THE ELECTRICAL CONDUCTIVITY OF BLOOD

II. Relation to Red Cell Count

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THE PRECEDING paper[1] describes an improved method of measuring the electrical conductivity of blood, and measurements are reported on normal human blood with the corresponding hematocrit and red cell counts. It was shown that for normal blood cells the conductivity method is a more accurate method for determining the red cell count than the conventional visual counting procedure. It is the purpose here to inquire into the relation between conductivity measurements and the erythrocyte concentration in a variety of clinical states, and to establish the validity of the conductivity method for converting such measurements on pathologic specimens into reliable clinical red cell counts.

Experimental Results

The procedure used was essentially that previously described, with heparin (0.2 mg. per cc. blood) added as anticoagulant, and the conductivity measured in the Ballard type cell at 30 C. In the visual blood count, standardized pipets and a Levy-Neubauer counting chamber were used, and the average of the two sides of a single counting chamber was taken as the red cell count for the pathologic specimens.

In the studies on normal blood, the Fricke equation[2] was found to relate accurately the visual count to the electrical conductivity within the limit of error of the visual count:

$$g = \frac{CX(K_0 - K)}{X K_0 + K} \qquad (1)$$

wherein g is the red cell count in millions per cu. mm.; C is a factor relating cell volume to count; X is a form factor which depends on the axial ratios of the cells, assuming them to be spheroidal; and K and K_o are the specific conductances of the whole blood and plasma respectively. With the serial dilution method,[3] the form factor for the normal human erythrocyte was found to be 1.39, and the most probable value of C was found to be 10.80 for normal bloods. On plotting the conductivity ratio, K/K_o, against the visual red cell count for the first 23 pathologic blood samples (fig. 1), wherein the best fitting curve for normal bloods is also shown, it was evident that the pathologic specimens tended to fall somewhat below the normal bloods. Figure 1 also includes more dilute and more concentrated samples

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This research was supported in part by grants from the Office of Naval Research (Contract No. N6-ONR-264 Task Order 10) and in part by grants from the Albert and Mary Lasker Foundation, the Lillia Babbitt Hyde Foundation and the Samuel H. Kress Foundation.

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of the pathologic bloods, made by centrifugation and by dilution with their own plasmas, in order to give points at either end of the curve. In table 1 are given the various pathologic conditions of the blood donors. In figure 2, K/K_o is plotted as a function of visual red cell count for the entire group of pathologic specimens, numbering 101 samples from 98 individuals. The tendency of the pathologic specimens to sag below the best fitting curve (A) for normal bloods is clearly evident.

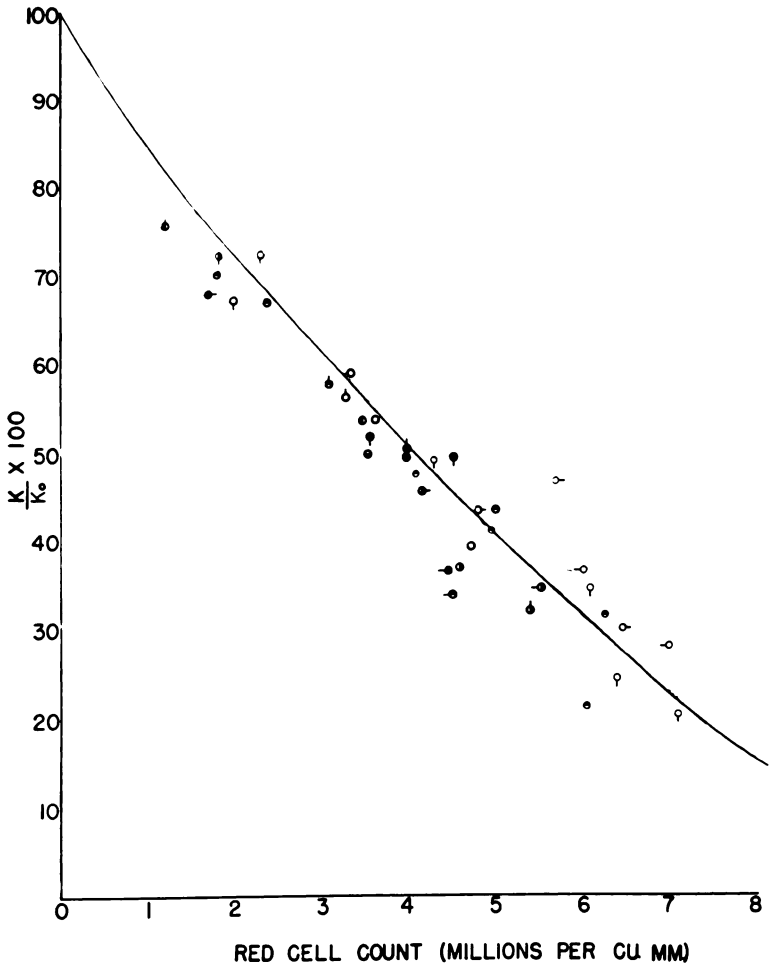

FIG. 1.—Variation of conductance ratio (K/K_o) as a function of red cell count for 23 pathologic specimens of blood, and of specimens made by dilution with their own plasma. Points with double circles represent undiluted specimens.

