A COMPARATIVE STUDY OF HEMOGLOBIN DETERMINATION BY VARIOUS METHODS.

BY FRIEDA S. ROBSCHEIT.

(From the George Williams Hooper Foundation for Medical Research, University of California Medical School, San Francisco.)

(Received for publication, November 3, 1919.)

The regeneration of hemoglobin and red cells following simple anemia has been studied in this laboratory for more than 2 years. A preliminary report of this work by Whipple and Hooper (13) has appeared and it is obvious that this curve of hemoglobin regeneration can be influenced by a number of diet factors. In this work it is essential that there be an accurate determination of hemoglobin. For this reason a comparative study of many hemoglobin methods was undertaken. It is believed that the method finally adopted for this work will be of value to other workers in the experimental field as well as to hospital and school laboratories where routine hemoglobin readings are so frequent. It need not be stated that much of the work expended upon routine hemoglobin determination is a total loss because the instrument used has not been standardized or the method is inaccurate. Too little is known as to the normal hemoglobin value in human beings as affected by age, altitude, climate, etc. No comparison is possible until some accurate standard is adopted for the routine work.

The earlier anemia work in this laboratory was done with Sahli's method using his modification of Gowers' instrument. It was soon apparent that incorrect results were being obtained and that the hemoglobin percentages were considerably higher than they should be when correlated with the other blood findings. The standard color tubes when checked against the oxygen capacity method of Van Slyke (12) showed great variations in color density. New tubes were purchased and when standardized showed much fading varying from 5 to 20 per cent. The
results obtained with these tubes were therefore sufficiently erroneous to warrant the discarding of this method for hemoglobin determination.

The Palmer carbon monoxide method was next tested and accurate results were obtained, provided the standard solutions were frequently checked.

Since the publication of Newcomer's method based on spectrophotometric data this method has also been carefully investigated, as well as a combination of Palmer's and Sahli's method with slight modifications described below.

**History of Methods.**

I. Acid Hematin Method.—Sahli (10), finding that methods employing artificial color standards were not satisfactory, brought forth the acid hematin method. Hemoglobin is converted into acid hematin by the addition of 0.1 N HCl and then compared with a standard of like material. Numerous criticisms of the method have appeared and as many modifications been offered. Berczeller (2) claimed that the presence of lipoids alters readings. Stäubli (11) called attention to the time factor for maximum color development. Palmer (9) claimed that the standard is not permanent, that there is considerable delay in maximum color development, and that the method is not applicable for blood of different species. Haessler and Newcomer (4) offered a modification in the instrument used, using eleven standard tubes of different concentrations for comparison. Lilliendahl-Petersen (7) employed Sahli's principle in a Tallquist form. Newcomer (8) recently published a method of hemoglobin determination by comparing an acid hematin suspension of blood with a piece of brown-colored glass of definite thickness. The method is based on spectrophotometric data. The comparison is made with a Duboscq type of colorimeter.

II. Carbon Monoxide Method.—Hoppe-Seyler in 1892 (6) published his procedure of accurately determining hemoglobin in the form of carbon monoxide hemoglobin. The technical difficulties involved were too numerous for general adoption of the method.
Haldane (5) 8 years later revived Hoppe-Seyler's principle of hemoglobin determination but in a much simpler form. He used Gowers' instrument.

Palmer (9) in 1918 published a method which has found much favor. The principle is that of Hoppe-Seyler's procedure; i.e., a color comparison of carbon monoxide hemoglobin solution with a standard of known hemoglobin content. Ammonia solution is used as a diluent instead of water. The color comparison is made in a Duboscq colorimeter.

EXPERIMENTAL.

