Alteration in Optical Density of Platelets Exposed to Hypertonic Solutions[1] (37477)

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When cells are introduced to a new osmotic environment, there may be an initial change in light scattering which has been ascribed to an alteration in cell volume (1–3) or conformation (4). Lovett (5) reported that bacteria exposed to urea underwent initial osmotic dehydration which resulted in a transient increase in optical density (OD). Following penetration by urea, osmotic equilibrium was regained and OD decreased to a point slightly below base line because of dilution. Exposure to sodium chloride, a nonpenetrating solute, produced a sustained increase in light scattering associated with the osmotic gradient to which the bacteria were exposed. Similar effects were reported by Fantl (2) and by Davis *et al.* (3) who found a transient increase in the OD of platelet-rich plasma (PRP) upon exposure to urea, while platelets exposed to sodium chloride or potassium chloride produced a sustained increase in OD. It was concluded that the transient or sustained increase in OD was due to osmotic shrinkage.

Similar conclusions have also been adopted in the analysis of the reverse phenomenon, *i.e.*, the abrupt change in OD followed by a return to base line values when platelets were exposed to hypotonic media. Many investigators (6–8) are using this observation as an assay of platelet function *in vitro* and are assuming that the initial alteration reflects platelet swelling and the return to base line represents the extrusion of water.

However, in all the references cited above no direct evidence has been presented to confirm that osmotic shrinkage is the cause of the increased OD. Since, in the case of hypotonic shock there is always a return to base line, we have chosen to investigate the optical response of platelets exposed to hypertonic stress. In this manner both permanent and temporary changes in OD can be obtained.

Materials and Methods. Units of platelet-rich plasma (PRP) were obtained from routine blood donations. The blood was collected in ACD (1:9) and centrifuged at 3000*g* for 90 sec at 10° to obtain PRP. Experiments were performed at room temperature and as soon as possible following collection (*i.e.*, 2–4 hr). Changes in optical density (*i.e.*, transmittance) were read in a Chrono-Log aggregometer (Chrono-Log Corp., Broomall, PA) and relayed to a Heath strip chart recorder (Model EV-20B) with a sensitivity of 10 mV.

One milliliter of PRP was placed in a test tube which contained a small magnetic stirring bar. The test tube was placed in the aggregometer and the contents were stirred at 1100 rpm. Solutions of sorbitol, urea, DMSO, ethylene glycol, NaCl, and KCl were made up in deionized distilled water and their osmolalities, which were determined by freezing-point depression, were adjusted by dilution. Fifty microliter quantities of solution were then added to the sample and the reaction was recorded.

Platelet volume was measured according to the method of Lundberg, Meryman and Estwick (9). Briefly, identical plastic tubes were filled with the sample, centrifuged at 40,000*g* for 4 min and the resulting packed volume was measured. This volume was corrected for trapped plasma on the basis of earlier

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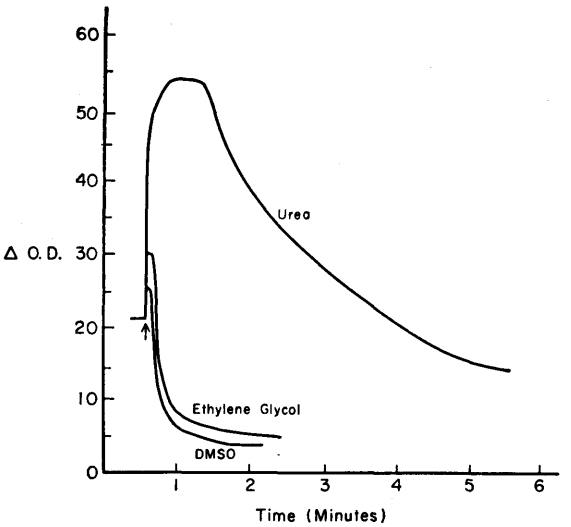

FIG. 1. Superimposed tracings showing change in OD following addition of penetrating compounds to platelet-rich plasma. Final osmolality with DMSO was 437 mOsm; ethylene glycol, 418 mOsm; and urea, 375 mOsm. Units of OD were arbitrarily assigned. Arrow indicates addition of chemical.

determinations with the same solutes [(9), unpublished data]. The sensitivity of the volume measurement is such that it will detect a volume difference of 3–5% between samples.

