A Colorimetric Method for Determination of Potassium with 0.2 c.c. of Blood.

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The precipitation of the potassium present by a solution of sodium cobalti-nitrite has already been applied to the colorimetric determination of potassium in body fluids. Takahashi's¹⁾ method reported from our pediatric laboratory, which was, as far as the author knows, the first paper dealing with the application, has as its basis the fact that a dark brown color is developed when potassium cobalti-nitrite previously dissolved in acid is reduced by ammonium sulphide. Lebermann²⁾ utilized the color of the cobalt salt dissolved in a concentrated hydrochloric acid. The present method of the author is another colorimetric application as will be related below.

Principle of the Method.

The potassium is precipitated as potassium cobalti-nitrite either in the acetic acid solution of blood ashed,³⁾ or in undiluted serum,⁴⁾ by adding an excess of the sodium cobalti-nitrite reagent. The precipitate is dissolved with nitric acid and then reduced with the addition of dimethylglyoxim and sodium sulphide.⁵⁾ The color thus developed is compared with the color of the standard.

1) Takahashi, H. Tohoku Igaku-Zasshi, 1923, **7**, 27. (Jap.)

2) Lebermann, F., Bioch. Zeitschr., 1924, **150**, 548.

3) Kramer, B. Journ. of Biol. Chem., 1920, **41**, 263.

4) Kramer, B. and Tisdall, F. F., Journ. of Biol. Chem., 1921, **46**, 339.

5) Feigl, F. u. Tutanowska, L. V., Berich. der Deutsch. Chem. Gesel., 1924, **57**,

The Material to be Used.

Blood is taken by venous puncture and pipetted into the Ernst pipette up to the mark of 0.2 c.c. As the anticoagulant lithium citrate is most convenient. In place of that puncture of the skin may be used, only, in this case no pressure should be applied to the part, thus allowing the blood to exude of itself.

Preparation of Reagents.

1. Sodium cobalti-nitrite reagent.

Solution A. 25 grms. of cobalt nitrate crystals are dissolved in 50 c.c. of water, and to this solution are added 12.5 c.c. of glacial acetic acid.

Solution B. 120 grms. of sodium nitrite (potassium-free) are dissolved in 180 c.c. of distilled water. To the whole of solution A are added 210 c.c. of solution B. Air is drawn through the solution until all the nitric oxide gas has passed off. The reagent thus prepared is kept in an ice chest and filtered each time before using. It will keep at least one month.

2. Potassium cobalti-nitrite.

Adding slowly 300 c.c. of sodium cobalti-nitrite solution (1) to 50 c.c. of 1% pure potassium chloride, the potassium cobalti-nitrite settle rapidly.

The precipitate is transferred to a Buchner funnel, washed three or four times with cold distilled water until the last washing fails to take color when a drop of ammonium sulphide is added. And then air-dried on the Buchner funnel.

3. Diluted nitric acid.

To 117 c.c. of concentrated nitric acid (spec. w. 1.317) is added 283 c.c. of distilled water.

4. Standard solution.

0.2905 gm. of potassium cobalti-nitrite (2) is dissolved in 250 c.c. of diluted nitric acid (3) by warming, then made up to 500 c.c. exactly with distilled water.

5. 1% alcoholic solution of dimethylglyoxim.

6. 1% sodium sulphide solution.

7. Glacial acetic acid.

8. Sodium acetate (powdered).

Procedure.

0.2 c.c. of blood is dried in a platinum dish or crucible over the

steam bath or in an incubator at 110°C . Then the dish or crucible is placed in a large porcelain dish (6 or 7 cms. in diameter.) with pieces of porcelain on the bottom. The porcelain dish and its contents are heated over a low flame until no more fumes come off. Then the porcelain dish with the crucible remaining inside is covered with a quartz or porcelain plate and now heated over a large burner until the blood is completely ashed. The platinum dish or crucible is removed and allowed to cool. The ash is dissolved in 0.5 c.c. of water with one or two drops of glacial acetic acid (7). 0.5 c.c. of sodium cobalti-nitrite reagent (1) is added drop by drop with constant shaking and then allowed to stand for at least 15 minutes. The precipitate is transferred into the centrifuge tube with the aid of a little water, and centrifuged at a speed of 2,000 revolutions per minute for 5 minutes.

The supernatant fluid is decanted and 5 c.c. of distilled water poured into the tube, which is centrifuged again. This procedure is repeated three times. After the last washing is decanted, 2 c.c. of diluted nitric acid (3) is added and warmed until the precipitate is dissolved. (Then follows the colorimetric technique below described.)

Direct Determination of Potassium in Serum.

According to the technique of Kramer-Tisdall¹⁾ the potassium is precipitated in undiluted serum by adding an excess of sodium cobalti-nitrite reagent.

Details of the Procedure. 1 c.c. of serum is put into a graduated centrifuge tube of 10 or 15 c.c. 2 c.c. of sodium cobalti-nitrite reagent (1) are then added drop by drop with constant stirring. At the end of 45 minutes 2 c.c. of water are added and the contents are again mixed and then centrifuged 15 minutes at a speed of 2,000 or 2,500 revolutions per minute. The supernatant fluid is decanted by means of centrifugation as above described, and the precipitate washed three times with water. The precipitate is then dissolved in 2 c.c. of diluted nitric acid.