I. Palmer Method.—We have used Palmer's method with only slight modifications. All experiments carried on during this investigation have been done on dogs. All blood is obtained by venous puncture. About 10 cc. of blood are drawn from the jugular vein with a glass syringe and emptied into a graduated centrifuge tube containing 2 cc. of a 1.6 per cent sodium oxalate solution. The plasma obtained by centrifugation is carefully pipetted off, the tube slightly tilted, and a 1 cc. calibrated pipette of small lumen quickly inserted with the finger closing the opening at the upper end. Blood is slowly drawn up to the 1 cc. mark, the pipette is thoroughly wiped on the outside, and its contents are transferred into a small test-tube. The pipette is carefully rinsed in 2 cc. of Na salt solution previously measured with this same pipette and emptied into a test-tube similar to the one containing the blood. This salt solution, now containing some red cells, is then carefully added to the 1 cc. of blood and the whole thoroughly mixed avoiding of course too vigorous shaking. Extreme care must be taken with this procedure so that the suspension of packed red blood cells is truly a dilution of one in three. The latter was ascertained to be the most convenient dilution, for the hemoglobin of our normal dogs is usually considerably over 100 per cent, frequently showing readings of 130 to 140 per cent. From the diluted blood cells suspension a 1 per cent solution of blood is made. 1 cc. of the diluted blood, 1:3, is drawn up into the same pipette used for diluting the packed cells as well as for measuring the original amount, is transferred to a 100 cc. volumetric flask containing
the 0.4 per cent ammonia solution, and made up to the 100 cc. mark with this same diluent. The solution is thoroughly mixed and at once saturated with carbon monoxide and read immediately. The percentage of hemoglobin obtained is multiplied by three—the packed red cells having been previously diluted 1:3—and this figure again multiplied by the red cell percentage of the blood computed from the hematocrit readings. In order to determine the accuracy of this apparently roundabout procedure, which is employed to prevent further bleeding for hemoglobin determination, readings were made from whole blood collected from the vein directly into a vessel containing sufficient dry sodium oxalate to prevent clotting and compared with the figures obtained by our means. Table I demonstrates the accuracy of the method as we employ it. It is obvious that by increasing the amount of hemoglobin used we tend to diminish any error of the method. 1 cc. of packed red cells should give more accurate readings than 20 mm. of whole blood.

Palmer's technique for the preparation of his standards has been closely adhered to. Palmer used either defibrinated ox or human blood. We have tried whole as well as defibrinated blood. Furthermore, blood obtained from different species has been investigated. In some cases blood has been obtained aseptically and the standard prepared from it with all aseptic precautions. With other standards only the usual cleanliness has been exercised.

The figures in Table II demonstrate the amount of fading of standards during the time of observation.

### TABLE I.*

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>per cent</td>
<td>per cent</td>
<td>per cent</td>
<td>per cent</td>
<td>per cent</td>
<td></td>
</tr>
<tr>
<td>69</td>
<td>207</td>
<td>46.0</td>
<td>95</td>
<td>94</td>
<td>+1</td>
</tr>
<tr>
<td>57</td>
<td>171</td>
<td>48.0</td>
<td>82</td>
<td>83</td>
<td>−1</td>
</tr>
<tr>
<td>67</td>
<td>201</td>
<td>40.0</td>
<td>80</td>
<td>80</td>
<td>0</td>
</tr>
<tr>
<td>59</td>
<td>177</td>
<td>47.2</td>
<td>84</td>
<td>84</td>
<td>0</td>
</tr>
<tr>
<td>62.1</td>
<td>186.3</td>
<td>50.5</td>
<td>94</td>
<td>94</td>
<td>0</td>
</tr>
<tr>
<td>76.9</td>
<td>230.7</td>
<td>42.6</td>
<td>98</td>
<td>97</td>
<td>+1</td>
</tr>
<tr>
<td>63.0</td>
<td>189</td>
<td>38.4</td>
<td>73</td>
<td>73</td>
<td>0</td>
</tr>
</tbody>
</table>

* This work has been repeated and similar results have been obtained.
## TABLE II.

