XXIX. DETERMINATION OF BLOOD POTASSIUM

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The authors' method [1936] for determination of potassium in 0·2 ml. of plasma gave fairly satisfactory results at room temperatures between 17 and 22°. Below 17° crystals of sparingly soluble $AgNO_2$ separated out, whilst above 22° the amount of precipitate recovered diminished rapidly. The method, therefore, was impracticable under New York summer conditions (temp. range 30–40°). Furthermore, its accuracy was low, for the maximum difference between duplicates was 10%. We have therefore examined each stage of the procedure with a view to rendering it independent of outside temperature variations and to increase its accuracy. Unless otherwise stated, all the results given below are the mean of two concordant values.

I. Critical analysis of the previous method

As previously described, the method consisted of the following stages:

A. Precipitation of K argenticobaltinitrite.
B. Washing of precipitate.
C. Decomposition of precipitate.
D. Colorimetry.

A. *Precipitation*

(1) *Ag content of systems.* The effect of varying the Ag content of the systems was studied at 0° and at 20°. The reagent was prepared by adding one volume of aq. $AgNO_3$ of different concentrations to ten of the sodium cobaltinitrite solution (see previous paper [1936]), shaking and filtering. 2 ml. portions of the filtrates were added to a series of 15 ml. graduated pyrex centrifuge-tubes, each containing 0·1 mg. K in 5 ml. water, giving a range of concentrations of 0·23–2·00% $AgNO_3$ in the final systems. The tubes containing 0·23–0·54% $AgNO_3$ were centrifuged after 2 hours at 0° and the higher $AgNO_3$ concentrations after 2 hours at 20°. Recovery was determined, taking the tubes containing 0·45% $AgNO_3$, and kept at 0° as standards. Crystals of $AgNO_2$ separated out in certain of the tubes, and these were not further analysed.

The results, given in Table I, show that recovery was greatest with 0·45–0·54% $AgNO_3$ and precipitation at 0°. Determination of potassium is not possible when the concentration of $AgNO_3$ exceeds 0·54% at 0° or 1% at 20°. This experiment shows, incidentally, that presence of Cl^- in the solution to be analysed would, by reducing the effective concentration of Ag, lead to low results being obtained. This might possibly be the case when a condition of hyperchloraemia exists.

Table I. *Effect of varying $AgNO_3$ concentration of systems*

Apparent recovery of K (mg.) at 0°	Apparent recovery of K (mg.) at 20°	Conc. of $AgNO_3$ %
0·072	—	0·23
0·1	0·01	0·45
0·1	—	0·54
($AgNO_2$)	0·037	0·64
—	0·045	0·73
—	0·049	0·91
—	0·049	1·0
—	($AgNO_2$)	1·25 and 2

(2) *Temperature of formation of precipitate.* K was determined in a number of systems, each containing 0·1 mg. K in 6 ml. solution, and 2 ml. reagent (0·45 % $AgNO_3$), which were maintained for 2 hours at 2–37° before being centrifuged. The final colorations obtained were compared with that given by the system maintained at 2°. It appears (Table II) that the recovery varies little from 2 to 8°, and then falls rapidly with increasing temperature to only 15 % at 37°.

Table II. *Effect of varying temperature of precipitation.*

All tubes contain 0·1 mg. of K.

Temp.	2°	8°	21°	30°	37°
Apparent K content	0·1	0·098	0·075	0·043	0·015

(3) *Prevention of flotation of precipitate.* The precipitate has a tendency to adhere to the walls of the tube, and to float on the surface of the solution, thereby leading to losses in the process of siphoning off. Breh & Gaebler [1930] recommended adding the reagent to warmed systems but did not exactly specify the temperature. Kerr [1926], in the determination of K as KNa cobaltinitrite, added octyl alcohol to the solutions before centrifuging. The following experiments were performed in order to determine the conditions under which flotation could best be eliminated.

2 ml. reagent were added to a series of tubes containing from 0·008 to 0·1 mg. K in 6 ml. solution. The temperature of the solutions at the moment of adding the reagent varied from 20 to 65°. The systems were allowed to stand 1 hour at room temperature (20°), and were then kept at 4° for 12 hours. 0·5 ml. saturated aqueous octyl alcohol was then added to certain of the tubes, and all tubes were centrifuged. The colorations obtained were compared with that given by a 0·1 mg. standard, precipitated at 65° without octyl alcohol. The results (Table III) indicate that apparent recovery varied inversely with initial

Table III. *Effect of varying temperatures at which cobaltinitrite reagent is added, and of adding octyl alcohol*

Standard: 3·1 mg. K, at 65°.

