LETTER TO THE EDITOR

# *Dependence of Platelet Volume Measurements on Heterogeneity of Platelet Morphology*

Dear Sir:

Critics of light microscopic measurements of platelet volume, for example Latimer (1979), have emphasized the theoretical limitations of this technique for platelet sizing over other methods such as microhematocrit (Born, 1970), electronic particle sizing (Grover et al., 1969; Laufer et al., 1979; Mundschenk et al., 1976) and light scattering (Latimer, 1979), even though in practice, mean volumes measured by these techniques for normal platelets in citrated platelet-rich plasma (PRP) appear to be in close agreement. For example, microhematocrit and light microscopic determinations of platelet mean volume apparently differ by <2% (microhematocrit mean volume of 5.8 $\mu m^3$ for 5 donors, range of means 4.1–6.9 $\mu m^3$ (Born, 1970) vs. microscopic mean of 5.9 $\mu m^3$ for 15 donors, range 4.4–8.0 $\mu m^3$). However, the consequences of a heterogeneity in platelet morphology on volume measurements have not generally been fully appreciated. Here we draw attention to a number of important consequences of a heterogeneity in platelet morphology on interpreting platelet volume measurements. These consequences are independent of the quantitative accuracy of the method utilized to measure platelet volume. The ability of the light microscope to consider platelet volume and morphology jointly may more than offset any of its quantitative disadvantages.

Any sample of platelets will contain a mixture of platelet morphologies ranging from large and small discoid-shaped cells (discocytes [D]) to others which are sphered to varying extents (discoechinocytes [DE], sphero-echinocytes [SE]) (Barnhart et al., 1972; Hattori et al., 1979; Milton and Frojmovic, 1979). Figure 1 *a* compares the overall distribution of platelet volume in PRP with the individual volume distributions of D, DE, and SE. Since the mean volumes of D, DE, and SE differ, it is clear that alterations in the relative proportion of these will be reflected by a change in the overall volume distribution.

Echinocytes (DE and SE) can arise from D after induction of shape change by an activator, such as ADP, according to the kinetic scheme (Milton and Frojmovic, 1979; Milton et al., 1980)

$$D \rightarrow DE \rightarrow SE \rightarrow DE \rightarrow D. \quad (1)$$

In addition, platelet morphology is sensitive to the method of PRP preparation (Sixma, 1978) and is altered in certain acquired disorders (Hattori et al., 1979). Moreover, certain acquired platelet disorders are associated with an increase in size of circulating platelets, eg. megathrombocytes (Garg et al., 1972), "stress" platelets (Penington, 1977). Since volume changes, for example, may reflect alterations in platelet morphology or be due to the appearance of increased numbers of megathrombocytes, it is necessary to know the underlying distribution of platelet morphologies if changes in platelet volume are to be interpreted in an unambiguous manner.

Coulter counter measurements of platelet mean volume are particularly sensitive to alterations in the heterogeneity of platelet morphology. The physical parameter measured by this technique is electrical size, which is equal to a shape factor times platelet volume; the shape factor is a function of the platelet axial ratio ($r_p$) and ranges from 1.18 for a D with $r_p = 0.26$, to 1.5 for a sphere, $r_p = 1.0$ (Laufer et al., 1979). Thus even a small increase in the frequency of SE can have a disproportionately larger influence on platelet mean volume. Attempts have been made to circumvent this problem by, for example, using EDTA as an anti-coagulant, thereby inducing platelet "sphering" (Mundschenk et al., 1976). However, volume determinations made in this way are difficult to interpret due to uncertain effects of these agents

on platelet volume and lack of precise definition, eg. $r_p$, of sphered platelets. More important, information concerning a change in morphology distributions as is seen in whole blood for certain acquired disorders (Hattori et al., 1979) and which may prove to be important diagnostically, is necessarily lost.

