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# THE ULTRASTRUCTURAL MORPHOLOGY OF AIR EMBOLISM: PLATELET ADHESION TO THE INTERFACE AND ENDOTHELIAL DAMAGE

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**Summary.—The pathogenesis of the ill effects following air embolism cannot be attributed solely to the space occupying and surface tension effects of the air bubbles altering the normal flow of blood through the vasculature. Decompression sickness was induced in rats and the following features of this process observed by electron microscopy in the vessels of the mesentery: imprisonment of blood elements (especially platelets) took place within the various enclosures created by the boundaries set up by different sized air bubbles between the layer of blood and the vessel walls, and the air/blood interface. Air bubble size and the thickness of the film of blood between bubbles varied enormously.**

**The air/blood interface had the following characteristics: (1) A surface associated protein layer measuring 20 nm which coated the air bubbles and which could slide off the bubble of origin and float freely in the blood. (2) Material morphologically similar to the surface layer was found away from the surface and included small lipid droplets between its layers, and platelets adhered to this to form small aggregates suspended from the interface. (3) The surface layer fused with like laminae and was found within the fluid blood in the vessel, sometimes with adherent platelet aggregates. (4) Platelet adhesion to the bubble interface with the formation of platelet aggregates of an early type i.e. without gross fibrin formation within the aggregates. (5) Pressure damage to underlying endothelial cells by the passage of air bubbles under pressure resulted in herniation of the endothelial cells through fenestrations in the more rigid structures of the vessel wall. (6) Deposits of fibrin on the walls of the vessels were noted after endothelial damage. (7) Lipid droplets were found attached to the surface associated protein on the air side of the air/blood interface and were also found incorporated within it, i.e. covered by this layer on both sides, in which case they took on an ellipsoidal shape.**

**These alterations would account for certain features of decompression sickness, such as the drop in platelets, even when the mechanical blockage by air bubbles was not of great significance.**

THE pathogenesis of the changes induced by air embolism includes effects other than those due to the simple arrest of flow in parts of the microcirculation due to impaction of bubbles. The reaction of the blood to the foreign surface presented to it by the air/blood interface gives rise to changes in the state of the circulating platelets, and in some regions the conversion of fibrinogen to fibrin.

Hartveit, Lystad and Minken (1968) examined by light microscopy the changes induced by venous air embolism in rabbits and mice. They found that the cause

of death was fibrin plugs in the terminal branches of the pulmonary arteries and that prior heparinization in mice increased the survival rate following embolism, and they concluded that the pathology of death due to venous air embolism was similar to that of pulmonary embolism due to a " clot ".

Investigation of the effects of a surfactant upon induced decompression sickness in animals was found to be beneficial and it was postulated that this action might be due to prevention of the protein coating of the air bubble (Malette, Fitzgerald and Eiseman, 1960). The alteration of the plasma components at the blood–gas interface has considerable practical and theoretical implications and, since platelets readily attach and spread on foreign surfaces, implications for the proclivity to thromboembolic phenomena and for the availability of platelets for normal functions. The delineation of the morphology of the changes induced in the vascular compartment and in the vessel wall itself in air embolism might indicate additional opportunities for therapy in decompression sickness, not at present taken.

Earlier work from this laboratory showed that there was a decrease in numbers of circulating platelets and a reduction of plasma lipids in experimental decompression sickness in rats (Philp, Gowdey and Prasad, 1967). Other evidence, including the observation that the incidence of bends was higher in rats with elevated platelet counts (Clark, Philp and Gowdey, 1969) and that the decrease in the numbers of circulating platelets during slow intravenous infusion of air into rabbits was augmented by agents which increase platelet adhesiveness (Philp, Schacham and Gowdey, 1971) led to the hypothesis that the surfaces of bubbles in the circulation possibly reacted with fibrinogen, lipids and platelets. This reaction at the interface could proceed to the formation of microthrombi and subsequent thromboembolism.

Observations by light and electron microscopy of vessels in stage- or explosively-decompressed rats have confirmed the presence of a layer of proteinaceous material at the air–blood interface, the adhesion of platelets and leucocytes to it and the occurrence of free circulating endothelial cells in these animals (Philp, Inwood and Warren, 1972).

This paper describes in detail some of the ultrastructural morphology of air embolism as seen in explosively decompressed rats. In particular, this includes the attachment of platelets to and eventual aggregation around the protein at the interface and the damage inflicted on the underlying endothelium by the pressure of the gas bubble.

## MATERIALS AND METHODS

*Animals and method of decompression.*—Five male Sprague-Dawley rats approximately 500 g in weight were compressed at 75 p.s.i.g. for 2 hours using a small animal hyperbaric chamber, after which time the pressure was reduced to ambient atmospheric pressure in 45 seconds. The animals which did not convulse and lose consciousness within 3 minutes were lightly anaesthetized with sodium pentobarbitone, 45 mg/kg body weight.

*Preparation of specimens for electron microscopy.*—Using an atraumatic technique and bloodless dissection, pudendal fat pad, mesenteric and retroperitoneal vessels and various organs were quickly isolated using a combination of sutures and fine artery clamps to prevent loss of vessel contents, *i.e.* blood and intravascular bubbles. During this initial dissection the abdominal contents were bathed in 3% buffered glutaraldehyde which afforded excellent *in toto* fixation of the smaller vessels.

Small segments of the isolated structures were subsequently removed and carefully transferred into fresh glutaraldehyde solution.