Change in Color Value of Palmer Standards of Hemoglobin.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Dog (whole)</td>
<td>100</td>
<td>99</td>
<td>90</td>
<td>75</td>
<td>73</td>
<td>74</td>
<td>74</td>
<td>72</td>
<td>71</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Discarded. Slight brownish tinge last 3 months.</td>
</tr>
<tr>
<td>II</td>
<td>Goat &quot; sterile.&quot;</td>
<td>100</td>
<td>85</td>
<td>80</td>
<td>73</td>
<td>74</td>
<td>74</td>
<td>75</td>
<td>75</td>
<td>75</td>
<td></td>
<td></td>
<td>26</td>
<td></td>
<td>Discarded. Slight brownish tinge last 3 months.</td>
</tr>
<tr>
<td>III</td>
<td>Dog (defibrinated)</td>
<td>100</td>
<td>99</td>
<td>80</td>
<td>87</td>
<td>86</td>
<td>86</td>
<td>80</td>
<td>81</td>
<td>82</td>
<td>82</td>
<td>82</td>
<td>20</td>
<td>16</td>
<td>No color change, &quot; &quot; &quot;</td>
</tr>
<tr>
<td>IV</td>
<td>Human &quot;</td>
<td>100</td>
<td>93</td>
<td>89</td>
<td>89</td>
<td>84</td>
<td>84</td>
<td>85</td>
<td>85</td>
<td>84</td>
<td></td>
<td></td>
<td>10</td>
<td>6</td>
<td>&quot; &quot; &quot;</td>
</tr>
<tr>
<td>V</td>
<td>Sheep &quot;</td>
<td>100</td>
<td>98</td>
<td>91</td>
<td>91</td>
<td>92</td>
<td>90</td>
<td>90</td>
<td>90</td>
<td>90</td>
<td></td>
<td></td>
<td>18</td>
<td></td>
<td>&quot; &quot; &quot;</td>
</tr>
<tr>
<td>VI</td>
<td>Dog (whole)</td>
<td>100</td>
<td>93</td>
<td>88</td>
<td>87</td>
<td>85</td>
<td>86</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td></td>
<td></td>
<td>15</td>
<td>0</td>
<td>Spoiled.</td>
</tr>
<tr>
<td>VII</td>
<td>&quot; (defibrinated)</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>96</td>
<td>96</td>
<td>95</td>
<td>5</td>
<td></td>
<td></td>
<td>0</td>
<td>5</td>
<td>No color change. &quot; &quot; &quot;</td>
</tr>
<tr>
<td>VIII</td>
<td>&quot;</td>
<td>100</td>
<td>99</td>
<td>99</td>
<td>96</td>
<td>96</td>
<td>95</td>
<td>95</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0</td>
<td>5</td>
<td>&quot; &quot; &quot;</td>
</tr>
</tbody>
</table>
Discussion of Table II.

No. I showed the greatest fading, 29 per cent in 8 months, the maximum change taking place during the first 2 months. The slight color change did not interfere with color comparison.

No. II was prepared from goat blood with aseptic precautions and kept sterile throughout the experiment. Neither the fragility of red cells nor bacterial decomposition seems to play an important role in the fading of color. The change was nearly as much as with No. I, the maximum fading taking place during the first 3 months.

The curve of No. III is more promising, defibrinated blood showing slightly better keeping qualities than whole blood. The fading was not so rapid nor so much as in Standards I and II, nor was any color change apparent.

No. IV prepared from pooled defibrinated human blood showed about the same stability of color as defibrinated dog blood. The greatest change took place during the first 2 months; after that the fading was slight.

We were more successful in keeping No. V, prepared from defibrinated sheep blood, for the maximum fading was but 10 per cent as compared with 16 and 20 per cent when using defibrinated human and dog blood.

No. VI, having as its source whole dog blood, faded 15 per cent during 6 months, not so much as Standards I and II also prepared from whole blood, but still slightly more than when the blood was previously defibrinated.

Standard VII again prepared from defibrinated dog blood was the only one which remained unchanged during the period of observation, 7 months.

Standard VIII, for which defibrinated dog blood was again employed, showed a fading of 5 per cent during 5 months. While more promising than the earlier ones, still the change is too much for accurate hemoglobin determinations.

Another standard prepared from whole dog blood to determine again the difference obtained with whole and defibrinated blood is but 2 months old. The fading already amounts to 7 per cent during this period.
II. Acid Hematin Method.—Because of the uncertain stability of carbon monoxide hemoglobin solutions we have attempted to solve the problem by searching for a more stable color standard. In view of Sahli’s work acid hematin was tried again. It offers an easier color comparison than do the reds of oxyhemoglobin and carbon monoxide hemoglobin. Sahli in his original work obtained satisfactory results, as did several of his coworkers. We have combined Palmer’s method with Sahli’s principle, that is, determined hemoglobin in the form of acid hematin, employing Palmer’s method of standardization. The sealed tubes containing dilute acid hematin suspension as purchased (Sahli instrument) are unsatisfactory as was pointed out in the history of the methods. The thought that acid hematin in a more concentrated form might not fade so readily arose, and therefore 5, 10, and 20 per cent suspensions were investigated. Our method of procedure was as follows:

