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OSMOTIC STABILITY OF BLOOD PLATELETS

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SUMMARY

1. Hypotonic solutions added to human platelet-containing plasma cause a transient decrease of absorbancy of light at 610 mμ which is followed by a gradual increase of absorbancy.

2. When platelets are stored for 7 hr at 4° C the absorbancy changes with variations of osmolarity and their aggregation with adenosine diphosphate (ADP) remain the same. However, the reversal of absorbancy declines during storage of platelet-containing plasma.

3. Platelets are not aggregated by stearate. Platelets appear to be only slightly affected by stearate concentration higher than 0·8 mM, but oleate has no effect.

4. Hypertonic solutions of NaCl and urea cause increase in absorbancy of platelet-containing human plasma. Hypertonic sucrose solutions produce no more change than isotonic solutions. Hypertonic NaCl produces permanent increases in absorbancy. In human platelet-containing plasma the increased absorbancy caused by hypertonic urea is transient and declines.

5. The osmotic platelet changes occur in isolated platelets as well as in platelet-containing plasma.

6. The absorbancy of frozen and thawed platelet-containing plasma is not significantly altered by hypotonic solutions but the absorbancy changes caused by hypertonic solutions are similar to that of unfrozen plasma.

7. The immediate absorbancy changes caused by hypo- and by hypertonic solutions are the same at 5° C and 30° C and are therefore probably of a physical nature. The reversal of absorbancy and aggregation of platelets by added adenosine diphosphate have $Q_{10} > 1$ and are therefore probably of a chemical-enzymic nature.

8. Divalent cations and contact activation are not required for the osmotic platelet changes and 10^{-3} M-Cu^{2+} and Zn^{2+} do not interfere. Inhibitors of oxidative phosphorylation, electron transfer, sodium, potassium activated adenosine triphosphatases and adenosine triphosphate do

not inhibit reversal of absorbancy of platelets exposed to hypotonic solutions. Cyanide, 5×10^{-3} M, fluoride, $1{\cdot}23 \times 10^{-2}$ M, iodoacetamide, 10^{-2} M, are moderately effective inhibitors. At hydrogen ion concentrations above pH 8, complete inhibition occurs.

9. N-ethyl-maleimide, 10^{-3} M, and mercloran inhibit completely reversal of absorbancy, indicating the necessity for sulphydryl compounds.

10. Intact platelets are essential for the reversal of absorbancy after hypotonic swelling. Osmotic changes by hypotonic solutions are independent of ADP aggregation of platelets.

INTRODUCTION

In this study, platelet changes caused by variations of osmolarity of platelet-containing human plasma were measured by light absorbancy and morphology. The results give some indication of platelet structure and the technique could be useful for the detection of differences of platelet activity in experimental and clinical conditions. It can also be applied to the characterization of platelets in different species.

METHODS

Blood was taken from healthy adult volunteers from an arm vein, by a two-syringe technique. One volume 0·13 M trisodium citrate solution or 1 vol. of a solution containing 0·027 M disodium ethylene-diaminetetra-acetate (EDTA) and 0·12 M sodium chloride was mixed with 9 vol. of blood. Platelet-containing plasma was obtained from blood centrifuged at 190 *g* for 10–15 min. Platelets were isolated from 10 ml. EDTA plasma by centrifuging at 2000 *g* for 25 min. The plasma was removed and the sediment suspended in 50 ml. 0·154 M-NaCl containing 0·0027 M EDTA and centrifuged at 2000 *g*. The sediment was then resuspended in veronal buffer containing 0·0027 M EDTA, pH 7·4. Centrifuging and storage of blood, plasma and platelets was carried out at 4° C.

Platelets were counted by the technique of Feissly & Lüdin (1949). Light absorbancy was measured in a Beckman DU spectrophotometer at 610 mμ in quartz cells of 1 cm light path using 2 ml. platelet-containing plasma and additions as indicated in Tables and Figures. The readings were compared with those obtained from mixtures of platelet-containing plasma and 0·154 M-NaCl. All specimens were read against 0·154 M-NaCl. The cell compartment was kept for most experiments at 30° C and occasionally at 5° C, covered with 'Parafilm' and inverted 6 times immediately before each reading. All solutions were freshly prepared with glass distilled water. The concentrations are given in m-osmoles. This was determined for plasma with an osmometer (Advanced Instrument Inc. Mass., U.S.A.).

The osmolarity of mixtures was calculated from $\Sigma vC/\Sigma v$, where v is the volume, and C the osmotic concentration (m-osmole/l.), of each component. C may be the measured osmotic concentration, or the osmotic concentration calculated from the molarity and osmotic coefficient (from Garner, 1928), e.g. 2 ml. of citrated plasma and 1 ml. of 0·03 M-NaCl when combined have a calculated osmotic concentration of

$$\frac{2 \times 302 + 2 \times 30 \times 0{\cdot}965}{3} = 221 \text{ m-osmole/l.}$$

The pH of the mixture was between 7·2 and 7·8 except where stated otherwise.