During the studies on normal blood,[1] it was found that, when the function $(K_o - K)/(K_o + K)$ was plotted as a function of the relative volume per cent red cells, as determined by a precise gravimetric process, an essentially linear relationship was observed (fig. 3). Since the volume per cent red cells is directly proportional to the red cell count, a similar relation was observed when the visual red cell count was plotted against $(K_o - K)/(K_o + K)$, although greater scattering of the points resulted from the lower precision of the visual count. This is equivalent to the Fricke equation above, with the

form factor assumed to be unity, and an appropriate best value for the constant of proportionality chosen. For comparison, the two equations are given:

$$g = C\frac{X(K_0 - K)}{XK_0 + K} \quad (1)$$

$$g = C_1\frac{K_0 - K}{K_0 + K} \quad (2)$$

TABLE 1.—*Data on Pathologic Conditions for Figure 1*

Subject	Notation in Fig.	Disease
Be		Acute Nephritis
Ca		Aplastic Anemia
Co		Sec. Polycythemia
De		Portal Cirrhosis
DeA		Sub-acute Bact. Endocarditis
Dem		Cancer of Lung
Fo		Bleeding Ulcer
Ga		Pernicious Anemia
Gr		Leukemia
Hi		Chronic Brucellosis
Jo		Bronchogenic Ca.
Ka		Hypochrom. Normocyt. Anemia
Ke		Acute Leukemia
Ky		Bleeding Ulcer
La		Rheumatic Heart
McG		Pneumoconiosis
McN		Lung Abscess
Me		Cirrhosis of Liver
Pa		Cirrhosis
Ro		Anemic Hypoplastic Marrow
St		Coronary Heart Disease
Sy		Chronic Arthritis
Ye		Periarteritis Nodosa

As can be seen in table 2, the former equation gives better agreement with the visual counts for normal blood than the latter, the standard deviations being 0.28 and 0.54 millions respectively. However, it was surprising to find Equation (2), with C_1 taken as 10.49, yielded better agreement than Equation (1).

In order to determine whether adjustment of the constant C of Equation (1) would improve the agreement between the conductivity and visual counts, the best value of C for the pathologic specimens was calculated and found to be 9.59, with X taken as 1.393. The standard deviation was reduced from 0.75 for C = 10.80 to 0.55 millions per cu. mm. for the pathologic samples, but increased from 0.28 to 0.75 millions per cu. mm. for the normal bloods. Thus, the Fricke equation did not appear to be as satisfactory empirically as Equation (2), which was consequently adopted as the preferable equation. This

superior agreement is fortuitous, and it is probable that some value of X other than unity might give a still better fit to the visual counts. However, since g is not very sensitive to the value of X, and only slight improvement could be expected, it was not deemed worth while to attempt determination of X as a function of the pathologic condition, particularly in view of the inaccuracies of the visual count with which the electrical count must be compared. It should also be borne in mind that the assumptions fundamental to the Fricke equation are not strictly true of erythrocytes, namely that they are uniform spheroids, and it is not beyond the bounds of possibility that the simpler equation might correspond more closely to the actual physical system being studied. This is at least suggested by the experimental observations.