The oxygen capacity of a sample of dog’s blood was determined by Van Slyke’s method and the hemoglobin content computed therefrom. An acid hematin standard in the form of a 20 per cent suspension, so diluted that a 1 per cent dilution prepared from it would read 100 per cent, was made. Because of our experience with whole and defibrinated blood in the preparation of standards for the carbon monoxide method we employed defibrinated blood for our first standard. It is well known and has been mentioned in the history of acid hematin methods that the time factor for allowing the maximum color of acid hematin to develop plays a very important rôle. This standard, after preparation and dilution, was allowed to stand 24 hours to insure correct readings. A 1 per cent dilution prepared from the 20 per cent suspension was of course employed for direct color comparison. The same time factor was used for the blood to be tested. A 1 per cent suspension of blood is used for the determination of hemoglobin. 0.1 N HCl is employed throughout the procedure, for the original standard suspensions as well as for all further dilutions. The strength of HCl used within a certain limit is immaterial. We tried N, 0.1 N, and 0.5 N and obtained identical readings. Stronger solutions than N caused precipitation. The entire amount of diluent is used at once, that is, 1 cc. of diluted packed red cells is discharged
into the volumetric flask containing about 50 cc. of 0.1 N HCl, then mixed, and the volume made up to the 100 cc. mark with 0.1 N HCl. The suspension is then allowed to stand 24 hours in the ice chest, thoroughly shaken again, for it must be remem-

<table>
<thead>
<tr>
<th>Palmer's method.</th>
<th>Author's modification.</th>
</tr>
</thead>
<tbody>
<tr>
<td>per cent</td>
<td>per cent</td>
</tr>
<tr>
<td>75</td>
<td>74</td>
</tr>
<tr>
<td>70</td>
<td>70</td>
</tr>
<tr>
<td>77</td>
<td>76</td>
</tr>
<tr>
<td>77</td>
<td>77</td>
</tr>
<tr>
<td>74</td>
<td>73</td>
</tr>
<tr>
<td>73</td>
<td>73</td>
</tr>
<tr>
<td>74</td>
<td>74</td>
</tr>
<tr>
<td>75</td>
<td>75</td>
</tr>
<tr>
<td>76</td>
<td>73</td>
</tr>
<tr>
<td>76</td>
<td>76</td>
</tr>
<tr>
<td>64</td>
<td>64</td>
</tr>
<tr>
<td>63</td>
<td>63</td>
</tr>
<tr>
<td>76</td>
<td>76</td>
</tr>
<tr>
<td>45</td>
<td>45</td>
</tr>
<tr>
<td>60</td>
<td>60</td>
</tr>
<tr>
<td>84</td>
<td>85</td>
</tr>
<tr>
<td>69</td>
<td>69</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>per cent</td>
<td>per cent</td>
<td>per cent</td>
</tr>
<tr>
<td>113</td>
<td>113</td>
<td>114</td>
</tr>
<tr>
<td>117</td>
<td>118</td>
<td>117</td>
</tr>
<tr>
<td>99</td>
<td>98</td>
<td>99</td>
</tr>
<tr>
<td>124</td>
<td>124</td>
<td>124</td>
</tr>
<tr>
<td>109</td>
<td>108</td>
<td>109</td>
</tr>
</tbody>
</table>

bered that we are dealing with a suspension and not a solution, and then read. The method of hemoglobin determination is exactly like that of Palmer's method and the readings obtained are almost identical as illustrated by the figures given in Tables III and IV.
The source of light used for color comparison makes little difference. Light from a northern exposure or that originating from a nitrogen-filled bulb filtered through “Daylite” glass gives equally good readings. The slight turbidity of the acid hematin suspension, and it is very slight in a 1 per cent dilution, does not in the least interfere with an accurate color comparison.

Different standards have been prepared, 5, 10, and 20 per cent suspensions, defibrinated as well as whole blood, also blood from different species. In fact, standards have been made up from the same sample of blood, one diluted for carbon monoxide hemoglobin determinations and the other in the form of acid hematin suspensions. The standards have been checked up once a month as those for Palmer’s method. Material for the 1 per cent dilution has been withdrawn from the stock bottles once each week, and the container resealed with paraffin. On prolonged standing some of the hematin settles to the bottom of the container but when the mixture is thoroughly shaken again the readings obtained are unchanged. It is of course very essential that all acid hematin suspensions, whether dilute or concentrated, are thoroughly mixed before using.

The keeping qualities, or rather stability of color density, are best demonstrated by Tables V, VI, VII, and VIII.

Standard IVa (Table V) faded 4 per cent during a time interval of 11 months. The change was apparent during the 1st month. In the same period of time the carbon monoxide standard had faded 16 per cent.

These tables (V, VI, VII, and VIII) demonstrate the keeping qualities of our acid hematin mixtures. Up to the present time the last three have remained practically unchanged. We shall continue to check up these suspensions once each month in order to determine just how long they will remain stable.