An attempt was made to keep the air bubbles in the same position throughout fixation as

they were *in vivo*. Some of the tissues were stored in a phosphate buffer in 5% glucose until it was convenient to continue the fixation procedure. The tissues were post-fixed in 1% osmium tetroxide in a barbiturate–acetate buffer for 1 hour. The specimens were further dehydrated in alcohols and ethanol/acetone mixture and finally embedded in "Spurr" embedding medium (Spurr, 1969).

Some of the blocks were stained with 0·5% uranyl nitrate and lead acetate at the ethanol/acetone stage of embedding.

The tissue blocks were cut with glass and diamond knives on an Ultratome III (LKB), mounted on supported copper grids and observed in a Philips 300 electron microscope using 60 or 80 kV. The thin sections were stained with 6% uranyl acetate and lead citrate in most instances.

## RESULTS

### *The microscopic appearance of air embolism following decompression and light microscopy of air embolism using toluidine blue stained half micron sections*

Intravascular frothing of the blood was observed and the vessels themselves were under increased tension. Great variation in the number and nature of the agglomerated air bubbles was apparent through the thin vessel walls (Fig. 1a). Enclosure of blood elements between the air bubbles took place within the various geometric forms created between the air bubbles, the blood and the vessel walls (Fig. 1b, c, d). There was great variation in the thickness of the layer of blood between the air bubble and the vessel wall (Fig. 1b, c, f). Fluid currents would be possible around the air bubbles and past the various interfaces. When extruded frothed blood was examined, platelets were seen to have adhered to the surface of the bubbles, and there was structural integrity (albeit fragile) of the air bubble-platelet/protein coating (Fig. 1e).

The air bubbles had curved surface in contact with the layer of blood next to the vessel wall and straight sides in contact with other bubbles. These profiles conform to the usual solid geometry of bubbles in foam (Matzke, 1946). Bubbles in a froth have various polyhedral forms, the limits of which appear to be the pentagonal dodecahedron and the truncated octahedron. In naturally occurring cells and grains (including bubbles in a froth) the ideal polyhedral properties lie close to the middle of this range (Underwood, 1970), and the number of edges per face is usually 5. The face that the bubble presents to the blood has oriented molecules projecting from it, *i.e.* hydrophilic portions of molecules face towards the plasma and hydrophobic towards the air.

### *The ultrastructure of the immediate environs of the bubbles: the thin films between contiguous bubbles and the structure of the "funnel"* (Fig. 2)

Adhesion of platelet aggregates to the "side wall" of the funnel between two air bubbles (Fig. 2a) frequently occurred. In other areas there were layers (up to 3) of platelets attached to each side of this region between air bubbles. The "funnel" region of the environs of the foam of air bubbles narrowed rapidly into a thin film between air bubbles.

The thin films between bubbles entrapped the various cellular elements of the blood, depending on the dimensions both of the blood cells involved and the thin film. Thus some blood cells, for example leucocytes, were caught in bubble films too small for their dimensions so that they bulged into the air spaces on either side and, in other profiles of the film between bubbles, red cells were strung out and caught between the interfaces like a string of sausages.

Since the morphology of the entrapped cells depended on the relative size of the thickness of the inter-bubble film and the size of the blood cell, relatively thin films therefore completely entrapped platelets (Fig. 2b, c, e).

The thin protein precipitate at the blood–air interface appeared capable of fusing with a like membrane from another film. This approximation was sometimes irregular and there was occasional buckling of one film upon the other (Fig. 2b, e). Although fibrin in any of its forms was absent in most of the blood films observed, strands were seen sometimes in association with platelets (Fig. 2c) and at other times attached to the precipitated protein of the interface (Fig. 2f). The film between adjacent air bubbles eventually became greatly attenuated (Fig. 2d). The extreme plasticity of this film and the constraints upon its contents were observed in the way this film and its surface protein restricted the platelets within it (Fig. 2e). Lipid droplets of varying sizes were seen adjacent to the surface protein and extended into the air portion of the blood–air interface in a spherical fashion (Fig. 2f).

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## EXPLANATION OF PLATES

FIG. 1.—Low power views of mesenteric vessels of rats suffering from decompression sickness.

(a) Mesenteric vessel with much frothed blood in its lumen. The vessel shows the variety of sizes of the air bubbles present and the great unevenness in the thickness of the intervening films of blood between the bubbles. × 13·5.

(b) Several air bubbles (B) have enclosed within their combined compass, a tricornered region in this plane. The vessel wall (VW) remains in contact with a relatively thick layer of blood. × 135.

(c) A further view of the blood caught between the bubbles and the funnelling down of this region into a thin film (TF). × 400.

(d) The air bubble (B) shown here is in proximity to the vessel wall rather than centrally placed. × 135.

(e) This photomicrograph illustrates the platelet coating (P) of a subsidiary or minor air bubble (A2) and that of the larger bubble (A1). This is the morphology of the coat of the air bubble in frothed blood removed from the vessel. × 540.

(f) In some instances the layer of cells and plasma close to the vessel wall (VW) was attenuated as in this example. A relatively thick " pillar " of red cells and plasma (R) is present between two air bubbles here. × 135.

FIG. 2.—The ultrastructure of the thin film between air bubbles.

(a) The funnel region between two air bubbles (A) contains a platelet aggregate (PA) without fibrin. A dense precipitate can be seen at the interface between the plasma and the air bubbles (PF). × 3600.

(b) Two thin films have partially fused. Within one film platelets (P1, P2, P3, and P4) are trapped. × 23,600.

(c) Within this thin film are platelets with pseudopodia, together with fibrin strands (F1, F2) and a " stringy " precipitate (SF). Portion of a red cell is present in the lower part of the photograph. × 12,000.