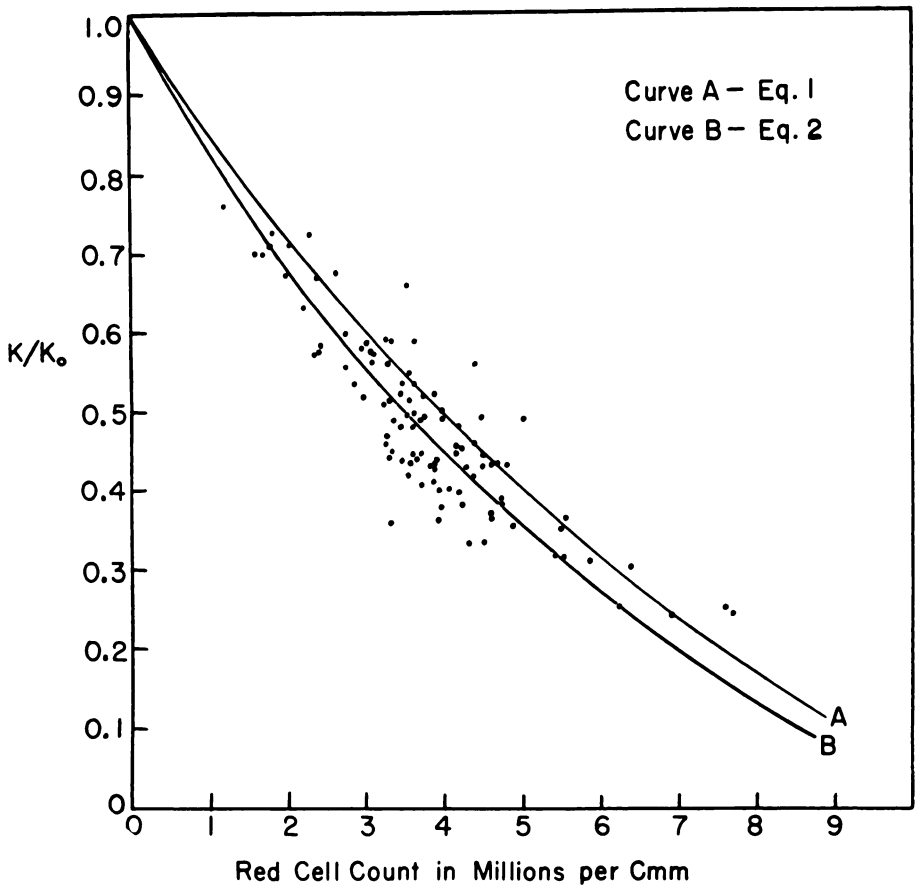

FIG. 2.—Variation of conductance ratio as a function of red cell count for 101 pathologic specimens

The value for C_1 was calculated for 157 blood specimens, including both normal and pathologic blood. The range was between 7 and 15.0 and the arithmetic mean was 10.49. By the use of this figure the visual counts were plotted against the calculated counts as shown in figure 4. The standard deviation between the two methods of estimating red cell count is ±0.50 millions per cu. mm. With Equation (2) the deviation is approximately equal in each of the classifications of specimens.

According to Magath,[4] the standard deviation of the visual count is about ±0.4 millions per cu. mm., so that the deviation between electrical count and visual count can, in the main, be reasonably attributed to the errors inherent in the visual counting method.

The experimental data are given in table 3, including clinical conditions, the visual red cell counts,

the count calculated by Equation (2) from the conductivity measurements, and the deviation between the two counts. In order to illustrate the range of blood counts for each group of diseases, the mean counts and the range of counts by both counting methods are given in table 4. The mean deviations for

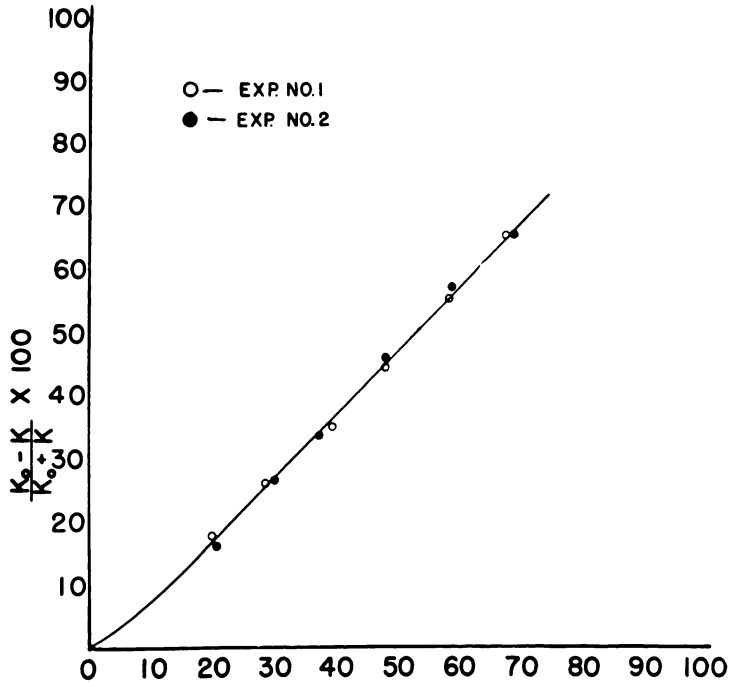

FIG. 3.—Graph showing essentially linear relationship between $\frac{K_o - K}{K_o + K}$ and volume per cent red cells obtained from serial dilution experiments with normal blood.