Temp.	mg. K found in systems containing 0·08	0·04	0·02	0·008	Remarks
65°	0·076	0·019	0·009	0	—
50°	0·082	—	—	—	—
40°	0·080	—	—	—	—
20°	0·086	—	—	—	—
65°	0·083	—	—	—	0·5 ml. saturated
40°	—	0·046	—	—	aqueous octyl
20°	0·088	—	—	0·010	alcohol added

temperature, and that the highest values were obtained by precipitation at room temperature with addition of octyl alcohol immediately before centrifuging.

(4) *Duration of precipitation.* It appeared from certain preliminary experiments that the duration of precipitation of 2 hours, as proposed by Breh & Gaebler, was inadequate. Moreover, it would be more convenient if this process were to take place overnight. Accordingly, 2 ml. reagent (0·45 % $AgNO_3$) were added to a number of tubes containing 0·04–0·1 mg. K in 6 ml. solution at 50°, and K was determined after 2 or 18 hours at 4°. The results (Table IV) show that practically quantitative recovery was obtained after 18 hours, but not after 2 hours.

Table IV. *Effect of varying duration of precipitation*

Standard: 0·1 mg. K, left at 4° for 18 hours.

	mg. K found with precipitation time of	
mg. K taken	2 hours	18 hours
0·1	0·069	0·1
0·06	0·0292	0·0592
0·04	0·0133	0·0349

(5) *Temperature of centrifuging.* K was determined in systems containing 0·04–0·16 mg. K in 6 ml., precipitated at 0° for 16 hours and centrifuged at 17, 21 and 28°. The differences between the results were negligible, showing that temperature of centrifuging does not significantly affect the results.

B. *Washing of precipitate*

Breh & Gaebler recommend three washes, centrifuging for 15 min. after addition of each portion of wash-water. The effects of shortening the time of centrifuging and of shaking the precipitate with the wash-water were investigated.

It was found that, taking 0·1 mg. K, only 0·075 mg. was recovered in shaken systems, and taking 0·06 mg. K, only 0·04 mg. It is important, therefore, when adding the wash-water, to disturb the precipitate as little as possible.

Reducing the duration of centrifuging from 15 to 5 min. did not affect the results obtained for 0·04–0·1 mg. K. If the wash-water is very carefully added, the centrifuging may even be entirely dispensed with, but with multiple determinations less time is required when the water is added more rapidly, and one batch of tubes is centrifuged whilst the other is being washed.

C. *Decomposition of precipitate*

The washed precipitate is heated with NaOH, in order to break up the complex and liberate the NO_2 groups as $NaNO_2$. The method previously used involving boiling the precipitate with 5 ml. 0·2*N* NaOH, cooling and diluting to 6 ml., was laborious and time-consuming. Robinson & Putnam [1936] advise heating at 100° for 10 min. This was found to give good results if the tubes are shaken before being placed in the water-bath. Thus, in a series of tubes containing 0·1 mg. K in 6 ml. solution and 2 ml. reagent (0·45 % $AgNO_3$), the recovery was 0·1 mg. in shaken, and 0·086 mg. in unshaken tubes.

D. *Colorimetry*

(1) *Reagents.* Robinson & Putnam recommended the addition of mixed Griess reagents for determination of HNO_2. It was found that equal coloration is obtained whether the Griess reagents are added separately or ready mixed, and since the latter procedure is the simpler, it was adopted.

(2) *Time necessary for development of colours, and permanence of coloration.* Griess reagents were added to a solution of HNO_2, and the coloration obtained was compared with that of a similar solution to which the reagents had been added an hour previously, and which had been set at a level of 20 in the colorimeter. The reading was 27·5 after 1 min., 22·5 after 2 min., 20 after 5 min. and 20 an hour later, indicating that full development of colour takes place within 5 min., and that the coloration does not change over 2 hours. The colour fades fairly rapidly when the solution is continuously exposed to intense illumination, as is shown by the following experiment. Griess reagents were added to a solution of HNO_2, and the coloration was read against itself after 5 min., and at intervals up to 60 min. The solution in one cup, with constant illumination, was kept as the standard (at 20), and that in the other cup was changed before each reading. The fading of the illuminated solution is illustrated by the following:

Duration of illumination	0	5	10	15	22	28	40	60 min.
Colorimeter reading of unilluminated solution	20	20	19·5	18·5	17·5	17·0	15·5	13·5

The intensity of coloration fell to 67·5 % of the initial value after 60 min. of illumination, but remained constant for at least 5 min. The standard solution should be replaced fairly frequently.

