

## USE OF GLASS FILTER AND URANYL-ETHANOL MIXTURE IN THE COLORIMETIC DETERMINATION OF SODIUM WITH URANYL ZINC ACETATE

SUSUMU SHIBATA AND WATARU MIZUTA

*Department of Clinical Pathology, Yamaguchi Medical School, Ube*

(Received April 10, 1954)

There are numerous modifications of basic procedure for the determination of sodium with uranyl zinc acetate reagent. The clinical laboratory requires a method that is simple in technic and that will yield reproducible results with an accuracy of clinical significance. Darnell-Walker's procedure<sup>1)</sup> was recommended by some workers<sup>2)3)</sup> on this basis, although it could not give results so quickly as did the flame-photometry. In that method sodium was precipitated from trichloroacetic acid filtrate of serum by uranyl zinc acetate reagent as sodium uranyl zinc acetate  $\text{Na}(\text{UO}_2)_2\text{Zn}(\text{CH}_3\text{CO}_2)_6$ . The precipitate was purified by repeated centrifugation and washing with acetic ether. After final washing with ethyl ether it was dissolved in water to be treated with sulfosalicylic acid solution. The yellow color thus produced which was stable for three hours or more was measured in a photoelectric colorimeter.

However, in our experience, the purification of precipitate was regrettably so tedious and required so much attention that the procedure could hardly comply with mass-treatment of various samples at a time.

In an attempt to overcome this short-coming we developed a simple manipulation of rolling and tapping for the complete production of sodium uranyl zinc acetate, and employed a glass filter which permits thorough washing instead of centrifugation.

It has been known that urine which had a disproportionately small amount of sodium as compared with potassium gave erroneously high value for sodium concentration when it was measured by uranyl method. Dilution of uranyl zinc acetate reagent with methyl and ethyl alcohols (u:m:e=5:3:2) has recently been recommended by Van Loon and others<sup>4)</sup> to remove the obstacle. We tested and affirmed the reliability of his prescription and, coupling it with our glass filter procedure, we devised a technique for the accurate determination of sodium in urine.

Our procedures to be presented below are suitable for routine work in the clinical laboratory, since they meet the demand of mass treatment and do not require extreme nervous tension.

### METHOD

*Reagent*

- 1) Aqueous solution of the trichloroacetic acid, 10g./dl. in concentration
- 2) Uranyl zinc acetate reagent: Two beakers a and b of one liter in size are calibrated to 500 ml. Crystals of purified uranium acetate  $\text{UO}_2 \cdot (\text{C}_2\text{H}_3\text{O}_2)_2 \cdot 2\text{H}_2\text{O}$  (77 g.), glacial acetic acid (14 ml.) and distilled water (400 ml.) are transferred into beaker a, while zinc acetate  $\text{Zn} \cdot (\text{C}_2\text{H}_3\text{O}_2)_2 \cdot 2\text{H}_2\text{O}$  (231 g.), glacial acetic acid (7 ml.) and distilled water (400 ml.) are introduced into beaker b. Both are warmed on an electric range and stirred with a glass rod to produce transparent solution. (It will take thirty minutes.) As soon as distilled water is added to the mark (500 ml.), the content of beaker b is transferred into beaker a under constant stirring. The resultant mixture, i. e., uranyl zinc acetate reagent, is cooled to room-temperature and preserved in a brown bottle. It becomes available for analysis after it has been allowed to stand overnight. Filter with a filter paper Toyoroshi No.7 before use. The reagent keeps indefinitely.
- 3) 95 per cent ethanol, prepared from absolute ethyl alcohol which has been redistilled from commercial quality.
- 4) Wash solution : A mixture of acetic ether (300 ml.) and glacial acetic acid (700 ml.)
- 5) Ethyl ether, redistilled from commercial quality
- 6) Aqueous solution of sodium acetate, 10 g./dl.
- 7) Aqueous solution of sulfosalicylic acid, 5 g./dl. in concentration.
- 8) Standard solution of sodium: Crystals of pure sodium chloride which has been dried at 100 C overnight (127.09 g.) is dissolved in distilled water contained in a 1000 ml. volumetric flask, and made to volume with distilled water. The resultant solution which has sodium in 500 mg./dl. is preserved in a pyrex glass bottle, and standard solutions of 50, 35, 25, and 20 mg./dl. Na are prepared by diluting it with distilled water on use.
- 9) Van Loon's uranyl zinc acetate reagent<sup>9)</sup>: Three volumes of methanol (redistilled) and two volumes of ethanol (redistilled) are added to five volumes of uranyl zinc acetate solution. They are mixed and filtered shortly before use.
- 10) Saturated alcoholic solution of zinc acetate: Crystals of pure zinc acetate (8 to 10 g.) are added to 90-96 per cent ethanol (redistilled from commercial quality; 200 ml.) and agitated to saturation. It is allowed to stand until a turbid solution separates over the layer of crystals. The supernatant is used for analysis.