TABLE 2.—*Standard Deviations between Electrical and Visual Counts for Various Equations (in millions per cu.mm.)*

Equation	Blood Specimens		
	Normal (33)	Pathologic (101)	Entire (157*)
1, with C = 10.80	0.28	0.75	
1, with C = 9.59	0.75	0.55	
2, with C_2 = 10.49	0.54	0.46	0.50

* This group includes 23 miscellaneous unclassified specimens.

each group are given, and the majority of these are within the accepted precision of the visual count. The fact that the electrical counts are neither uniformly higher nor lower than the visual counts for any group of diseases indicates that the errors in the visual counting method can account for the main portion of the deviation.

Discussion

The fact that the Fricke equation with form factor 1.393 did not fit the pathologic specimens as well as the empiric relation implies a form factor of unity. The use of a form factor requires much inconvenience in determining its value, so it has been considered preferable to regard Equation (2) as merely an empiric equation with one arbitrary parameter, which can readily be evaluated. No physical

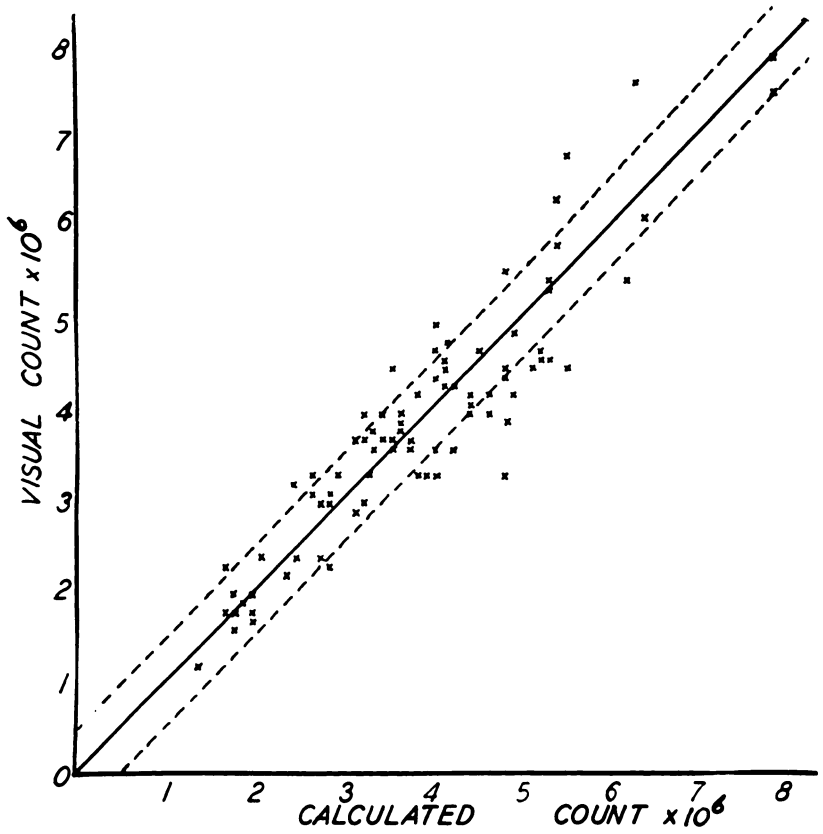

Fig. 4.—Comparison of visual counts with calculated counts for 101 pathologic specimens

significance should be attached to the fact that this equation is equivalent to the Fricke equation with a form factor of unity, because the calculated count is insensitive to the value of X. Therefore, it is incorrect to conclude from a theoretic point of view that the form factor is necessarily being considered to have unit value. In order to establish X precisely, it is necessary to use the method of Velich and Gorin,[3] which requires elaborate procedures of serial dilution for each range of blood counts. In addition, the inaccuracy of the visual counts is such that it is impractical to evaluate X from the presently available data by any empiric method, such as the method of least squares. Therefore, Equation (2), with only one arbitrary constant, is considered satisfactory.

