Turbidometric Evaluations of Platelet Activation: Relative Contributions of Measured Shape Change, Volume, and Early Aggregation

JOHN G. MILTON AND MONY M. FROJMOVIC

The relative contributions of ADP-induced changes in mean platelet axial ratio (R), mean volume (V_T), pseudopod formation, refractive index, and early platelet aggregation to the changes in light transmission (%T) through stirred platelet suspensions (aggregometer) are determined. Measurement of the change in R within the first 10s of ADP addition indicates that the slope of the initial decrease in %T (5s) is in good agreement with that predicted from the theory of Latimer et al. (1977). However, when the changes in %T due to changes in V_T are also considered, the observed rate of change of %T is shown to be 20–210% larger than predicted. These observations point to an additional process which produces a decrease in %T. It is shown that platelet aggregation occurring on this early time scale does not contribute to the initial changes in %T. Aggregation does lead to an increase in %T once there are ≥30–40% singlet platelets aggregated which occurs at ≥4–5s for 1–10 μM ADP. In the absence of aggregation, the extent of %T decrease at >40s is >25% smaller than predicted from the change in R and V_T. It is suggested that at early times, changes in platelet refractive index produce significant decreases in %T which are on average offset by comparable increases due to the early volume increase, whereas at later times pseudopod formation has an important influence on %T.

Key Words: Light transmission; Adenosine diphosphate; Platelet aggregation; Platelet shape change; Platelet volume

INTRODUCTION

Changes in percent light transmission (%T) through stirred platelet-rich plasma (PRP) are widely used to monitor drug-platelet interactions (for a recent review see Frojmovic and Milton, 1982). It is widely held that whereas the initial decrease in %T following addition of drugs to stirred PRP is largely due to changes in platelet shape, the subsequent increases in %T arise from platelet aggregation (Born, 1970; Frojmovic, 1978; Latimer et al., 1977). Measurements of these changes in %T form the basis of numerous investigations into the kinetics and mechanisms of drug-platelet interactions.

Recent studies have suggested that significant platelet aggregation occurs along

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with shape change before increases in %T are detectable (Borne and Hume, 1967; Gear, 1982; Milton et al., 1981a). Indeed, it has been suggested that microaggregate formation may cause, in whole or in part, the initial decrease in %T (Kitek and Breddin, 1980), though this suggestion has been controversial (Breddin, 1981; Patschke, 1981). On the other hand, other studies have shown good correlation between microscopically- and turbidometrically-measured shape change (Milton et al., 1980) and between observed changes in %T and those predicted from measured mean platelet axial ratio (R) on the basis of conventional light scattering theory (Latimer et al., 1977).

The aggregating agent, adenosine diphosphate (ADP), is the most widely used activator of platelets for in vitro evaluations of platelet pharmacology and physiology, as it is considered to be both directly and indirectly associated with most in vivo mechanisms of platelet (patho) physiology (Frojmovic and Milton, 1982). Here we reassess the relative contributions of platelet shape and aggregation to the initial changes in %T following ADP addition to stirred PRP. The relative contribution of two other factors which can influence initial %T are also examined: 1) volume changes (Latimer, 1975a; Latimer and Pyle, 1972; Latimer et al., 1968), and 2) pseudopod formation (Latimer et al., 1977).

MATERIALS AND METHODS

Preparation of Platelet Suspensions

Normal donors were chosen from healthy men and women between the ages of 18 and 30 years. Blood was drawn by venipuncture into 3.8% citrate (1 vol to 9 vol blood) and platelet-rich plasma (PRP) prepared as described previously (Tang and Frojmovic, 1977). Washed platelets were prepared by the method of Mustard et al. (1972), using three washes and bovine-albumin-Tyrodes containing calcium and magnesium.

Platelet morphology was classified from the appearance of platelets under phase-contrast microscopy (Frojmovic and Panjwani, 1976; Frojmovic and Milton, 1982): 1) edge-on discocytes (D) appear ellipsoid with a clear center, whereas face-on they appear circular with dark center, 2) sphero-echinocytes (SE) appear circular with a white center, and 3) disco-echinocytes (DE) are all those platelets which do not satisfy the criteria for D and SE. Geometric criteria corresponding to D, DE, and SE have been published previously (Milton and Frojmovic, 1979a). In determining the fraction of D, DE, SE, i.e., respectively f_D, f_{DE}, f_{SE}, 400 platelets were classified. The reproducibility of platelet morphology determined in this way was to within <5% D, <5% DE, <1% SE between several investigators in the same or different laboratories counting the same sample and <2%D, <2% DE, <1% SE for a single, experienced investigator.

Platelet mean axial ratio (R) was determined from the relation

$$R = f_D(\bar{r}_p)_D + f_{DE}(\bar{r}_p)_{DE} + f_{SE}(\bar{r}_p)_{SE} \quad (1)$$

where r_p is the axial ratio, i.e. ratio of thickness (t) and diameter (d), and $(\bar{r}_p)_D$, $(\bar{r}_p)_{DE}$, and $(\bar{r}_p)_{SE}$ are, respectively, the mean axial ratios of D, DE, and SE. Criteria

for determining t and d for D, DE, and SE have been published previously (Frojmovic and Panjwani, 1976; Frojmovic et al., 1978). R was estimated to be accurate to within 2%. Hydrodynamic axial ratios predicted from R agree well with those measured by rheo-optical methods (Frojmovic et al., 1976).