Results. The results of the experiments described below represent one of at least four experiments performed in each group. Figure 1 shows that when either urea, DMSO, or ethylene glycol (all nearly equal in osmolality) was added to platelets, there was a rapid but transient increase in OD. The initial increase, however, varied for each agent. The return of the tracing to below base line suggests cellular penetration by these agents as well as sample dilution. Figure 2 illustrates the change in OD with addition to PRP of approximately equiosmolal solutions of two nonpenetrating salts and a nonpenetrating sugar alcohol. Although under these conditions the increase in OD is permanent, the extent of the change is not directly related to osmolality. Furthermore, platelet volume following exposure to KCl or sorbitol was identical, indicating that osmotic shrinkage was not directly related to the increase in OD. In none of these experiments was there any evidence of platelet aggregation. Table I summarizes the data concerning the addition of either NaCl, KCl or sorbitol to PRP. The change in platelet volume after exposure to sorbitol was not significantly different ($p > 0.05$) from that of the other two compounds. However, the change in OD caused by either salt was significantly greater ($p < 0.01$) than that of sorbitol. Figure 3 depicts OD changes when samples of PRP were exposed to KCl or NaCl (final osmolality of 423 and 403, respectively) and to a 50:50 mixture of both with an osmolality of 410 mOsm. Once again, the increase in OD was not proportional to solution osmolality. In other experiments a correlation between osmolality and OD change could not be made, however, there was a linear relationship between osmolality and volume when the osmolality of the solution was between 300–600 mOsm.

Discussion. These experiments confirm earlier studies which showed an increase in OD when hypertonic solutions were added to PRP. However, the extent of the increase in OD was different for each agent, electrolyte or nonelectrolyte, even though the solution osmolalities were nearly identical. Furthermore, the fact that the resulting volumes of platelets exposed to osmotically similar solution of KCl, NaCl or sorbitol were identical

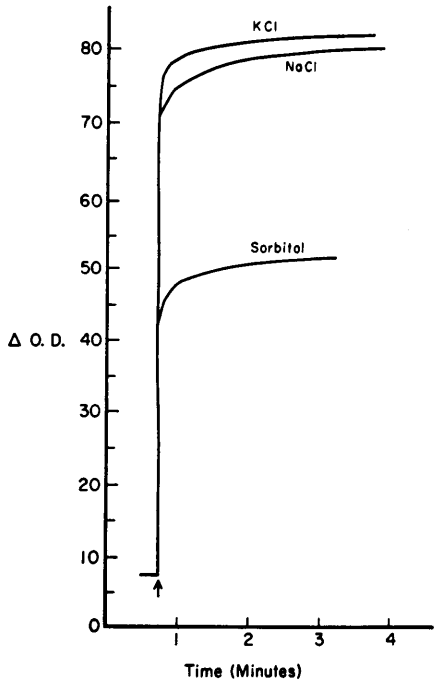

FIG. 2. Superimposed tracings showing change in OD following addition of sorbitol, NaCl, or KCl to platelet-rich plasma. Final osmolality was between 383 and 423 mOsm. Units of OD were arbitrarily assigned. Volume measurements made before and after addition of sorbitol and KCl indicated a volume reduction of 12.5% with both agents.

is further evidence that osmotic dehydration was not the sole cause of the increase in OD. One explanation for these observations is that the increase in OD may have been due in part to an alteration of the refractive index of the platelet–liquid mixture, which varied depending on the agent introduced. Since the phenomenon of light scattering occurs when the refractive index of two components of a system (*e.g.*, platelets and plasma) is different, an alteration in the refractive index of either component (*e.g.*, the plasma) will also alter the degree of light scattering. The relative speed with which the new equilibrium values were reached may reflect the relative speed of penetration of the compounds involved. Alternatively, specific platelet configurations or changes in internal structure