Purification of commercial uranium acetate (or nitrate) and revival of staled uranyl zinc acetate reagent: A sufficient amount of caustic ammonia is added to uranium acetate (or nitrate) dissolved in the smallest amount of concentrated hydrochloric acid or to a staled solution of uranyl zinc acetate which has been collected in a brown bottle after use. The mixture separates into a clear supernatant and a heavy precipitate which is brownish yellow in color, while it is allowed to stand. (If separation is incomplete, another amount of caustic ammonia is required). The mixture is transferred into a filter which has filter papers Toyoroshi No.2 in duplicate, and aspirated under negative pressure until the layer of brownish yellow precipitate which is formed on the filter produces slight fissures. Then the precipitate is washed away from the filter with a small volume of caustic ammonia into a beaker, and brought into solution with the smallest amount of concentrated hydrochloric acid. The resultant yellow solution is filtered through two sheets of Toyoroshi No. 7 filter paper to get clear filtrate. This is again treated with caustic ammonia and the resultant precipitate is collected on a filter, aspirating in the same manner as before. It is dissolved in the smallest sufficient amount of 30 per cent acetic acid (glacial acetic acid 30:distilled water 70), filtered under negative pressure, and then transferred into a 1 liter beaker. The beaker is placed in a boiling water bath until its content evaporates to a concentrated syrup, which yields crystals of uranium acetate while it is allowed to cool at room temperature. Commercial uranium acetate (or nitrate)

requires purification before it is used for analysis and, in our experience, no chemicals of any factory were an exception.

### Instruments

Round-bottomed centrifuge tubes (15 mm.  $\times$  10 cm.) of pyrex glass and instruments depicted in Figures 1, 2 and 3. The glass wares washed thoroughly with running tap water, rinsed repeatedly with redistilled water and dried after they were soaked in potassium bichromate sulphuric acid. Glass filters No. 4 for microanalysis.

### Procedure

#### A. Determination of sodium in serum

(1) Volumes of 0.8 ml. of trichloroacetic acid are dropped into separate test tubes containing 0.2 ml. aliquots of serums  $S_1, S_2, \dots$  under constant agitation, 1.0 ml. of redistilled water is added to each and mixed. The mixtures are filtered through filter papers Toyoroshi No. 7 to obtain clear filtrates (ten-fold dilutions of original serums) after they were allowed to stand for five minutes.

(2) Aliquots of 0.2 ml. of 35 mg./dl. Na solution, 25 mg./dl. Na solution, serum filtrate  $S_1$ , serum filtrate  $S_2, \dots$  are introduced into round-bottomed centrifuge tubes A, B, C, D,  $\dots$ , respectively, and 1.0 ml. of uranyl zinc acetate reagent and 0.5 ml. of ethanol are added to each. The test tubes are subjected to rolling and tapping for five minutes to precipitate