Platelet mean volume (V_T) was determined from the relation

$$V_T = f_D\bar{V}_D + f_{DE}\bar{V}_{DE} + f_{SE}\bar{V}_{SE} \quad (2)$$

where, respectively, $\bar{V}_D$, $\bar{V}_{DE}$, $\bar{V}_{SE}$, are the mean volumes of D, DE, SE. Calculation of $\bar{V}_D$, $\bar{V}_{DE}$, $\bar{V}_{SE}$ is the same as described previously (Milton and Frojmovic, 1979a; Milton et al., 1981b). Typically, 80D, 50–60 DE, and 60–80 SE were measured in determining, respectively, $\bar{V}_D$, $\bar{V}_{DE}$, and $\bar{V}_{SE}$. V_T was accurate to within 3%.

Glutaraldehyde Fixation

Platelets were fixed by the addition of four volumes 0.8% (v/v) glutaraldehyde (Polysciences, Inc., EM Grade) in Ca, Mg-free Tyrodes, pH 7.4, 37°C to the platelet suspension. This fixative procedure does not significantly alter platelet morphology (Frojmovic and Panjwani, 1976) or alter the extent of platelet aggregation (Nichols and Bosmann, 1979). Simultaneous addition of 100 μM ADP and glutaraldehyde solution to PRP produced no alteration in platelet morphology. Since at 1 sec post-100 μM ADP addition we typically observe a 10–20% decrease in D, this observation implies that the fixation time is much less than 1 sec.

Platelet aggregation (%PA) at time t was determined by counting the number of nonaggregated platelets (N_t) in with a hemocytometer and utilizing the relation

$$\%PA = \left(1 - \frac{NT}{No}\right) \times 100\% \quad (3)$$

where No is the total nonaggregated platelet count (Benner et al., 1980; Milton et al., 1981a).

Changes in light transmission (%T) were monitored using the aggregometer apparatus described previously with a rapid chart speed (10 inches/min) and amplifying the changes in %T threefold (Tang and Frojmovic, 1980). 1–3 μl of aggregating agent were quickly injected, using a Hamilton syringe, into 0.4 ml platelet suspension which was contained in a siliconized cuvette (6.9 mm × 45 mm) with stir bars (6 mm × 1 mm) spun at 1000 rpm, 37°C. The mixing time was less than 1 sec (Born, 1970; Milton et al., 1980).

Kinetic studies for early platelet aggregation were done with 0.1 ml PRP contained in the above cuvettes with stir bars spun at 1000 rpm in order to decrease the mixing time. The mixing time was estimated by monitoring the changes in %T following addition of 1–3 μl 0.4% Trypan Blue stain (Grand Island Biological Co.) to stirred PRP. The smallest volume for which this could be done was 0.3 ml, for which the mixing time was <0.5 sec. Determinations of mixing time as a function of volume in the cuvette and extrapolation to 0.1 ml suggested a mixing time of about 200 msec. The process was terminated by addition of 0.4 ml 0.8% glutaraldehyde (V/V) and %PA determined as described above. The time courses for the changes in platelet morphology, R and V_T, were done in a similar manner except that aggregation

was minimized by stopping the stirrer after allowing 1 sec for mixing. Although under these conditions ≤10–20% of the platelets occurred in small aggregates (mostly doublets and triplets), there was no difficulty in assessing morphology, R, and V_T even for the aggregated platelets.

Reagents Trisodium citrate (J. T. Baker Co., Phillipsburg, N.J.), ADP (Sigma), and epinephrine (Sigma) were freshly prepared in modified Tyrodes, pH 7.4; apyrase was a gift from Dr. M. A. Packham (Toronto, Canada) and was used at a concentration of 0.1 units per ml washed platelets; porcine heparin was used at 50 units per ml washed platelets using Hepalean (10,000 units ml^{-1}) (Harris Labs, Brandford, Conn.), bovine albumin, fraction V (Sigma); fibrinogen, human lyophilized grade L (Kabi AB, Montreal, Canada) was purified free of plasminogen with lysine-sepharose columns, then concentrated with glycine precipitation, dialyzed overnight with Tyrodes, pH 7.4, and divided into aliquots at a final concentration of 10 mg ml^{-1} (>96% clottable) and stored at −75°C; ethylenediaminetetraacetic acid (EDTA) (Fisher Scientific Co., Montreal, Canada) was used as a 5% solution in double distilled water at pH 9.0 with <12 μl added to 0.4 ml PRP at pH 7.4 with no significant effect on pH of PRP.

RESULTS

ADP-Induced Aggregation

A. Influence of Early Platelet Aggregation on %T

Figure 1 compares the time course for the changes in %T, platelet shape (expressed as the increase in the fraction of echinocytes (f_E)), and platelet aggregation (%PA) following addition of 1 μM ADP. Both f_E and %PA rapidly increase over the first 6 sec concomitantly with the %T decrease.

