obtained on one of 4 cases of obstructive jaundice with a hemorrhagic diathesis are presented in the table for comparison The results clearly show that an excess of calcium as well as an insufficient amount of calcium prolonged the time required for the coagulation of normal and pathological plasma. The optimal amount of calcium required varied among normal individuals. Usually recalcification with 0.1 cc of a 0.0025 M calcium chloride solution resulted in a minimal coagulation time of 10 seconds. However, in certain instances a minimal coagulation time occurred when recalcification was carried out with 0.01, 0.005, and 0.00125 M calcium chloride solutions.

It is well known that an excess of any neutral salt preserves the fluidity of the blood. Horne,[5] Sabbatini,[6] Rettger,[7] and Stassano and Daumas[8] observed the anticoagulant properties of large amounts of calcium salts.

If the quantitative determination of prothrombin is to be reliable there must be no variables except the prothrombin. The present studies indicate that calcium is a variable and that in order to obtain a minimal coagulation time (true prothrombin time) the optimal amount of calcium necessary for recalcification must be determined in each instance.

10263

Use of Cyanide in the Determination of Ascorbic Acid.

M. CUSHMAN AND ALLAN M. BUTLER. (Introduced by James L. Gamble.)

From the Department of Pediatrics, Harvard Medical School, and the Infants' and Children's Hospitals, Boston, Mass.

Though blood plasma or serum inhibits the catalytic oxidation of ascorbic acid as shown by De Caro and Giani[1] and Mawson,[2] Barron, Barron, and Klemperer[3] found that this protective property of plasma did not completely prevent such oxidation. Kellie and

5 Horne, R. M., *J. Physiol.*, 1896, **19**, 356.

6 Sabbatini, L., Abstr. in *Mosso's Arch. ital. de biol.*, 1901, **36**, 416.

7 Rettger, L. J., *Am. J. Physiol.*, 1909, **24**, 406.

8 Stassano, H., and Daumas, A., *Compt. rend. Acad. Sc.*, 1924, **150**, 937.

1 De Caro, L., and Giani, M., *Z. f. physiol. Chem.*, 1934, **228**, 13.

2 Mawson, C. A., *Biochem. J.*, 1935, **29**, 569.

3 Barron, E. S. G., Barron. A. G., Klemperer, F., *J. Biol. Chem.*, 1936, **116**, 563.

Zilva[4] and Barron, De Meio, and Klemperer[5] reported complete inhibition of the catalytic oxidation of ascorbic acid by the addition of potassium cyanide. Pijoan, Townsend and Wilson[6] reported that the concentration of ascorbic acid in plasma and in the metaphosphoric acid filtrate of plasma decreased significantly over short time intervals. To prevent such loss during plasma ascorbic acid determinations Pijoan and Klemperer[7] advocated the addition of approximately 1 mg of potassium cyanide per cc of blood. Pijoan and Eddy[8] in attempting to prevent the loss of ascorbic acid during the analysis of whole blood added as much as 2 mg of potassium cyanide per cc of whole blood. Mindlin and Butler[9] added 1 drop of 5% KCN to 5 cc of blood (approximately 0.6 mg of potassium cyanide per cc) in the determination of plasma ascorbic acid. Friedman, Rubin, and Kees[10] showed that in the absence of cyanide there was no significant loss of reducing power in plasma standing in an ice box for 3 hours. Moreover, they observed that the addition of 2.5 mg of potassium cyanide per cc of blood caused a decolorization of 2-6-dichlorophenolindophenol, corresponding to an ascorbic acid concentration of approximately 0.1 mg %. They were unable to explain the fact that the addition of the same amount of potassium cyanide to bloods of very high ascorbic acid content caused a slight decrease in the apparent ascorbic acid concentration. They concluded that there was no reason for the addition of potassium cyanide to blood for the determination of plasma ascorbic acid. Farmer and Abt[11] in studying the effect of potassium cyanide on the decolorization of 2-6-dichlorophenolindophenol concluded that its use invalidated plasma ascorbic acid values. Furthermore, they observed that during a 2-hour period there was a gradual and variable fall in the plasma ascorbic acid values when whole blood, plasma, or plasma filtrate stood at room temperature both in the presence and in the absence of cyanide.

This paper presents further evidence concerning the effect of potassium cyanide on 2-6-dichlorophenolindophenol, the stability

[4] Kellie, A. E., and Zilva, S. S., *Biochem. J.*, 1935, **29**, 1028.

[5] Barron, E. S. G., de Meio, R. H., Klemperer, F., *J. Biol. Chem.*, 1936, **112**, 625.

[6] Pijoan, M., Townsend, S. R., and Wilson, A., PROC. SOC. EXP. BIOL. AND MED., 1936, **35**, 224.

[7] Pijoan, M., and Klemperer, F., *J. Clin. Invest.*, 1937, **16**, 443.

[8] Pijoan, M., and Eddy, E., *J. Lab. and Clin. Med.*, 1937, **22**, 1227.