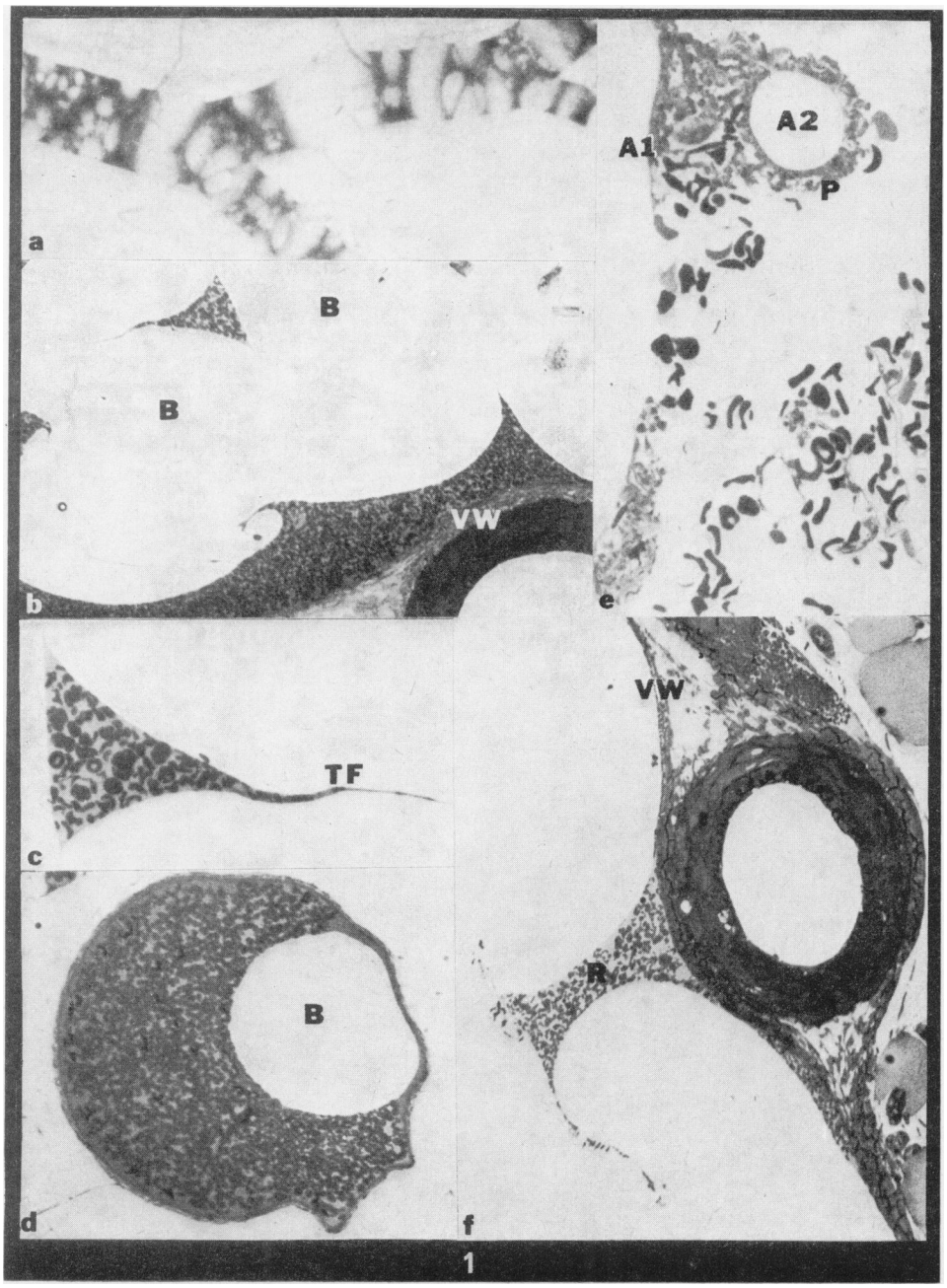
(d) This electron micrograph shows the extreme form of thin film (T) between bubbles. × 23,600.

(e) The film which encloses two platelets (P1, P2) is bent back upon itself. It illustrates both the plasticity of the platelet and the constraints of the enclosing protein precipitate upon the platelet. × 14,400.

(f) Fibrin (F) in strands is present in this thin film which also possesses a lipid droplet (LD) on its surface. × 23,600.

FIG. 3.—Surface associated protein precipitate (possibly fibrin in some form).