The effect of platelet aggregation on changes in %T is examined in Figure 2. Inhibition of platelet aggregation with EDTA (Figure 2a) and absence of fibrinogen (Figure 2b) results in an augmentation of the extent of the total decrease in %T without affecting the initial rate of %T decrease. It follows that platelet aggregation produces an increase in %T and that the decrease in %T in the first 4–5 sec following ADP addition reflects changes in platelet shape. The earliest time at which the influence of platelet aggregation on %T can be detected is 4–5 sec. At 5 sec post-1 μM ADP, 40% of the platelets are singlets and 25% are in large aggregates (>7 platelets per aggregate), whereas at 4 sec post-10 μM ADP, 30% are singlets and 60% occur in large aggregates. Thus there does not seem to be a simple correlation between the appearance of an increase in %T and the underlying aggregate size distribution. Nichols and Bosman (1979) have also reported a lack of correlation between %T and aggregate size distributions occurring at greater than 10 sec post-ADP addition.

Platelet shape change as measured from echinocyte formation (f_E) can include changes in the mean platelet axial ratio (R), mean volume change (V_T), pseudopod production, changes in refractive index, and/or plasma membrane reorganization (Frojmovic and Milton, 1982). We next consider each of the remaining factors.

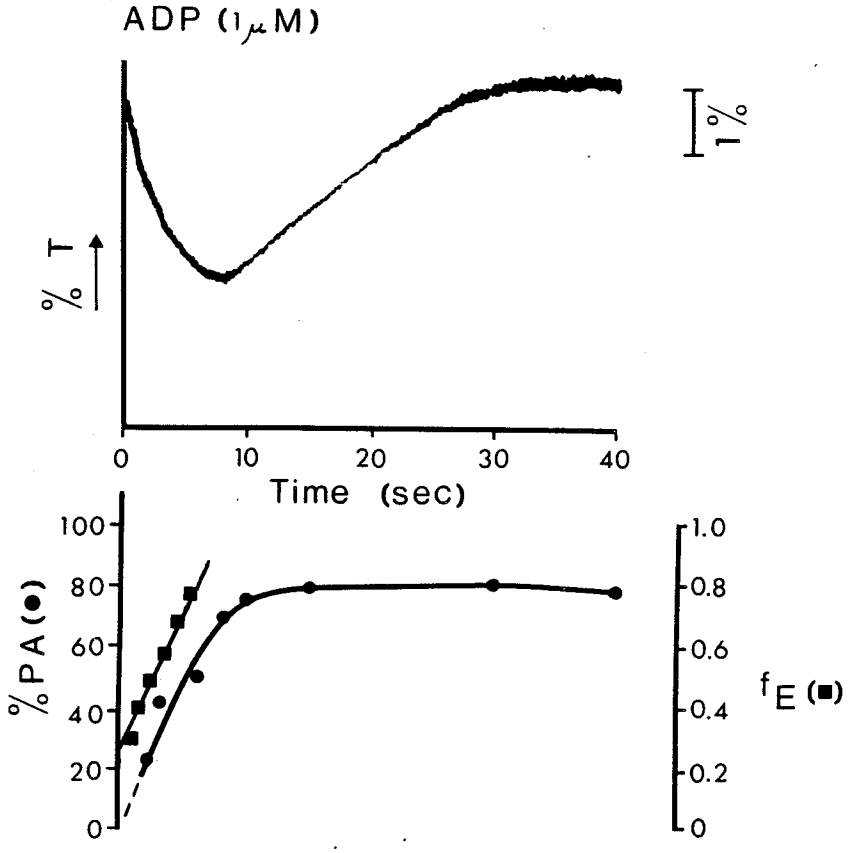

FIGURE 1. Percent light transmission changes (%T; top figure), microaggregation (●) and shape change (■) (bottom figure) as a function of time following addition of 1 μM ADP. %PA was determined from equation (3), and is data for 2 donors and represented as mean values. The same donors were used for the %T and %PA data.

B. Influence of V_T on %T

Figure 3 plots V_T as a function of time for the first 10 sec following addition of 1 μM and 10 μM ADP to stirred PRP. There is a rapid increase in V_T of 12–13% in the first sec, followed by a gradual decrease. The predicted changes in %T due to these changes in V_T can be determined by the methods in the Appendix and are shown in Table 1. As can be seen, the volume would be expected to be associated with an increase in %T of 0.2–2.9% sec^{-1}.