TABLE 3.—*Experimental Data*

Subject	Diagnosis	K_0 (mhos/cm.) $\times 10^3$	K (mhos/cm.) $\times 10^3$	Visual Count (millions per cu.mm.)	Calc. Count (millions per cu.mm.)	Dev. (millions per cu.mm.)
		Cardiovascular Diseases				
LA	Rheum. heart disease	13.30	6.41	3.98	3.76	−0.31
McC	Rheum. heart disease	13.50	5.82	3.84	4.17	+0.33
KL	Rheum. heart disease	13.30	5.87	3.65	4.07	+0.42
SHA	Rheum. heart disease	13.90	4.66	4.31	5.22	+0.91
SH	Rheum. heart disease	13.70	5.20	3.96	4.72	+0.76
BAR	Rheum. heart disease	13.66	5.92	3.88	4.15	+0.27
SO	Coronary heart disease	12.86	4.32	4.51	5.21	+0.70
DeA	Subacute bact. endocarditis	13.50	7.95	3.33	2.76	−0.57
HL	Subacute bact. endocarditis	12.60	7.31	2.95	2.79	−0.16
VE	Subacute bact. endocarditis	12.70	6.64	3.88	3.29	−0.59
JAC	Thrombophlebitis	13.50	4.92	3.92	4.89	+0.97
BRO	Thrombophlebitis	13.07	6.80	3.74	3.31	−0.43
VI	Thrombophlebitis	13.50	5.17	4.23	4.68	+0.45
FA	Thrombophlebitis	13.30	5.95	3.70	4.01	+0.31
SL	Arteriosclerosis obliterans	13.30	5.43	3.71	4.41	+0.70
MU	Arteriosclerosis obliterans	13.40	5.84	3.57	4.12	+0.55
LAC	Arteriosclerosis obliterans	13.50	5.67	3.54	4.28	+0.74
SL	Thrombangiitis obliterans	13.00	4.64	4.88	4.97	+0.09
YE	Periarteritis nodosa	12.90	5.04	4.73	4.60	−0.13
MI	Periarteritis nodosa	13.30	6.25	3.26	3.78	+0.52
BAB	Periarteritis nodosa	12.70	7.15	3.08	2.93	−0.15
FOX	Dermatomyositis	13.12	7.53	3.09	2.85	−0.24
AR	Dissem. lupus erythem.	12.60	8.52	2.62	2.02	−0.60
		Hypochromic and Other Anemias				
KA	Hypochr. normocyt. anemia	13.50	9.43	1.69	2.00	+0.31
GRA	Hypochr. normocyt. anemia	12.90	7.87	3.23	2.54	−0.69
JON	Hypochr. normocyt. anemia	13.10	6.41	3.36	3.60	+0.24
PRI	Hypochromic anemia	12.90	7.09	3.56	3.05	−0.51
HIC	"Refractory" anemia	13.40	9.40	1.58	1.84	+0.26
RO	Hypoplastic anemia	13.50	7.57	3.28	2.95	−0.31
CA	Aplastic anemia	13.02	9.89	1.19	1.43	+0.24
GAN	Aplastic anemia	12.90	8.15	2.20	2.37	+0.17
GO	Splenic anemia	13.00	7.69	3.26	2.69	−0.57
HOG	Banti's syndrome	13.30	6.57	3.75	3.55	−0.20

TABLE 3.—*Continued*

Subject	Diagnosis	K_0 (mhos/cm.) $\times 10^3$	K (mhos/cm.) $\times 10^3$	Visual Count (millions per cu.mm.)	Calc. Count (millions per cu.mm.)	Dev. (millions per cu.mm.)
			Renal and Miscellaneous Diseases			
BE	Acute glom. nephritis	14.40	10.1	1.79	1.84	+0.05
BE	Acute glom. nephritis	14.10	9.44	2.37	2.08	−0.29
OT	Chronic nephritis	13.32	7.40	2.75	3.00	+0.25
OT	Chronic nephritis	11.10	6.36	2.34	2.85	+0.51
WA	Nephrotic syndrome	11.60	5.01	4.47	4.16	−0.31
SY	Rheumatoid arthritis	14.82	6.78	4.16	3.90	−0.26
JAN	Rheumatoid arthritis	12.80	6.16	3.60	3.67	+0.07
CL	Cushing's syndrome	13.20	5.31	3.97	4.47	+0.50
JA	Diabetes mellitus	12.86	5.60	4.41	4.13	−0.28
McN	Lung abscess	11.31	4.91	5.02	4.14	−0.88
HI	Chronic Brucellosis	13.56	4.93	4.46	4.90	+0.44
WE	Epidermophytosis	13.70	5.89	3.63	4.10	+0.47
			Neoplastic Diseases			
KC	Acute leukemia	13.40	9.00	1.98	2.06	+0.08
GR	Acute leukemia	13.40	6.60	4.53	3.57	−0.96
KE	Acute leukemia	13.00	9.43	2.29	1.67	−0.62
KAT	Acute lymph. leukemia	12.30	8.75	2.02	1.77	−0.25
GAL	Acute lymph. leukemia	13.40	8.03	2.74	2.63	−0.11
MO	Chronic lymph. leukemia	13.00	6.68	3.30	3.33	+0.03
SA	Chronic myelog. leukemia	13.00	5.57	3.89	4.20	+0.31
DAG	Chronic myelog. leukemia	13.30	5.11	4.75	4.67	−0.08
QU	Lymphoblastoma	13.10	4.15	5.53	5.44	−0.09
HIR	Lymphosarcoma	13.40	6.44	3.45	3.68	−0.07
McCA	Lymphosarcoma	13.70	6.83	3.62	3.51	−0.09
HA	Follicular lymphosa	13.50	6.02	3.60	4.02	+0.42
JAC	Giant follicular lymphoma	13.50	5.44	4.06	4.46	+0.40
NA	Hodgkin's disease	12.90	7.53	2.42	2.50	+0.08
POS	Hdogkin's disease	12.50	5.41	4.62	4.15	−0.47
FOR	Hodgkin's disease	12.90	5.13	4.20	4.52	+0.32
KAH	Multiple myeloma	11.40	5.23	3.25	3.89	+0.64
KAH	Multiple myeloma	11.80	5.22	3.29	4.06	+0.77
DEM	Bronchogenic carcinoma	12.60	4.62	4.60	4.81	+0.21
JO	Bronchogenic carcinoma	12.51	6.46	3.56	3.25	−0.21
COW	Bronchogenic carcinoma	13.36	4.82	4.39	4.93	+0.54
RY	Carcinoma of stomach	12.90	5.55	4.26	4.18	−0.08
MEI	Adenoca. of stomach	13.10	5.84	4.50	5.57	+1.07
BI	Abdominal carcinomatosis	13.40	5.83	4.68	4.13	−0.55
			Hepatic and Gastro-intestinal Diseases			
DEF	Hepatitis	12.93	4.66	3.32	4.94	+1.61
DUR	Biliary cirrhosis	13.36	6.01	3.33	3.98	+0.65
ME	Portal cirrhosis	13.10	7.02	3.47	3.17	−0.30

