Colorimetric Determination of Potassium by Folin-Ciocalteu Phenol Reagent

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A method for the colorimetric estimation of manganese by reduction of the phosphomolybdic-phosphotungstic acid phenol reagent has been described (Abul-Fadl, 1948). Cobalt was also found to give a similar colour reaction in the presence (but not in the absence) of amino-acids. Conversion of cobalt into the cobaltinitrite did not affect its response to this reaction.

As methods for the estimation of potassium in biological fluids depend on precipitation as cobaltinitrite, and subsequent estimation of one of the constituents of the precipitate, it was thought that this colour reaction might be useful in this connexion.

Amongst colour reactions hitherto used for the microdetermination of potassium are those of Doisy & Bell (see Briggs, 1923), and of Looney & Dyer (1942). Both reactions are based on the formation of an azo compound by the nitrite radicals of cobaltinitrite. Theoretically, those methods which determine directly a stable constituent of the precipitate, e.g. cobalt, are preferable to those which depend upon the unstable nitrite radical. Breh & Gaebler (1930) described a method in which cobalt is determined as the thiocyanate. This method involves the precipitation of potassium as the silver cobaltinitrite complex which is more sparingly soluble than the customary $K_2NaCo(NO_2)_6$. The method is, however, more troublesome to execute and the colour reaction involved is not entirely satisfactory, since it depends upon such factors as temperature, ethanol concentration, etc. Jacobs & Hoffman (1931) introduced a reaction between alkaline ferrocyanide and cobalt compounds in the presence of choline hydrochloride, which gives a stable green colour.

EXPERIMENTAL

Solutions

Sodium cobaltinitrite reagent (Kramer & Tisdall, 1921). As described by King (1947).

Standard potassium solution. K_2SO_4 (0·2228 g. A.R.) is dissolved in 500 ml. of water, giving a solution which contains 20 mg. K/100 ml.

Glycine (1 M). 7·5 g./100 ml. water. The solution is filtered and preserved with a few drops of chloroform.

Sodium carbonate solution (25% w/v). Anhydrous Na_2CO_3 (25 g.) is dissolved in warm water and made to 100 ml. This solution is kept in a warm place.

Phenol reagent of Folin & Ciocalteu (1927). For method of preparation see King (1947). This reagent is diluted for use (1 vol. reagent + 2 vols. water).

Method

Principle. Alkaline solutions of cobalt salts, in presence of a trace of amino-acid (glycine or alanine), reduce the phosphomolybdic-phosphotungstic acid phenol reagent to a blue colour, the intensity of which is directly proportional to the amount of cobalt present, and hence, if potassium has been precipitated as cobaltinitrite, to the amount of potassium in the original solution.

Procedure. The precipitation of potassium from serum is carried out in principle according to the method of Kramer & Tisdall (1921) as adapted by King (1947). In a 15 ml. conical centrifuge tube, marked at 6 ml., is placed 0·2 ml. of serum.

In a similar tube is placed 0·2 ml. of standard potassium solution (containing 20 mg. K/100 ml.). To each tube is slowly added 0·5 ml. of filtered sodium cobaltinitrite reagent with constant shaking. After 45 min. 1 ml. of water is added, and the contents are mixed and centrifuged at moderate speed for 15 min. The tubes are then inverted and briefly drained on filter paper; 2 ml. of water are added down the side of each tube without disturbing the precipitate. The tubes are again centrifuged for 5 min., inverted and thoroughly drained. The precipitates are washed with 5 ml. 70% ethanol, which is blown into the tubes so as to stir up the precipitates. After centrifuging and draining thoroughly, 2 ml. of water are added to each tube, and the tubes placed in a boiling water bath until dissolution is complete. A third tube containing 2 ml. distilled water is used as a blank.