C. Influence of R on %T

Figure 4 shows that R increases linearly for 10 sec following addition of 1 μM ADP. The relative extinction (E_R) as a function of R, i.e., the extinction relative to that predicted for a sphere with R = 1, has been calculated by Latimer et al. (1977). The

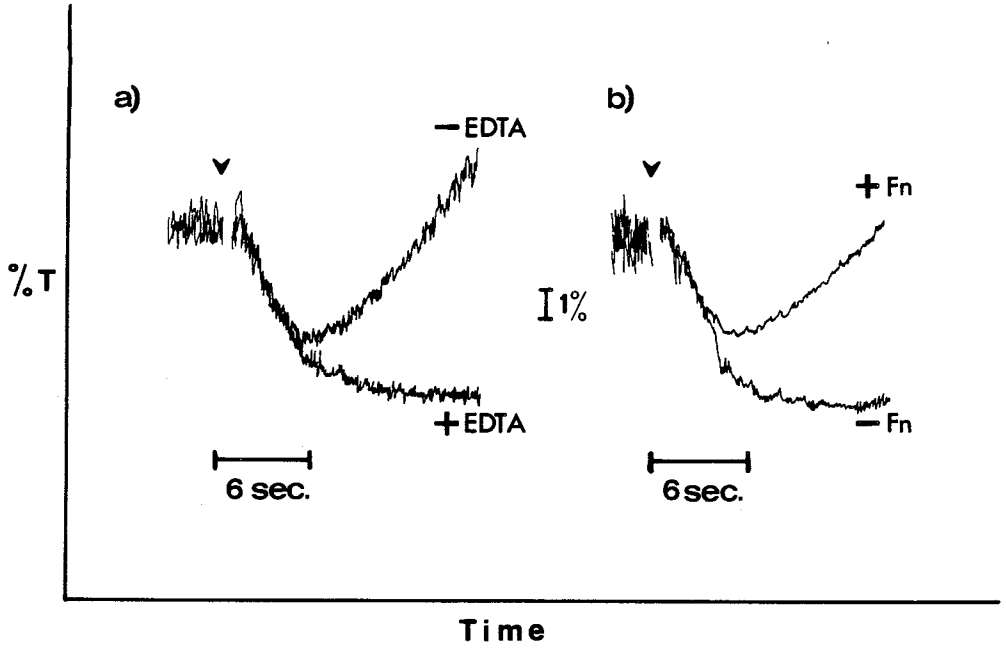

FIGURE 2. Effect of inhibition of microaggregation by 5 mM EDTA and absence of fibrinogen on the early changes in light transmission following addition of ADP (▼) to stirred platelet suspensions. In (a) the platelet suspension is PRP and ADP = 1 μM. In (b) the platelet suspension is washed platelets and ADP = 10 μM. Fibrinogen final concentration was 0.5 mg ml^{-1}. Axes as in Figure 1A but 3× expansion for %T.

relative transmittance (T_R) can be calculated as $T_R = 100 \exp(-E_R)$. As can be seen, a linear increase in R over 10 sec leads to a predicted linear decrease in T_R over 3–5 sec. Table 1 compares the rate of change of T_R to the observed rate of change of %T for a number of donors for 1–10 μM ADP. As observed by Latimer et al. (1977) there appears to be excellent agreement (within 10%) between theory and experiment. However, when the effect of the observed changes of V_T on %T are taken into account we see that the predicted rate of change of %T is actually 20–210% smaller than is observed. These observations imply the occurrence of an additional process which produces a decrease in %T.

D. Influence of Pseudopods and V_T on %T

Figure 5(a, b) plots platelet morphology, R and V_T, as a function of time following addition of 10 μM ADP. R attains a maximal value after 40 sec. Therefore T_R should attain its minimum value at 40 sec. However, minimal values of measured %T occur within 10 sec (See Figure 2) and thereafter increase slowly, 1 %T increase by 40 seconds for 10 μM ADP in presence of 5 mM EDTA (2 donors). Using the measured values of R at t = 0, 10 sec, and 40 sec (see Figure 4b), it can be determined from the calculations of Latimer et al. (1977) that 75% of the total decrease in T_R has occurred at 10 sec. The average decrease in %T observed at 10 sec was 5% (mean

TABLE 1 Comparison of Measured and Predicted Initial Rate of Change of %T and of Changes in Mean Platelet Volume (V_T) and Mean Platelet Axial (R)

ADP Concentration	Mean Platelet Axial Ratio (R)		Mean Platelet Volume (V_T)		Initial Rate of %T Change (%sec^{-1})				
						Predicted			
	Initial Value	Rate of Change (sec^{-1})	Initial Value (μm^3)	Rate of Change[a] (sec^{-1})	Measured[b]	From V_T[c]	From R	Total	% Difference Between Measured and Predicted
1 μM									
Donor 1[d]	.25	.025	5.3	−.23	−1.7	.9–1.4	−1.8	−(0.4–0.9)	20–50
Donor 2[d]	.35	.026	4.3	−.04	−1.3	.2–0.3	−1.2	−(0.9–1.0)	70–80
Donor 3	.30	.013	7.0	−.29	−0.8	1.1–1.7	−0.9	+(0.2–0.8)	125–200
Donor 4	.32	.021	6.0	−.32	−1.2	1.2–1.6	−1.1	−(0.1–0.5)	110–140
Donor 5	.33	.022	7.0	−.44	−1.4	1.7–2.5	−1.3	+(0.4–1.2)	130–190
10 μM									
Donor 4	.32	.026	6.0	−.35	−1.5	1.3–1.8	−1.3	+ (0–0.5)	100–130
Donor 5	.33	.024	7.0	−.52	−1.4	2.0–2.9	−1.3	+(0.7–1.6)	150–210
Donor 6	.31	.020	6.5	−.39	−1.3	1.5–2.2	−1.3	+(0.2–0.9)	115–170

[a] The rate of change of V_T was taken as linear between 1–6 sec (see Figure 3). V_T was measured at 1 sec, 3 sec, and 6 sec, except for donor 2 (3 sec and 6 sec) and donor 3 (2 sec, 4 sec, 6 sec).