These 6 electron micrographs demonstrate a protein precipitate (SF) which occurs at the air–blood interface. This material was observed separate from other blood components (Fig. 3(a)) and in combination with platelets (P) in thin films (Fig. 3(b), (f)) between air bubbles (A). Lipid droplets (LD, L) were observed caught up in this material (Fig. 3(c), (d), (e)). Occasionally a complex deposit of this material (M) was present and in Fig. 3(c) such a complex is seen close to several red cells (RC). Branching of the material was observed. (a) × 5600, (b) × 12,600, (c) × 3800, (d) × 25,500, (e) × 25,500, (f) × 12,600. (e) is a higher magnification of (c).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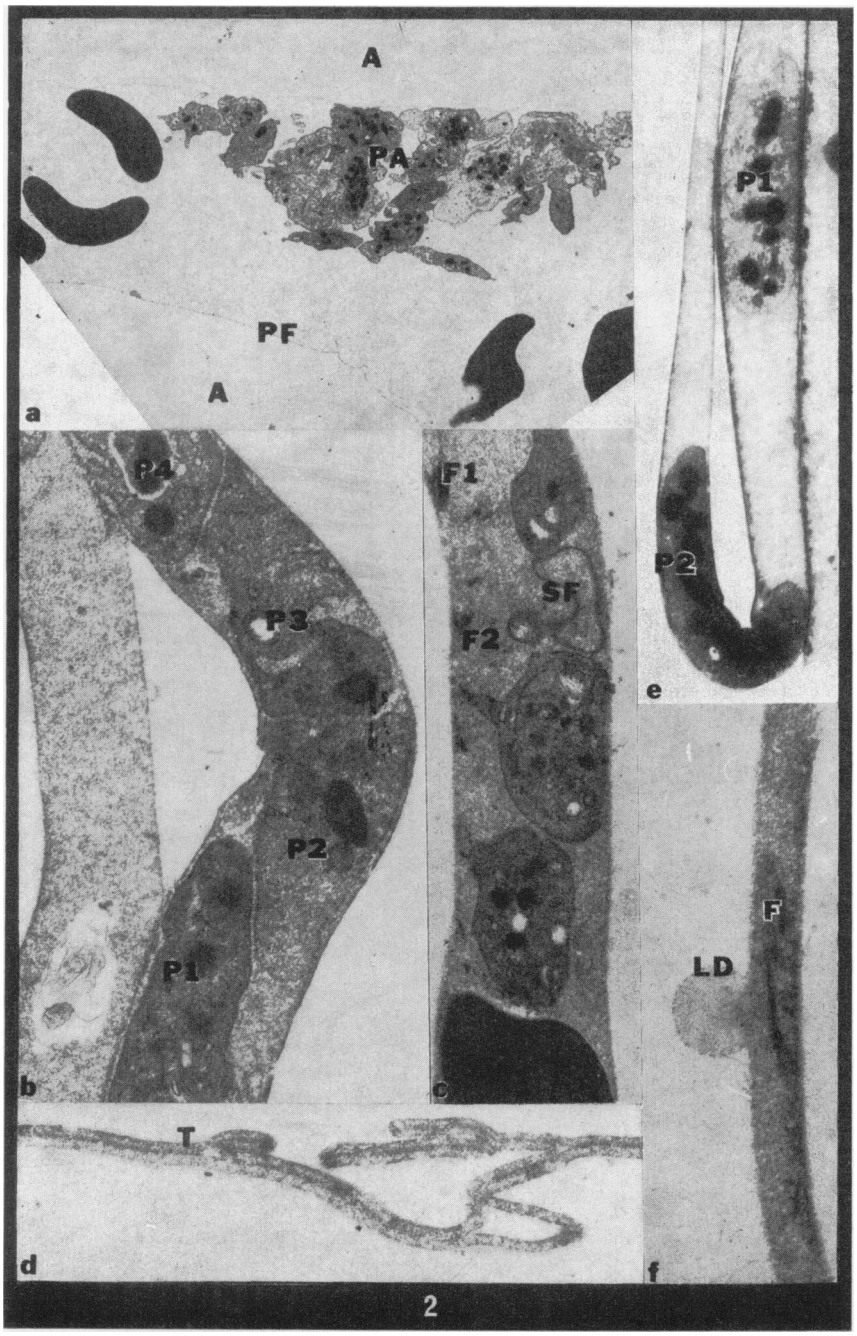
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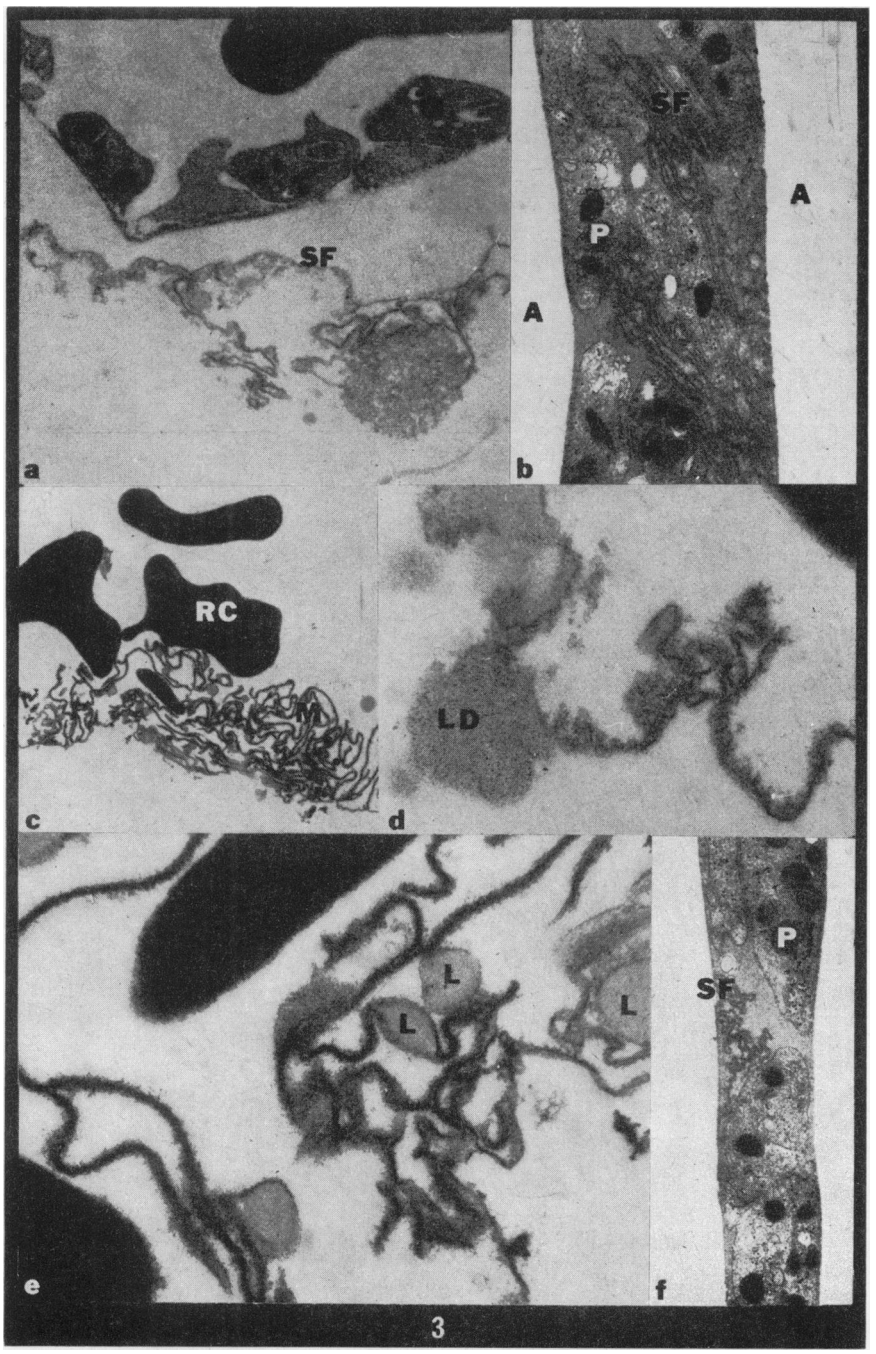
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*The interposition of lipid droplets within the precipitated protein leaves of the bubble coat* (Fig. 3)