To each of the three tubes, while still hot, 1 ml. glycine solution (7·5%) and 1 ml. Na_2CO_3 solution (25%) are added and thoroughly mixed. 1 ml. diluted Folin-Ciocalteu phenol reagent is then added to each, the contents are mixed again, and the tubes are allowed to stand in a water bath at 37° for 10–15 min. After cooling to room temperature the volume is accurately adjusted to 6 ml. in each tube, and the colours are read in a photoelectric colorimeter, using a red filter and setting the zero with the blank. The colours are stable for several hours.

RESULTS

The effect of glycine on the blue colour reaction is shown in Fig. 1. Reduction of the phosphotungstic-phosphomolybdic acids by cobalt salts could not

be effected in the absence of amino-acids. As little as 0·005 M-glycine is enough to develop the colour reaction in solutions containing 0·01 mg. Co. The colour, however, is rapidly and optimally obtained in the presence of 0·2–0·5 M-glycine. Higher concentrations, on the other hand, reduce the colour intensity. Other amino-acids are also effective, although those of higher molecular weight than alanine give with the phenol reagent in the absence of cobalt blue colour reactions, the intensity of which increases with the rise in their molecular weight.

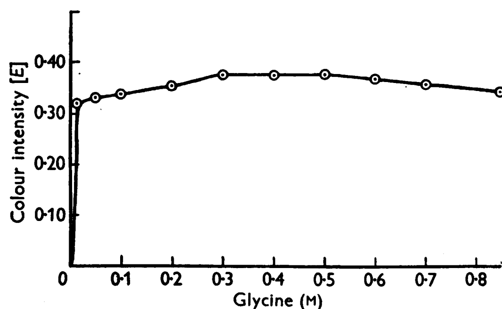

Fig. 1. Effect of glycine concentration on blue colour development in the estimation of 55 μg. K as cobaltinitrite by Folin-Ciocalteu reagent.

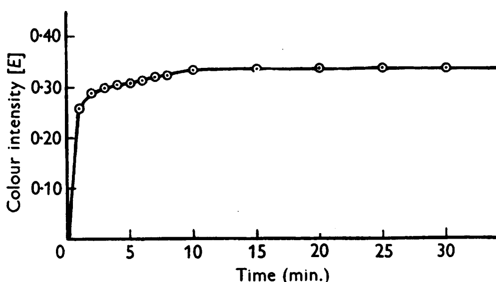

Fig. 2. Rate of Folin-Ciocalteu blue colour development at room temperature. 50 μg. K in 0·2 M-glycine.

Fig. 2 shows the rate of the blue colour development at room temperature in 0·2 M-glycine. The colour attains its maximum intensity in about 15–20 min. at room temperature, but much more rapidly at 37°. The blue colour, however, once formed, is stable for several hours and only starts to fade very slowly after 24 hr.

A strict correlation between the intensity of the blue colour and the quantity of potassium present as cobaltinitrite complex is shown in Fig. 3. The relative intensities of the blue colour obtained by the new method and of the green colour of the choline-ferrocyanide are shown on the same figure. It is evident that the new colour reaction provides an accurate and more sensitive method for estimation of potassium.

This is further illustrated in Tables 1 and 2 which give results of potassium determinations in normal and pathological sera by the new method, by that of Jacobs & Hoffman (1931) and by the flame

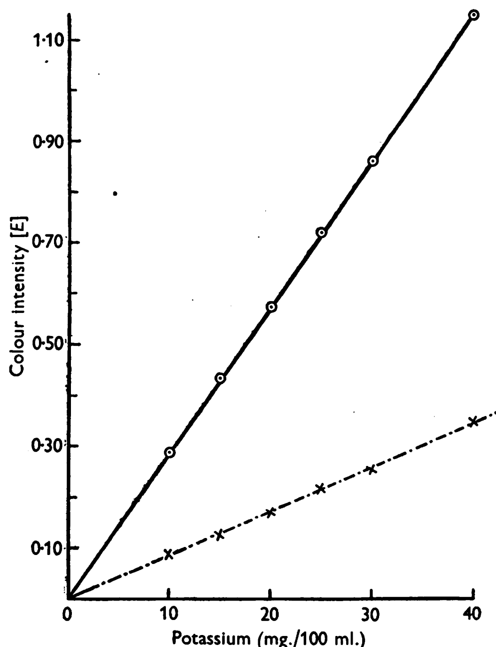

Fig. 3. Colorimetric estimation of potassium in 0·5 ml. potassium sulphate standard solutions by the new (Folin-Ciocalteu) method and by that of Jacobs & Hoffman (1931). The colour intensities were measured in a Hilger Spekker absorptiometer. —— Folin-Ciocalteu blue colour (Ilford spectrum red filter, no. 608); –·–·– choline-ferrocyanide green colour (Ilford spectrum orange filter, no. 607).

