A Specific Gravity Method for Determining Hematocrit in Rabbits

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I. Introduction

The procedures and results in this paper are presented to record an adaptation and application of a clinical technique developed during World War II. The extreme desirability of a quick, yet accurate method of making such blood determinations as hematocrit, hemoglobin, and plasma proteins is readily apparent, especially in making appraisal of physiological state with respect to hemorrhage, shock, and other conditions resulting from human participation in modern warfare. A quick and accurate method for determining these blood statistics was developed by Phillips, Van Slyke, et al (1), and used by the armed forces.

The method of Phillips, Van Slyke, et al., is frequently designated the "copper sulphate method" since the crucial operation in the method is to permit drops of whole blood or plasma to fall into solutions of copper sulfate. On entering the sulfate solution the drop becomes encased in a film of copper proteinate which maintains the integrity of the drop up to 20 seconds. On losing initial dropping momentum the drop will either remain at rest, rise, or descend, depending on its specific gravity relative to that of the surrounding copper sulfate solution. Thus the expenditure of only a few drops each of whole blood and plasma suffices for the determination of the specific gravities of each if there is available a series of copper sulfate solutions of finely graded and known specific gravities.

A second foundation of the method consists of the use of certain equations whereby the desired blood statistics can be calculated by use of the determined specific gravity values of whole blood and plasma. The speed and desirability of the method, however, inhere in the fact that calculation by equation is not necessary. Phillips, Van Slyke, et al. have provided a cleverly designed line chart which enables, by use of a straight edge, the direct reading of plasma protein, hematocrit, and hemaglobin, once the specific gravities of whole blood and plasma are known.

The many advantages of the method, as used clinically, at once suggested the possibility of its application to investigations as a research tool. The observations reported herein were planned to apply the method to a laboratory animal and to check the values obtained by the use of the conventional methods. Thus far the method has been applied only to the rabbit as a laboratory form and to the hematocrit as a blood statistic.

For the hematocrit values, Phillips, Van Slyke, et al. utilized an equation formulated by Ashworth and Tiggertt (2), and the derivation can be presented as follows: Let G_B, G_C, and G_P represent the specific

gravities respectively of whole blood, centrifuged cells, and plasma. Let H represent the hematocrit (cc cells per 100 cc whole blood). Then 100 G_B would represent the weight of 100 cc of whole blood. This weight may be equated to the weight of the constituent centrifuged cells plus the weight of the plasma, or

(1) $100G_B = HG_C + (100 - H)G_P$ and (2) $100G_B = HG_C + 100G_P - HG_P$

(3) Then $HG_C - HG_P = 100G_B - 100G_P$ and (4) $H(G_C - G_P) = 100(G_B - G_P)$

(5) Then $$H = \frac{100(G_B - G_P)}{G_C - G_P}$$

(6) Equation (3) may be rearranged: $HG_C = 100G_B - 100G_P + HG_P$

(7) Then $$G_C = \frac{100(G_B - G_P) + HG_P}{H}$$

By measurement of G_B, G_P, and H, both Phillips, Van Slyke, et. al., and Ashworth and Adams (3) arrived at the value 1.097 for G_C, which permits the substitution of this value in equation (5) which then becomes:

(8) $$H = \frac{100(G_B - G_P)}{1.097 - G_P}$$

The line chart of Phillips, Van Slyke, et. al. is based on this equation.

It was the purpose of the study reported herein to apply the copper sulfate method to be determination of the specific gravities of whole blood and plasma from rabbits then to determine the hematocrit from the line chart of Phillips, Van Slyke, et al. and to check this determination by the conventional method of centrifuging whole rabbit blood in Wintrobe Tubes.

II. Material and Procedures

A. Materials

The copper sulfate solutions used in our study consisted of 68 bottles of 4 ounce capacity (approximately 100 cc), with screw caps. This constitutes a "complete set", ranging from specific gravities of 1.008 to 1.075 inclusive by steps of 0.001. Preparation of this series began with 4 pounds of finely pulverized copper sulfate, and necessary large bottles, filtering equipment, volumetric flasks, graduated cylinders, and distilled water. The full bottles were labelled with the specific gravities of their contents, and the entire array placed in four rows in elevated steps such that the full length of each bottle was fully visible.