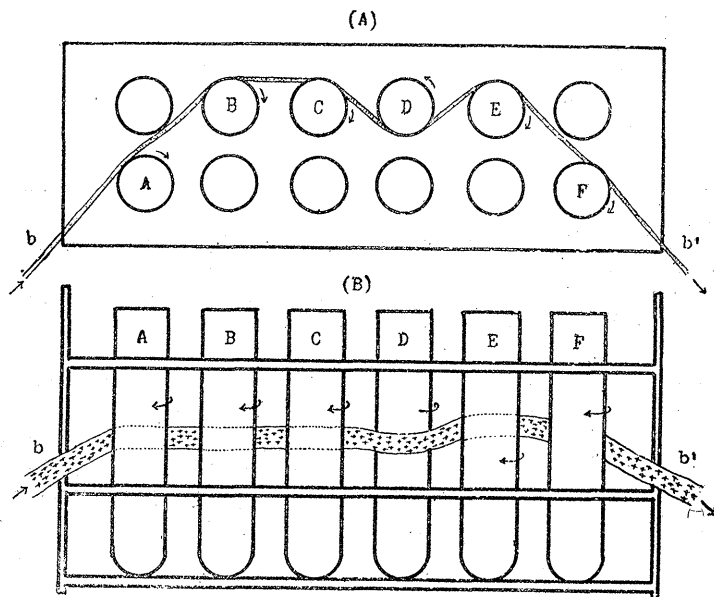


Fig. 1. Test tube rack for rolling and tapping. (A): upward view, (B): lateral view. Rolling and tapping are carried out in a test tube rack made of metal which has a metal wire netting plane at its bottom. After the rack was firmly settled on a desk the round-bottomed centrifuge tubes A, B, C,  $\dots$  are inserted in it. A strip of bandage  $bb'$  is passed between the tubes, as shown in this figure, and this is pulled alternately in the directions  $bb'$  and  $b'b$ , 120 times per minute. The centrifuge tubes are, in this way, tapped on the metal wire netting while they are rolled by the bandage. The tubes are treated at a time with ease.

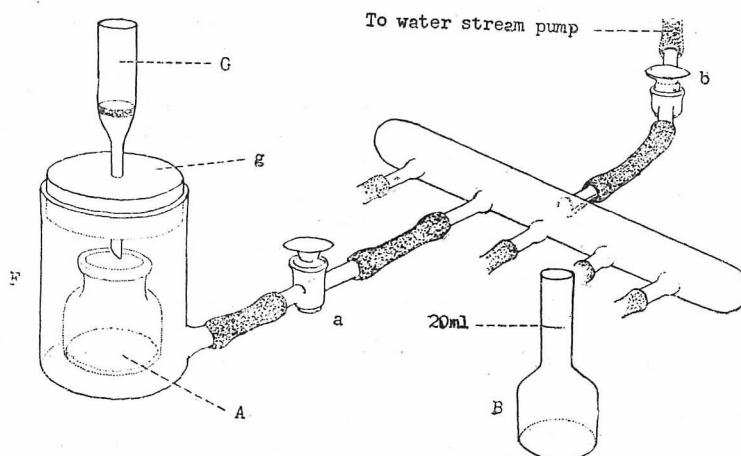


Fig. 2. Apparatus for filtration under negative pressure. Aspiration jar F which has vial A within is plugged with a rubber stopper g that is equipped with glass filter G, and it is connected with a water stream pump through stop-cocks a and b. B is a 20 ml. volumetric flask which is to be exchanged for vial A when the precipitate of sodium uranyl zinc acetate is dissolved in hot water.

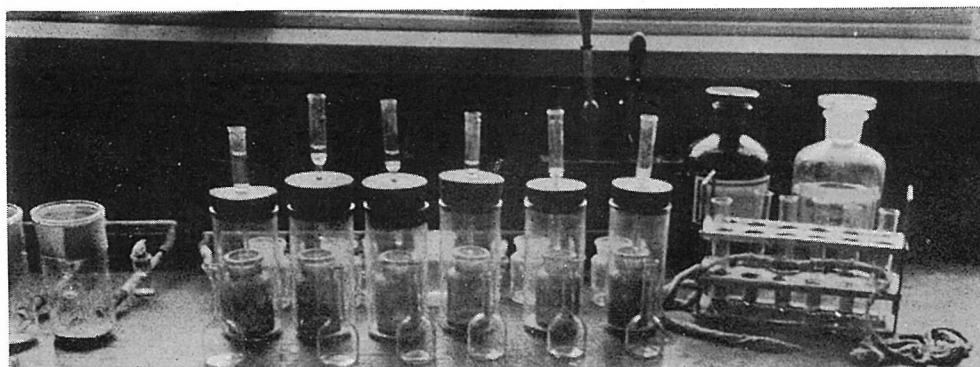


Fig. 3. Assembly of the filtration apparatus with a test tube rack for rolling and tapping.

sodium uranyl zinc acetate after they were mounted on a metal test tube rack as shown in Figure 1.