A 1 per cent standard diluted from Standard VIIa at the time of preparation, April 1, 1919, and simply kept in an Erlenmeyer flask in the ice chest still read 100 per cent on October 21, 1919. Another 1 per cent suspension diluted from the defibrinated sheep blood standard and kept under the same conditions as the one mentioned above also remained unchanged during the period of observation, 4 months. For exactly how long a period the 1 per cent dilution would remain stable we do not at present
Hemoglobin

know. We consider it safe to make up our 1 per cent suspensions from the stock mixture once each month. It may be of some importance that all these standards were preserved in an ice chest with fairly constant temperature, fluctuations rarely exceeding 1–4°C.

### TABLE V.

**Standard IVa; Acid Hematin. Defibrinated Human Blood Prepared Nov. 25, 1918.**

<table>
<thead>
<tr>
<th>Date</th>
<th>New standard prepared</th>
<th>Standard IVa</th>
<th>Amount of fading</th>
<th>Amount of fading of carbon monoxide standard</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>1918</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dec. 24</td>
<td>100</td>
<td>97</td>
<td>3 per cent</td>
<td>7 per cent</td>
<td>Carbon monoxide standard was prepared from the same sample of blood.</td>
</tr>
<tr>
<td>1919</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jan. 24</td>
<td>100</td>
<td>97</td>
<td>3 per cent</td>
<td>11 per cent</td>
<td></td>
</tr>
<tr>
<td>Mar. 5</td>
<td>100</td>
<td>96</td>
<td>4 per cent</td>
<td>11 per cent</td>
<td></td>
</tr>
<tr>
<td>Apr. 1</td>
<td>100</td>
<td>97</td>
<td>3 per cent</td>
<td>16 per cent</td>
<td></td>
</tr>
<tr>
<td>May 15</td>
<td>100</td>
<td>96</td>
<td>4 per cent</td>
<td>16 per cent</td>
<td></td>
</tr>
<tr>
<td>June 23</td>
<td>100</td>
<td>97</td>
<td>3 per cent</td>
<td>16 per cent</td>
<td></td>
</tr>
<tr>
<td>Aug. 6</td>
<td>100</td>
<td>96</td>
<td>4 per cent</td>
<td>15 per cent</td>
<td></td>
</tr>
<tr>
<td>Sept. 7</td>
<td>100</td>
<td>96</td>
<td>4 per cent</td>
<td>15 per cent</td>
<td></td>
</tr>
<tr>
<td>Oct. 21</td>
<td>100</td>
<td>96</td>
<td>4 per cent</td>
<td>16 per cent</td>
<td></td>
</tr>
</tbody>
</table>

### TABLE VI.

**Standard Va; Acid Hematin. Defibrinated Sheep Blood Prepared Jan. 24, 1919.**

<table>
<thead>
<tr>
<th>Date</th>
<th>New standard prepared</th>
<th>Standard Va</th>
<th>Amount of fading</th>
<th>Amount of fading of carbon monoxide standard</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>1919</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mar. 5</td>
<td>100</td>
<td>100</td>
<td>0 per cent</td>
<td>2 per cent</td>
<td>Carbon monoxide standard was prepared from the same sample of blood.</td>
</tr>
<tr>
<td>Apr. 1</td>
<td>100</td>
<td>100</td>
<td>0 per cent</td>
<td>9 per cent</td>
<td></td>
</tr>
<tr>
<td>May 15</td>
<td>100</td>
<td>100</td>
<td>0 per cent</td>
<td>9 per cent</td>
<td></td>
</tr>
<tr>
<td>June 23</td>
<td>100</td>
<td>100</td>
<td>0 per cent</td>
<td>8 per cent</td>
<td></td>
</tr>
<tr>
<td>Aug. 6</td>
<td>100</td>
<td>100</td>
<td>0 per cent</td>
<td>10 per cent</td>
<td></td>
</tr>
<tr>
<td>Sept. 7</td>
<td>100</td>
<td>100</td>
<td>0 per cent</td>
<td>10 per cent</td>
<td></td>
</tr>
<tr>
<td>Oct. 21</td>
<td>100</td>
<td>100</td>
<td>0 per cent</td>
<td>10 per cent</td>
<td></td>
</tr>
</tbody>
</table>
TABLE VII.
Standard VIa; Acid Hematin. Whole Dog's Blood Prepared Mar. 5, 1919.