Fatty acids were suspended in sufficient NaOH for 75–78 % neutralization. The suspensions were warmed until all particles were dissolved and 1 vol. was mixed with 1 vol. veronal buffer at 37° C to give a pH of 7·8 and 1 vol. of this mixture was added to either 10 vol. distilled water or 10 vol. 0·154 M-NaCl at 37° C. These suspensions, because of their instability, were used immediately after preparation. The experiments were carried out as follows: to 2 ml. of citrated or EDTA plasma containing $35–80 \times 10^4$ platelets/mm^3 and stored between 70 min and 3 hr at 4° C, was added 1–1·4 ml. water or aqueous solution to obtain the desired osmolarity. The absorbancy of these mixtures was measured against time. When four or more repetitive experiments were carried out as in Figs. 1–4, from the absorbancies measured at 2, 5, 10 and 15 min, S.E. of the mean was calculated and the t test applied. In Figs. 5–10 and Tables 1 and 2, two or three repetitive experiments were carried out. The variation from the mean was not greater than ± 5 %.

The abbreviations used are: Pl.c. plasma for platelet-containing plasma; ADP for disodium adenosine-5-diphosphate; ATP for disodium adenosine-5-triphosphate; NEM for N-ethyl maleimide; mercloran for 3-chloromercuri 2-methoxypropyl urea; A_{610} for absorbancy at 610 mμ.

RESULTS

Influence of hypotonic solutions on absorbancy of platelet-containing plasma. Pl.c. plasma was diluted with solutions of differing osmolarity and the change in absorbancy was measured. Results are given in Fig. 1, where it is seen that addition of isotonic NaCl (154 mM = 286 m-osmoles) produced little change in A_{610} during 15 min. By lowering the osmolarity a drop in A_{610} occurred which was followed by a gradual rise. Hypotonicity of comparable osmolarity produced by several inorganic salts ($MgCl_2$, $SrCl_2$ and PO_4^{3-}) gave similar reductions of absorbancy. The absorbancy of the hypotonic pl.c. plasma could be increased to its initial value by restoring the isotonicity with NaCl (see Figs. 9 and 10).

Influence of fatty acids on absorbancy of platelet-containing plasma. In other experiments, suspensions of sodium stearate-stearic acid or sodium oleate-oleic acid were added to human plasma at various osmolarities. In Fig. 2 are recorded experiments in which the absorbancy of a mixture of 0·37 mM stearate and human pl.c. plasma was determined. Aqueous stearate suspensions resulting in 201 m-osmoles produced a reduction in absorbancy very similar to that obtained with distilled water. Addition of stearate suspended in 0·154 M-NaCl resulting in 296 m-osmoles produced a similar reduction of absorbancy than 0·154 M-NaCl alone.

In circulating blood, non-esterified fatty acids are not present in the free form but are bound in the albumin and lipoprotein fractions. The effect of an albumin stearate complex on absorbancy of pl.c. plasma was, however, not greatly different from that of inorganic solutions of the same osmolarity. In Fig. 3 are recorded experiments in which 0·82 mM stearate was present in the mixture. At this stearate concentration there was still a drop in absorbancy when the osmolarity was lowered from 296 to 180 m-osmoles. However, at this stearate concentration the absorbancy was

initially higher at 296 m-osmoles than in the absence of stearate and it rapidly fell over 15 min when compared with suspensions in the absence of stearate.

In Fig. 4 it is seen that 0·9 mM oleate, whether suspended in water or 0·154 M-NaCl, or bound to albumin at 200 and 294 m-osmoles, had the same effect on absorbancy of pl.c. plasma as inorganic salts of equal osmolarity.

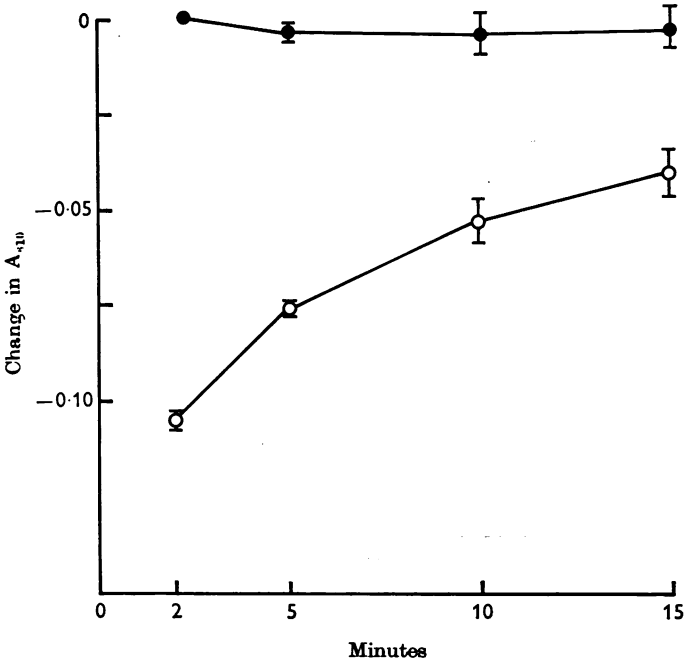

Fig. 1. Mixtures of pl.c. plasma and: NaCl, 294–296 m-osmoles –●—●–; H_2O, 200–204 m-moles –○—○–. Each point is the means of 10 experiments. The bars indicate the S.E. of the mean.

The influence of hypertonic solutions on absorbancy of platelet-containing plasma. Further experiments were carried out with hypertonic solutions. From Fig. 5 it is seen that while hypertonic solutions of NaCl caused the biggest increase in absorbancy, urea followed closely the NaCl effect; hypertonic sucrose had no greater effect on absorbancy than isotonic solutions.

