Slyke (1932). No attempt is made in these early methods to separate sodium from potassium in a complex solution such as blood serum. In general, the procedures follow the scheme in classical analytical chemistry, according to which heavy elements and the alkaline earths are removed prior to the ignition; the sodium and potassium are then weighed as the combined sulphates or chlorides. Potassium is then estimated in the redissolved salts by precipitation as the chloroplatinate, K_2PtCl_6 , or the perchlorate, $KClO_4$, and the sodium calculated by difference. The disadvantage of such methods is

that when sodium is present in much smaller amounts than potassium, all errors are heaped on the small amount of sodium. Since sodium is always present in large excess over potassium in human serum, this error can be neglected in serum analyses. These methods are, in fact, the most accurate available to the present day, and are used in certain research problems. Being long and tedious, however, they seriously limit a study of sodium metabolism. Furthermore, 15 ml. or more of blood are required, an amount frequently unavailable, particularly in the case of infants.

Later methods depend upon the relative insolubility of certain sodium salts and the solubility of the corresponding potassium salts in various solutions.

The first of such methods, proposed by Fenton in 1898, was quite unsatisfactory. It was based on the precipitation of sodium as the salt of dihydroxy-acetone. The latter was not sufficiently insoluble for exact work, and the reagent was difficult to prepare. The method was useful, nevertheless, in that it pointed the way to a new approach in sodium determinations.

Subsequent methods in which sodium is precipitated as a complex nitrite or acetate, or as the pyroantimonate, have been more successful.

In the following review, only the more important methods are dealt with. They are grouped according to the

reagent which is used to precipitate the sodium, namely caesium bismuth nitrite, potassium pyroantimonate, and uranyl zinc acetate. Since methods using the latter have been so widely used they are considered in more detail.

Methods Using Caesium Bismuth Nitrite

By the method of Doisy and Bell (1920), sodium is precipitated as the complex sodium caesium bismuth nitrite, which has the formula, $9 \text{CsNO}_2 \cdot 6 \text{NaNO}_2 \cdot 5 \text{Bi}(\text{NO}_2)_3$. This compound can be estimated by gravimetric, volumetric or colorimetric means. Weighing was not recommended by the authors due to contamination of the precipitate by a scum of bismuth subnitrite and also by potassium nitrate which crystallizes out under the conditions of the procedure. Although they also described a colorimetric estimation, based on a coupling reaction using α -naphthylamine and sulphanic acid, the authors preferred the volumetric procedure in which the nitrite is oxidized to the nitrate on titration with standard permanganate. Despite the fact that only one ml. of serum, urine or whole blood is required for an analysis, the method has not achieved much popularity. A precipitation period of at least 24 hours at 0°C . is required. The caesium bismuth nitrite reagent, furthermore, was found to be quite unstable even if stored in an inert atmosphere at 1°C . Finally, some workers reported considerable technical difficulties,

particularly in the rather complicated titration.

Methods Using Potassium Pyroantimonate

Kramer and Tisdall in 1921 proposed a gravimetric method for the estimation of sodium in serum and other biological materials based on the precipitation of sodium as the sparingly soluble sodium pyroantimonate. According to Peters and Van Slyke (1932), however, difficulties were encountered in applying the method. These were apparently caused by the tendency for the finely divided precipitate to pass through filters, the inconstant amount of water of crystallization, and the inclusion with the precipitate of other substances which are also insoluble in alkaline media.

Independent attempts were made by Balint (1924) and Kramer and Gittleman (1924) to overcome these objections by iodometric titration of the antimony in the precipitate.

Balint also studied the optimum conditions for quantitative precipitation of sodium pyroantimonate in serum analysis. He concluded that preliminary ashing of serum was necessary; this finding was confirmed by Eisenman (Wakeman, Eisenman and Peters, 1927). Rourke (1928), notwithstanding, found ashing to be unnecessary if precipitation of proteins is avoided by adding alcohol to the serum at a low temperature. Potassium, magnesium, calcium, sulphate, phosphate and chloride were shown not to interfere.

Most of the methods described in the above paragraphs have yielded more or less useful clinical results. In a critical study of various methods, Liegeois (1937) found that the antimoniate methods were less accurate than the triple acetate methods to be described next.

Methods Using Uranyl Zinc Acetate

Most recent procedures for the determination of sodium are based on the precipitation of sodium as one of the triple acetates first reported by Streng in 1886. The reagent most frequently employed is uranyl zinc acetate, in some modification of Barber and Kolthoff's (1928) gravimetric method.