In the ebb and flow of microturbulence around air bubbles within the vasculature it would be unlikely that a protein precipitate would remain firmly affixed to its original bubble in all instances. Separate profiles of this protein precipitate were observed in various positions in relation to the air bubbles (Fig. 3). In some instances, convoluted material was within the air bubble (Fig. 3a) while in others it was caught between air bubbles in films of different sizes (Fig. 3b, f). The material itself contained lipid droplets (Fig. 3c, e) and these were applied to its surface (Fig. 3d). The material resembled a " stringy " form of fibrin.

The lipid droplets appeared spherical if they were attached to the inner aspect of the interface and ellipsoidal if they were covered on both sides by protein precipitate, and enmeshed in it. Scarborough *et al.* (1969) examined blood–solid interfacial reactions and found a protein precipitate at the interface which was similar to that found here. Hartveit *et al.* (1968) in their investigation of air embolism detected fat droplets in association with fibrin in the coronary veins.

The appearances of the lipid droplets enmeshed in protein layers gives rise to the speculation that there may be " stabilization " or orientation in a hydrophobic–hydrophilic fashion of protein moieties of the plasma around the lipid droplets.

*Platelet adhesion to the surface layer of protein precipitate*

Platelets were observed adhering to each side of the surface protein layer (Fig. 4a) and to have undergone shape change with aggregation. In other instances the string-like profile of this surface layer was seen within small platelet aggregates (Fig. 4b, e). The surface protein precipitate adhered to itself when the opportunity arose (Fig. 4c), resulting in a double layer (Fig. 4d) of the material.

There appeared to be 3 types of primary adhesion of platelets to the air-bubble interface (Table), and these were: contact with the interface by platelet plasmalemma covering the long axis of the platelet, *i.e.* the platelet's long axis was parallel or tangential to the interface; attachment to the interface by dendritic platelet pseudopodia, and contact with the interface by plasmalemma over the rim of the platelet.

The type of contact that a platelet made with the blood/air interface depended to some extent on the number of platelets in the area. Where the platelets were numerous, they tended to align themselves like shingles on a roof so that the short axis attachment predominated. Where platelets were relatively scarce, there was greater frequency of long axis attachment.

This shingling, or shelving, effect was especially noted where a group of platelets had been caught in a thin film between 2 air bubbles. In the sample counted the proportions of the various types of contact were: long axis parallel, 37%; pseudopodial contact, 31% and short axis parallel, 32%.

Multivesicular sacs were seen in association with the pseudopodia and expulsion of the secondary vesicles occurred in some instances into the bubble itself. This type of formation may be associated with the biochemical release reaction of the platelet (Warren and Vales, 1972). Platelet pseudopodia tended to contact the interface and to run along it. The platelets attached to the surface were sometimes contorted into grotesque shapes, with their granule-containing region localized

TABLE.—*The Mode of Contact of Platelets with Air Bubbles*

| | Mode of contact of platelets with interface | | | |
|---|---|---|---|---|
| EM film (Column 1) | Long axis of platelet parallel to interface (Column 2) | Contact of interface by pseudopodia (Column 3) | Short axis* parallel to interface (Column 4) | Fat droplets (Column 5) |
| 1 | 27 | 18 | 11 | 8 |
| 2 | 13 | 19 | 20 | 3 |
| 3 | 16 | 6 | 9 | 0 |
| 4 | 22 | 14 | 16 | 0 |
| 5 | 19 | 25 | 32† | 3 |
| 6 (4)‡ | 21 | 15 | 15 | 0 |
| Totals | 118 | 97 | 103 | 14 |
| Percentages | 37 | 31 | 32 | |

Total platelet contact regions counted = 318

The table above is the result of the examination of a number of " survey " 35 mm rolls of film taken in a Philips EM 300 with 31–35 frames in each. The mode of contact of platelets immediately beneath the air/blood interface is divided into contact with the surface by means of the plasmalemma of the platelet covering its long axis (column 2), pseudopodia (column 3) and plasmalemma covering the rim of the platelet (column 4). The number of fat droplets found at the interface is shown in column 5.