II. Improved method

On the basis of the above study, the following method was elaborated whereby 48 potassium determinations may be performed during 8 hours.

A. *Reagents.* These are the same as previously reported, except that 1 volume of 40 % $AgNO_3$ is added to 20, instead of 10, volumes of the Na cobaltinitrite solution, and the 3 ml. of Griess reagents are mixed before adding, instead of adding each separately.

B. *Procedure.* 2 ml. of freshly prepared cobaltinitrite reagent are added to 48 tubes each containing 5 ml. of the solution to be analysed at room temperature. The tubes are placed in a water-bath; an hour later ice is added, and the water-bath is left in a refrigerator overnight. In the morning 0·5 ml. of saturated aqueous octyl alcohol is added to all tubes. Twenty-four tubes are centrifuged for 15 min. at 2500 r.p.m. The supernatant fluid is removed, 0·2 ml being left behind, and 7 ml. of water are added thereto. The remaining 24 tubes are centrifuged and washed, the washing, alternately with the first batch, being repeated three times. To the washed precipitate 4–5 ml. of 0·2*N* NaOH are added, the tubes are shaken, placed in a water-bath at 100° for 10 min. and then cooled to room temperature. The volume is made up to 6 ml., and the tubes are again centrifuged for 5 min. It is advisable to compare the amount of precipitate in the tubes with that in the standards before adding NaOH, as this allows one to estimate the amount of final centrifugate which should be taken for colour development. 1 ml. of the standards (0·1 mg. K) and 0·5–5 ml. of the other solutions are transferred to a series of 50 ml. volumetric flasks containing approximately 15 ml. 10 % acetic acid, 3 ml. of mixed Griess solution are added, and the volume is made up to 50 ml. The colorations are read against the 0·1 mg. standard, set at 20, changing the standard after every six determinations and illuminating only during the actual reading.

It will be seen from Table V that theoretical results were obtained for amounts of from 0·01 to 0·1 mg. K, duplicate determinations not varying by more than ± 2 % from the mean.

Table V. *Results obtained using revised method*

No. of determinations		12	8	2	8	10	4	2
mg. K	Taken	0·10	0·060	0·050	0·040	0·020	0·010	0·008
	Found	0·10	0·060	0·050	0·040	0·021	0·010	0·007
Max. variations		0·091–0·11	0·054–0·071	—	0·037–0·044	0·019–0·023	—	—

C. *Application to determination of K in blood.* 0·2 ml. plasma is transferred to a tube containing 10·1 ml. of water, 0·3 ml. of 10 % Na tungstate, 0·3 ml. of 2/3 N H_2SO_4, and 0·1 ml. of 5 % $AgNO_3$ are added. The mixture is shaken and centrifuged, and two 5 ml. samples of centrifugate are taken for K determination, as above. The K content per 100 ml. of plasma is given by $110\,S/RV$, where S is the level at which the standard is set, R is the reading and V is the number of ml. NaOH centrifugate taken for colour development.

Determinations of the K content of 12 samples of heparinized plasma from 6 normal human subjects (from the finger or ear) gave a mean value of 19·9 mg. per 100 ml. (varying from 16·7 to 23·0 mg.). This result is substantially identical with that found by other authors, using different methods.

The amount of K present in 0·2 ml. normal plasma is approximately 0·04 mg., so that 5 ml. of centrifugate would contain about 0·02 mg. Since 0·01 mg. K may be determined with sufficient accuracy, it follows that in cases of necessity as little as 0·1 ml. of plasma might be taken for the duplicate determination.

Summary

1. A method is described whereby amounts of from 0·01 to 0·1 mg. K may be determined, with practically theoretical recovery.

2. Application of this method to blood makes possible the determination of K in 0·1–0·2 ml. plasma (duplicate determinations). Blood from the finger or ear may therefore be used for the purpose.

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