TABLE 3.—*Continued*

Subject	Diagnosis	K_0 (mhos/cm.) $\times 10^3$	K (mhos/cm.) $\times 10^3$	Visual Count (millions per cu.mm.)	Calc. Count (millions per cu.mm.)	Dev. (millions per cu.mm.)
		Hepatic and Gastro-intestinal Diseases—Continued				
PA	Portal cirrhosis	13.40	6.64	3.53	3.54	+0.01
DE	Portal cirrhosis	13.14	7.57	3.08	2.83	−0.25
ROB	Portal cirrhosis	13.55	7.81	2.39	2.82	+0.43
WEL	Portal cirrhosis	13.40	5.12	4.20	4.69	+0.49
AL	Portal cirrhosis	11.50	6.15	2.84	3.18	+0.36
COR	Hepatomegaly	13.50	5.93	3.92	4.09	+0.17
KY	Bleeding duod. ulcer	13.50	7.23	3.62	3.17	−0.45
FO	Bleeding duod. ulcer	13.60	6.83	3.99	3.48	−0.51
KY	Bleeding duod. ulcer	13.80	5.98	4.81	4.15	−0.66
BR	Diverticulosis	13.10	5.40	3.87	4.37	+0.50
BA	Diverticulosis	13.20	6.85	2.97	3.32	+0.35
LOT	Ulcerative colitis	12.80	6.27	3.70	3.59	−0.11
WI	Ulcerative colitis	13.80	8.10	3.01	2.85	−0.16
		Macrocytic Anemias				
RI	Macrocytic anemia	13.50	6.05	4.16	3.88	−0.28
GA	Pernicious anemia	12.40	8.99	1.81	1.67	−0.14
HO	Pernicious anemia	13.40	5.61	4.28	4.30	+0.02
WA	Pernicious anemia	13.20	6.90	3.45	3.29	−0.16
DU	Pernicious anemia	13.70	5.09	4.60	4.83	+0.23
MAY	Pernicious anemia	13.30	5.82	3.46	4.10	+0.64
CU	Pernicious anemia	13.00	4.62	4.23	4.99	+0.76
		Polycythemia				
COS	Polycythemia vera	12.70	3.07	6.91	6.41	−0.50
PV	Polycythemia vera	12.40	3.16	6.23	6.45	+0.22
SCH	Polycythemia vera	13.40	3.39	5.49	6.25	+0.76
KI	Polycythemia vera	12.80	3.91	6.39	5.58	−0.81
WEB	Polycythemia vera	13.80	4.32	5.86	5.49	−0.37
BRI	Polycythemia vera	13.80	3.49	7.61	6.25	−1.36
ROW	Polycythemia vera	13.10	3.22	7.70	6.35	−1.35
McG	Secondary polycythemia	12.24	3.90	5.42	5.42	0.00
CO	Secondary polycythemia	13.02	4.76	5.55	4.87	−0.68