[b] Variability of measured rates of decrease of %T was ± 10%.

[c] Calculated for N equal to 200,000 μl^{-1} and 400,000 μl^{-1}.

[d] Donors 1 and 2 are same as in Figure 4.

for 5 donors) suggesting that the total decrease in %T that should be expected at 40 sec is ~6.7%. This suggests that the observed decrease in %T at 40 sec is >25% smaller than would be predicted on the basis of the changes in R (%T has increased by ~2.7% by 40 sec, i.e., 1.7% plus the observed 1% increase in %T). Latimer et al. (1977) observed a similar discrepancy and suggested that it represented the existence of an additional process affecting %T which produced increases in %T.

There is little significant change in V_T between 10–40 sec (see Figure 5b). Thus, the observation that the total decrease in %T is too small cannot be accounted for by changes in V_T.

From Figure 5a it is clear that at times greater than 10 sec there is a progressive increase in more sphered platelets, i.e., SE and advanced DE. These morphologies are typically associated with increased numbers of pseudopods (Frojmovic and Mil-

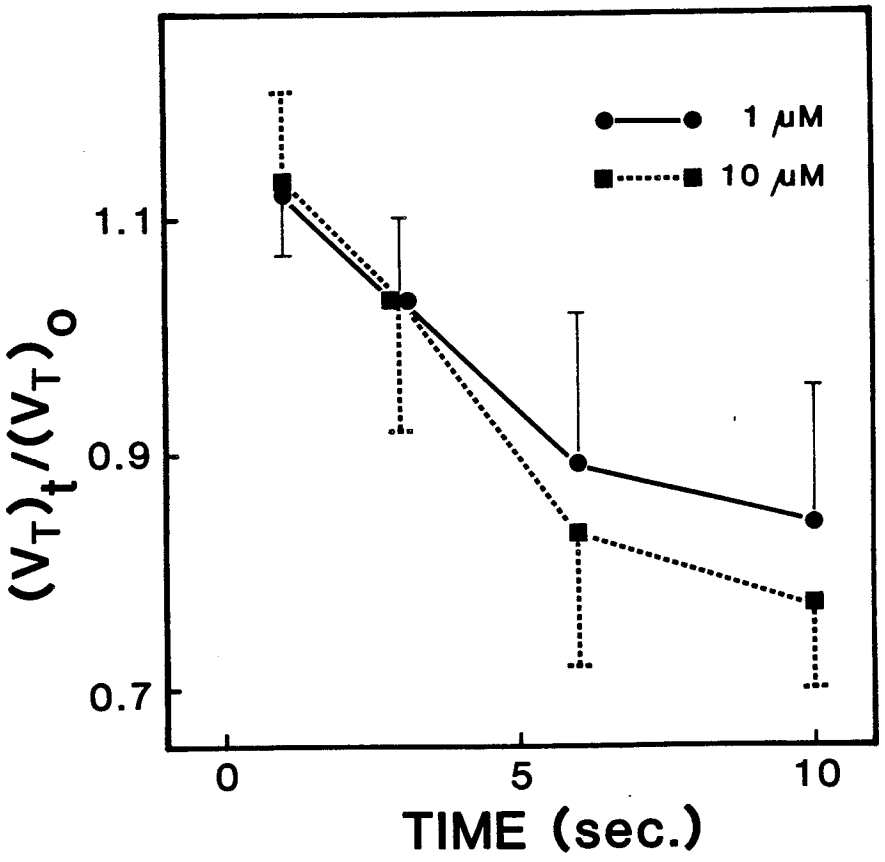

FIGURE 3. Changes in platelet mean volume (V_T) over the first 10 sec following addition of 1 μM ADP and 10 μM ADP. V_T measured at any given time $(V_T)_t$ has been normalized to that obtained prior to addition of ADP, $(V_T)_o$. V_T has been determined from equation (2). Data for 1 μM ADP (n = 5) and 10 μM ADP (n = 3) is represented as mean ± 1 SD.