<table>
<thead>
<tr>
<th>Date</th>
<th>New standard prepared</th>
<th>Standard VIa</th>
<th>Amount of fading (per cent)</th>
<th>Amount of fading of carbon monoxide standard (per cent)</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>1919</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apr. 1</td>
<td>100</td>
<td>100</td>
<td>0</td>
<td>7</td>
<td>Carbon monoxide</td>
</tr>
<tr>
<td>May 15</td>
<td>100</td>
<td>100</td>
<td>0</td>
<td>12</td>
<td>standard prepared</td>
</tr>
<tr>
<td>June 23</td>
<td>100</td>
<td>99</td>
<td>1</td>
<td>13</td>
<td>prepared from the</td>
</tr>
<tr>
<td>Aug. 6</td>
<td>100</td>
<td>100</td>
<td>0</td>
<td>15</td>
<td>same sample of</td>
</tr>
<tr>
<td>Sept. 7</td>
<td>100</td>
<td>100</td>
<td>0</td>
<td>14</td>
<td>blood.</td>
</tr>
<tr>
<td>Oct. 21</td>
<td>100</td>
<td>100</td>
<td>0</td>
<td></td>
<td>Spoiled.</td>
</tr>
</tbody>
</table>

TABLE VIII.

<table>
<thead>
<tr>
<th>Date</th>
<th>New standard prepared</th>
<th>Standard VIIa</th>
<th>Amount of fading (per cent)</th>
<th>Amount of fading of carbon monoxide standard (per cent)</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>1919</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>May 15</td>
<td>100</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>Carbon monoxide</td>
</tr>
<tr>
<td>June 23</td>
<td>100</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>standard prepared</td>
</tr>
<tr>
<td>Aug. 6</td>
<td>100</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>prepared from the</td>
</tr>
<tr>
<td>Sept. 7</td>
<td>100</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>same sample of</td>
</tr>
<tr>
<td>Oct. 21</td>
<td>100</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>blood.</td>
</tr>
</tbody>
</table>

III. Newcomer Method.—While this investigation was being carried on Newcomer published his new method of estimation of hemoglobin, details of which have been described above. A piece of this brown semaphore glass 0.96 mm. in thickness was tested and gave most satisfactory readings when compared with Palmer's figures as shown in Table IX.

A Duboscq colorimeter is used in this laboratory and the brown glass inserted above the plunger. The corresponding cup is partially filled with distilled water for the reasons mentioned by Newcomer. As a source of light for these hemoglobin determinations either the lamp containing "Daylite" glass or light from a northern exposure gave equally satisfactory results. While the colors of the glass 0.96 mm. in thickness and the acid hematin suspension of the blood to be tested matched satisfac-
torily, the color is very light, almost a lemon-yellow when matched. It is therefore quite evident that this is somewhat of a disadvantage for darker shades are more easily matched and of course there is no means of regulating the depth of standard color as one does when using liquid mixtures. For example, with our liquid standards set at 10 the resulting readings of the test fluids range usually between 10 and 13, while with the colored glass, at least with the piece of this particular thickness, the readings are around 5 to 7 and are not so accurate as those around 10 or 12 with the Duboseq instrument.

TABLE IX.

<table>
<thead>
<tr>
<th>Palmer's method</th>
<th>Newcomer's method (glass 0.96 mm. thick)</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>per cent</td>
<td>per cent</td>
<td></td>
</tr>
<tr>
<td>113</td>
<td>114</td>
<td>+1</td>
</tr>
<tr>
<td>116</td>
<td>114</td>
<td>−2</td>
</tr>
<tr>
<td>99</td>
<td>100</td>
<td>+1</td>
</tr>
<tr>
<td>107</td>
<td>108</td>
<td>+1</td>
</tr>
<tr>
<td>98</td>
<td>101</td>
<td>+3</td>
</tr>
<tr>
<td>117</td>
<td>118</td>
<td>+1</td>
</tr>
<tr>
<td>100</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>100</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>100</td>
<td>101</td>
<td>+1</td>
</tr>
<tr>
<td>100</td>
<td>103</td>
<td>+3</td>
</tr>
<tr>
<td>100</td>
<td>99.2</td>
<td>−0.8</td>
</tr>
</tbody>
</table>

Average difference ................................ | +0.73

The added advantage of course is that all artificial standards like this glass are supposedly permanent in color and this obviates the necessity of liquid standard preparations. Because of the pale color of the standard glass we purchased another piece somewhat thicker—1.02 mm.—hoping to obtain easier readings. While the color was slightly darker in this new piece our readings were not so accurate as is evident from the figures cited (Table X).