The absorbancy caused by hypertonic NaCl in pl.c. plasma changed with time to the same degree as mixtures with isotonic NaCl. In contrast the increase in absorbancy caused by hypertonic urea was transient and declined during the next 15 min (see Fig. 7). A more detailed account of the influence of urea on platelets is given elsewhere (Fantl, 1966).

Influence of storage on platelet osmotic reactivity. Osmotic platelet changes occur only for a limited time. During storage the absorbancy of pl.c. plasma decreases; results with such plasma stored for 7 hr at 4° C are given in Fig. 6. The reactivity of platelets to ADP remained unaltered for more than 7 hr storage.

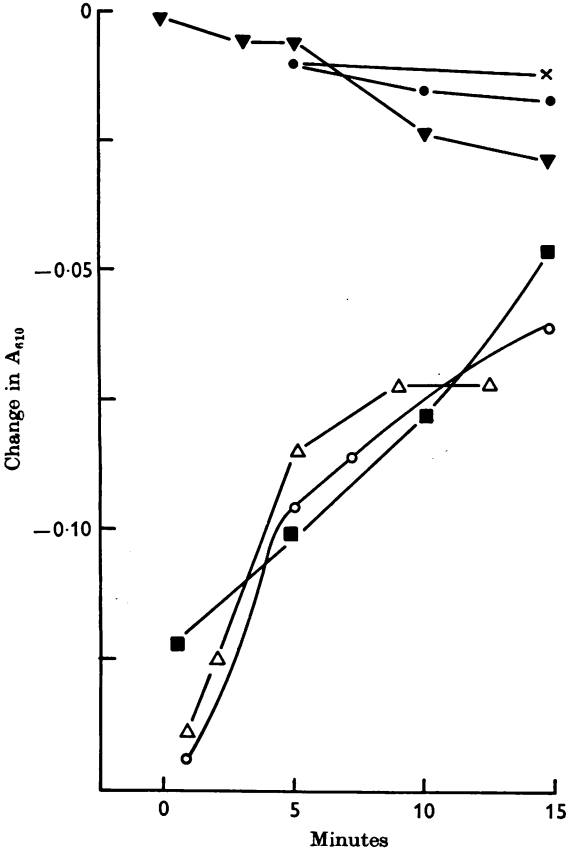

Fig. 2. Mixtures of pl.c. plasma and: NaCl, 296 m-osmoles −●—●−; 200 m-osmoles −○—○−; 0·37 mM stearate, 201 m-osmoles −△—△−; 0·37 mM stearate, 0·154 M-NaCl, 296 m-osmoles −▼—▼−; 0·37 mM stearate, 1·2% human cryst. albumin, 202 m-osmoles −■—■−; 0·37 M stearate, 0·154 M-NaCl, 1·2% human cryst. albumin, 297 m-osmoles −×——×−.

Whether plasma components influence the osmotic platelet changes was determined in isolated platelets as well as in pl.c. plasma. The results given in Fig. 7 show that platelets isolated and stored for 1 hr at 4° C reacted to 180 and 1000 m-osmoles in a similar manner to pl.c. plasma.

Platelets were disrupted by freezing and thawing of pl.c. plasma. The absorbancy of the thawed suspension was lower than the original pl.c.

plasma. Reduction from 294 to 180 m-osmoles caused little change in absorbancy. With hypertonic NaCl or urea the absorbancy of the thawed pl.c. plasma increased; the secondary decrease with urea occurred as in unfrozen plasma. Addition of ADP to frozen and thawed citrate pl.c. plasma did not alter the absorbancy.