Colorimetric Technique. 1.5 grms. of sodium acetate is put into a 50 c.c. volumetric flask and then the potassium cobalti-nitrite from blood or serum dissolved as above mentioned is poured together with the rinsings of the centrifuge tube. 4.0 c.c. of standard solution (4) (2.0 c.c. for serum) are pipetted into another 50 c.c. volumetric flask already containing 1.5 grms. of sodium acetate. To both flasks about 20 c.c. of water, 5 c.c. of 1% dimethylglyoxim solution (5) and 2 c.c. of 1% sodium sulphide are added. After 3 minutes both flasks are put in an incubator (110°C .) and kept there

for 15 minutes and are then cooled, or they may be dipped into boiling water up to the depth of the neck and kept there for 15 minutes and then cooled in running water. Then water is added up to the mark for colorimetric determination. The colors of the contents of both flasks are compared in a Dubosq colorimeter, the standard being set at 20 mms.

Calculation is as follows :

$\frac{\text{Reading of standard}}{\text{Reading of unknown}} \times 0.4 \times 500 = \text{mgrms. of K in 100 c.c. blood.}$

$\text{Reading of unknown} \times 0.2 \times 100 = \text{mgrms. of K in 100 c.c. serum.}$

Discussion.

The following results, which have been obtained upon a purely theoretical solution of potassium chloride, demonstrate the accuracy of the method. The average deviation from the theoretical is $\pm 1.0\%$ (Table I).

TABLE I.

	K present	K found	Error
1	0.400 mgrm.	0.404 mgrm.	+ 1.0 %
2	"	0.395	- 1.0
3	"	0.388	- 3.0
4	"	0.402	+ 0.5
5	"	0.415	+ 3.0
6	"	0.398	- 0.5
Average			± 1.6

The analysis of potassium in blood and serum are shown in Table II and III.

TABLE II.

	K found by Kramer-Tisdall's method using 5 c.c. of serum (per 100 c.c. serum)	K found by author's method using 1.0 c.c. of serum (per 100 c.c. serum)	Difference
1	17.0 mgrms.	17.3 mgrms.	+ 1.8 %
2	16.8	17.2	+ 1.2
3	19.0	19.6	+ 3.2
4	20.8	21.5	+ 3.3
5	22.4	21.0	- 6.2
6	19.7	20.4	+ 3.5
7	19.4	18.8	- 3.1
8	15.2	15.1	- 0.65
9	14.5	15.2	+ 5.0
10	17.0	16.3	- 4.1
11	16.8	16.1	- 4.2
12	16.8	17.3	+ 3.0
Average			± 3.0

Used horse serum.

TABLE III.

	K found by K ramer's method using 2 c.c. of blood (per 100 c.c.)	K found by author's method using 0.2 c.c. of blood (per 100 c.c.)	Difference
1	224 mgrms.	231 mgrms.	+ 3.0 %
2	195	199	+ 2.0
3	203	210	+ 3.4
4	225.5	235	+ 4.0
5	230	228	- 0.9
6	206	208	+ 1.0
7	168	172	+ 2.4
8	183	176	- 3.8
9	180	182	+ 1.1
10	194	200	+ 3.1
11	156	160	+ 2.6
12	138	136	- 1.5
Average			± 2.4

Used rabbit blood.

The control determinations were in all cases made by Kramer's method.³⁾ The figures show the difference of $\pm 3\%$ on an average.

Table IV demonstrates that known amounts of potassium added to blood may be quantitatively recovered by the author's method.

TABLE IV.

Recovery of potassium added to blood.

	K present	K added	Total K present	Total K found	Error
1	0.329 mgrm.	0.200 mgrm.	0.592 mgrm.	0.585 mgrm.	-1.2%
2	"	0.200	0.592	0.590	-0.4
3	"	0.200	0.592	0.580	-2.0
4	"	0.100	0.492	0.510	+3.6
5	"	0.100	0.492	0.497	+1.0
6	"	0.100	0.492	0.500	+1.7
7	"	0.100	0.492	0.510	+3.6
8	"	0.040	0.432	0.435	+0.7
9	"	0.040	0.432	0.438	+0.9
10	"	0.040	0.432	0.445	+3.0
11	"	0.040	0.432	0.440	+2.0
12	"	0.040	0.432	0.446	+3.0

The amounts of potassium found in the blood of normal adults and children suffering from anaemia and poliomyelitis are given in Table V.

TABLE V.

Potassium content of blood in normal and pathological conditions.

	Age	Diagnosis	K per 100 c.c. blood
1	7 yrs. 7 mos.	Poliomyelitis	205 mgrms.
2	6 yrs. 5 mos.	Anaemia (Sahli 40)	180
3	3 yrs.	Anaemia (Sahli 15)	162
4	4 yrs. 2 mos.	Anaemia (Sahli 45)	200
5	Adult	Normal	212
6	Adult	Normal	197

Conclusions.

1. A simple micro method for the determination of potassium in blood and serum has been described.

2. Using the method described above potassium may be determined on 0.2 c.c. of blood or on 1.0 c.c. of serum with an error not greater than $\pm 5\%$.

3. Results obtained by Kramer's and the author's method for potassium are within an average deviation of $\pm 3\%$.

4. Potassium added to serum may be recovered almost quantitatively.

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