* This type of contact with the interface involved contact between the region of the platelet which involved the circumferential microtubules, *i.e.* contact of the blood/air interface by the rim of the platelet.

† This film included a region in which platelets were arranged in a shingle fashion with the microtubular bundles of the platelets closest to the blood/air interface; hence the large number with their short axis parallel to the interface.

‡ Film 6 (4) line includes observations in 4 separate films which have been added together. These films included studies of a few platelets at high magnification.

If a pseudopod contacted the surface and the platelet's long axis surface was also adherent to the interface then that cell was counted in both columns 2 and 3.

## EXPLANATION OF PLATES

FIG. 4.—Platelet aggregates found within the vasculature in rats suffering from decompression sickness.

Platelet aggregates (A1, A2) adhering to surface associated protein precipitate (Fig. 4(a)). This precipitated film at the interface between air bubbles (A) and plasma could fuse with a similar precipitate to form strands within the plasma (SF).

A high power view of two fused layers (Fig. 4(b), (c), (e)) is shown in Fig. 4(d). The centre dark line is assumed to be the plane of fusion. (a) × 7450, (b) × 4950, (c) × 3350, (d) × 42,700, (e) × 17,300.

FIG. 5.—Damage to endothelium in air embolism.

(a) The localized increased intravascular pressure induced by the occurrence of air emboli within the vessel appears capable of herniating endothelial cell nuclei (E1, E2) through the fenestrae of the elastic laminae (EL) of the intima (Fig. 5(a)).

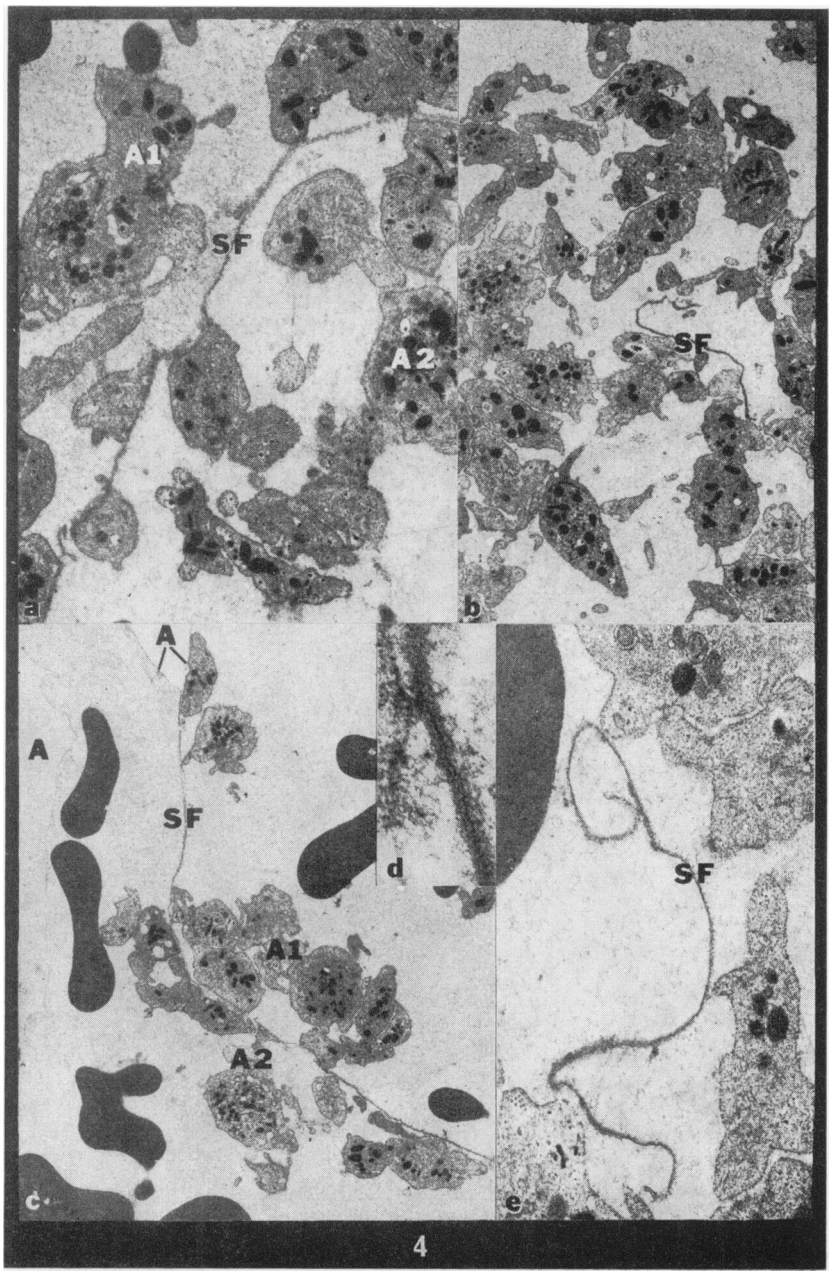
(b) In some instances a portion of the cytoplasm of the endothelial cell was also pushed back into the interstitial space of the vessel wall through the gap in the elastic lamina (EL) (Fig. 5(b)). In this figure the red cell (RC) is in close contact with the endothelial cell (E) which is displaced outward by the same forces which are acting on the red cell. (a) × 11,100, (b) × 16,800.

(c) In this electron micrograph can be seen strands of fibrin (F) some distance away from the endothelial lining of the vessel, beneath a platelet (P) adhering to the surface of an air bubble. (c) × 16,800.