Blood was drawn with 10 ml syringes fitted with 22 gauge needles; heparin was used as an anticoagulant. A wooden "rabbit holder" was found very useful. Further manipulation of the blood was accomplished by using pipettes made from glass tubing 7 mm in diameter. Each pipette was approximately 20 cm long of which about 15 cm was slimly tapered tip; the opening at the tip was approximately 1 mm in diameter.

B. Procedure

The preparation of the solutions of copper sulfate of graded specific gravities is not difficult and requires little apparatus. The solutions

are all prepared by volumetrically diluting a standard of high specific gravity, the standardization of which is contingent upon the fact that the specific gravity of a saturated solution can be computed if its temperature at the time of the saturation is known. Phillips, Van Slyke, et al. (1) give temperature-specific gravity computation tables and dilution ratios in their monograph. The diluted solutions of copper sulfate of known specific gravity can be made conveniently in 100 ml aliquots and bottled in standard 4 ounce prescription bottles. One hundred gravity determinations can be made in each 100 ml of copper sulfate solution before the specific gravity of the solution is appreciably altered.

A determination of the hematocrit in the rabbit necessitates the use of a heparinized blood specimen. Some experimentation was necessary in the selection of a desirable anticoagulant. Phillips, Van Slyke, et al. (1) suggest the use of a mixture of ammonium oxalate and potassium oxalate with the application of a correction factor to the determined gravities to compensate the alteration of the actual gravity by the oxalate. Rabbit blood specimens oxalated with the ammonium-potassium oxalate mixture would not show consistant gravities and the correction factor was not applicable to the results. Heparin was found to be satisfactory as an anticoagulant. The amount of heparin needed to prevent the coagulation of 2 ml of rabbit blood, which was the average amount drawn for each gravity determination, was very small and the use of a correction factor was not necessary.

In obtaining and testing a blood specimen the following procedure was observed. About one ml of heparin (10 mgm per one ml of water) was drawn into a hypodermic syringe. The syringe was rotated so that the wall of the barrel was "wet" with the solution. The excess heparin was returned to the ampule. This procedure left a film of the solution on the inside of the syringe sufficient to prevent the coagulation of a two or three ml sample. All blood samples were drawn from the marginal ear vein of the rabbit. Immediately following with withdrawal, the syringe was agitated for several minutes to insure uniform and complete heparinization.

The specific gravity of the whole blood was determined by dropping it into the copper sulfate solutions from an average height of 10 mm. Blood dropping from this height hit the copper sulfate with sufficient momentum to break into it as a discrete drop and not spread out as a surface film or plunge to the bottom of the bottle in one rapid movement. As previously mentioned, blood dropped into the copper sulfate will either rise, descend or remain suspended depending upon its specific gravity relative to that of the solution. It was assumed that a drop which remained suspended for 15 to 20 seconds had a specific gravity equivalent to that of the solution into which it was dropped.

After the whole blood gravity was determined the remaining blood sample was divided into two portions. One portion was centrifuged and the plasma thus separated from the formed elements. The plasma was pipetted off, and its specific gravity was determined in the same manner as was that of whole blood.

TABLE 1. Rabbit Hematocrits Based on Whole Blood and Plasma Specific Gravities and the Line Chart, Checked by Centrifuging in Wintrobe Tubes.

