Electronic Measurement of Cellular Volumes. II. Frequency Distribution of Erythrocyte Volumes

By C. C. Lushbaugh, N. J. Basmann and B. Glascock

THE APPARATUS, technic, and calibrations described in the first paper of this series,[1] were used to study frequency distribution of erythrocyte volumes in man and in other animals. These analyses were made in order to determine whether such population curves varied in a manner, either theoretically or practically, useful for clinical or descriptive hematology.

Methods

Blood samples were obtained in dry heparinized capillary tubes. Five λ of the sample was diluted with 10 ml. of saline. One-half ml. of this suspension was diluted with 200 ml. of saline, agitated thoroughly, and counted routinely in the particle counter. The second dilution was changed if the resulting count did not fall between 3000 and 4000 cells per 0.5 ml. in 13 seconds. When the standard counting rate was obtained, frequency distribution of RBC volumes was determined using the 100-channel pulse height analyzer unit.[1] The resulting curves were compared visually and then analyzed as a spectral curve and as a composite of two populations of normally distributed (Gaussian) particles.

A stylized population profile (frequency distribution of volumes) for circulating mammalian erythrocytes has been drawn (fig. 1), which defines the terms used in the first type of analysis. The curve was considered a spectral peak whose resonance or resolution was expressed mathematically by its modal frequency, mode, mean, width at half-height, and fractional width. "Fractional width," less confusing as a term than spectrographic "resolution," was determined by dividing width by mode. These numbers were used as analyzer channel volts without conversion to μ^3.

An IBM Model 704 computer* performed the second type of mathematical analysis. After proper programming, the computer fit two normal Gaussian curves to the population profile curve as obtained by the 100-channel analyzer. The data were then expressed graphically (figs. 2 and 3) and mathematically as proportional areas under the total curve with standard deviations of the Gaussian curves enclosing these areas.

Results

Results of spectral analysis of these population profiles are given in tables 1 and 2. Their fractional width varied between 0.40 and 0.60 in healthy normal humans, mice, monkeys, chickens, frogs, and toads. In several anemic horses, as well as in 12 patients with abnormal blood pictures, width of RBC population profile increased by a skewing of the curve to the large side of the mode so that a large increase in fractional width occurred. The hematologic details of the 12 abnormal humans are shown in table 2. Fractional width of these RBC profiles was greater than 0.6; the mean W/M was 0.80. As the mode became smaller its frequency increased, the width of the peak narrowed, and the

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This work was performed under the auspices of the U. S. Atomic Energy Commission. Submitted Jan. 3, 1962; accepted for publication Apr. 17, 1962.

*The computer program was arranged and operated by H. Israel of LASL Group H-6.

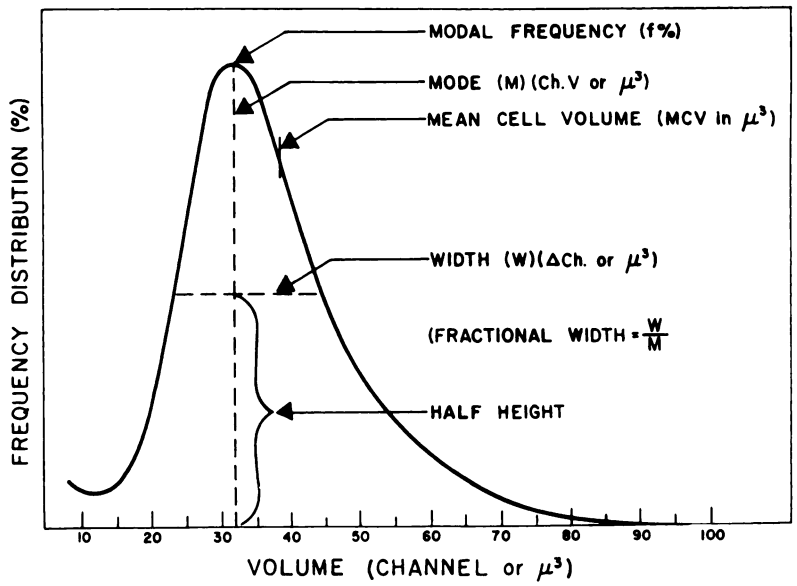


Fig. 1.—Frequency distribution of erythrocyte volumes, defining terms used in the "spectral" or fractional width analysis.

mean moved closer to the mode. The MCV never coincided with the mode because of the skew to the larger volume. In birds and reptiles especially (fig. 3), but occasionally in ill human beings, this skew seemed visually to result from two overlapping populations of cells with modes of different size. Examination of Wright-stained blood films of such specimens showed that if two such populations did exist, they could not be differentiated microscopically. This study indicated that the bulge to the right was not caused by leucocytes.