(d) In continuity with the damaged endothelium (DE), which in another part of the same section showed herniation through the elastic lamina (EL) are deposits of fibrin (F) attached to the vessel wall.

(e) This mural deposit of fibrin was evident in other areas not obviously in continuity with damaged endothelium. (d) × 16,800, (e) × 11,100.

(f) This shows the edge of an air bubble and the subjacent endothelial layer (E) of the vessel wall. There is a relatively large lipid droplet (LD) on the air side of the air/blood interface. This interface has fine strands of fibrin (F) attached to it. Platelets (P) are present between the endothelium and the air bubble. (f) × 13,200.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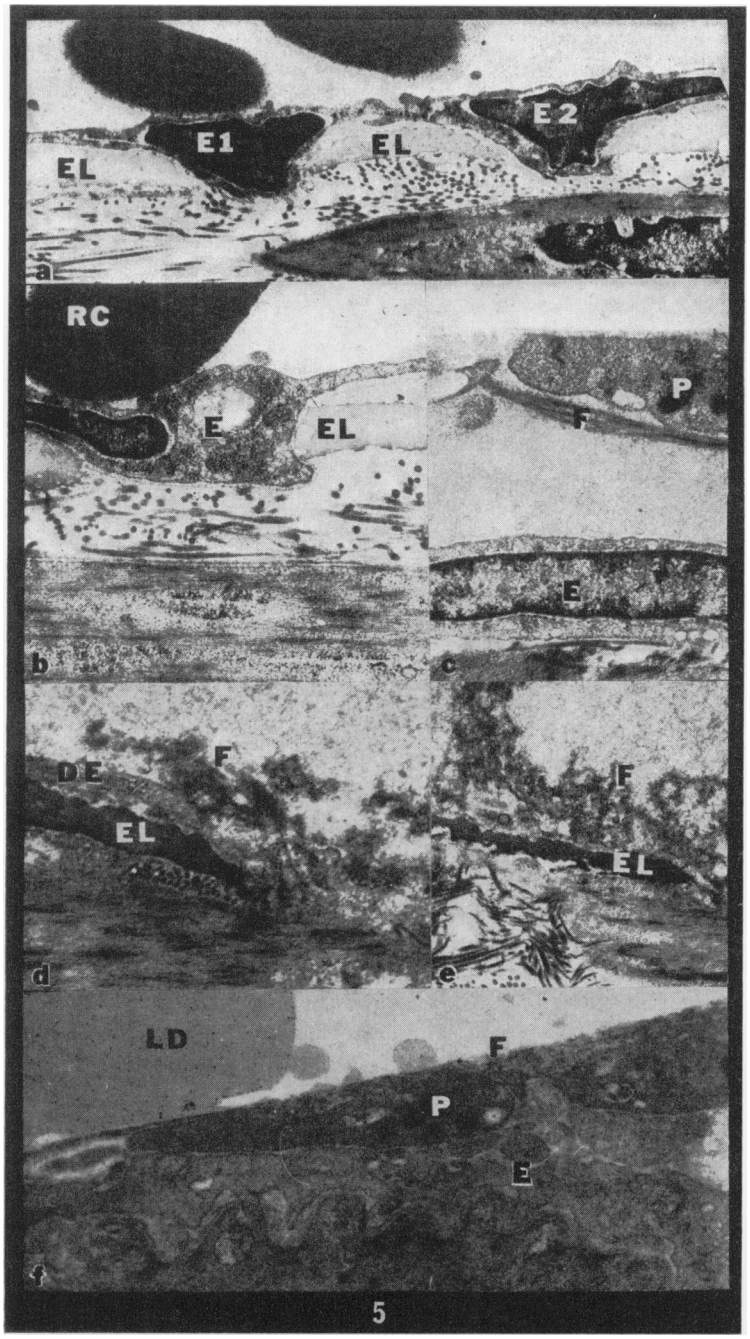


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at one pole of the cell and a granule-free bulbous process comprising the remainder of the cell in that profile.

*The mechanical damage produced by air embolism and the formation of deposits of mural film*

The endothelium was damaged in these experiments. In certain areas there was a reversal of the normal situation whereby the basement membrane of the endothelial layer is smoothly continuous and the endothelial nuclei protrude into the lumen. In such preparations the nuclei of the endothelial cells had herniated through the fenestrae of the elastic laminae of the vessel wall to result in a smooth wall surface and a damaged inner endothelial one (Fig. 5a, b). Fibrin was observed both in strands (Fig. 5c) and in a flocculent form adjacent to remnants of damaged endothelium (Fig. 5d). In other regions there were deposits of mural fibrin (Fig. 5e). Occasionally the film between the air bubble and the endothelium would be thin. This thin film in some instances showed lipid droplets of different sizes on its air surface and fibrin and platelets on its deep surface (Fig. 5f). Layering of platelets on the endothelial lining with retention of granules within the platelets was observed. Vesicular damage to the endothelium was noted in some areas and there were gaps between endothelial cells. Where there was no pliable underlying structure the endothelial surface became attenuated over the part.

## DISCUSSION

*The air–blood interface: surface associated protein*

It is interesting to note that in the standard model concerning the hydrated lamellar crystal which is formed by layers of " aqueous solution " and the amphipathetic lipid, the hydrophilic heads are exposed to the adjacent aqueous layer whereas the hydrophobic tails are protected (Mysels, 1968).

Dynamic processes in soap films have been discussed by Mysels (1968), who described the differences between rigid and mobile films and their interconversion, the origin and measurement of film elasticity, the effect of rate of formation on film thickness and the evidence against the existence of thick, rigid, water layers at the surface.