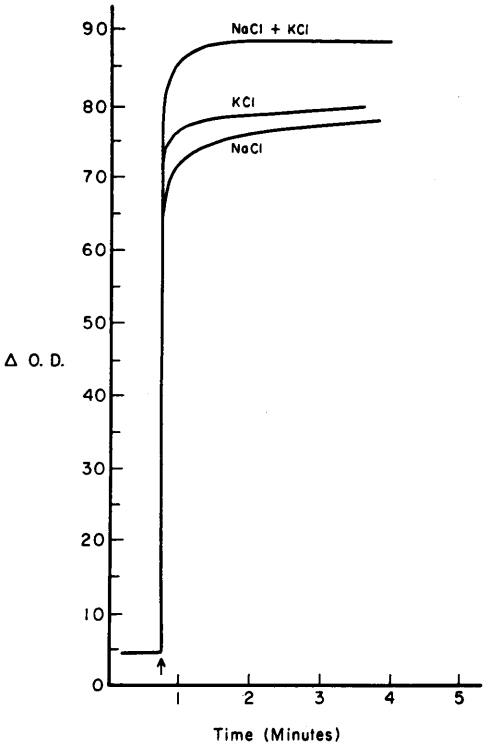

FIG. 3. Superimposed tracings showing change in OD following the addition of hypertonic NaCl or KCl and a 50:50 mixture of each to platelet-rich plasma. Note that the combination of the two salts resulted in a greater increase in OD with no concomitant increase in osmolality.

TABLE I. The Effects of Either NaCl, KCl or Sorbitol on Platelet Volume and Optical Density.

Compound added	No. of expts	Osmolality (mOsm ± SD)	ΔOD ± SD[a]	ΔVol ± SD[b] (%)
KCl	8	410 ± 18.4	56 ± 24	18.9 ± 2.5
NaCl	7	402 ± 2.2	54 ± 20	24 ± 5.2
Sorbitol	10	395 ± 5.4	34 ± 11	20.3 ± 3.1

[a] Units of OD were arbitrarily assigned.

[b] Volume is expressed as a percentage reduction from an isotonic control sample.

dependent on the nature of the suspending solution could be responsible for the different optical densities observed, although this seems unlikely considering the compounds used.

The observations reported here may also extend to the change in OD following hypotonic shock. Lundberg, Meryman and Estwick (9) found that platelet volume remained unchanged until the osmolality of the solution became very hypotonic (150 mOsm), yet investigators measuring OD changes in response to hypotonic shock always stay above this osmolality. Therefore, it is possible that the changes in OD produced by hypotonic shock may also reflect changes in solution refractive index and not simply changes in platelet volume.

Summary. Following addition of several compounds to platelet-rich plasma, there was an initial increase in optical density which may or may not be permanent. The increase varied from compound to compound and was unrelated to the osmolality of the medium as well as the resulting platelet volume. It is postulated that the change in OD may not be due entirely to osmotic dehydration as suggested by others, but to alterations in the refractive index of the platelet suspension.

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1. Mitchell, P., Bacterial Anatomy. Symp. Soc. Gen. Microbiol. **6**, 150 (1956).

2. Fantl, P., Aust. J. Exp. Biol. Med. Sci. **44**, 451 (1966).

3. Davis, J. W., McField, J. R., Phillips, P. E., and Graham, B. A., Blood **39**, 388 (1972).

4. Latimer, P., Moore, D. M., and Bryant, D. F., J. Theoret. Biol. **21**, 348 (1968).

5. Lovett, S., Proc. Soc. Exp. Biol. Med. **120**, 565 (1965).

6. Handin, R. I., Fortier, N. L., and Valeri, C. R., Transfusion **10**, 305 (1970).

7. Hardeman, M. R., Meer, J., and Vreeken, J., Abstr. Congr. Int. Soc. Thromb. Haem. 3rd, Washington, DC., p. 202 (1972).

8. Kim, B. K., and Baldini, M. G., Proc. Soc. Exp. Biol. Med. **142**, 345 (1973).

9. Lundberg, A., Meryman, H. T., and Estwick, N., Amer. J. Physiol. **222**, 1100 (1972).

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