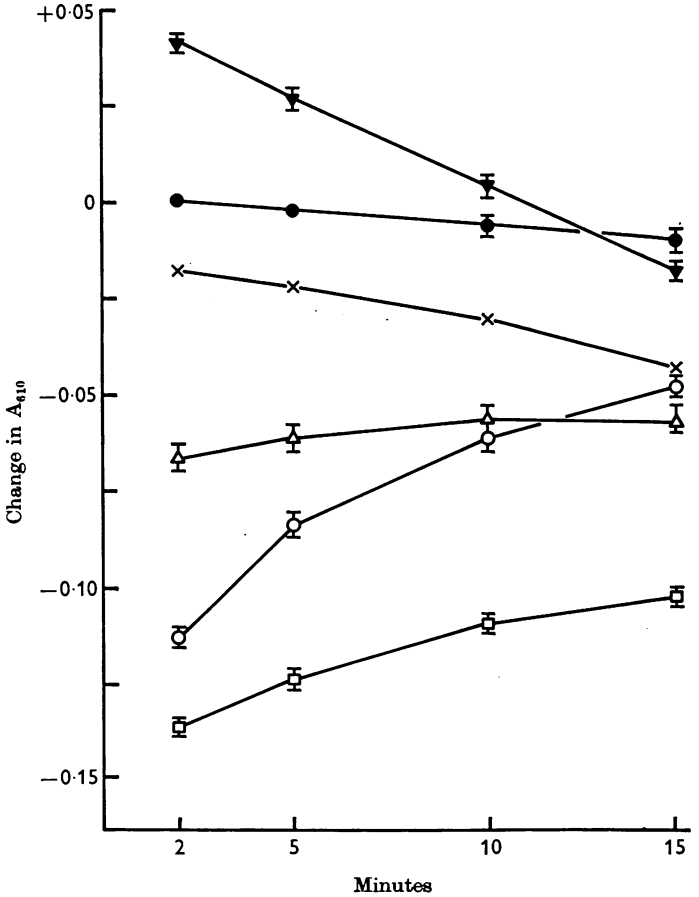

Fig. 3. Mixtures of pl.c. plasma and: NaCl, 296 m-osmoles –●—●–; H_2O, 180 m-osmoles –○—○–; 0·82 mM stearate, 0·154 M-NaCl, 296 m-osmoles –▼—▼–; 0·82 mM stearate, 1·3 % bovine serum albumin, 0·154 M NaCl, 296 m-osmoles –×—×–; 0·82 mM stearate, 180 m-osmoles –Δ—Δ–; 0·82 mM stearate, 1·3 % bovine serum albumin, 180 m-osmoles –□—□–.

The pronounced changes which platelets undergo during osmotic alterations of plasma made an investigation of the underlying mechanisms desirable.

Mechanism of osmotic platelet change. Mixtures with differing osmolarities

were observed at 5 and 30° C respectively. The results given in Table 1 show that osmotic platelet changes are connected with two processes: lowering the osmolarity from 297 to 201 m-osmoles produced the same drop in absorbancy at both temperatures. Therefore, the physical process of altering the osmolarity is probably not associated with a chemical

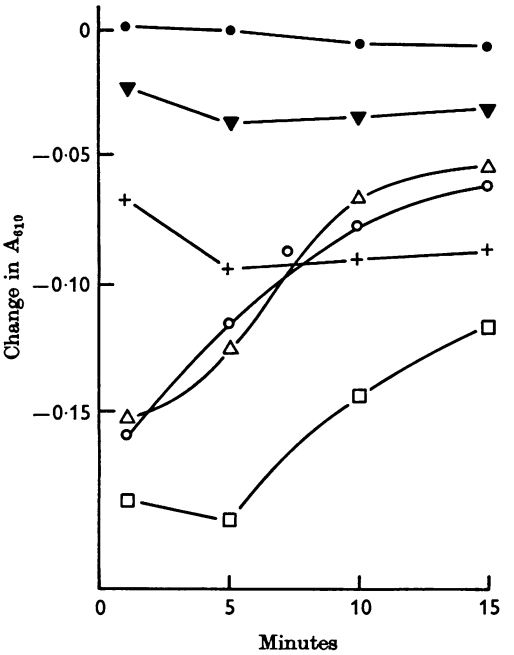

Fig. 4. Mixtures of pl.c. plasma and: NaCl, 294 m-osmoles -●—●-; H_2O 200 m-osmoles -○—○-; 0·9 mM oleate, 0·154 M-NaCl, 295 m-osmoles -▼—▼-; 0·90 mM oleate 201 m-osmoles -△—△-; 0·9 mM-oleate, 1 % human cryst. albumin, 0·154 M-NaCl, 295 m-osmoles +—+; 0·90 mM oleate, 1 % human cryst. albumin, 201 m-osmoles -□—□-.

reaction. The secondary increase of absorbancy of platelets exposed to hypotonic solutions and the secondary reduction of absorbancy of platelets exposed to hypertonic urea both have Q_{10} values calculated from Table 1 between 1·45 and 1·7. These platelet changes are therefore probably connected with chemical or enzymic reactions. The aggregation of platelets by added ADP at 304 m-osmoles had a Q_{10} of 1·75.

The Q_{10} values should not be considered as constants, because volume changes of particles in a suspension measured by light absorbancy are only approximate since there is not a linear relation between the apparent absorbancy of suspensions and number or particles; errors are also introduced from the clumping of platelets.

In order to find any connexion between osmotic and chemical changes experiments with inhibitors were carried out. From the results in Table 2 it is seen that the mechanism associated with the reversal of absorbancy which occurs when the osmolarity of pl.c. plasma is reduced from 296 to 200 m-osmoles does not require divalent cations for activity or contact activation, remains unaffected by 10^{-3} M-Cu^{2+} or Zn^{2+} ions and by in-

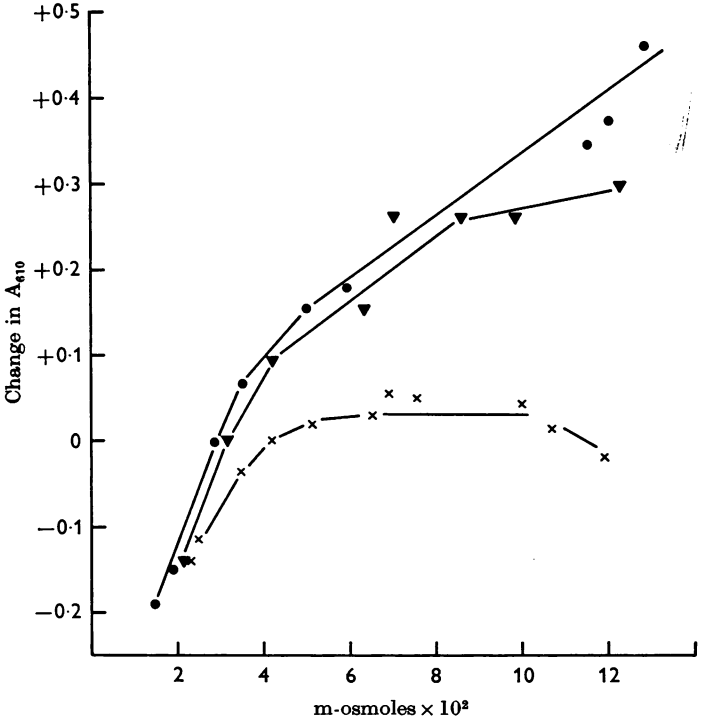

Fig. 5. Mixtures of pl.c. plasma and: NaCl, -●—●-; urea, -▼—▼-; sucrose, ×—×. A_{610} was determined 2 min after mixing. Temp. 30° C. Each point on the curves represents average results from 3 experiments.