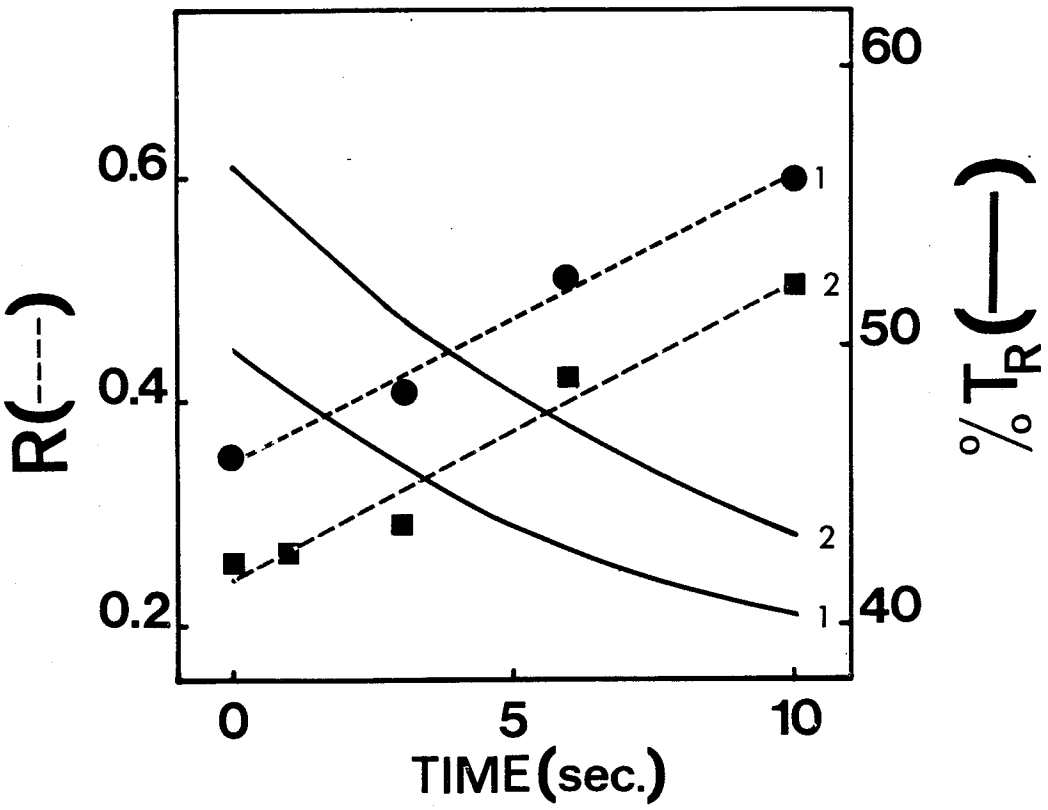

FIGURE 4. Platelet mean axial ratio (R) and relative transmittance (T_R) as a function of time following addition of 1 μM ADP to PRP for 2 donors shown in Figure 3. A linear regression analysis was used to fit the relation for R. Values for the relative extinction as a function of R (from linear regression analysis) were taken from Figure 4 in Latimer et al. (1977) ("stirred line") and T_R calculated as described in text. Values for the initial rate of change of T_R, dT_R/dt, are reported in Table 1.

ton, 1982). Thus, one explanation for why the observed change in %T at 40 sec is too small is that pseudopod formation is associated with an increase in %T. This latter conclusion was also suggested by Latimer et al. (1977). An alternate explanation is that this discrepancy reflects a change in platelet refractive index independent of pseudopod formation (Frojmovic, 1978; Lindner et al., 1977).

In the first 3 sec post-ADP addition, very few platelets (≤10–20%) acquire 1–3 long pseudopods and the dominant morphological change in increased membrane roughness (Milton et al., 1980), presumably reflecting the formation of short blunt pseudopods. If this pseudopod formation is also associated with an increase in %T, then the discrepancy between the observed rate of change of %T and that predicted from changes in R and V_T would be greater than was apparent in section C.