In addition to the thinning forces which occur in a film there are others, such as double layer electrical interaction and steric interaction of adsorbed surfactants, which come into play when the film is reduced to the appropriate thinness (Mysels, 1968).

In a study of black lipid membranes (BLM) at bifaces Ti Tien (1968) considered that when the membrane was relatively thick—0·1–1·0 $\mu$m there was an organic phase between 2 adsorbed monolayers of lipid molecules at the surface. At distances greater than 10 nm the attraction of lipid monolayers across the biface was small. He considered that the chief cause of thinning occurred because of the presence of a Pateau–Gibbs border, *i.e.* at the edge where the membrane ended. He found that BLM less than 9 nm were useful models of biological membranes. Ti Tien considered that the lipid molecules would possess a higher degree of order in the BLM state than in the lipid solution.

Scarborough *et al.* (1969) described and illustrated both attached and detached " adsorbate " which formed at glass–blood and silicone–blood interfaces. The folded detached adsorbate, and in the instance of the glass–blood adsorbate its

trilaminar structure, were similar to the surface associated protein precipitate found in our experiments. They noted platelet adhesion to the adsorbate in a fashion resembling that found here.

An analysis of the rheology of the circulation during air embolism, stressed as it would be by the occurrence of air bubbles, would be complex. However, it is evident that at an early stage in the production of air bubbles bolus flow would become instituted with laminar near-wall drag on the annulary flow between the " boli " of agglomerated bubbles or single large bubbles, as discussed by Whitmore (1968). Movement in the films of blood between the air bubbles would be induced by this as well as other forces, possibly including gravity, to create layered flow around the bubble. Detachment of surface associated protein precipitate from its bubble of origin would be easily accomplished under these circumstances.

*Platelet adhesion to the foreign surface of air/blood interface*

The gas bubbles in decompressed animals have been likened to foreign bodies in the vascular system, with reactions to these intruders by the circulating platelets and leucocytes (Adebahr, 1971).

An analysis of platelet interaction with artificial surfaces with regard to consequences of flow, exposure time and surface nature has been made by Friedman and Leonard (1971). They examined the surfaces of glass, siliconized glass, fluorinated ethylene propylene, polyamide and polyvinyl chloride. Their results led them to conclude that initial platelet adhesion was a function of flow rate and exposure time but generally independent of the foreign surface studied. The explanation given for the fact that at long exposure times platelet adhesion became independent of flow rate was that the surface became " saturated ".

Friedman *et al.* (1970) considered that quantitative analysis of platelet adhesion to foreign surfaces required investigation of the effects of flow on convection and diffusion of platelets, platelet and foreign surface reactivities and surface coverage due to progressive platelet adhesion. The phenomenon of platelet adhesion to the air bubbles could be considered therefore in the 3 steps proposed by Friedman and his co-workers. These were (1) the " natural " state of the platelets in the flowing blood, *i.e.* platelets in stream lines parallel to the surface, (2) the diffusion of platelets at right angles to these flow streamlines and (3) adhesion at a rate controlled by the platelet–surface reactivity:

(1) It is evident that the turbulence resulting from the existence of gas bubbles in the blood will disrupt all streamlines in the vessels so involved. Microturbulence between separate air bubbles will result in several vortices with their outer rims moving in the same direction as the blood flow in the vessel. The microturbulence in the funnel area of the environs of the bubble will create conditions conducive to platelet adhesion and aggregation. (2) This second step is critical and, even if the subsequent step involving platelet surface reactivity (*i.e.*, the reactivity is high) it is governed by the effect of " platelet diffusion " (Friedman *et al.*, 1970). (3) The rate of platelet adhesion to the bubble in the gas/blood system would appear to remain dependent upon the flow rate and the platelet–surface reactivity as in other situations (Friedman *et al.*, 1970).

The role of platelets in blood–surface interactions has been reviewed by Salzman (1971). He considered that the adhesion of platelets to many foreign surfaces required an intermediate bridging layer which might be composed of both

the external " halo " coating around the platelet and a plasma protein cofactor attached to the foreign surface.

In studies of the nature of adhesion of platelets to fibrin, the nature of. and the adsorbed material incorporated in. the fibrin were held to be important factors in the induction of adhesion. Thus, in contradiction to ready adhesion to collagen fragments, adhesion to fibrin in which the thrombin had been removed or neutralized did not induce platelet adhesion or the release phenomenon (Hovig *et al.*, 1968). However, polymerizing fibrin and the intermediate products of incubation of fibrin with reptilase or thrombin readily induced platelet aggregation (Niewiarowski *et al.*, 1972).

A clearer definition of the role of platelets in the contact phase of blood coagulation has been attempted by Walsh (1972). He considered that platelets enhance the reactions involving factors XII and XI to form contact activation product in the absence of calcium and he termed this activity contact product forming activity (CPFA). This activity was specifically and rapidly stimulated by adenosine diphosphate (ADP) in physiological concentrations, was reversible, and when induced by ADP was accompanied by a reversible change in shape typical of the usual response of platelets to ADP.

Walsh regarded " contact product forming activity " not as a " platelet factor " but rather as a property associated with the platelet surface which was altered under certain conditions, especially after exposure to ADP. A comparison of a phospholipid mixture (Folch) possessing PF3 activity with freshly collected intact platelets showed that this phospholipid mixture was inactive in all test systems for CPFA (Walsh, 1972). The evidence at this juncture seems to point towards the activity being associated with the reaction of viable cells, which by their reaction, modify the micro-environment.

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