The IBM computer analysis produced two Gaussian population fits to the asymmetrical distribution curves (figs. 2 and 3). Figure 2 shows two overlapping populations of RBC in a normal young girl, a picture which has become the rule as these analyses continue, with population A comprising 45.6 per cent and population B 54.4 per cent of the total area or number of RBC analyzed. In table 3, other parameters of these two normal human RBC populations are listed. Population A appears more uniform and volumetrically smaller than population B, although it comprises about half the total circulating RBC.

A similar analysis is depicted of the blood of a normal chicken (fig. 3). The mode of population B is so far to the right of that of population A that the presence of this symmetrical population is well defined by the total curve. It is evident that the two populations of chicken RBC have fewer cells with identical volumes than is the case with human blood.

The results of a study of a case of congenital spherocytosis are shown in table 4 and in figure 4 in order to illustrate how these methods and technics might be used in hematology. The patient, a 24 year old farm laborer living at 8,000 feet altitude, received pronounced subjective improvement following a splenectomy. The objective results one month afterward were increases in RBC and hemoglobin without a change in MCV. The fractional width of the

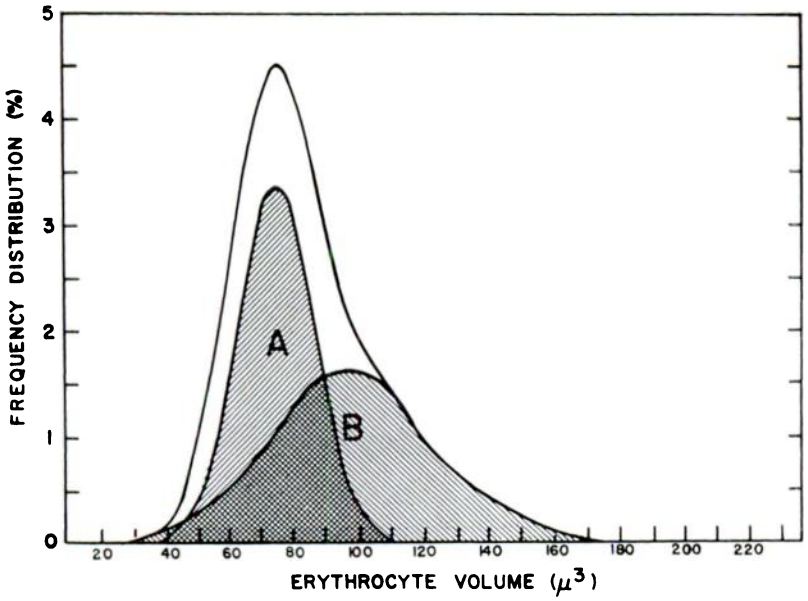


Fig. 2.—Frequency distribution curve of erythrocyte volumes of human blood and its representation by two cell populations (A and B), each with a normal Gaussian distribution.

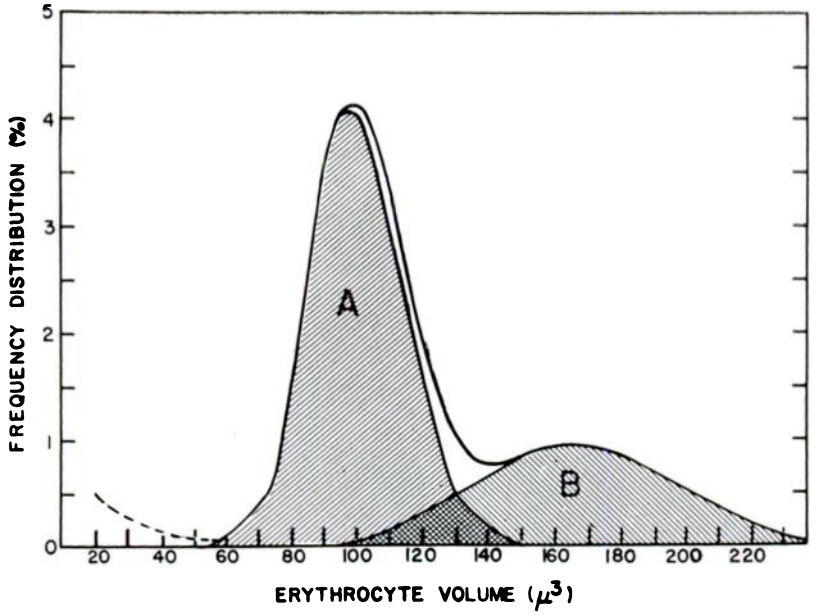


Fig. 3.—Frequency distribution curve of erythrocyte volumes of chicken blood and its representation by two cell populations (A and B), each with a normal Gaussian distribution.

