picture of leukemia. These clinical conditions will be discussed elsewhere.

6697

Determination of Serum Total Base.

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Practically all the modern methods of determining serum total base are modifications of Fiske's[1] procedure for total base in urine. The modification proposed by Stadie and Ross[2] has until recently received the widest use. In this method, the serum is ashed directly without removal of phosphate, and the base in the ash, which is present as sulfate, is analyzed by the indirect titration method of determining the sulfate as benzidine sulfate. A correction is added for the amount of base present as metaphosphate.

Recently Peters and Van Slyke[3] have published a method combining the procedures of Fiske and of Stadie and Ross. The objection to the original method of Stadie and Ross is chiefly that the results obtained are too low and too variable because sulfates are not completely precipitated in the presence of phosphate and because the method of correcting for base bound to phosphate by adding the number of milliequivalents of inorganic phosphate in the serum is not accurate. Peters and Van Slyke's procedure avoids this difficulty by a preliminary removal of the phosphate as ferric phosphate by addition of ferric alum and then ammonia to precipitate the excess iron.

In the author's experience, Peters and Van Slyke's method has a number of objectionable features. First, the procedure is tedious and time-consuming. As described, a single determination requires 24 hours or longer. Secondly, the technique is so difficult and so liable to errors by loss of material that it could not be entrusted to the average hospital technician. Thirdly, the blank is high and variable. Finally, an error is produced by a precipitation of from 1 to 2

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* This investigation was begun when the author was a National Research Council Fellow in Medicine at Rush Medical College.

[1] Fiske, C. H., *J. Biol. Chem.*, 1922, **51**, 55.

[2] Stadie, W. C., and Ross, E. C., *J. Biol. Chem.*, 1925, **65**, 735.

[3] Peters, J. P., and Van Slyke, D. D., Quantitative Clinical Chemistry, Vol. II —Methods, Baltimore, 1932.

m.eq. of calcium along with the ferric phosphate and ferric hydroxide. This loss is shown in Table I, where only 75% of the calcium was recovered in the filtrates after removal of phosphate.

TABLE I.

Comparison of Calcium Analyses on Filtrate Obtained by Author's Method with those on Filtrate by Method of Peters and Van Slyke.

Material Analyzed	Calcium per liter.		
	Direct Analysis	Author's Filtrate	Peters and Van Slyke's Filtrate
	m.eq.	m.eq.	m.eq.
Serum	5.38	5.19	3.72
"	5.30	5.22	3.43
"	5.25	5.20	3.84
Inorganic solution	6.00	6.20	5.10
" "	6.00	6.15	4.85

The author has determined serum total base by a modification of his method for urine.[4] The procedure used avoids the aforementioned objections and combines accuracy with simplicity of technique. This is all the more important in that the clinical significance of serum total base determinations has become so well recognized that it is desirable to have a method available for the routine hospital chemist.

Method. Reagents. Ferric Chloride. Four cc. of a stock solution of 10% $FeCl_3$ in 0.2 N HCl are diluted to 100 cc.

Ammonium acetate-ammonium hydroxide solution. One gm. of ammonium acetate and 73.5 cc. 0.1 N NH_4OH are made up to a liter.

Benzidine solution. In a 250 cc. volumetric flask, 4 gm. of benzidine of highest purity are added to 45 cc. of normal HCl, diluted with water to 150 cc. and agitated until dissolved. The solution is made up to the mark and filtered. Its titration equivalent is determined as follows: 2 cc. of the benzidine solution are diluted with exactly 15 cc. of water and filtered through an ashless filter paper. 15 cc. of the filtrate are titrated with 0.02 N NaOH to neutrality to phenolphthalein. The titration figure obtained is multiplied by 17/15 to give the titration equivalent of 2 cc. of benzidine solution. The solution should be refiltered every few days and restandardized. Sulfuric acid 4.0 N. Phenolphthalein, 1% in alcohol.

Sodium hydroxide 0.02 N is prepared from concentrated, carbonate-free sodium hydroxide by dilution with CO_2-free water. If used in an automatic "refill" burette, its container should be pro-

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[4] Hoffman, W. S., *J. Biol. Chem.*, 1931, **93**, 787.

tected from CO_2 and moisture by a soda lime tube. It should be frequently restandardized.

One cc. of serum, prepared carefully to avoid any hemolysis of red cells, is measured into a 25 cc. volumetric flask and diluted with about 10 cc. of water. To it are added first one cc. of ferric chloride solution and then one cc. of ammonium acetate-ammonium hydroxide solution, and then water to the mark. The contents are thoroughly shaken and poured into a large (and preferably long) Pyrex tube, which is then heated in a free flame until the first indication of boiling. At this point, there is seen a flocculent brownish yellow coagulum which contains all the protein and fat, all the phosphate, both inorganic and organic, as the ferric salt, and all the excess of iron as the basic ferric acetate. This material is immediately filtered through an ashless filter paper in a 5 cm. funnel into a 25 cc. test tube. If the proportions of reagents have been correct, the filtration is very rapid, and the filtrate is water clear. If, however, the filtration of 20 cc. of this liquid requires more than one minute, and if the filtrate is slightly cloudy, then the quantity of reagents must be adjusted. Usually this can be accomplished by using a trace more or less than the stipulated one cc. of ammonium acetate-ammonium hydroxide reagent. One or two trials will usually suffice to discover the optimum proportions; and once the required quantity is determined, it can be used indefinitely, provided that the ammonia content of the reagent is not allowed to decrease.