(3) The whole contents of A, B, C, D, . . . are transferred to individual glass filters which are equipped with aspiration jars, each storing vials inside, as illustrated in Figure 2, and they are aspirated under negative pressure of Bunsen's water stream pump to separate the precipitate of sodium uranyl zinc acetate from the excess of uranyl reagent which is filtered down in the vials.

(4) Portions of 0.5 ml. of wash solution are poured into centrifuge tube A, B, C, . . . with a Komagome pipette whose tip is drawn out to a fine tube, allowing them to flow from orifice to bottom so as to rinse the inner surface of the centrifuge tubes entirely and they are

transferred to corresponding glass filters A, B, C, . . . . ., respectively. The washing is repeated once more.

(5) The glass filters are washed with two portions of 0.5 ml. of ether in the same manner as in (4), and dried at 37 C for about five minutes until ether no longer smells. The glass filters are again installed in the aspiration jars after the vials stored therein was exchanged for 20 ml. volumetric flasks.

(6) The centrifuge tubes A, B, C, . . . are washed with 1 ml. portions of hot redistilled water to dissolve all the crystals of sodium uranyl zinc acetate adhering to the walls. The solutions thus obtained are at once transferred to the corresponding glass filters A, B, C, . . . , and aspirated under slightly negative pressure so that the precipitate on the filters may also be brought into solution and allowed to flow into the volumetric flasks. The procedure is repeated thrice, and the glass filters are again washed with cool redistilled water until the contents in the volumetric flasks attain about 10 ml. in amount.

(7) Aliquots of 0.8 ml. of sulfosalicylic acid solution and 0.8 ml. of sodium acetate solution are introduced to the volumetric flasks and mixed by agitation to obtain yellowish brown coloration which is quite limpid. The colored solutions A, B, C, D, . . . are made to a volume of 20 ml. with distilled water, and mixed by inversion.

(8) The absorbance a, b, c, d, . . . for the colored solutions A, B, C, D, . . . , respectively, is measured in a photoelectric colorimeter at 430  $m\mu$  (Filter S 43), and a rectilinear line which passes the points (a, 350 mg./dl. Na) and (b, 250 mg./dl. Na) is plotted on a rectangular coordinate graph. The sodium concentration of serum  $S_1, S_2, \dots$  is read by the comparison of absorbance b, c, . . . . . with this line. (Dividing by 2.3 enables the conversion of the concentration expressed in mg./dl. into that in m. Eq./l.)

#### B. Determination of sodium in urine.

(1) Aliquots of 0.5 ml. of urines  $U_1, U_2, \dots$  are introduced into separate test tubes graduated to 5.0 ml., 1.0 ml. of redistilled water and 3.5 ml. of zinc acetate solution are added and mixed. The mixtures are heated in a water bath at 80-90 C for ten to fifteen minutes, and they are made to a volume of 5.0 ml. with 95 per cent ethanol after they were cooled to room temperature. Clear protein- and phosphate-free filtrates which are tenfold dilution of the original urines are obtained by filtration with Toyoroshi No. 7.

(2) Into separate round-bottomed centrifuge tubes A, B, C, D, E, F, . . . are introduced 0.2 ml. aliquots of 50, 35, 20, and 0 (blank) mg./dl. Na solutions as well as the filtrates  $U_1, U_2, \dots$ , and 2.0 ml. portions of Van Loon's or its modified reagent are added. The precipitate of sodium uranyl zinc acetate is formed, filtered, washed, dissolved, colorized, and measured in a photoelectric colorimeter in the same way as described in A (procedure for serum sodium).

The reliability of these procedures was examined as follows:-

1) Observation 1. Obedience to Beer's law: Standard Na solutions of 50, 100, 150, 200, 250, 300 and 350 mg./dl. were subjected to the procedure A, and their absorbance was measured in Erma's photoelectric spectrophotometer over the whole range of visual spectrum (400-700  $m\mu$ )

2) Observation 2. Interference by potassium ion: Van Loon and his co-workers recommended a mixture of uranyl reagent, absolute methanol and 95 per cent ethanol in the proportion 5:3:2 which was allegedly able to obviate the undesirable precipitation of potassium. Sham urines of K/Na (in mg.) ratio 1, 5, 10, 15, 20 and 25 were prepared with 300 mg./dl. Na solution and 1000 mg./dl. K (potassium chloride) solution, and they were treated with Van Loon's mixture

to verify if they were recovered exactly, regarding them to be ten-fold dilution of urines in the procedure B. The recovery by the mixtures (uranyl reagent:methanol:ethanol=5:5:0, 5:4:1, and 5:2:3) were also studied.