[9] Mindlin, R. L. M., and Butler, A. M., *J. Biol. Chem.*, 1938, **122**, 673.

[10] Friedman, G. J., Rubin, S. H., and Kees, W., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 358.

[11] Farmer, C., and Abt, A., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 399.

of reduced ascorbic acid in whole blood, plasma, and plasma filtrate, and the question of whether potassium cyanide should or should not be used in plasma ascorbic acid determinations.

The effect of potassium cyanide on decolorization of 2-6-dichlorophenolindophenol in metaphosphoric acid solutions: Table I shows that the decolorizing effect of potassium cyanide on the dye depends upon, one, the lot of potassium cyanide, two, the concentration of potassium cyanide, and three, the pH of the reaction mixture. The decolorizing effect was greater when the reaction mixture was pH 2, which is the pH of the titration procedures in common use, than at the pH of 4 which is used in the method of Mindlin and Butler.[9]

TABLE I.

Effect of Different Lots of KCN on Decolorization of 2-6-dichlorophenolindophenol in Metaphosphoric Acid Solutions Expressed as the Ascorbic Acid Equivalent in mg %, Had the Determinations Represented Plasma Analyses.

Lot No. KCN	Mg KCN in sample analyzed	decolorization as mg % ascorbic acid	
		pH 2	pH 4
M 1	0.6	0	0
"	2.0	0	0
"	4.0	0.2	0.1
P 1	0.6	0.15	0.15
"	2.0	0.55	0.40
"	4.0	1.00	0.60

Recrystallization of potassium cyanide from a saturated water solution with or without the addition of alcohol has enhanced rather than reduced the decolorizing property of a poor lot of the salt. Precipitation of cyanide from a solution of the salt by the addition of a slight excess of silver nitrate and filtration gave a solution that did not decolorize the dye. Acidification of a solution of potassium cyanide with H_2SO_4, evaporation to dryness at room temperature, and addition of water to the original volume gave a solution whose decolorizing power was approximately the same as that of the original potassium cyanide solution.

Table II shows the importance of the 3 factors mentioned above on the effect of potassium cyanide in the determination of solutions of known ascorbic acid concentration.

Table III shows the range of error introduced into the determination of plasma ascorbic acid when the 2 brands of potassium cyanide referred to in Tables I and II are used at the pH 2 and 4. The data show that the use of cyanide, in the amounts recommended by Pijoan and Klemperer[7] and Mindlin and Butler,[9] in plasma ascorbic acid determinations does not necessarily invalidate them. Depend-

TABLE II.
Effect of Different Lots of KCN on Apparent Ascorbic Acid Concentration of a 1 mg % Ascorbic Acid Solution at pH 2 and pH 4.

		pH 2				pH 4	
		Titration[9]		Photoelectric Col.		Photoelectric Col.[8]	
Lot No. KCN	Mg KCN in sample analysed	mg% found	% error	mg% found	% error	mg% found	% error
	0	1.0	0	1.0	0	1.0	0
M 1	0.6	1.0	0	1.0	0	1.0	0
"	2.0	1.1	+ 10	1.0	0	1.0	0
"	4.0	1.2	+ 20	1.1	+10	1.1	+10
P 1	0.6	1.1	+ 10	1.1	+10	1.1	+10
"	2.0	1.5	+ 50	1.4	+40	1.3	+30
"	4.0	2.0	+100	1.8	+80	1.4	+40

ing upon the lot of cyanide there may be no error whatever, or an error amounting to 0.1 mg % in the case of the Mindlin and Butler[9] procedure, and 0.1-0.2 mg % in the case of the titration procedure.

TABLE III.
Effect of Different Lots of KCN upon Determination of Plasma Ascorbic Acid. Measurements Were Made by Means of Photoelectric Cell at pH 2 and 4.

	Lot No. KCN	Mg KCN in sample analyzed	Mg% ascorbic acid	
			pH 2	pH 4
Plasma 1	0	0	0.6	0.6
	P 1	0.6	0.8	0.7
	M 1	0.6	0.6	0.6
" 2	0	0		0.9
	P 1	1.0		1.0
" 3	0	0	0.5	0.5
	P 1	0.6	0.6	0.6
	P 1	1.0	0.8	0.7

This is an error which is not of clinical significance in a method in which the error is plus or minus 0.1 mg %. In our hands the limit of error of the method in which 2 cc of plasma is used is 0.2 mg % when the pH is less than 2.5, and 0.1 mg % when the pH is between 3.5 and 4.2. As shown by Mindlin and Butler[9] the greater experimental error at the more acid pH is due to the unequal rates of fading in the blank-dye solution and in the plasma-dye solution. When the pH of the final solution is between 3.5 and 4.2 this difference in the rate of fading is almost eliminated.