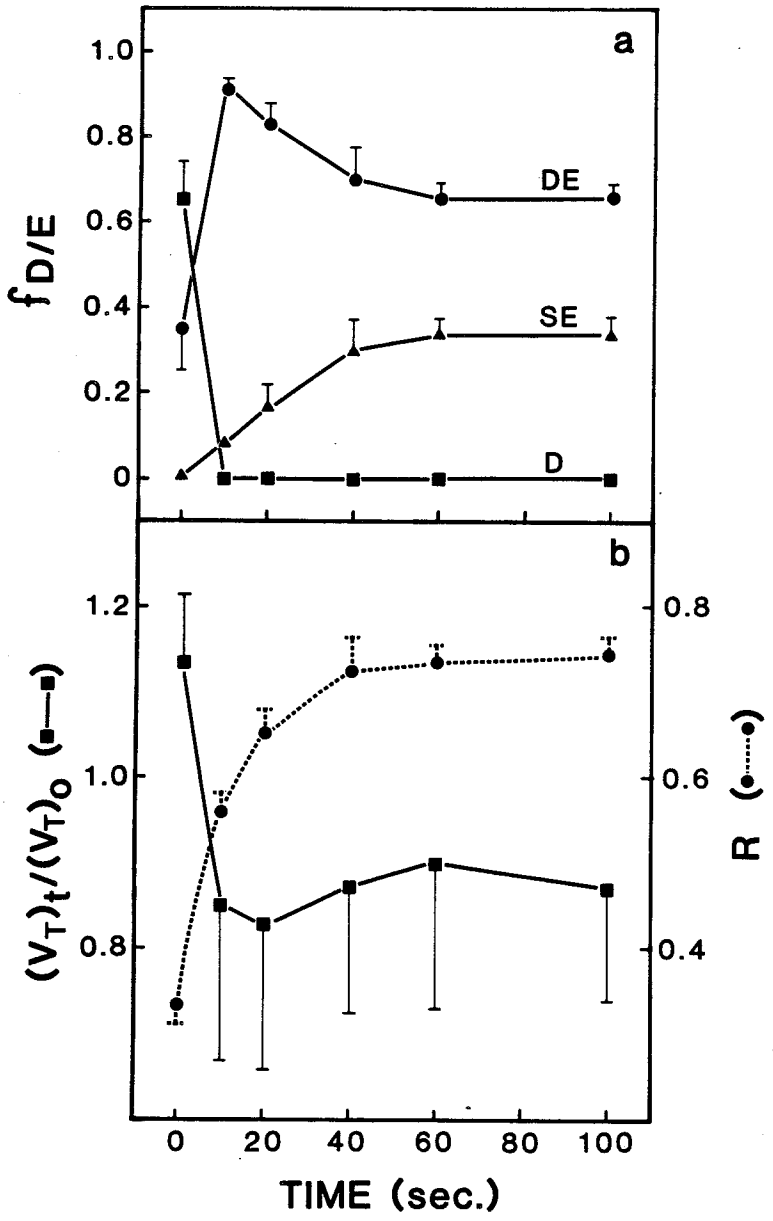

FIGURE 5. Comparison of a) the time course of the change in platelet morphology with b) the time course for platelet mean volume (V_T) and mean axial ratio (R), following addition of 10 μM ADP to PRP. R was determined from equation (2). $f_{D/E}$ equals the fraction of platelets present as discocytes (D), and disco (DE)- or sphero (SE)-echinocytes. All values are for 4 donors and are represented as mean ± 1 SD.

DISCUSSION

We have examined the relative contribution of platelet axial ratio (R), mean platelet volume (V_T), pseudopod formation, and aggregation to the initial change in %T following addition of ADP to stirred platelet suspensions. The initial rate of decrease of %T appears very similar to that predicted from the changes in R. However, when the changes in %T due to changes in V_T are also considered, it is found that the observed rate of decrease in %T is 20–210% larger than would be expected from the changes in R and V_T. These results suggest the presence of an additional process which produces a decrease in %T. In contrast to the prediction of Kitek and Breddin (1980), platelet aggregation has no detectable influence on the initial changes in %T ($t \simeq 3$–5 sec). Moreover, pseudopod formation is predicted to produce an increase in %T (Latimer et al., 1977). In view of these observations, we suggest that the most likely process which produces the initial additional decrease in %T is related to changes in platelet refractive index, N_c. At later times ($t \geq 10$ sec) %T is more positive than would be expected in view of the changes in R or V_T. This may reflect a %T increase associated with pseudopod formation and/or changes in N_c.

Changes in %T are inversely related to changes in N_c (Frojmovic, 1978; Lindner et al., 1977). Three possible mechanisms which can alter N_c are: 1) influx/efflux of water (Gear, 1981), 2) evagination of internalized platelet membrane occurring during platelet shape change (Frojmovic and Milton, 1982; Milton and Frojmovic, 1979b; Morgenstern and Kho, 1977), and 3) alterations in the intrinsic properties of the membranes (Frojmovic and Milton, 1982). In the case that volume changes are solely related to the flux of water, it has been predicted that N_c and V_T are inversely related (Latimer and Pyle, 1972). Gear (1981) has suggested that the rapid initial increase in V_T arises from an influx of water in response to changes in ionic gradients. Since the increase in V_T occurs within 1 sec (see Figure 3), there should be an initial increase in %T occurring at this time. With our present apparatus and techniques we are unable to reliably measure %T changes that occur within 1 sec due to %T artifacts introduced during ADP addition. However, back extrapolation of the changes in %T to zero time (see Figure 2) does seem to suggest the possibility of a %T increase. Thereafter, the decrease in V_T would increase N_c, and hence decrease %T.

The initial increase in V_T which occurs with little change in R (compare Figures 3 and 4) implies an increase in surface area. It has been predicted that an evagination of internalized platelet membranes occurs during platelet shape change (Frojmovic and Milton, 1982; Morgenstern and Kho, 1977). It is possible that the subsequent decrease in V_T is associated with the rearrangement of this newly evaginated platelet membrane into the more complex morphologies associated with DE and SE. The influence of these membrane rearrangements on N_c is unknown.

Our observations for the aggregometer indicate that early platelet aggregation has no effect on %T until the percent nonaggregated platelets is 40%, and thereafter results in an increase in %T. This observation is in contrast with two previous predictions. Chang and Robertson (1976) have shown that aggregation of spherical

particles by laminar flow and Brownian motion leads to a rapid increase in %T, reflecting the fact that the scattering cross section of N aggregated particles is less than that for the individual N particles (50% reduction on doublet formation). On the other hand, Kitek and Breddin (1980) have observed a decrease in %T for ristocetin-induced agglutination of formalin-fixed platelets and have suggested that similar changes might occur for early platelet aggregation induced by ADP. Recently, Latimer and Wamble (1982) have predicted, with an anomalous diffraction theory, that the direction of the changes in %T associated with early platelet aggregation, i.e., $\leq$10 platelets per aggregate, is a function of the optical geometry of the instrument used to measure %T. However, the differences between our observations and those of Latimer and Wamble cannot be solely due to differences in instrumentation. Although a laser beam rheo-aggregometer showed a greater sensitivity for detecting early platelet aggregation, only increases in %T were observed (Yung and Frojmovic, 1982).