The change in platelet size and shape during the initial step of hemostasis (Born, 1970) and during storage (Holme and Murphy, 1979) is of considerable interest. Accurate determination of the volume change that accompanies platelet shape change has obvious mechanistic importance. Measurements of overall platelet volume by centrifugal methods (Born, 1970), electronic particle sizing (Laufer et al., 1979) and microscopic methods (Table I) suggest that ADP-induced platelet shape change of normal platelets is approximately isovolumetric. However, a second consequence of the influence of platelet morphology on volume measurements is that changes in platelet volume accompanying shape change must be underestimated. This is because before shape change the sample of platelets will already contain a certain fraction of DE and SE (Fig. 1 *a*) and their presence will diminish the observed change in volume on shape change. The extent to which the volume change will be underestimated is a function of the fraction of D ($f_D$) in the initial PRP. Indeed if the overall mean volume ($\overline{V}_T$) is

$$\overline{V}_T = f_D \overline{V}_D + (1 - f_D) \overline{V}_E \tag{2}$$

where $\overline{V}_E$ is the mean volume of echinocytes, i.e., DE and SE, $\overline{V}_D$ is the mean volume of D and the symbol $\overline{V}$ indicates arithmetic mean volumes, then we see that

$$R = \frac{(\overline{V}_T)_{+ADP}}{(\overline{V}_T)_{-ADP}} = \left[1 + f_D\left(\frac{\overline{V}_D}{\overline{V}_E} - 1\right)\right]^{-1} \tag{3}$$

where, for simplicity, it has been assumed that $\overline{V}_E$ before and after addition of ADP is unchanged (i.e., $\overline{V}_{E+ADP} = (\overline{V}_E)_{-ADP}$) and the fraction of D following addition of ADP is zero [i.e., $(f_D)_{+ADP} = 0$]. As $f \rightarrow$

TABLE I

COMPARISON OF VOLUME CHANGES OF PLATELETS IN PRP ESTIMATED FROM OVERALL MEAN VOLUME ($V_T$)* AND $V_{SE}/V_D$ MEASUREMENTS

| Donor | Method of Calculation‡ | $(V_T)_{-ADP}$ | $(V_T)_{+ADP}$ | $V_D$ | $V_{SE}$ | $\frac{(V_T)_{+ADP}}{(V_T)_{-ADP}}$ | $\frac{V_{SE}}{V_D}$ |
|---|---|---|---|---|---|---|---|
| | | $\mu m^3$ | $\mu m^3$ | $\mu m^3$ | $\mu m^3$ | | |
| Normal§ | Arithmetic | 5.4 | 5.4 | 5.2 | 4.7 | 1.0 | 0.9 |
| | Geometric | 4.8 | 4.7 | 4.8 | 4.4 | 1.0 | 0.9 |
| BSS‖ | Arithmetic | 17.1 (9.3)¶ | 15.6 | 6.4 | 12.7 | 0.9 (1.7) | 2.0 |
| | Geometric | 15.8 | 14.3 | 6.1 | 10.8 | 0.9 | 1.8 |

*$(V_T)_{+ADP}$, $(V_T)_{-ADP}$, $V_D$, $V_{SE}$ are, respectively, the overall platelet mean volume ± ADP, mean volume of D and SE.

‡Geometric mean volumes were determined from a log-probability plot as described by Smith and Jordan (1964). Arithmetic mean volumes were determined from the relationship (Milton and Frojmovic, 1979) $\overline{V}_T = f_D\overline{V}_D + f_{DE}\overline{V}_{DE} + f_{SE}\overline{V}_{SE}$, where, respectively, $\overline{V}_D$, $\overline{V}_{DE}$, $\overline{V}_{SE}$ are the arithmetic mean volumes of D, DE, SE and $f_D$, $f_{DE}$, $f_{SE}$ are the fraction of the total platelet volume distribution contributed by each morphology ($f_D + f_{DE} + f_{SE} = 1$).