hibitors of oxidative phosphorylation, electron transfer or by Na^+, K^+ activated ATPase. It is also independent of ADP activity. Cyanide and fluoride inhibited moderately, and considerable or almost complete inhibition occurred at pH > 8.

From experiments given in Fig. 8 it is seen that the reversal of absorbancy was only slightly reduced by 10^{-2} M iodoacetamide and not at all by 4×10^{-3} M ATP. However, the latter two compounds inhibited the platelet aggregation by added ADP.

In Fig. 9 and Fig. 10 are given results with sulphydryl inhibitors. It is seen that $2{\cdot}4 \times 10^{-3}$ M NEM (Fig. 9) and $3{\cdot}3 \times 10^{-3}$ M mercloran (Fig. 10),

inhibit completely reversal of absorbancy. Lower concentrations of these two inhibitors are less active but they still inhibit ADP platelet aggregation.

Morphological platelet change. Simultaneously with the photometric measurements microscopic observations of platelets were made in mixtures prepared as for light absorbancy. Whereas platelets treated with ADP

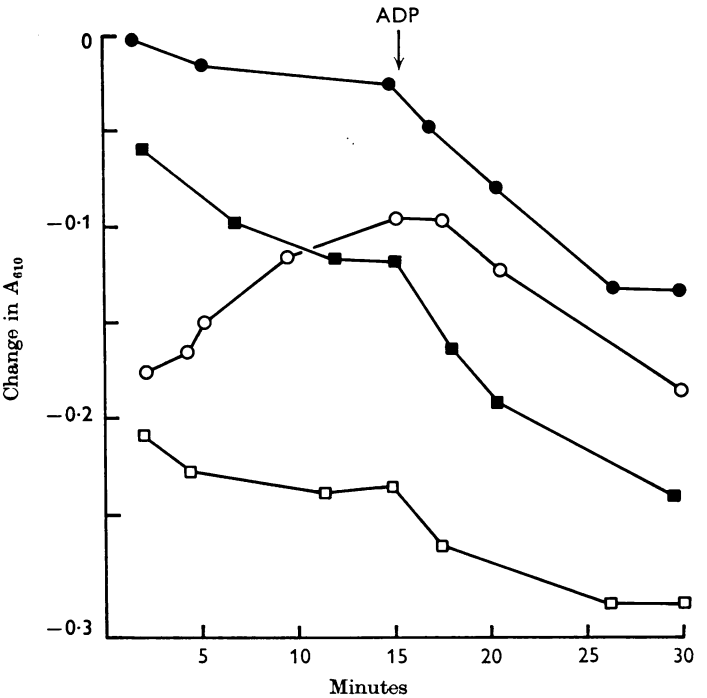

Fig. 6. Mixtures of pl.c. plasma 65 min after collection, stored at 4° C and: NaCl, 294 m-osmoles −●—●−; 200 m-osmoles −○—○−. Pl.c. plasma 7 hr after collection stored at 4° C and: NaCl, 294 m-osmoles −■—■−; 200 m-osmoles −□—□−. After 15 min indicated by the arrow 5·7 μg ADP in 0·2 ml. 0·154 M-NaCl was added.

aggregated (Gaarder, Hellem, Jonsen, Laland & Owren, 1961), this aggregation was considerably less or even absent with specimens treated with hypotonic solutions or stearate suspensions.

DISCUSSION

In microscopical studies Nelken, Gilboa-Garber & Gurevitch (1961) found that isolated human platelets disintegrated in hypotonic solutions in a characteristic manner but that the morphological changes were partly reversible when there was a return to isotonicity. Baserga & Ballerini

(1957) measured turbidity of suspensions of isolated platelets with a photometric technique and Rogner (1963) observed that platelets became more transparent in a hypotonic milieu.

In the present experiments hypotonic solutions of inorganic salts added to pl.c. human plasma produced a drop in absorbancy which was followed by a gradual rise if the pl.c. plasma was stored for no longer than 7 hr at 4° C. Under these conditions the bulk of the platelets remained as isolated bodies with very little clumping.

