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A NEW PERMANENT STANDARD FOR ESTIMATION OF HEMOGLOBIN BY THE ACID HEMATIN METHOD.

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While investigating various hemoglobin methods, we found the acid hematin modification[1] of Palmer's method[2] very satisfactory in as far as accuracy of estimation is concerned. The preparation of an accurate stock solution of the strong acid hematin standard is a difficult task for those who are not research biochemists. Since the solution must be prepared about every 4 months, the method is hardly suitable for general use by physicians. We realized that a permanent standard is required to make the method universally available.

Newcomer's method,[3] using a colored plate of glass as the standard, seemed very attractive. But we found, as did Miss Robscheit,[1] that the color is too pale to give consistent results at least with workers whose eyes are not sensitive to very slight changes of pale colors.

We have, therefore, devised a standard solution containing inorganic material only, which if set at 15 mm. in a colorimeter will match the color of a 1 per cent acid hematin solution set at 10 mm. (a 1 per cent solution being a 1 in 100 dilution in weak HCl of blood containing 13.8 gm. of hemoglobin in 100 cc.). The intensity of the color varies with change in temperature, but this is taken into account in the calculation (see below). The final standard prepared by us contains 32 gm. of ferric sulfate and 80 mg. of chromic sulfate in 100 cc. Unfortunately, the ferric

[1] Cohen, B., and Smith, A. H., *J. Biol. Chem.*, 1919, xxxix, 489. Robscheit, F. S., *J. Biol. Chem.*, 1920, xli, 209.

[2] Palmer, W. W., *J. Biol. Chem.*, 1918, xxxiii, 119.

[3] Newcomer, H. S., *J. Biol. Chem.*, 1919, xxxvii, 489.

sulfate on the market is not uniform in color, we found a great difference in the character of the color of several samples secured from the same manufacturer. We made our ferric sulfate from recrystallized ferrous sulfate by the usual method, but we carefully regulated the amount of heat used.[4] We have every reason for expecting our standard solution to keep indefinitely. We have been unable to detect any change in color during 10 months.

TABLE I.

Comparison of Estimations of Hemoglobin by the Proposed Method and by Van Slyke's Method.

Blood No.	Percentage of hemoglobin.	
	New method.	Van Slyke's method.*
1	29.7	30.6
2	37.0	36.8
3	43.7	42.9
4	49.7	49.0
5	56.0	56.0
6	61.0	61.3
7	65.7	66.0
8	72.3	74.0
9	80.4	79.8
10	90.8	91.9
11	96.2	96.7
12	100.5	100.0
13	104.8	104.6
14	117.3	116.8
15	123.2	122.6
16	131.3	132.2

* We found it necessary to take the mean of several Van Slyke estimations in order to keep the probable error below 1 per cent. The results by our method given above are also the mean of several determinations.

Estimations made by using this standard agree very closely with the results by Van Slyke's method[5] as will be seen by examination of Table I. No other method that is adapted to clinical use gave results within 5 per cent of the Van Slyke estimations.

[4] Because of this difficulty in reproducing our standard we shall check the solution before it is distributed. It may be secured from Hynson, Westcott and Dunning, Baltimore.

[5] Van Slyke, D. D., *J. Biol. Chem.*, 1918, xxxiii, 127. Van Slyke, D.D., and Stadie, W. C., *J. Biol. Chem.*, 1921, xlix, 1.

Technique.—If the patient bleeds freely from a skin puncture, 0.05 cc. of blood may be taken with an accurate 0.1 cc. pipette. After wiping off the tip of the pipette the blood is quickly blown out into 2.45 cc. of water in a test-tube and mixed at once. The tube may be set aside (corked) until a convenient time for estimation, or the work may proceed as soon as the blood is fully laked.

TABLE II.

Per Cent of Hemoglobin Corrected for the Temperature of the Standard, Corresponding to Various Colorimeter Readings.