frequency distribution of RBC volumes, which was exceptionally large before splenectomy, was normal one month later. As shown by the analysis of the two populations (table 4-C and fig. 4), this result was due to a proportionally greater increase in population A than in population B, and a decrease in the

Table 1.—*Fractional Widths of RBC Population Profiles (ACS-5)*

Species	No.	Frequency (per cent)	Width (△Ch.)	Mode (Ch.)	Mode (μ^3)	Fractional Width (W/M)
Normal man	14	4.5	17.3	33.0	70.6	0.52
Pathologic man	12	3.6	26.2	33.3	71.6	0.80‡
Normal mouse	8	7.8	11.0	18.0	38.5	0.61
Normal chicken	13	4.0	16.3	38.6	82.6	0.42
Normal monkey	1	5.6	16.0	27.0	57.8	0.59
Normal frog*	1	3.1	22.0	40.0	85.6	0.55
Normal toad*	1	3.8	18.0	39.0	83.5	0.46
Normal (?) horse	5	6.8	12.9	12.8	27.4	1.00
	3†	4.0	21.0	28.0	28.0	0.75

*ACS = 3.
†ACS = 6.
‡Mean of W/M in 12 cases shown in detail in table 2.

Table 2.—*Fractional Width Analysis of RBC Volumetric Population Profiles in 12 Human Cases with Abnormal Blood Picture*

Diagnosis	Sex	Age	RBC	Hmct	MCV	Hgb	Fractional Width Analysis F %	W (ch)	M (ch)	W/M
Acute leukemia	F	12	2.0	14	70	6.5	3.9	20	23	0.87
Chronic myelogenous leukemia	F	30	3.1	28	90	10.0	4.9	28	30	0.93
Polycythemia R. V.	M	45	7.8	59	75	19.8	4.3	21	29	0.72
Polycythemia R. V.	F	62	6.5	52	80	17.0	4.3	32	37	0.87
Polycythemia R. V.	M	55	7.1	64	88	20.0	3.3	30	38	0.79
Hypochromic anemia	F	40	3.8	28	74	8.0	4.0	20	30	0.66
Microcytosis	F	16	5.5	40	71	13.0	4.6	18	24	0.75
Macrocytosis (L. cirrhosis)	M	50	2.6	33	126	12.4	2.9	30	43	0.70
Prostatic carcinomatosis	M	60	4.2	34	78	10.2	2.5	34	37	0.92
Erythroblastosis*	M	0	6.3	64	102	17.0	3.2	26	34	0.76
Erythroblastosis	M	0	5.8	64	114	16.0	3.6	23	30	0.77
Neonatal jaundice (Coombs negative)	M	0	4.3	44	102	13.0	3.7	30	34	0.88

*Depicted in fig. 5.

modal volume of population B. These effects would logically result from the expected decrease in RBC destruction and production following splenectomy.

Discussion

Although the possibility that two distinct populations of RBC circulate at one time in the same individual has been surmised previously on the basis of different sensitivities to various hemolysins,[2] good morphologic support has never been obtained. If the two populations of RBC demonstrated here mathematically correspond physiologically with the two kinds of cells differentiated by hemolysins, one would expect population A to consist of older, more fragile cells than population B, since it is well known that reticulocytes and relatively new red cells are larger than senile cells which are also hemolysin-sensitive. If true, this method should show a shift in favor of population B in hemolytic anemia. This conjecture is substantiated in figures 5 and 6, which depict two cases of a hemolytic anemia, erythroblastosis fetalis, before and

Table 3.—*Average Parameters of Erythrocyte Populations in Normal Humans*

Population	(per cent)	σ (Width) (μ^3)	Mode (μ^3)
A	45.6	11.7 ± 2.4	72.8 ± 11.6
B	54.4	28.5 ± 5.4	95.0 ± 14.8

B/A = 1.36 ± 0.07.