3) Observation 3. Interference by phosphate ion: Phosphate was supposed to interfere with the determination, because it was also precipitated by uranyl zinc acetate solution. In our procedure for the determination of sodium in urine the phosphate was removed in step (1) with zinc acetate solution so as to be exempted from this kind of interference. Sham urines of P/Na (in mg.) ratio 0.8, 4, 8, 12, 16 and 20 were prepared with 300 mg./dl. Na solution and potassium hydrogen phosphate ( $\text{KH}_2\text{PO}_4$ ) solution, and examined for precipitation.

4) Observation 4. Accuracy of the estimation: The recovery of sodium was studied on two serums and eight urines. An amount of 3% of sodium was added to 0.2 ml. portions of serums to raise their sodium concentration by 50 mg./dl., and as for urine the redistilled water in step (1), procedure B, was replaced by 1.0 ml. of 15 mg./dl. Na solution in order to elevate calculated concentration of urine sodium by 30 mg./dl.

#### RESULTS AND DISCUSSION

Darnell and Walker<sup>1)</sup> advocated a rather cumbersome and delicate operation for the precipitation of sodium uranyl zinc acetate. "To one ml. of the trichloroacetic acid filtrate solution prepared by wet-ashing was added 5 ml. of freshly filtered uranyl zinc acetate. At five minutes intervals, seven 0.3 ml. portions of ethyl alcohol were added. These additions should have taken thirty minutes and may have taken longer. After each of the first five additions of alcohol, the liquid in the tube was mixed, first by tapping the bottom of tube, producing rotary motion in the upper part of the liquid, then by rolling the tube between the palms, thus mixing the contents in the lower part of the tube. The last two additions of alcohol served to wash down the walls of the tube were allowed to remain layered on the solution." In our method this time-consuming process was displaced by the efficiency of rolling and tapping in a test tube rack, which facilitated simultaneous precipitation of as many as ten samples within five minutes. The manipulation requires no skill as easily perceived from Figure 1.

Another improvement in our procedure is concerned with the purification of precipitate. Darnell and Walker employed centrifugation and decantation for this purpose. "After the last addition of alcohol, the mixture was centrifuged at 2000 r.p.m. for 10 minutes. The supernatant fluid was decanted, and the tube was inverted and drained for five minutes. The mouth of the tube was wiped dry. The precipitate was agitated blowing on it a fine stream of about two ml. of ethyl acetate-acetic acid wash liquid. The wall of the tube was washed down with a small amount of the liquid. The tube was centrifuged for ten minutes,

drained for five minutes, and the mouth of the tube was wiped as before. The precipitate and the wall of the tube were washed with about 5 ml. of ether. The tube was centrifuged for about five minutes, the supernatant fluid was decanted, and the tube was drained for one minute only. The precipitate was washed a second time with 5 ml. of ether. The tube was centrifuged for five minutes and the supernatant fluid was decanted. The tube was drained for one minute. The tube was placed in the incubator (37 C) for five minutes to evaporate the last trace of ether."

It is evident from the foregoing description that the accuracy of the determination is entirely dependent upon the exact performance of all the steps of centrifugation and decantation, since the loss of any one crystal of sodium uranyl zinc acetate during decantation as well as the slightest insufficiency in washing the precipitate may entail an appreciable error. This compels us to pay meticulous attention throughout the process. Glass filters were introduced in our procedure in the hope that the precipitate might be washed completely with a sufficient amount of wash solution, because filtration with a glass filter was superior to centrifugation and decantation in the rapidity and ease of manipulation, and assured a perfectly purified precipitate without demanding any such nervous tension as was felt in the process of purification which was specified in the original method.

The absorption spectra of the purified precipitate (sodium uranyl zinc acetate) which was dissolved in hot water and then treated by sulfosalicylic acid and sodium acetate are presented in Figure 4 (observation 1).