The precipitate formed when an aqueous solution containing sodium is treated with Barber and Kolthoff's reagent is uranyl zinc sodium acetate which has the formula, $(\text{UO}_2)_3\text{ZnNa}(\text{CH}_3\text{COO})_9 \cdot 6\text{H}_2\text{O}$. This triple salt is particularly well suited for sodium estimations. Because of its great weight, 67 times that of the sodium present, it readily lends itself to gravimetric micro-analysis. Volumetric and colorimetric adaptations have also appeared due to the ease with which some component (and hence sodium) in the precipitate can be measured.

For convenience, the uranyl zinc acetate methods are reviewed according to the technique employed in the final

stage of the analysis, namely, gravimetric, volumetric or colorimetric estimation.

Gravimetric methods. Next to the early classical methods, the gravimetric uranyl zinc acetate procedures are the most accurate available. Among the chief modifications are those of Butler and Tuthill (1931), Consolazio and Dill (1941), and Hald (1939). The first two methods differ only in the way the serum is ashed, the former employing concentrated sulphuric and nitric acids in a wet-ashing technique, whereas the latter ash serum to dryness in crucibles heated in an electric furnace. Hald, however, prefers to remove phosphate prior to ashing, a precaution of doubtful value in serum analyses because of the small amount of phosphate present.

These gravimetric procedures are much used in research and for comparison purposes when testing new methods, but their application to routine clinical work is limited by the time required for the ashing and gravimetric steps.

Volumetric methods. One of the best-known titrimetric procedures for serum sodium is that of Weinbach (1935), in which sodium is precipitated by uranyl zinc acetate from a trichloroacetic acid filtrate of serum. After washing with a solution of acetone (saturated with triple salt), the precipitate is dissolved and titrated with standard alkali.

A somewhat similar procedure has been reported by Dreguss (1939), the special feature of which is the simultaneous removal of phosphate and protein by means of alcoholic zinc acetate.

An entirely different technique was introduced by Ball and Sadusk (1936) for the volumetric estimation of the triple salt precipitated from solutions of serum. It involves the reduction of hexavalent uranium in the triple salt to the quadrivalent state, and subsequent quantitative oxidation with dichromate to the hexavalent condition.

Some objections to these methods have been noted. Velarde (1947), for example, found errors ranging from -3.30 to +4.59 per cent in recovery experiments by Weinbach's method. Furthermore, in the method of Ball and Sadusk very careful control of the reduction and oxidation process is needed to obtain accurate results.

In general, the use of volumetric methods in clinical work is limited by the fact that they are inconvenient and time-consuming when numerous samples are estimated.

Colorimetric methods. Most colorimetric procedures which have been described for the estimation of serum sodium consist of the following four stages:

(1) Deproteinization

(2) Precipitation of uranyl zinc sodium acetate

- (3) Washing of the precipitated triple salt
- (4) Colorimetric determination of one of the constituents of the dissolved precipitate

Certain other methods include a fifth step, the removal of phosphate, but this is of no importance in serum estimations, as will be seen later. Furthermore, two methods which do not follow this general scheme have also been noted.

(1) Deproteinization - With few exceptions, all methods have employed either trichloroacetic acid as a protein precipitant or an ashing procedure for the destruction of serum protein. Some methods, for example those of Hoffman and Osgood (1938) and Darnell and Walker (1940), give directions for both techniques.

It has been pointed out that trichloroacetic acid filtrates yield values up to 3 per cent higher than those obtained from ashed serum. This has been attributed by Ball and Sadusk (1936) to a volume displacement of the precipitated protein, the result being an increased concentration of sodium in the filtrate. The remedy suggested by the latter investigators is either to precipitate the proteins in such a way as to cause the least bulk of precipitate to be formed, or else to add a large proportion of trichloroacetic acid so as to minimize the effect due to volume displacement. The latter alternative was utilized

by Hoffman and Osgood (1938) and by Stone and Goldzieher (1949) with good results.

(2) Precipitation of uranyl zinc sodium acetate -

Among the reagents used for the precipitation of sodium are uranyl zinc acetate solutions containing acetic acid and, in some cases, ethanol in varying amounts. McCance and Shipp (1931), for example, use a 50 per cent alcoholic solution, whereas Darnell and Walker (1940) prefer an aqueous reagent (Weinbach). Other investigators such as Stone and Goldzieher (1949) compromised by adding a small proportion of alcohol to their reagent. The purpose of the alcohol in all cases was to effect a more complete precipitation of the triple salt.