Table 4.—*RBC Volumetric Analyses in Congenital Spherocytosis before and after Splenectomy*

A. Hematologic parameters

	RBC	Hmct	MCV	Hgb	MHC
Pre-splenectomy	4.1	36	88	12.6	30.5
Post splenectomy	5.8	51	88	16.7	28.5

B. Fractional width analysis

	F %	W (ch)	M (ch)	W/M
Pre-splenectomy	3.0	44	35	1.26
Post splenectomy	4.5	17	32	0.53

C. Large and small population analyses

	%	F %	W (μ^3)	M (μ^3)
Pre-splenectomy				
Population A	32.6	1.8	49	77
Population B	67.4	1.8	73	104
Post splenectomy				
Population A	36.3	3.0	30	75
Population B	63.7	2.1	68	95

after exchange transfusions with normal adult blood. The apparent preponderance of cells of large volume before transfusion and their proportional diminution afterward can be seen in both cases. In the more severely affected case (fig. 5), only population B seemed to be present before the exchange transfusion.

The hypothesis that young, newly formed cells comprise population B is susceptible to experimental verification, and such experiments are in progress. If this hypothesis proves to be true, the size of population B could be used to evaluate bone marrow response to disease, since it would measure the relative proportion of young RBC in the circulating blood. An increase in the relative size of population B seems to explain the increase in fractional width we have seen in the hematologic diseases that we have studied. A decrease in fractional width is to be expected where population B decreases in proportional size or where there is an increase in modal volume of population A without an accompanying increase in its standard deviation or width. Thus, in the one case of Addisonian pernicious anemia studied by this method, a fractional width of 0.45 was found because of the large mode of population A (85.6 μ^3, or 40 volts) and a width of only 15 volts.

The concept that population A is older than population B implies that B replenishes A as its cells become smaller and older. Both populations contain cells of identical volume, which must differ from each other in some other morphologic characteristic, such as thickness in relation to diameter. The