Both microscopic (Figures 3 and 5) and resistive particle counter (Gear, 1981) measurements indicate that there is an early increase in V_T following ADP addition. However, the resistive counter measurements suggest that this increase in V_T is maintained for at least 2 minutes, whereas microscopic methods suggest that V_T decreases over this time interval (Figures 3 and 4b). The discrepancy between these two measurements of V_T is a consequence of the fact that the physical parameter measured by the resistive particle counter is electrical size. Electrical size is equal to a shape factor times platelet volume; the shape factor is a function of the platelet r_p and ranges from 1.20 for a D with $r_p = 0.3$ (average value for D), to 1.48 for a sphere with $r_p = 0.95$ (average r_p for SE) (Laufer et al., 1979). The increase in R, and hence the shape factor, largely offsets the V_T decrease, and consequently means that electrical size is little affected. Thus, resistive particle counter measurements would not be expected to show the decrease in V_T shown in Figure 5b.

The effects of V_T changes on %T observed here are opposite to those predicted from theory (Latimer, 1975a; Latimer and Pyle, 1972; Latimer et al., 1968). For example, Latimer (1975a) predicts that a 1% decrease in V_T should decrease %T by 0.3% and, moreover, that this effect should be even larger when %T is measured with instruments with poorly collimated optical geometries, e.g. aggregometer (Latimer, 1975b). Our calculations (see Appendix) show that a decrease in V_T should produce an increase in %T. This difference largely arises because in the theoretical treatments, changes in V_T are mathematically equated with changes in N_c, whereas in our measurements (i.e. calculation of dk/dV_T) there is essentially no change in N_c occurring with the change in V_T.

Changes in %T are routinely used to monitor changes in platelet shape (Frojmovic, 1978; Frojmovic and Milton, 1982). It is clear that changes in R, V_T, N_c, and pseudopod formation are reflected by changes in %T. However, it is not certain whether changes in N_c are invariably associated with changes in platelet shape. In view of this observation, we suggest that predictions concerning changes in platelet shape arising from interpretation of changes in %T should be confirmed by direct examination of platelet morphology by the appropriate microscopic method, as reported for a correlative study between initial rate of %T decrease and rate of dis-

cocyte disappearance for ADP treatment of PRP (Milton et al., 1980). Finally, early platelet aggregation is more appropriately studied by monitoring the disappearance of nonaggregated platelets, either by the use of a hemocytometer (Born and Hume, 1967; Milton et al., 1981a; Siversten, 1976), or possibly with a suitable adaptation of resistive particle counters (Benner et al., 1980; Lumley and Humphrey, 1981; Gear and Lambrecht, 1981; Gear, 1982).

APPENDIX

Determination of Effect of Changes of V_T on %T

It has been shown that for platelets (Frojmovic and Panjwani, 1975; Latimer, 1975b)

$$\%T = 100 \exp(-Ba_s^2) \tag{A-1}$$

where

$$B = \Pi NLK,\ a_s = \left(\frac{3V_T}{4\Pi}\right)^{1/3}$$

and a_s is the radius (cm) of a sphere with volume V_T, k is the particle optical efficiency, L is the light path length of the cuvette, and N is the platelet concentration (number cm^{-3}). The effect of changes in V_T on %T is given by

$$\frac{d\%T}{dV_T} = -100\frac{B}{k}\left\{\frac{k}{2\Pi}a_s^{-1} + a_s^2\frac{dk}{dV_T}\right\}\exp(-Ba_s^2) \tag{A-2}$$

where the dependence of the particle optical efficiency on V_T is dk/dV_T.

From equation (A-2) it follows that determination of $d\%T/dV_T$ requires an evaluation of V_T, k, and dk/dV_T. k can be evaluated from the slope of a plot of log %T versus N (slope is equal to k/0.434 AL where A is the projected surface area, $A = \Pi a_s^2$). For a well-stirred platelet suspension in an aggregometer it is found that for human platelets (V_T 6.9 μm^3) k is 0.054 and for rabbit platelets (V_T 4.1 μm^3) k is 0.043 (see Figure 2 in Frojmovic and Panjwani, 1975). Assuming that the differences in k arise solely from differences in V_T (that is, for example, that the refractive indices of rabbit and human platelets are similar), it can be seen using these values for k and V_T that dk/dV_T is small (0.0036 μm^{-3}) and positive. The values for dT/dV_T shown in Table 1 have been calculated from equation (2) using an average value of k of 0.05, the above estimate of dk/dV_T, and measured values of N, L, and V_T.

The influence of the changes in V_T on %T as a function of time, d%T/dt, was determined from the relation

$$\frac{d\%T}{dt} = \frac{dT}{dV_T}\cdot\frac{dV_T}{dt} \tag{A-3}$$

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