The stability of reduced ascorbic acid in whole blood, plasma, or metaphosphoric acid plasma filtrate at room temperature with and

without potassium cyanide. Table IV shows the mg % of plasma ascorbic acid found after samples of whole blood, plasma, or metaphosphoric acid plasma filtrate stood for specified time intervals. In those samples to which potassium cyanide was added, 0.6 mg of potassium cyanide (lot M 1) was added per cc of whole blood. The data show that whole blood and metaphosphoric acid plasma filtrates stood as long as 24 hours without significant change in ascorbic acid concentration. When plasma stood at room temperature for over 4 hours, there was a significant progressive fall in ascorbic acid concentration. Although the different effects of standing observed by others[6, 11] may have been due to the presence of a catalyst, it is interesting to note that we made no particular attempt to eliminate such substances. The data also show that although the addition of potassium cyanide did not alter the observed concentrations of ascorbic acid, it did not prevent the progressive loss from the samples that stood as plasma.

TABLE IV.

Effect of Time of Standing at Room Temperature of Whole Blood, Plasma, or Metaphosphoric Acid Plasma Filtrates with or without KCN on Plasma Ascorbic Acid Concentration. Results are given as mg% of plasma ascorbic acid.

Blood No.	Sample standing	Time of standing in hr				
		0	2	4	6	24†
4	Whole blood	1.9	1.8	1.8		
	Plasma	1.9	1.8	1.7		
	" + KCN*	1.8	1.7	1.7		
	Filtrate	1.9	1.9	1.9		
5	Whole blood	1.8		1.8	1.8	1.7
	" " + KCN*				1.7	1.6
	Plasma	1.8			1.5	1.2
	" + KCN*	1.7			1.5	1.1
	Filtrate	1.8			1.8	1.7

*0.6 mg KCN lot M 1 per cc whole blood.
†First 6 hours at room temperature, last 18 hours in ice box.

Conclusions. The use of potassium cyanide as recommended in plasma ascorbic acid analyses[7, 9] does not necessarily invalidate the results and may cause no error. Certain lots of potassium cyanide may decolorize 2-6-dichlorophenolindophenol, the decolorizing power of a particular lot depending upon the concentration of the salt and the pH of the dye-salt solution. Reduced ascorbic acid is stable in whole blood or metaphosphoric acid plasma filtrates for as long as 24 hours. There is a significant loss of reduced ascorbic acid when plasma stands at room temperature for more than 4 hours. This loss is not prevented by the presence of potassium cyanide in con-

centrations recommended in plasma ascorbic acid analysis. These observations indicate that there is no reason for the addition of potassium cyanide in the determination of plasma ascorbic acid by the methods mentioned.

10264

Immunological Differentiation of Shigella Alkalescens from *Shigella paradysenteriae* Flexner, V, W, X, Y, and Z.

ERWIN NETER.

From the Laboratories of the Children's Hospital, and the Department of Pathology and Bacteriology, University of Buffalo School of Medicine, Buffalo, N. Y.

When Andrewes[1] described for the first time a new member of the Shigella group that is referred to today as *Shigella alkalescens,* his main purpose was to differentiate this microörganism from true dysentery bacilli. The purpose of this differentiation still holds true, since *Shigella alkalescens* does not cause epidemic or endemic forms of bacillary dysentery in man as does the closely related *Shigella paradysenteriæ* Flexner. Both microörganisms show similarities, not only in cultural characters but also in antigenic structure: *Shigella alkalescens* may be agglutinated by anti-Flexner sera and anti-*Shigella alkalescens* sera may agglutinate certain strains of *Shigella paradysenteriæ* Flexner as demonstrated by Andrewes,[1] Smith and Fraser,[2] Popoff and Spanswick,[3] Welch and Mickle,[4] Gilbert and Coleman,[5] Neter and Rappole,[6] and Neter.[7, 8] Recently, it was found that type-specific anti-*Shigella paradysenteriæ* Flexner sera differ markedly in their content of cross-agglutinins for *Shigella alkalescens.* While anti-*Shigella paradysenteriæ* Y sera strongly agglutinated *Shigella alkalescens,* and anti-*Shigella paradysenteriæ* V and X sera contained a moderate titer of agglutinins for this microörganism, anti-*Shigella paradysenteriæ* W and Z sera may lack agglutinins for *Shigella alkalescens* in spite of a high titer of

1 Andrewes, F. W., *Lancet,* 1918, **194**, 560.
2 Smith, J., and Fraser, A. M., *J. Path. and Bact.,* 1928, **31**, 511.
3 Popoff, N. W., and Spanswick, M. P., *J. Lab. and Clin. Med.,* 1931, **16**, 437.
4 Welch, H., and Mickle, F. L., *Am. J. Publ. Health,* 1934, **24**, 219.
5 Gilbert, R., and Coleman, M. D., *Am. J. Publ. Health,* 1934, **24**, 449.
6 Neter, E., and Rappole, F., *Arch. Path.,* 1938, **25**, 298.
7 Neter, E., *J. Bact.,* 1938, **35**, 202.
8 Neter, E., *J. Immunology,* 1938, **35**, 339.