The triple salt is precipitated in various ways. McCance and Shipp treated 1 part of aqueous test solution with 10 parts of alcoholic uranyl zinc acetate at 0°C for 1 hour, whereas Albanese and Lein (1948) used 1 to 4 parts of aqueous solution and 10 parts of alcoholic reagent at 4°C for 1 hour. Darnell and Walker treated 1 part of aqueous solution with 5 parts of an aqueous reagent (Weinbach), followed by the addition of 2.1 parts of ethanol over a period of 30 minutes. Bradbury (1946), however, simplified the technique of Darnell and Walker by using 1 part of aqueous solution, 4 parts of aqueous reagent (Weinbach) and 2 parts

of 95 per cent alcohol, allowing the mixture to stand at room temperature for 20 to 30 minutes. Fowweather and Anderson (1948) found it necessary to alter the method of Bradbury by treating 1 part of the sodium-containing solution with 2 parts of aqueous reagent, 3 parts of absolute alcohol, and 2 parts of water at 3°C. for 2 hours. In the method of King et al (1942), 1 part of aqueous solution is treated with 2 parts of aqueous reagent and 5 parts of ethanol at 0°C. for an overnight period.

(3) Washing of the precipitated triple salt - Among the wash reagents which have been used in colorimetric methods to remove excess reagent from the precipitated triple salt are the following: (i) 95 per cent alcohol (Albanese and Lein, 1948), (ii) absolute alcohol, saturated with triple salt to prevent re-solution of the precipitate (King et al, 1942), (iii) glacial acetic acid, saturated with triple salt (Salit, 1932), (iv) ethyl acetate - acetic acid solution (Darnell and Walker, 1940), (v) 95 per cent alcohol - glacial acetic acid solution, saturated with triple salt (Hoffman and Osgood, 1938; Stone and Goldzieher, 1949).

It was shown by Hoffman and Osgood in 1938 that saturated solutions of the triple salt in alcohol, glacial acetic acid or acetone produced precipitates of uranium

salts when added to uranyl zinc acetate. Since the intensity of the colour of the dissolved triple salt is dependent upon the amount of uranium present, a slight positive error is produced because of extra precipitation of the triple salt. Hoffman and Osgood (1938) claim that in their method this positive error is balanced by an equal negative error due to incomplete precipitation of the triple salt. This type of compensation no doubt also operates to a certain extent when using the other wash reagents mentioned above.

The chief disadvantage of the Hoffman -Osgood and Darnell-Walker methods is the fact that two additional washings with ether are required to remove the acetic acid left after the first washing, thus greatly lengthening the procedures. This difficulty is overcome in the Stone and Goldzieher procedure where the washed triple salt is dissolved in strongly alkaline solution (thereby neutralizing any acid from the wash reagent) prior to further treatment with hydrogen peroxide.

(4) Colorimetric determination of one of the constituents of the dissolved precipitate - Most colorimetric uranyl zinc acetate methods depend upon the measurement of the colour produced when the dissolved triple salt is treated with one of the following: potassium ferrocyanide, sulphosalicylic acid or hydrogen peroxide. In a number of

other methods no further reagent is used to intensify the weak yellow colour of the uranyl ion.

The use of potassium ferrocyanide in sodium methods originated with Barrenscheen and Messiner (1927), who applied the well-known colour reaction between uranium salts and the ferrocyanide ion (in weakly acid solution) to the colorimetric estimation of the uranyl ion (and hence sodium) in the triple salt. The deep, plum-red colour produced was more easily and accurately estimated when visual colorimetric instruments were used than was the untreated solution of triple salt.

Numerous modifications using ferrocyanide have appeared, notably those of McCance and Shipp (1931), Salit (1932) and King et al (1942). All these investigators were aware of the fact that the colour produced due to uranyl ferrocyanide was not stable, but that after some minutes it became darker owing to the formation of colloidal zinc ferrocyanide. No serious errors due to this cause were observed so long as the colours of the test and standard solutions were compared simultaneously in a visual colorimeter within 10 or 15 minutes after the addition of ferrocyanide. When photoelectric colorimeters were introduced, however, this colour instability assumed greater significance (Fowweather and Anderson, 1948) since in these instruments no direct, simultaneous comparison between test

and standard solutions is made. Other objections to the use of ferrocyanide have been reported, such as sensitivity to changes in pH, temperature and reagent concentrations.

Darnell and Walker (1940) treated the dissolved triple salt with sulphosalicylic acid and sodium acetate. The deep yellow colour obtained is stable, but does not obey Beer's law exactly, and a calibration curve must be used.

A number of investigators, including Krakusin (1948) and Albanese and Lein (1948) simply dissolved the triple salt in water and measured the intensity of the yellow solution. These methods, in general, lack sensitivity and are subject to large experimental errors. The Krakusin method is examined in greater detail in Section III.

Stone and Goldzieher (1949) treated the dissolved precipitate with hydrogen peroxide in alkaline solution to give an orange-red colour. This method also is discussed more fully in Section III.

The two methods which differ fundamentally from the others are due to Bradbury (1946) and Fowweather and Anderson (1948). It occurred to Bradbury that since the uranyl ion made the reagent a bright yellow, the supernatant should lose colour in proportion to the amount of triple salt precipitated. He found this to be the case; his method, therefore, and a modification by Fowweather and