Rabbit	Specific Gravities		Hematocrit	
Number	Whole Blood	Plasma	Line Chart	Centrifuge
73	1.049	1.023	35.1	35.0
83	1.046	1.022	32.2	32.2
76	1.051	1.024	37.5	39.7
88	1.050	1.024	35.9	35.9
72	1.051	1.021	39.5	39.5
73	1.052	1.025	37.6	38.0
74	1.054	1.023	42.2	42.0
76	1.050	1.023	36.6	36.6
85	1.054	1.023	42.1	42.0
74	1.049	1.022	36.0	36.2
73	1.049	1.022	36.0	35.0
83	1.046	1.023	31.4	31.4
1	1.047	1.025	31.0	32.0
2	1.044	1.024	27.4	28.0
3	1.052	1.023	39.2	38.0
4	1.052	1.024	38.5	37.5
5	1.047	1.022	33.5	36.0
6	1.055	1.025	42.0	42.0
8	1.052	1.024	38.5	38.0
9	1.043	1.021	29.0	31.0
10	1.050	1.022	37.5	36.0
11	1.049	1.023	35.0	36.0
12	1.045	1.020	32.5	33.0
13	1.048	1.021	36.0	38.0
14	1.051	1.023	38.0	38.0
15	1.050	1.023	36.7	37.0
16	1.049	1.021	36.8	35.0
17	1.043	1.020	30.0	34.0
18	1.052	1.025	38.0	40.0
19	1.055	1.027	40.0	40.0
20	1.048	1.019	37.0	40.0
21	1.046	1.020	34.0	34.0
22	1.053	1.026	38.0	37.0
23	1.050	1.024	36.0	38.0
24	1.054	1.023	42.5	44.0
25	1.049	1.021	37.0	38.0
26	1.054	1.025	41.0	45.0
27	1.045	1.019	33.5	34.0
28	1.055	1.024	42.4	40.0

A line chart devised by Phillips, Van Slyke, et al. (1) was used to compute the hematocrit values. This chart consists of two parallel, vertical lines some distance apart. On one line whole blood gravities are plotted as points. On the other line plasma gravities are similarly plotted. Between these two lines is an oblique line upon which are plotted the hematocrit values. A straight edge connecting the determined whole blood and plasma gravities intersects the oblique line at the hematocrit value.

A Wintrobe tube was filled from the remaining part of the blood specimen. After centrifuging it the hematocrit value was read directly, and this value was used as a criterion for evaluation of the hematocrit by the specific gravity method.

III. Results

Quantitative data on the 39 determinations of the rabbit blood hematocrit that have been made are listed in Table I. A case by case comparison of the values derived by the use of the specific gravities and line chart and those derived from centrifuging shows close agreement in most cases. The range of values of the hematocrit by the two methods are:

By line chart	27.4 to 42.5
By centrifuge	28.0 to 45.0

Strangely enough, the mean hematocrit is identical for the two methods, at a value of 37.0. Lest this be misunderstood, it should be pointed out that there is a *mean difference* between the values by the two methods of 1.13 units. Such indicates that were a given rabbit blood sample tested by both line chart and centrifuge the values would on the average differ by 1.13 units. (ml per 100 ml whole blood). As a further test the coefficient of correlation was calculated by the method of rank differences (rho) with the result that rho equalled 0.9077, this revealing a high correlation between results derived by the two methods.

IV. Conclusions

(1) Mean hematocrit value for rabbit blood in 39 determinations by the centrifuge method was 37.0.

(2) An identical mean (37.0) was obtained in 39 determinations of the hematocrit by the specific gravity method.

(3) The mean difference in hematocrit as determined by the two methods was 1.13.

(4) The coefficient of correlation (as determined by the method of rank differences) between the specific gravity and centrifuge hematocrit values was 0.9077.

V. Literature Cited

(1) Phillips, Robert A., and Donald D. Van Slyke, Vincent P. Dole, Kendall Emerson, Jr., Paul B. Hamilton, and Reginald M. Archibald. Copper Sulfate Method for Measuring Specific Gravities of Whole Blood and Plasma, with Line Charts for Calculating Plasma Proteins, Hemoglobin, and Hemotocrit from Plasma and Whole Blood Gravities. (From the United States Navy Research Unit at the Hospital of the Rockefeller Institute for Medical Research. The work described in this paper was done under a contract

between the Office of Scientific Research and Development and the Hospital of the Rockefeller Institute for Medical Research.)

(2) Ashworth, C. T. and W. D. Tiggertt. 1941. A Simple Rapid Method for Determining Relative Blood Volume Changes by Specific Gravity. Jour. Lab. and Clin. Med. 26:1545-1552.

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