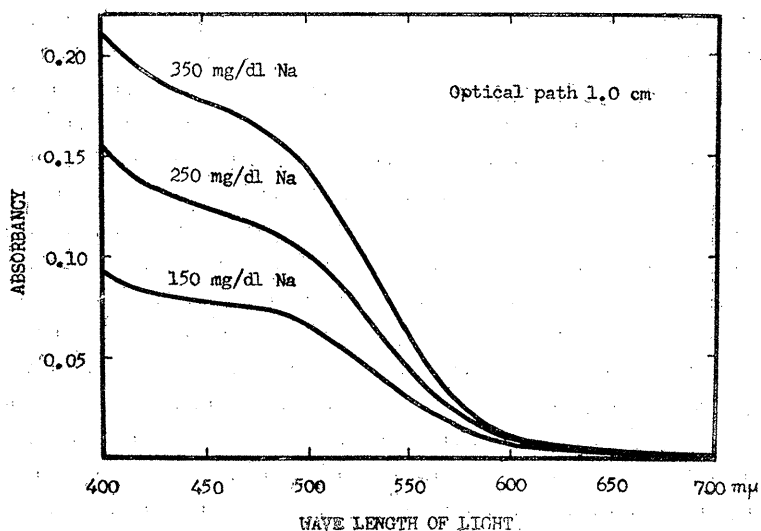


Fig. 4. Light absorption of the colorized solution.

This discloses a conspicuous absorption in the range below  $500\text{ m}\mu$  of wave length. That Beer's law is exactly obeyed within the limits between  $400\text{ m}\mu$  and  $540\text{ m}\mu$  is clearly demonstrated by Figure 5 in which relation of absorbance to sodium concentration is summarized with respect to various wave lengths.

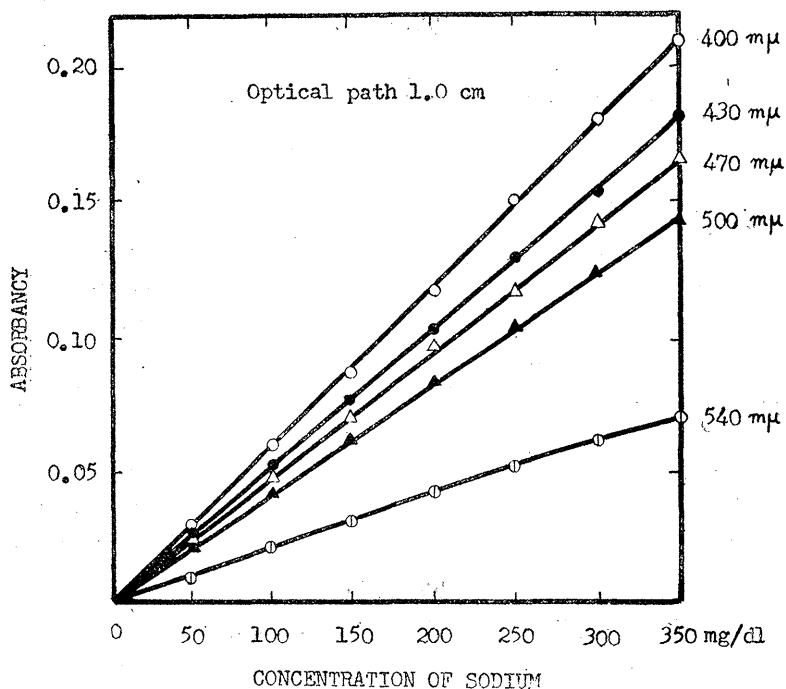


Fig. 5. Obedience to Beer's law.

Table I (a) shows the interference by potassium (observation 2). Van Loon's mixture<sup>4</sup>) prevents the determination from the undesirable effect of potassium so long as K/Na (mg.) ratio of the sample remains within 10, probably because the amount of methanol contained in the mixture is effective enough to keep potassium uranyl zinc acetate in solution. For the samples of K/Na ratio of more than ten a mixture of uranyl reagent:methanol:ethanol=5:5:0 or 5:4:1 may be preferred to Van Loon's mixture. However, as a matter of fact this constitutes an omnipotent reagent for the determination of sodium in various biological fluids, since K/Na ratio seldom exceeds 10 even in urines which are extremely rich in potassium.