photometer (cf. Klyne, 1948). Table 3 gives the results of analyses carried out by the new method and by the flame photometer on a serum to which known amounts of potassium had been added.

DISCUSSION

The method described above suffers from the disadvantage, common to all other methods involving the indirect estimation of potassium by precipitation in different complex forms and estimating some constituent other than potassium, viz. that the complex may vary in composition according to conditions of precipitation. These methods, however, are still in general use, although the flame photometric methods may take their place (Barnes, Richardson, Berry & Hood, 1945; Hald, 1947; Domingo, Klyne & Weedon, 1948; Klyne, 1948).

The method, nevertheless, offers a simple and accurate means for the micro-estimation of potassium in biological fluids. The determination has

Table 1. *Determination of serum potassium by different methods*

Serum			Results: Folin-Ciocalteu method		Results: Jacobs & Hoffman method	
Sample no.	Diagnosis	Vol. taken (ml.)	Photometric readings* (E)	Found (mg. K/100 ml. serum)	Photometric readings* (E)	Found (mg. K/100 ml. serum)
1	Normal	0·2	0·270	20·0	—	—
		0·2	0·265	19·7	—	—
		0·5	0·685	20·3	0·175	20·0
		0·5	0·690	20·4	0·180	20·3
2	Normal	0·2	0·245	18·0	—	—
		0·5	—	—	0·160	18·2
3	Acute nephritis	0·2	0·400	29·6	—	—
		0·5	1·000	29·4	0·258	29·4
		0·5	—	—	0·265	30·2
4	Periarteritis nodosa	0·2	0·370	27·5	—	—
		0·2	0·375	27·7	—	—
		0·5	0·950	27·9	0·247	27·5
		0·5	0·960	28·2	—	—
5	Alkalosis	0·2	0·310	22·8	—	—
		0·2	0·310	22·8	—	—
		0·5	—	—	0·205	22·8
6	Acute nephritis	0·2	0·295	21·9	—	—
		0·2	0·300	22·2	—	—
		0·5	0·770	22·6	0·190	21·1
		0·5	0·760	22·4	—	—
7	Arthritis	0·2	0·300	22·2	—	—
		0·2	0·295	21·8	—	—
		0·5	0·740	21·8	0·200	22·2
		0·5	0·750	22·1	—	—
8	Addison's disease	0·2	0·255	19·0	—	—
		0·2	0·260	19·2	—	—
		0·5	0·660	19·4	0·170	19·4
9	Meningitis	0·2	0·275	20·5	—	—
		0·2	0·280	20·7	—	—
		0·5	0·700	20·6	0·190	21·1
		0·5	0·708	20·8	—	—
10	Nephritis	0·2	0·290	21·5	—	—
		0·5	0·730	21·4	0·187	21·0
11	Alkalosis	0·2	0·270	20·0	—	—
		0·2	0·270	20·0	0·175	19·7
12	Nephritis	0·2	0·250	18·5	—	—
		0·5	0·610	17·8	0·150	17·3

* Ilford tricolour red light filter.

Table 2. *Determination of potassium in normal sera by the new Folin-Ciocalteu method and the flame photometer*

No. of specimens examined ...	22
	K (mg./100 ml.)
Average value by new method	17·6
Range	15·5–21·0
Average value by flame photometer	17·7
Range	15·3–21·4
Root mean square difference between the two methods	1·7

been conveniently carried out on 0·2 ml. of serum instead of 0·5 ml., the minimum required for Jacobs & Hoffman's (1931) method. It has been successfully applied to routine work.