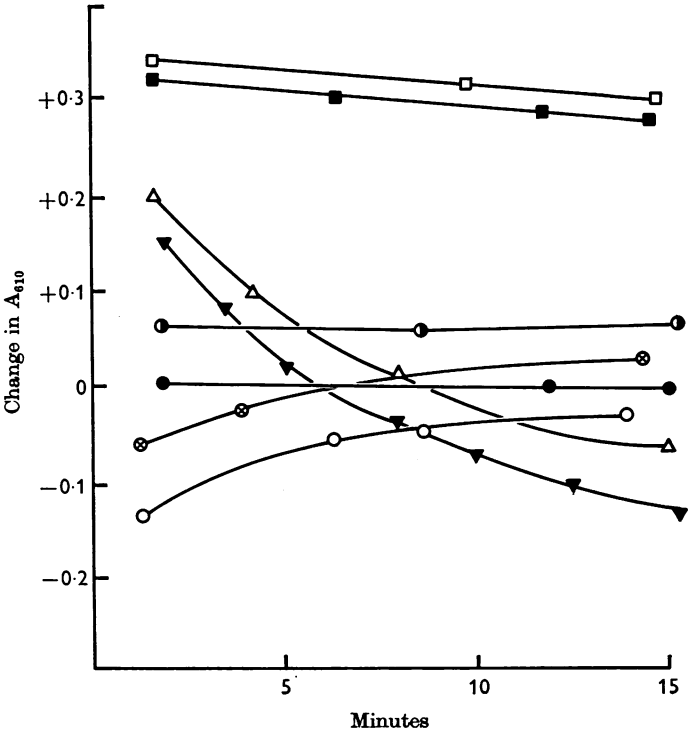

Fig. 7. Mixtures consisted of EDTA pl.c. plasma 60 min after collection stored at 4° C and : NaCl, 294 m-osmoles –●—●–; H_2O, 180 m-osmoles –○—○–; NaCl, 1000 m-osmoles –■—■–; urea, 1080 m-osmoles –▼—▼–. Isolated platelets and: NaCl, 295 m-osmoles –◑—◑–. H_2O, 180 m-osmoles –⊗—⊗–; NaCl, 1000 m-osmoles –□—□–; urea 1080 m-osmoles –△—△–.

Because it has been hypothesized that certain fatty acids can enhance platelet aggregation and so enhance thrombosis (Poole, 1958) experiments were carried out with stearate and oleate. Suspensions of 0·37 mM stearate produced the same optical changes in pl.c. plasma as hypotonic solutions of inorganic salts of similar osmolarity. Suspensions of 0·82 mM stearate were perhaps slightly toxic to the platelets. However, the imme-

diate drop in absorbancy of pl.c. plasma after addition of any of the hypotonic suspensions of fatty acids below 300 m-osmoles was unimpaired. Oleate (0·9 mM) had the same effect on pl.c. plasma as inorganic salts of equal osmolarity. Microscopical observations showed that 0·82 mM stearate,

TABLE 1. Temperature coefficients of platelet reactions

	201 m-osmoles		297 m-osmoles		868 m-osmoles	
Osmolarity	I	II	I	II	I	II
Temp. 5° C 0 min	0·613	0·497	0·771	0·649	1·070	0·898
Plus ADP, temp. 5° C 15–30 min	—	—	0·787	0·651	0·955	0·747
Temp. 30° C 0 min	0·591	0·472	0·750	0·630	1·040	0·858
Plus ADP, temp. 30° C 15–30 min	—	—	0·670	0·553	0·692	0·564
Difference in A_{610} between 297 and 201 m-osmoles temp. 5° C 0 min	−0·158	−0·152	—	—	—	—
Difference in A_{610} between 297 and 868 m-osmoles temp. 5° C 0 min	—	—	—	—	+0·299	+0·249
Difference in A_{610} between 297 and 201 m-osmoles temp. 30° C 0 min	−0·159	−0·158	—	—	—	—
Difference in A_{610} between 297 and 868 m-osmoles temp. 30° C 0 min	—	—	—	—	+0·290	+0·288

To 2 ml. citrated human platelet-containing plasma was added: 1 ml. H_2O, 201 m-osmoles; 1 ml. 0·154 M-NaCl, 297 m-osmoles; 1 ml. 2 M urea, 868 m-osmoles. After 15 min 5·6 μg ADP in 0·2 ml. 0·154 M-NaCl was added. The absorbancy A_{610} is corrected for 3 ml. Plasma I contained $8{\cdot}5 \times 10^4$ platelets/mm^3 and plasma II 5×10^4 platelets/mm^3. The experiments were carried out between 70 and 155 min after blood collection. The temperatures were 5 and 30° C respectively.

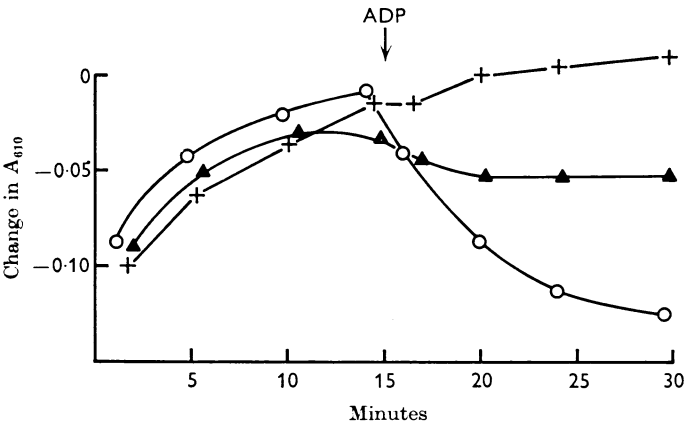

Fig. 8. Mixtures of pl.c. plasma and: H_2O, 200 m-osmoles −○—○−; 10^{-2} M iodoacetamide, 210 m-osmoles −▲—▲−; 4×10^{-3} M ATP, 204 m-osmoles +—+. After 15 min indicated by the arrow 5·7 μg ADP in 0·2 ml. H_2O was added.