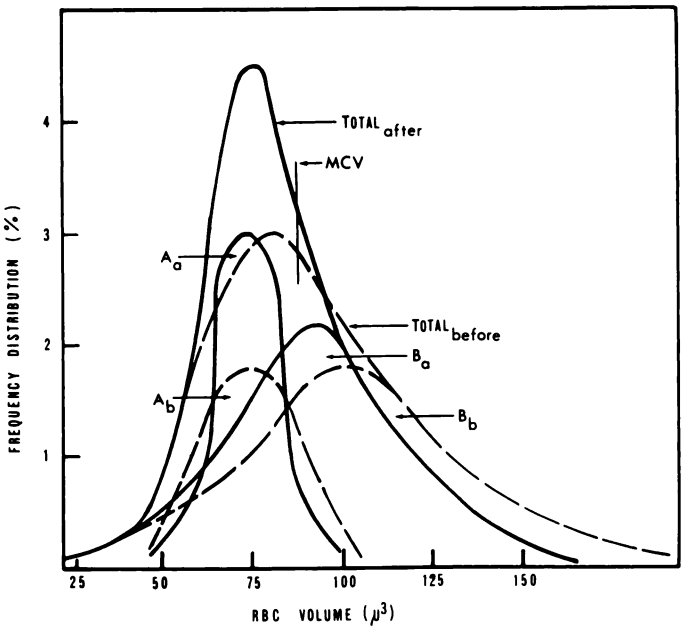


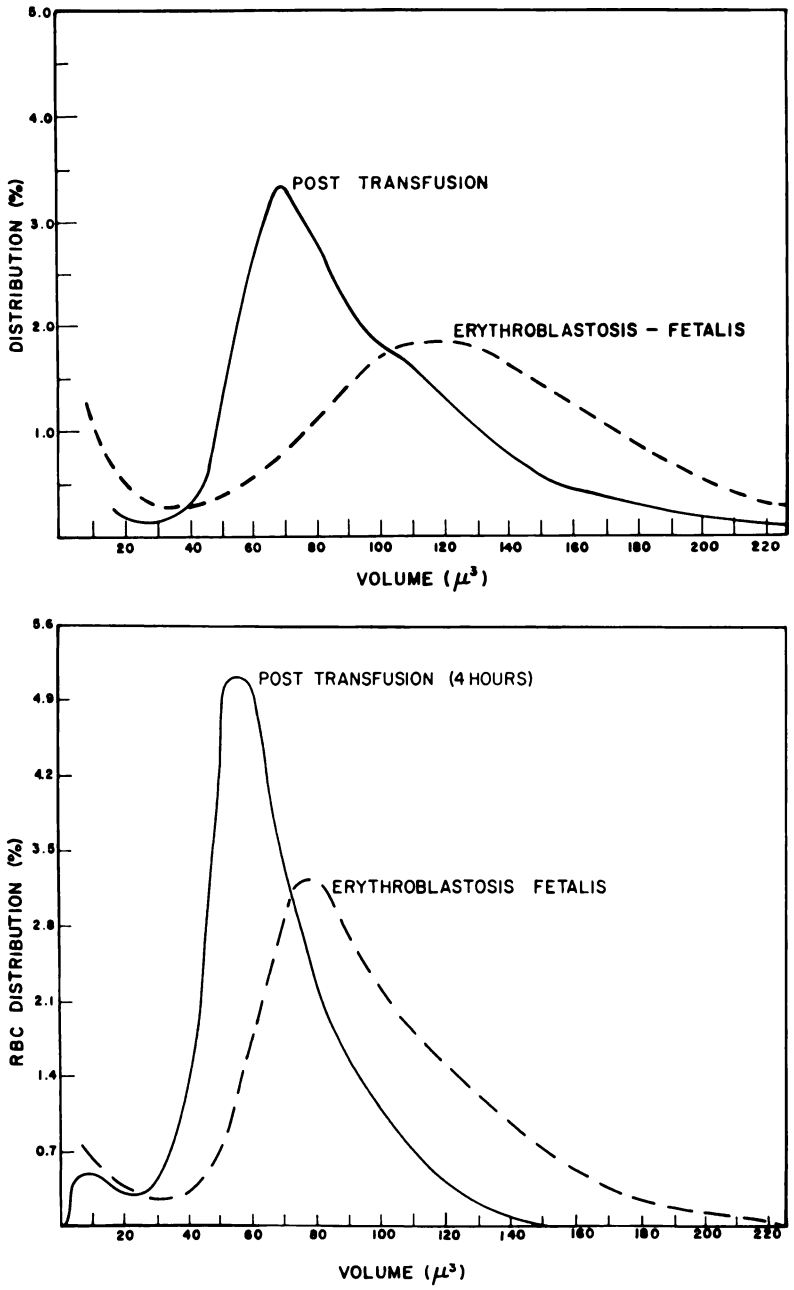
Fig. 4.—Pre- and postsplenectomy study of the frequency distributions of RBC volumes (shown as total, A and B populations) from a case of congenital spherocytosis.

apparent existence of the small and large but overlapping populations in the case of congenital spherocytosis described here suggests that different shapes do not define the two kinds of cells and that some other explanation is needed.

The fractional width method of analysis, while mathematically simple, seems usable only as a screening method for evaluating quickly the normality of RBC volumes and their distribution. An abnormal fractional width could be used to indicate the need for the more complicated fitting of two Gaussian distribution curves to the RBC profile. The latter method of analysis seems to have more potential usefulness hematologically than the other.

Summary

The frequency distribution of erythrocyte volumes of animals and man, determined electronically, showed a skew toward large volumes. Simple mathematical and computer analyses of these curves seemed to reveal the presence of two populations of erythrocytes with Gaussian distribution. One consisted of cells of relatively small volume and the other of cells of large volume. Study of avian erythrocytes revealed that in these species the two populations were sufficiently different volumetrically that their distributions did not completely overlap and their presence was not obscured in the total distribution curves. Analysis of pathologic states showed that, in most, the volumetric distributions shifted toward large volume as the disease developed. In severe erythroblastosis, for example, only the large population was demonstrable. Consequently, the larger-volumed population appeared to be composed of reticulocytes and young erythrocytes. These studies and those in progress indicate that this technic may afford another useful dimension to investigative and therapeutic hematology.



Figs. 5 & 6.—Pre- and posttransfusion RBC profiles in two erythroblastosis fetalis cases showing large cell preponderance in the presence of active hemolytic disease.

Summario in Interlingua

Le curva de distribution de frequentia del volumines erythrocytic in animales e homines, electronicamente determinate, monstrava dissymmetria in favor de grande volumines. Simple analyses mathematic e analyses a computator de tal curvas pareva revelar le presentia de duo populationes de erythrocytos

con un distribution gaussian. Un de iste populationes de erythrocytos consisteva de cellulas de volumine relativemente micre, le altere de cellulas de grande volumine. Le studio de erythrocytos avian revelava que in iste species le duo populationes de cellulas esseva sufficientemente differente in quanto a lor volmines pro que lor distributiones non esseva completemente imbricate e lor presentia non esseva obscurate in le curva total. Le analyse de statos pathologic monstrava que, in le majoritate de illos, le distributiones volumetric se displaciava verso le grande volumines a mesura que le morbo progredeva. Per exemplo, in sever erythroblastosis, solmente le population de grande erythrocytos esseva demonstrabile. Consequentemente, le population de erythrocytos de grande volumine pareva esser componite de retinculocytos e juvene erythrocytos. Iste studios e alteres currentemente in progresso indica que le presente technica promitte devenir un utile instrumento in le recerca e therapia hematologic.

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