§Data from Fig. 1, where in the absence of ADP, $f_D = 0.76$, $f_{DE} = 0.19$, $f_{SE} = 0.05$ and in the presence of ADP $f_D = 0.0$, $f_{DE} = 0.40$, $f_{SE} = 0.60$. This data was chosen to illustrate a situation where shape change is isovolumetric according to $V_T$ measurements. More generally the change in arithmetic mean volume ($\overline{V}_T$) upon addition of ADP varies from a decrease of 20% to an increase of 10% (data from seven donors). This variation is in large part due to differences in the heterogeneity of the platelet morphologies in the PRP before and after addition of ADP.

‖This data is for donor B who has been described in Frojmovic et al. (1978) and Milton et al (1979). For PRP in the absence of ADP, $f_D = 0.30$, $f_{DE} = 0.68$, $f_{SE} = 0.02$, and 30 s following addition of 10 $\mu$m ADP, $f_D = 0.0$, $f_{DE} = 0.60$, $f_{SE} = 0.40$.

¶Data in parentheses are for platelet volume in whole blood in which for this donor $f_D = 0.8$.

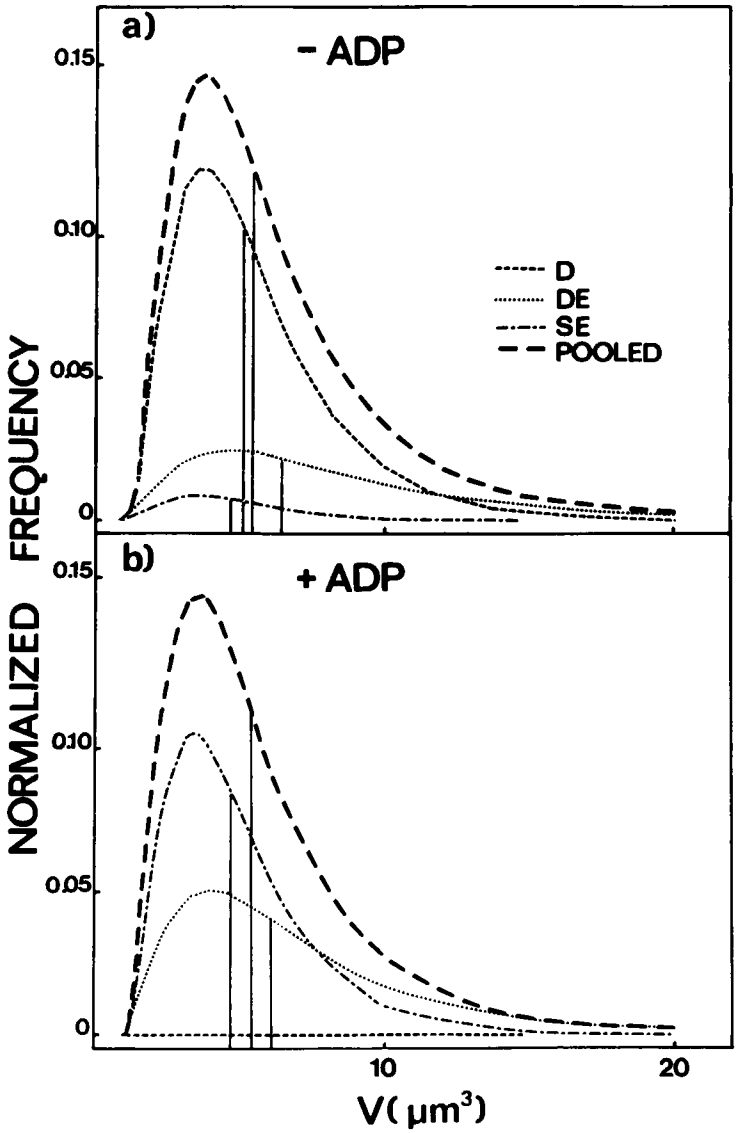


FIGURE 1 Comparison of the overall distribution of platelet volumes with the volume distributions of D, DE, and SE for (*a*) normal PRP and (*b*) 30 s after addition of 10 μM ADP to PRP. Normalized log-normal distributions for D, DE, and SE were calculated from experimental data by the method of Smith and Jordan (1964) and the mathematical distribution used to construct the above volume distributions. The distributions for D, DE, and SE have been weighted by the relative frequency of these morphologies and the overall volume distribution curve obtained by summation. Arithmetic mean volume is indicated by the solid vertical lines. Volumes of DE and SE have been corrected for the contribution due to pseudopods in the following manner: the summated length of pseudopods for DE was estimated from the data of Holme et al. (1977) to be ~10 μm and that for SE from the data of Barnhart et al. (1972) to be ~20 μm. For a maximal pseudopod radius of 0.1 μm, this corresponds to a volume correction of 0.3 $\mu m^3$ and 0.6 $\mu m^3$, respectively. It should be noted that normal PRP typically contains 15–35% DE and <2% SE; hence these corrections affect overall mean volume by <2%.

0, $R \rightarrow 1$ and hence does not indicate the true volume change on shape change, i.e., $\overline{V}_E/\overline{V}_D$. Only in the unlikely situation that $f_D = 1$ is the true volume change accompanying shape change measured (for PRP prepared from normal donors, $f_D = 0.65$–$0.85$).