The difference in readings (Table X) was more than with the piece 0.96 mm. in thickness, an average of 0.73 higher with the former glass as compared to 2.4 points lower with the thicker one. We feel certain that this larger difference is due to the
fact that with the piece 1.02 mm. thick the color, although
darker, is not so readily matched. The suspension of acid
hematin demonstrates of course a very slight turbidity, the lack
of which is very noticeable when using the heavier glass. The
color of the latter is a clear yellowish brown while the acid hematin
because of its slight opaqueness seems a somewhat different
shade of brown. Some of the readings cited were made by dif-
ferent workers in the laboratory and the same difficulty was
voiced by all that the colors do not seem to be quite the same.
Two pieces of glass placed one on top of the other, each 1.02 mm.
in thickness, increased the difference in readings considerably, as

<table>
<thead>
<tr>
<th>Palmer's method</th>
<th>Newcomer's method (glass 1.02 mm. thick)</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>per cent</td>
<td>per cent</td>
<td></td>
</tr>
<tr>
<td>64</td>
<td>62</td>
<td>-2</td>
</tr>
<tr>
<td>63</td>
<td>60</td>
<td>-3</td>
</tr>
<tr>
<td>76</td>
<td>72</td>
<td>-4</td>
</tr>
<tr>
<td>45</td>
<td>47</td>
<td>+2</td>
</tr>
<tr>
<td>60</td>
<td>56</td>
<td>-4</td>
</tr>
<tr>
<td>84</td>
<td>81</td>
<td>-3</td>
</tr>
<tr>
<td>69</td>
<td>66</td>
<td>-3</td>
</tr>
</tbody>
</table>

Average difference .................................. -2.4

well as the difficulty of exact color match. Newcomer (8) states
that it is impossible to secure an artificial color match which
runs true through a range of thicknesses. While the actual
difference in thickness of the pieces of glass purchased—using of
course only one at a time—seems slight, a decided difference in
color match is apparent.

In view of the above mentioned difficulties we prefer using the
liquid acid hematin standards.

DISCUSSION.

In summarizing our observations with the Palmer method it
is evident that as long as the standard solutions are prepared
once a month very accurate results may be obtained. The
method itself is certainly simple and easily carried out by even
comparatively inexperienced laboratory workers. The main disadvantage lies in the color fading of the standard solutions. Among nine different standards observed for a period of from 2 to 13 months we have found but one solution which for nearly 7 months remained unchanged. The remaining eight all faded sufficiently to prohibit their use for accurate work, with one exception possibly—Standard VIII—which demonstrated a fading of only 5 per cent during a 5 months period. Considering the figures presented it seems that better results were obtainable with blood previously defibrinated than with whole blood. For a time interval of 3 months the standard solutions prepared from defibrinated blood demonstrated a fading of from 0 to 13 per cent as compared with 13 to 27 per cent evident in those mixtures originating from whole blood. We have never encountered any difficulty with reference to a true change of color, at least during a period of 6 months. During the last few months of observation a very slight brownish tinge was noticeable but never sufficient to interfere seriously with a color comparison, excepting perhaps Nos. I and VI, which were discarded after 8 months observation. The greatest change in color density in standards prepared from whole blood apparently takes place during the first 2 months and reaches its maximum during the 3rd month. From then on the change is but comparatively little and remains so, in some instances for a year or over: With the defibrinated blood, I think we have a standard of better keeping qualities; the change is not quite so pronounced. Although two of the defibrinated blood mixtures, Nos. III and IV, showed a fading of 11 per cent each during a time interval of 2 months, we have three others where the change was considerably less, one solution demonstrating but a 5 per cent loss of color within 5 months, another remaining unchanged for nearly 7 months, and a third fading 10 per cent in 9 months.

The type of hemoglobin evidently plays no important rôle, dog's blood apparently giving as satisfactory results as goat's, sheep's, or human blood; or rather the other species mentioned offer no more stable hemoglobin solutions than dog's blood.

Bacterial decomposition does not seem to be a very important factor, for blood obtained aseptically and the hemoglobin solution prepared with sterile precautions demonstrated no more
stable qualities than did those standards prepared with only the usual care and cleanliness.

It should be mentioned here that all these stated observations pertain to the stock solutions, the 20 per cent dilutions. We have never attempted to keep the 1 per cent dilutions prepared from the concentrated mixtures for more than a week, at least under experimental conditions existing here. A 1 per cent solution prepared will not fade within a week if kept on ice and resaturated with carbon monoxide each time the container is opened. After that time interval a change does take place which although not apparent to the eye is readily demonstrable when checked against the oxygen capacity method. Appleton (1) states that the 1 per cent solutions prepared by her began to deteriorate in from 2 to 4 weeks. During her investigation the diluted solution was kept saturated by a continual flow of gas. Why such a difference in stability occurs we do not know. Frequent opening of containers and resaturation with CO seem to give no better results than resaturation once a month. While a standard is being used for routine work it is necessary to open the container once each week in order to procure the necessary material for one 1 per cent dilution.