TABLE 2. Influence of inhibitors on reversal of absorbancy of citrated human platelet-containing plasma subjected to hypotonic solutions

Substance or conditions	M-osmoles of mixture	Molar concn. in mixture	Increase in A_{610} during 15 min		Type of inhibition expected	% inhibition found
			Control	Experiment		
EDTA	203	5×10^{-3}	0·065	0·064	Divalent cations	0
Silicone surface	200	—	0·081	0·074	Contact activation	10
Cu^{2+}, Zn^{2+}	202	1×10^{-3}	0·065	0·070	—SH	0
2,4,Dinitrophenol	205	1×10^{-2}	0·073	0·075	Uncoupling phosphorylation	0
	204	9×10^{-3}				< 10
Sodium azide	204	1×10^{-2}	0·063	0·064	Electron transfer	0
ATP	203	4×10^{-3}	0·073	0·070	ADP aggregation. (Born, 1962)	0
Ouabain	204	9×10^{-3}	0·078	0·082	Na^+, K^+ activated ATPase (Schatzmann, 1953)	0
Sodium cyanide	203	5×10^{-3}	0·081	0·067	Electron transfer	20
Oligomycin	202	Approx. 1×10^{-3}	0·110	0·077	Coupled phosphorylation, electron transfer (Lardy, Johnson & McMurray, 1958)	< 30
Antimycin A	202	Approx. 6×10^{-4}	0·110	0·079	—	< 30
Sodium fluoride	206	$1{\cdot}23 \times 10^{-2}$	0·028	0·019	Carbohydrate metabolism	32
			0·104	0·052		50
			0·037	0·014		62
OH^- ions	205	pH 8·36	0·080	0·017	H^+ ions	80

The mixtures consisted of 2 ml. citrated human platelet-containing plasma and 1 ml. of the additions as indicated.
Experimental mixtures had the same osmolarity as the controls produced by dilution of platelet-containing plasma with water or NaCl.
Oligomycin and antimycin A had to be used as suspensions because of their low solubility in water. Temp. 30° C.

which is an abnormally high concentration, did not produce clumping. Kerr, MacAuley, Pirrie & Bronte-Stewart (1965) added fatty acids as lecithin sols to pl.c. plasma and assumed that aggregation of platelets occurred. Although the conditions in the two sets of experiments are not

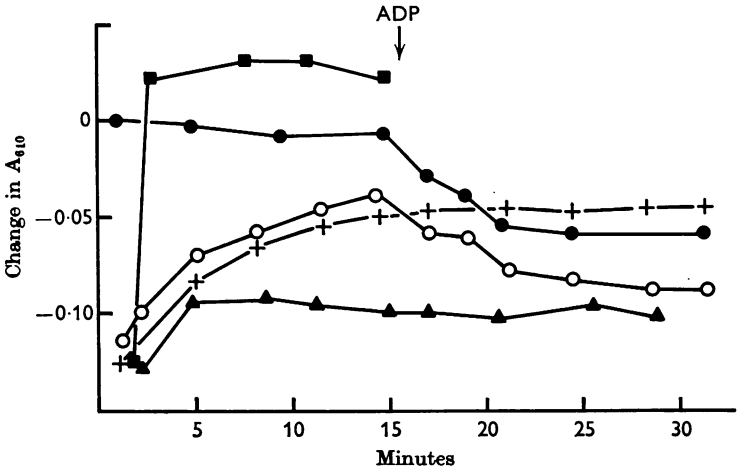

Fig. 9. Mixtures of pl.c. plasma and NaCl, 291 m-osmoles −●—●−; H_2O, 200 m-osmoles −○—○−; H_2O, after 2 min, 9 mg NaCl was added 295 m-osmoles −■—■−; $2{\cdot}4 \times 10^{-4}$ M NEM, 200 m-osmoles +—+; $2{\cdot}4 \times 10^{-3}$ M NEM, 202 m-osmoles −▲—▲− after 15 min indicated by the arrow 5·7 μg ADP was added.

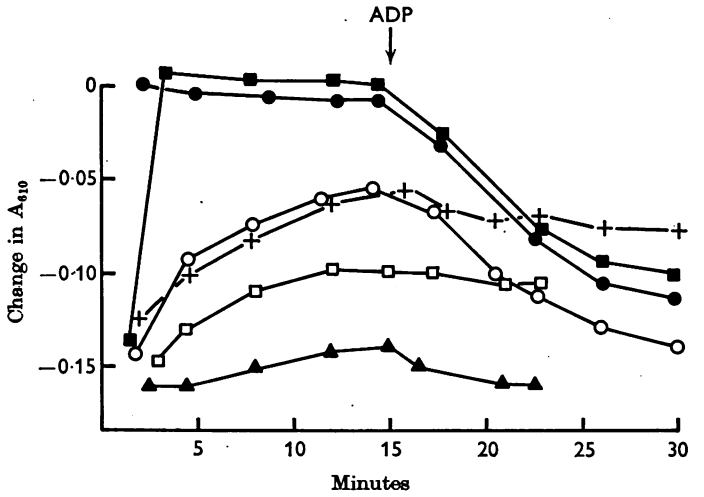

Fig. 10. Mixtures of pl.c. plasma and: NaCl 296 m-osmoles −●—●−; water, 200 m-osmoles −○—○−; H_2O at 2·5 min 9 mg NaCl was added, 296 m-osmoles −■—■−; $3{\cdot}3 \times 10^{-4}$ M mercloran, 200 m-osmoles +—+, $6{\cdot}6 \times 10^{-4}$ M mercloran, 201 m-osmoles □—□; $3{\cdot}3 \times 10^{-3}$ M mercloran, 203 m-osmoles −▲—▲−. After 15 min indicated by the arrow 5·7 μg ADP was added.