The filtrate is cooled under the tap to room temperature. Twenty cc., equivalent to 0.8 cc. of serum, are transferred to a 25 cc. lipped platinum crucible and treated with one cc. of 4 N H_2SO_4. The contents are evaporated on a steam bath until charring occurs, which usually requires about one and a half hours. The crucible is now transferred to a Rogers ring burner equipped with a platinum wire sling. A small flame is allowed to play over the rim of the crucible until a gentle ebullition occurs. After several minutes the liquid begins creeping up the sides of the crucible. The flame is now increased but still kept at the rim, so that evaporation takes place from the sides of the vessel without active boiling. At this point, there is little danger of loss, and the flame can be slowly increased until the upper fourth of the crucible is at a dull red. Soon there is left only a minute quantity of a viscous liquid on the bottom of the crucible which is evaporated by allowing the flame to hit lower and lower. When all the liquid is evaporated, the full force of an oxidizing flame is allowed to play over the whole crucible, keeping it at red heat for one minute. Thus the last traces of carbon are oxidized, and all sulfuric acid is removed. The whole ashing procedure can be completed in about 10 minutes.

Into the cooled crucible, exactly 15 cc. of water are measured, followed by exactly 2 cc. of the benzidine solution. The mixture is thoroughly stirred with rubber-tipped glass stirring rod until it is certain that all the inorganic sulfate has been dissolved and reprecipitated as benzidine sulfate. The latter is filtered off on an ashless filter. 15 cc. of the filtrate are titrated to neutrality to phenolphthalein with 0.02 N NaOH.

A blank determination is run on the reagents at frequent intervals. Its value is usually less than 0.05 cc. of 0.02 N NaOH.

The calculation can be best illustrated by an example. The final titration required 10.60 cc. of 0.02 N NaOH. The blank was 0.05 cc. 2 cc. of benzidine had a titration equivalent of 18.30 cc. of 0.02 N NaOH. $(18.30 - .05 - (10.60 \times 17/15) \times 5/4 \times 1/50 \times 1000) = 156$ m.eq. per liter of serum. The whole determination can be completed in little more than 2 hours.

The results obtained are about 1% too high, because of the slight change in volume during the removal of phosphate and protein at the boiling point. If, therefore, 1% is subtracted from the figure obtained by the above calculation, the correct value for serum total base concentration is obtained.

Table I shows that no calcium is lost in the precipitation of phosphate. Table II illustrates the accuracy of the determinations on inorganic solutions. In Table III are presented the results of determinations of serum total base on 15 normal men and women by both the author's method and that of Peters and Van Slyke. The average uncorrected value obtained by the author's method was 156.9 m.eq. per liter, as compared with 154.2 by the method of Peters and Van Slyke. If a correction of 1% is subtracted from the former and if 1% is added to the latter to correct for the lost

TABLE II.
Determination of Total Base in Inorganic Solutions.

Inorganic Solution m.eq. per liter			Total Base per liter	
			Author's Method	Peters and Van Slyke's Method
			m.eq.	m.eq.
Na	145	Total 156	156.1	152.5
K	5		156.1	154.7
Ca	6		156.6	154.7
P	15		156.6	155.5
			156.1	153.3
			157.5	155.2
Aver.			156.5	154.3

TABLE III.
Determination of Serum Total Base in Normal Serum.

		Total Base		
Case No.	Age and Sex	Author's Method*	Peters and Van Slyke's Method*	Stadie and Ross's Method
		m.eq. per liter	m.eq. per liter	m.eq. per liter
1	M 25	157.2	154.0	152.6
2	M 23	156.6	153.8	151.8
3	M 23	155.4	152.8	
4	M 32	157.5	151.0	
5	F 24	154.6	154.0	
6	F 28	156.1	155.0	
7	M 24	158.0	156.6	
8	M 25	158.5	155.7	
9	F 35	156.6	154.0	150.4
10	M 28	155.5	153.2	150.2
11	M 22	154.2	152.8	149.8
12	M 35	160.2	157.5	154.6
13	M 22	159.6	154.6	153.2
14	F 22	156.9	154.0	
15	M 24	156.6	153.8	
Aver.		156.9	154.2	
Min.		154.2	151.0	
Max.		160.2	157.5	
Corrected Aver.*		155.3	155.7	

* The values given are uncorrected. To obtain the correct value 1% is subtracted from the value by the author's method and 1% is added to the value by Peters and Van Slyke's method.

TABLE IV.
Recovery of Base Added to Serum.

No.	Total Base in Serum	Added Base as K_2SO_4	Total Base	
			Calculated	Found
	m.eq. per l.	m.eq. per l.	m.eq. per l.	m.eq. per l.
Exp. A.				
1	158.8	4.6	163.4	163.9
2	158.8	13.8	172.6	171.8
3	158.8	23.0	181.8	182.3
Exp. B.				
1	156.1	4.6	160.7	161.6
2	156.1	13.8	169.9	171.0

calcium, the results agree. Table IV presents data showing the accuracy of recovery of added base to serum.