Table 3. *Recoveries of potassium added to normal serum*

Potassium added to serum (mg./100 ml.)	Total potassium found (mg./100 ml.): New method*	Total potassium found (mg./100 ml.): Flame photometer†
0	19·5	19·7
	19·0	
5	25·7	24·1
	24·9	
10	32·0	29·0
	30·0	
15	34·2	34·9
	34·2	
20	39·0	39·7
	40·0	

* 0·2 ml. serum used. † 1·0 ml. serum used.

SUMMARY

1. A micromethod is described for the estimation of potassium in sera and biological fluids.

2. The method depends on the precipitation of potassium as cobaltinitrite, and the colorimetric estimation of the cobalt in the latter by the reduction of the Folin-Ciocalteu phosphomolybdic-phosphotungstic phenol reagent.

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The Nicotinamide-Saving Action of Tryptophan and the Biosynthesis of Nicotinamide by the Intestinal Flora of the Rat

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Tryptophan can replace nicotinamide in the diet of the rat (Krehl, Sarma, Teply & Elvehjem, 1946; Singal, Sydenstricker & Littlejohn, 1947*a*), the dog (Singal, Sydenstricker & Littlejohn, 1947*b*), the pig (Luecke, McMillen, Thorp & Tull, 1947) and the rabbit (Wooley, 1947). It increases the urinary elimination of nicotinamide metabolites in man (Perlzweig, Rosen, Levitas & Robinson, 1947; Sarett & Goldsmith, 1947), dog (Singal *et al.* 1947*b*), rat (Rosen, Huff & Perlzweig, 1946; Singal, Briggs, Sydenstricker & Littlejohn, 1946; Schweigert & Pearson, 1947), mouse (Schweigert & Pearson, 1947), cotton rat (Schweigert, Pearson & Wilkening, 1947) and horse (Schweigert *et al.* 1947). The mechanism of the replacement of nicotinamide by tryptophan is obscure. The fact that tryptophan increases the elimination of nicotinamide metabolites favours the conception of a direct conversion. However, kynurenic acid (Ellinger, 1904), kynurenine (Kotake, 1931) and xanthurenic acid (Lepkowsky, Roboz & Haagen-Smit, 1943) are the only known end products of tryptophan metabolism in mammals. Kynurenic acid has been shown unable to replace nicotinamide (Rosen, Huff & Perlzweig, 1947) and being an α-substituted pyridine derivative cannot be directly converted into nicotinic acid. Kynurenine and xanthurenic acid are found in the urine of mammals mainly in pyridoxin deficiency. Beadle, Mitchell & Nyc (1947) believe they have shown that nicotinic acid can be formed from kynurenine by a mutant strain of Neurospora. Biosynthesis of nicotinamide is known to occur in numerous mammals and might take place either in the mammalian tissues or in the intestinal tract by the activity of the intestinal flora. It is of interest to study the mechanism of the tryptophan conversion, to find out whether the intestinal bacteria are involved in this phenomenon, and if so to find the way in which the conversion takes place. Participation of the intestinal flora is indicated by the work of Schweigert & Pearson (1947), who showed that the increase of nicotinamide methochloride elimination is more marked after oral than after parenteral administration of tryptophan to rats.

Biosynthesis of trigonelline in plants (*Trigonella foenum graecum*) is stimulated by administration of ornithine, arginine, proline or glutamic acid and further increased by hexamethylene tetramine (Klein & Linser, 1932, 1933*a*, *b*). It is possible that one of the above amino-acids might be an intermediate between tryptophan and nicotinamide. Possible pathways from ornithine to nicotinic acid have been suggested by Klein & Linser (1932) and by Guggenheim (1940). In the present paper the role of the intestinal flora in the conversion of tryptophan into nicotinamide was studied in rats *in vivo*;

* Some of the results presented in this paper have been briefly reported (Ellinger & Abdel Kader, 1947, 1948).