From the tables of acid hematin standards, it is readily seen that, while these acid hematin mixtures may change in time, they certainly have proved themselves to be much more stable than the carbon monoxide solutions. One may with perfect safety and with complete assurance of obtaining accurate results employ these acid hematin suspensions for 6 months at least. The stock mixtures above mentioned will of course be observed to determine just how long they actually remain unchanged.

The method of preparing blood for hemoglobin determinations in the form of acid hematin is slightly simpler than the carbon monoxide method, for the former makes unnecessary the extra step of saturation with carbon monoxide. The slight disadvantage is the time interval necessary for the maximum color of acid hematin to develop. In using large quantities of blood (at least much greater amounts than are used clinically) we have allowed 24 hours. This, however, is not necessary as a 1 hour interval gives accurate reading. We have observed no difference between figures obtained after 60 minutes standing and 24 hours.
The latter time happened to be more convenient in our experimental work. Newcomer published a table with his method showing the exact percentages of color development of acid hematin in given periods of time. He considers 40 minutes as safe.

Palmer in his publication states that blood of different species cannot be used for hemoglobin determinations in the form of acid hematin. As will be seen from our tables, we have standards prepared from human, dog's, and sheep's blood and have compared human blood with both dog's and sheep's blood standards without encountering any difficulty whatsoever. The comparisons have been made in all combinations possible with our material and readings have checked accurately. The figures presented in the tables readily answer the question of stability. While the latter may not be permanent or remain unchanged indefinitely, still it is much less time-consuming to prepare a fresh standard once every 6 months instead of once each month as we have had to do when employing the carbon monoxide method.

The Newcomer method would of course be the best solution of the entire problem, but as mentioned before the color match is not exact. The use of as simple a standard as a piece of colored glass certainly is a great advantage. The difference in color may not be so apparent to all eyes. The table accompanying the glass standard is an asset, as it definitely settles the question of time interval for development of the maximum color of acid hematin, and thus does away with one of the disadvantages of the earlier acid hematin methods.

Since the completion of this work a communication of Cohen and Smith (3) has appeared which confirms much of this work. They suggest the same standard solution because of its stability under army camp conditions.

CONCLUSIONS.

1. The Sahli hemoglobin method when using the color tubes accompanying the instrument gives very inaccurate results because of the decided variance in color density of the standard tubes, due to fading.
2. The Palmer method offers very satisfactory means of hemoglobin determinations if the standard solutions are freshly prepared. The method itself is very simple and may be successfully carried out by anyone familiar with colorimetry. The standard solutions prepared in the laboratory although carefully made have not been sufficiently stable to insure accurate determinations over periods of more than 3 to 4 weeks.

3. Newcomer's method obviates many difficulties heretofore observed with other procedures and gives good results with the glass 0.96 mm. in thickness, although the color is quite pale. When using the heavier glass, 1.02 mm. in thickness, the color match is only approximate and the figures obtained are not so satisfactory as those resulting from use of the thinner piece.

4. A method is presented applying Palmer's procedure to Sahli's principle which has proved most satisfactory. It removes the difficulty we encountered with Palmer's method, the lack of stability of color in the standard solutions. It has the advantage of an easier color match than that of red tint. The standards prepared have remained sufficiently unchanged for a period of 11 months to insure accurate hemoglobin determinations during this long period.

It may be suggested that for routine hospital work an acid hematin standard prepared in this way and kept at relatively constant temperature will remain unchanged for 8 months or longer. 1 per cent solutions may be prepared from time to time from the standard concentrated solution and this 1 per cent solution can be used to fill the standard tube of the common Sahli hemoglobinometer. This insures an accurate base line for hemoglobin determinations and with refilling of the Sahli tubes once a month will give accurate clinical determinations. Such clinical determinations are not the rule and are much to be desired.

BIBLIOGRAPHY.

Hemoglobin

A COMPARATIVE STUDY OF HEMOGLOBIN DETERMINATION BY VARIOUS METHODS
Frieda S. Robscheit


Access the most updated version of this article at http://www.jbc.org/content/41/2/209.citation

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/41/2/209.citation.full.html#ref-list-1