The use of mean volume measurements to estimate the volume changes accompanying shape change is further complicated by the fact that even after maximal activation a variety of platelet morphologies exist (Fig. 1 *b*). Since SE represents the most advanced form of platelet shape change (see Eq. 1), it would seem reasonable to consider the volume change that occurs on passing from D to SE. Table I compares the volume changes estimated from overall mean volume measurements to those estimated from the mean volumes of SE and D. Clearly the volume change with shape change can be much greater than would be anticipated from overall mean volume measurements. This conclusion is true whether arithmetic or geometric mean volumes are considered.

The importance of taking into consideration platelet morphology in interpreting platelet volume measurements is underlined by problems encountered with platelet sizing in hereditary giant platelet syndromes (HGPS). For certain HGPS, in particular Bernard Soulier syndrome (BSS) (Frojmovic et al., 1978; Milton et al., 1979) and Montreal platelet syndrome (Milton and Frojmovic, 1979), the D are more normal-sized and there is a hypervolumetric shape change, i.e., the mean volume of SE is 1.8–2.4 times that of D. It is apparent from Table I that on the basis of only overall mean volume measurements for platelets in PRP for a BSS donor, one would expect neither the existence of more normal-sized discocytes nor a hypervolumetric shape change. Indeed the overall mean volume change upon addition of ADP actually decreases! This apparent contradiction arises from the fact that, typically, the predominant platelet morphology in PRP prepared from HGPS donors is DE with only 10–30% D. Since the mean volume of DE exceeds that of SE (see Fig. 1), shifting the distribution of platelet morphologies towards SE will result in a decrease in overall mean volume. As is shown in Table I, if overall mean volumes were measured for platelets in whole blood, where for this donor $f_D$ is 0.8, then the more correct conclusions would be obtained (data in brackets).

Conceptually the measurement of platelet volume requires a parallel identification of morphology and size for each platelet in the sample. Whereas microscopic methods are without equal in terms of measuring platelet morphology, other methods, such as small-angle light scattering, are superior in terms of providing a quantitative measurement of size (Latimer, 1979). At present, light microscopic analysis of the type we have described (Frojmovic and Panjwani, 1976; Milton and Frojmovic, 1979) is the only method available to perform both tasks. It is quite possible that by combining, for example, cell sorting techniques with light scattering techniques (Mullaney et al., 1976) or perhaps by appropriate analysis of Coulter counter volume distributions, that a more suitable and convenient method of platelet sizing will be developed. It is hoped that our comments will spark interest into the design and development of better methods for accomplishing these tasks.

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