identical it is apparent from the presented evidence that platelet changes are dependent upon osmolarity of plasma and that photometric measurements give no indication of morphological platelet changes, because reduction of absorbancy of pl.c. plasma can be associated either with ADP aggregation (Gaarder *et al.* 1961) or with swelling induced by hypotonic solutions.

In vitro experiments with free non-esterified fatty acids have no physiological counterparts, since in circulating blood non-esterified fatty acids do not occur in the free form but are present as albumin and lipoprotein complexes. Haslam (1964) noted that the absorbancy of washed human platelets was decreased by behenate, but that this did not occur in the presence of albumin, and Tompkins & Dayton (1965) pointed out that variations in the concentration of free fatty acids in circulating blood have no influence on blood clotting time.

The absorbancy of platelet-containing plasma subjected to hypertonic solutions depended upon the source of platelets and the compounds used. With the three compounds tested, hypertonic NaCl gave the largest increase in absorbancy. This was followed by urea and no increase was observed with hypertonic sucrose. With human platelets hypertonic NaCl gave immediate and permanent increase in absorbancy; changes due to urea were transient and declined. Animal platelet-containing plasma did not show the same plattern as human plasma (Fantl, 1966).

The mechanism connected with the osmotic platelet changes is complex. The drop in absorbancy associated with swelling of platelets caused by hypotonic solutions and increase of absorbancy associated with shrinking caused by hypertonic solutions gave the same difference in absorbancy between 5 and 30° C. Further, hypotonic swelling of platelets was not influenced by any of the inhibitors used. Therefore the immediate absorbancy changes of platelets due to osmotic variations are probably of physical nature.

Apparently in a hypotonic environment water diffuses rapidly into platelets and in response to hypertonic environment water leaves the platelets quickly. The platelet reacts as a perfect osmometer but the reversal of absorbancy with a $Q_{10} > 1$ suggests a connexion with enzymic processes and not a mere physical syneresis of a gel-like structure.

Experiments with appropriate inhibitors have shown that divalent cations and contact activation are not necessary for the reversal of absorbancy following hypotonic swelling. The experiments do not indicate that oxidative phosphorylation, electron transfer or sodium potassium activated ATPases are associated with osmotic platelet changes. They also excluded influence of adenosine disphosphate as an explanation of the osmotic changes. Gaarder & Laland (1964) have suggested that ADP

induced platelet aggregation is due to hydrogen bonding. However, in view of the Q_{10} being greater than 1 a chemical reaction seems to be involved (Salzman, Chambers & Neri, 1965; Spaet, 1965).

Pl.c. plasma and isolated platelets show the same osmotic changes. This is in contrast to ADP aggregation of platelets which requires a plasma component. Moderate inhibition of reversal of absorbancy following hypotonic swelling was seen in the presence of cyanide, fluoride and iodoacetamide. Complete inhibition occurred at pH > 8. Blockage of –SH groups by N-ethyl-maleimide or mercloran inhibited the reversal of absorbancy, indicating the necessity for –SH radicals.

Skålhegg, Hellem & Ödegaard (1964) found that 8×10^{-4} M iodoacetic acid inhibited ADP induced platelet aggregation.

Frozen pl.c. plasma has a lower absorbancy than the unfrozen plasma and little reduction of absorbancy occurs in a hypotonic environment. However, increase of absorbancy after addition of hypertonic solutions and reversal of absorbancy in the presence of urea still takes place. This suggests that the reversal of absorbancy after hypotonic swelling and the reversal after hypertonic shrinkage are connected with different platelet components.

There is a superficial similarity between volume changes of platelets and of mitochondria. Although platelets contain enzymes and substrates of oxidative phosphorylation, respiration is a minor factor in platelet activity in contrast to mitochondria, for which oxidative phosphorylation is essential (Chapell & Greville, 1963). In mitochondria swelling can be induced in an isotonic fluid but platelets swell mainly in hypotonic environment. It appears that platelets have compartments, probably with membranes, which can undergo reversible volume changes at different osmolarities. It is undecided whether a contractile protein (Bettex-Galland & Lüscher, 1961), contractile vacuoles or crystae participate in osmotic changes. The reversal of absorbancy following hypotonic swelling occurs only with intact platelets during a limited time. This suggests lability of structure, enzyme or substrate connected with osmotic changes. The substrate could well be a labile ester such as ATP of which hydrolysis of 1 mole can extrude between 400 and $2\text{–}5 \times 10^3$ M water (Lehninger, 1959; Chapell & Greville, 1963).

Mr M. E. Matthaei, Microscopy Laboratory of University of Melbourne, carried out the phase contrast microscopy, Dr R. J. Sawers, Haematology Department, Alfred Hospital, Melbourne, carried out the platelet counts and Mrs B. Skym carried out the statistical calculations.

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