TABLE 4.—*Range of Blood Counts*

Clinical Condition	No.	Mean visual RBC in mil/mm.^{3}	Range Visual RBC	Mean cond. RBC in mil/mm.^{3}	Range cond. RBC	Mean Dev.	No. cond. RBC higher	No. cond. RBC lower
Cardiovascular	23	4.09	2.62–4.88	3.95	2.02–5.22	0.476	14	9
Liver and G.I.	16	3.50	2.39–4.81	3.63	2.82–4.93	0.440	9	7
Macrocytic anemia	7	3.71	1.81–4.60	3.87	1.67–4.99	0.320	4	3
Polycythemia	9	6.35	5.42–7.70	6.08	4.87–7.95	0.560	3	5
Hypochromic anemia	10	2.71	1.19–3.75	2.50	1.43–3.60	0.360	5	5
Neoplasma	24	3.69	1.98–5.53	3.80	1.67–5.57	0.360	13	11
Renal and misc.	12	3.58	1.79–5.02	3.61	1.84–4.90	0.360	7	5

Before making any decision as to the suitability of either equation, a large number of samples from a variety of clinical conditions, including anemias and polycythemias, were measured by both counting procedures. A total of 101 pathologic, 33 normal, and 23 miscellaneous specimens were counted, yielding visual counts in the range of 1.19 to 7.70 millions per cu. mm. This number and range, it is reasonable to conclude, constituted a thorough test of the conductivity method.

The curve (B) in figure 2 is a plot of Equation (2), and it is clear that it is a better fit than Equation (1), and a "scattergram" comparing the electrical count by Equation (2) and the visual count is shown in figure 4. The standard deviation between the two methods of counting was 0.45 million. Thus, 68 per cent of all

TABLE 5.—*Two Independent Measurements on Same Blood Sample*

Subject	No.	$Kx10^3$	K_0x10^3	g	Dev.
Ca	1	4.94	12.6	4.60	0.05
	2	4.98	12.6	4.55	
So	1	5.00	12.6	4.50	0.04
	2	4.87	12.6	4.46	
Ra	1	4.53	12.6	4.90	0.10
	2	4.61	12.6	4.80	
St	1	4.98	12.6	4.50	0.00
	2	5.07	12.6	4.50	
So	1	4.75	12.6	4.70	0.05
	2	4.80	12.6	4.65	
Ra	1	4.75	12.8	4.70	0.00
	2	4.72	12.8	4.70	
Ov	1	4.61	11.9	4.60	0.10
	2	4.52	11.9	4.50	
Le	1	5.36	12.2	4.20	0.10
	2	5.52	12.2	4.10	
To	1	5.55	12.5	4.10	0.00
	2	5.56	12.5	4.10	
Fa	1	5.08	12.4	4.50	0.00
	2	5.08	12.4	4.50	

comparisons between visual and calculated counts will agree within 0.45 million, while 95 per cent will agree within 0.9 million cells.

Magath et al.[4] have shown that the standard error of a red cell count made by enumerating the cells in 80 squares of a single chamber is about ± 0.405 million cells per cubic centimeter. The coefficient of variation is ± 8 per cent. Although this appears to make red cell counting a very inexact procedure, Ponder[5] agrees that most clinical laboratories count red cells similarly. Ponder divides the errors of the visual red count into three: those of the pipet, those of the chamber, and those which arise from an imperfect distribution of the cells on the squares of the chamber, i.e., the field error. Many chamber and pipet errors can be almost eliminated by experienced investigators, and with only the field error remaining, precision of ± 3 per cent can be achieved. However, Ponder counts 8 samples of 80 squares to achieve these results. Due to the errors inherent in the red cell count by the usual visual methods, no significance can be attached to two counts done

on the same individual which differ from each other by less than 16 per cent.[4] Thus, in the normal range of 5.0 million cells per cu. mm., differences must be greater than 0.80 millions per cu. mm. to be significant. Nearly 95 per cent of the electrical and visual counts are within this margin of agreement. In order to test the