Phosphate interferes with the determination, yielding unreasonably small value for sodium concentration especially in case of urine samples of large P/Na (mg.) ratio, because sodium uranyl zinc acetate is not dissolved in hot water on



account of its strong affinity to the precipitate of uranyl zinc phosphate which is produced by the interaction of uranyl reagent and phosphate. Estimation decreases in exponential curve, as listed in Table I (b), with increase in P/Na ratio (observation 3). Preliminary removal of phosphate by zinc acetate is accordingly indispensable for exact determination of sodium in urine.

TABLE I  
*Use of uranyl reagent-methanol-ethanol mixtures to avoid the interference by potassium and phosphate*

Recovery of sodium in per cent						
(a)					(b)	
K/Na † of sham urine	5:5:0	5:4:1	5:3:2*	5:2:3	P/Na † of sham urine	5:3:2*
1	76	95	99	102	0.8	96
5	96	97	100		4	78
10	97	102	101		8	62
15	98	103	106		12	55
20	104	106	110		16	35
—	—	—	—		20	47

\* Van Loon's mixture

† K/Na and P/Na are expressed in mg./dl.

5:5:0, 5:4:1, 5:3:2 and 5:2:3 indicate the composition of the mixture which was used to precipitate the sodium. These are expressed in the ratio of uranyl reagent:methanol:ethanol

TABLE II  
*Duplicate determination and recovery test*

A. duplicate determination (mg./dl.)

Serum: 343.5, 347.2	Urine: 466.0, 452.0	387.0, 380.0
317.5, 317.5	347.0, 349.0	346.0, 348.0
312.6, 310.0	320.0, 316.0	312.0, 314.0
310.0, 309.8	308.0, 308.0	300.0, 300.0
310.0, 303.1	292.0, 294.0	290.0, 298.0
	231.0, 234.0	141.0, 144.0
	137.0, 135.0	133.0, 133.0

B. Recovery test (3γ of sodium was added to 0.2 ml. of serum; 5γ of sodium was added to 0.5 ml. of urine)

Na concentration of serum or urine (mg./dl.)	Na concentration of serum or urine after sodium was added (mg./dl.)	Theoretical concen- tration of sodium after the addition (mg./dl.)	Recovery of the added sodium in percentage
Serum 311.0	367.0	361.0	112.0
312.4	367.0	362.4	109.0
Urine 320.0	352.0	350.0	107.0
322.0	352.0	352.0	100.0
466.0	493.0	496.0	107.0
452.0	489.0	482.0	102.0
231.0	260.0	260.0	97.0
234.0	265.0	264.0	103.0
97.0	127.0	127.0	100.0
97.0	125.0	127.0	93.0

The results of duplicate determination and recovery test (observation 4) are presented in Table II. The divergence between two determinations on the same samples was between 0 and 3 per cent, and the recovery amounted to 103 per cent on the average. The normal range of serum sodium was 310-345 mg./dl. (135-150 mEq./l.).

#### SUMMARY AND CONCLUSION

A simple method for the determination of serum sodium by means of uranyl zinc acetate reagent was developed, introducing a convenient and efficient manipulation of rolling and tapping for the precipitation of sodium as well as employing a glass filter which permits complete purification of the precipitate. This was also applied to the determination of urine sodium, using a uranyl reagent to which methanol was added in combination with ethanol so as to avoid the obstructive effect of potassium after urine was subjected to the preliminary removal of phosphate by zinc acetate solution.

The divergence in duplicate determination was zero to two per cent for serum sodium and zero to three per cent for urine sodium. The recovery test amounted to 103 per cent on the average, ranging from 93 per cent to 112 per cent.

#### REFERENCES

- 1) DARNELL, M. C. J. R., AND WALKER, B. S. : Determination of sodium in biological fluids. *Ind. & Eng. Chem. (Anal. Ed.)* 12: 242-244, 1940.
- 2) SAVAGE, M. S., AND MAGINUS, J. E. : Sodium metabolism in health and disease, and a photometric method for its determination in biological fluid. *Amer. J. Med. Tech.* 16: 169-180, 1950.
- 3) SHUKERS, C. F. : Review of estimation of serum sodium and potassium. *Amer. J. Clin. Path.* 22: 606-615, 1952.
- 4) VAN LOON, E. J., LEIN, M. R., AND SEGER, A. J. : The determination of biologic sodium. *J. Lab. & Clin. Med.* 39: 148-152, 1952.