Colorimeter reading.	Temperature.										
	15.5°	16.5°	17.5°	18.5°	19.5°	20.5°	21.5°	22.5°	23.5°	24.5°	25.5°
mm.											
8.0	131.5	134.1	136.7	139.5	142.0	144.7	147.4	150.8	154.0	157.0	160.6
8.5	123.7	126.2	128.6	131.1	133.6	136.2	139.2	142.0	144.8	148.0	151.2
9.0	116.9	119.2	121.3	123.8	126.2	128.8	131.4	133.9	136.5	139.1	142.7
9.2	114.5	116.7	119.0	121.2	123.5	125.9	128.3	131.3	133.9	136.9	139.8
9.4	111.9	114.1	116.3	118.5	120.8	123.3	125.8	129.4	131.2	133.9	136.6
9.6	109.4	111.6	113.9	116.1	118.4	120.6	122.8	125.4	128.5	131.1	133.8
9.8	107.2	109.3	111.4	113.6	115.8	118.1	120.5	123.3	126.2	128.6	131.0
10.0	105.0	107.1	109.2	111.5	113.5	115.8	118.2	120.5	123.1	125.6	128.2
10.2	103.0	105.0	107.0	109.1	111.2	113.5	115.9	118.1	120.5	123.1	125.7
10.4	101.0	103.0	105.0	107.0	109.0	111.1	113.2	115.6	118.0	120.6	123.2
10.6	99.0	101.1	103.0	105.0	107.0	109.0	111.0	113.3	116.0	118.4	120.8
10.8	97.4	99.4	101.4	103.3	105.2	107.2	109.3	111.6	113.9	116.2	118.7
11.0	95.8	97.6	99.4	101.2	103.0	105.0	107.0	109.2	111.5	114.0	116.5
11.5	91.3	93.2	95.1	96.9	98.8	100.5	102.5	104.7	106.9	109.1	111.5
12.0	87.8	89.5	91.2	92.9	94.7	96.5	98.4	100.4	102.5	104.6	106.8
12.5	84.2	85.9	87.6	89.2	91.2	92.8	94.5	96.4	98.5	100.5	102.5
13.0	80.8	82.5	84.3	85.9	87.5	89.2	91.0	92.8	94.7	96.8	98.9
13.5	78.0	79.5	81.0	82.6	84.2	85.8	87.5	89.4	91.3	93.2	95.1
14.0	75.2	76.7	78.2	79.7	81.3	82.8	84.3	86.1	88.0	89.9	91.8
15.0	70.2	71.6	73.0	74.4	75.8	77.3	78.6	80.4	82.2	83.9	85.4
16.0	65.8	67.1	68.4	69.7	71.0	72.4	73.8	75.4	77.0	78.6	80.3

Add exactly 2.5 cc. of 0.2 N HCl (18 cc. of C.P. acid per liter are close enough). The volume of the mixture is 5 cc., and the dilution of the blood is 1 part in 100. Warm the tube in a water bath at 55–60° (often hot tap water is satisfactory) for 7 minutes or more. This develops the maximum color of the acid hematin (we did not find 1 hour standing at room temperature sufficiently reliable). In the meantime the colorimeter has been set and tested

for uniformity of the light on both sides. Put the standard solution in the left hand cup and set at 15 mm. Cool the blood mixture and put it in the other cup. Make several readings, being careful to avoid eye-strain. Determine the temperature of the standard at once by inserting a thermometer in the liquid.

No calculation is necessary since the percentage of hemoglobin can be read directly from the table using the average reading and the temperature. Table II is an abbreviation of the complete table that will be furnished with the solution. An estimation of 100 per cent indicates that the blood contains 13.8 gm. of hemoglobin in 100 cc. Normal bloods will, of course, give a distinctly higher estimation.

It is more satisfactory to use oxalated venous blood. In this case measure exactly 1 cc. into a 100 cc. flask, containing about 40 cc. of water. After laking has occurred add 50 cc. of 0.2 N HCl while mixing, and dilute to the mark. To cut the foam use a drop of alcohol (caprylic alcohol must not be used). Mix well, and heat some of the solution in a test-tube as described above.

In cases of marked anemia use double quantity of blood, and divide the estimated per cent of hemoglobin by 2. If 0.1 cc. is taken for the micro method 2.45 cc. of HCl are to be used instead of 2.5 cc.

Preservation of the Standard.—The solution is kept in a Non-sol or Pyrex flask, corked with a rubber stopper (if a new stopper is used, scour off the powdered material). The standard after being used is stored in a second flask. Before the main stock of standard is exhausted check up the used standard against it by making estimations with both, using the same acid hematin preparation. If they agree, pour the used standard into the other flask and use again. We have found that with reasonable care no change in the solution is noticed during months of repeated use. Of course, the cup and plunger of the colorimeter must be clean and dry before the standard is put in the cup. If dried salts collect on the stopper and on the mouth of the flask, wipe off with a damp cloth.

Advantages of the Method.—The technique is quick, simple, and accurate. Special apparatus is not required. The standard is permanent.