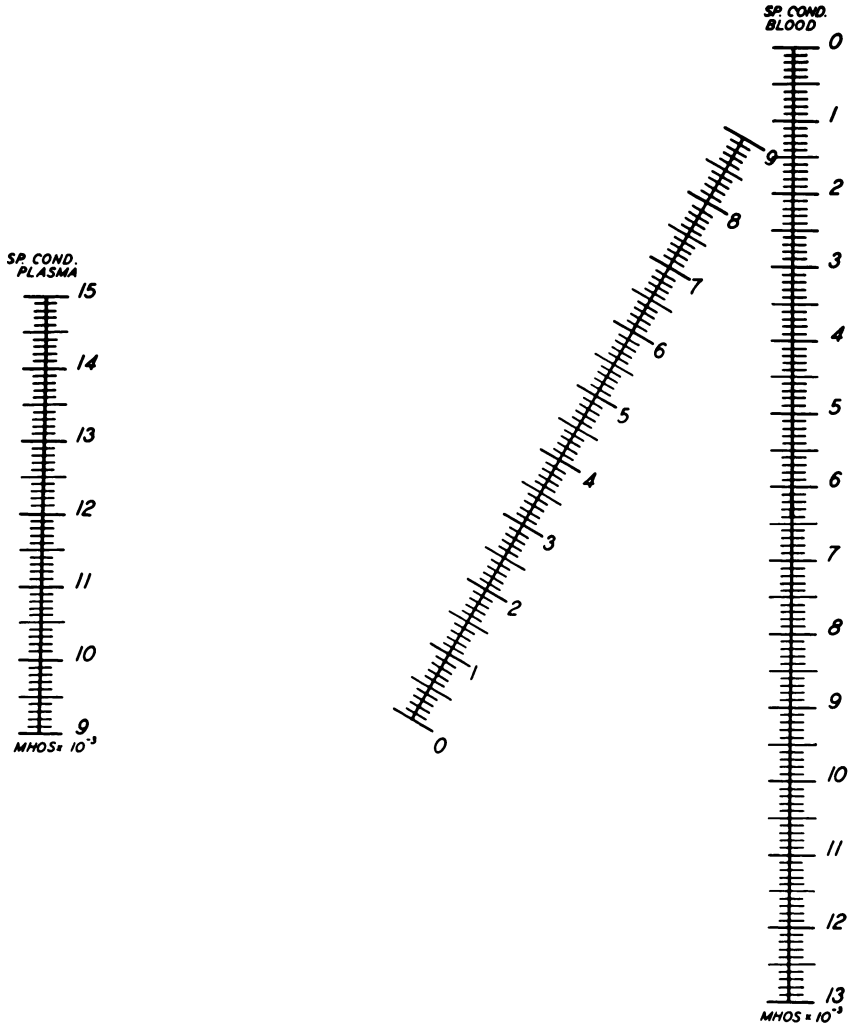

FIG. 5.—Nomograph for calculating red blood cell counts from the specific conductance of plasma and of whole blood.

reproducibility of electrical counts, two independent measurements were made on the same blood sample. Sometimes these two counts were made within a matter of hours of each other; on other occasions as much as a day and a half separated the two counts. The data from these studies are presented in table 5. The standard deviation was ±0.06 million cells per cu. mm., and the coefficient of variation 1.2 per cent. Thus, in the normal range of 5.0 million cells per cu. mm., counts

varying from each other by 0.24 million cells per cu. mm. represent actual differences in the number of circulating erythrocytes in the peripheral blood. It would appear, then, that by using the electrical method an important increase in the precision with which blood counts are made can be achieved.

It is apparent that the employment of conductivity measurements for red cell counting avoids several of the sources of error encountered in visual method. There is no dilution, the quantity of the blood sample is not critical, and the visual and other errors of the enumerating method are eliminated.

Although the value of 10.49 for C_1 in Equation (2) has been shown to give the most satisfactory agreement over the entire range of red cell counts, further studies may show the desirability of making modifications in the equation or in the value of C_1 as more data are assembled.

It is of interest to point out that the erythrocyte volume per cent is equal to $100\,(K_0 - K)/(K_0 + K)$, as shown in figure 3. Thus the volume per cent is shown experimentally to be measured by the conductivity, except for a small error at low concentrations as is evident in figure 3. It is probable that such a determination is more precise than the volume per cent obtained by centrifugation. It has been repeatedly observed that centrifugation does not completely separate the corpuscles from the plasma,[6, 7] and attempts to measure the fluid left in the sediment have not given reproducible results.[8]

For convenience in obtaining cell counts from the conductivity data, a nomograph has been constructed, which is given in figure 5. By placing a straightedge across the measured plasma and whole blood conductances, the red cell count may be read directly with sufficient precision from the diagonal line.

Summary

1. The blood and plasma conductance was measured in 157 blood samples. Of these, 101 were from hospital patients with a variety of clinical conditions, 33 were from normal individuals, and 23 were unclassified.

2. Blood counts were done on the same specimens.

3. An empiric equation, $g = C_1 \frac{K_0 - K}{K_0 + K}$, was found to give the red cell count from the conductivity measurements, wherein $C_1 = 10.49$, and g is in millions per cu. mm.

4. A nomograph is presented to simplify the calculations of the red cell count.

ACKNOWLEDGMENTS

Appreciation is here expressed to Dr. D. P. Barr and Dr. P. Reznikoff of New York Hospital, Cornell Medical Center, to Dr. J. S. LaDue of Memorial Hospital, and to Capt. W. H. H. Turville and Capt. H. L. Weaver of the U. S. Naval Hospital, St. Albans for their kind cooperation and for making available clinical material. Appreciation is also expressed to Col. J. R. Wood, Chief, Medical Division, Army Chemical Center for making available certain laboratory facilities.

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