J. Physiol. (1972), **223**, *pp.* 229–242
With 1 *plate and* 6 *text-figures*
Printed in Great Britain

THE INITIATION OF PLATELET THROMBI IN NORMAL VENULES AND ITS ACCELERATION BY HISTAMINE

BY NICOLA A. BEGENT, G. V. R. BORN AND D. E. SHARP

From the M.R.C. Thrombosis Research Group, Department of Pharmacology, Royal College of Surgeons of England, Lincoln's Inn Fields, London, W.C. 2

(*Received 24 December 1971*)

SUMMARY

1. In cheek pouch preparations of anaesthetized hamsters, platelet thrombi or 'white bodies' were produced in venules by the micro-iontophoretic application of adenosine diphosphate (ADP). Currents of 10–400 nA were passed through micropipettes containing 10 mM-ADP, the tips of which were less than 5 μ from the outer wall of the venule. The effect was quantitated by determining the time between starting the currents and the first appearance of platelets adhering inside the venule opposite the tip of the micropipette.

2. Repeated applications of ADP to the same site on a venule caused the appearance of white bodies after intervals which were almost constant for up to 3 hr.

3. The time to first appearance of a white body was inversely related to the iontophoretic current between about 10 and 200 nA. Currents smaller than 10 nA had no effect on the platelets. With currents of 200 nA or more the time remained at a minimum of less than 20 sec.

4. With currents of about 300 nA the minimum time increased little as the pipette tip was withdrawn up to 20 μ from the venule; with greater distances the time increased progressively.

5. Histamine caused gaps to appear between endothelial cells in cheek pouch venules. Histamine at a concentration of 10 mM in micropipettes was applied iontophoretically by currents of 300 nA to venules at the same sites as ADP. Histamine alone had no effect on circulating platelets. When applied before and together with ADP, histamine decreased the time to first appearance of white bodies by up to 40 % below that determined with ADP alone. Iontophoretically applied histamine did not alter the mean blood flow velocity in the venules.

6. After stopping the application of histamine, the time to first appearance of white bodies produced by ADP increased again in about 5 min to the control values.

7. Bradykinin, which does not cause endothelial gaps in cheek pouch venules, did not accelerate the induction of white bodies like histamine.

8. There were no microscopic abnormalities in venules in which white bodies had formed. Venules exposed to histamine accumulated circulating carbon particles in discrete wall areas.

9. It is concluded that adhering white bodies can be induced repeatedly in normal venules by direct action of externally applied ADP on circulating platelets and that the accelerating effect of histamine on white body formation is due to the separation of endothelial cells which accelerates the inward diffusion of ADP.

INTRODUCTION

The main function of the blood platelets is to adhere to the walls of injured blood vessels and to each other as aggregates which initiate haemostasis. The mechanism of platelet aggregation *in vitro* is being elucidated by methods applicable to suspensions of platelets in plasma or in physiological saline solutions (for reviews see Mustard & Packman, 1970; Born, 1971). It is uncertain whether the process demonstrable *in vitro* accounts for the adhesion and aggregation of platelets *in vivo*. Adhesion and aggregation can be experimentally induced in living animals by injuring vessels in various ways, ranging from the macroscopic mechanical damage caused by a cut to the microscopic thermal damage caused by a laser beam. However, for a complete analysis of the thrombogenic process it is necessary to establish how the initiation and growth of intravascular platelet aggregates depend quantitatively on the following variables: (1) the cellular and geometrical properties of the blood vessel at the site of the thrombogenic stimulus; (2) the nature, duration and strength of that stimulus; (3) the concentrations in the blood of calcium and fibrinogen which are known to be necessary for platelet aggregation *in vitro* (Born & Cross, 1964; Cross, 1964) as well as of other proteins, including Hageman factor and immunoglobulins which influence the rate of platelet aggregation (Bang, Heidenreich & Matsudo, 1970); (4) the concentration, distribution and reactivity of platelets in the flowing blood; and (5) the properties of the blood flow, particularly its velocity profile.

We have already described a method which makes possible the production of platelet thrombi or 'white bodies' *in vivo* under conditions in which these variables can be measured or controlled (Begent & Born, 1970*a*). The principle is to apply a specific substance as thrombogenic stimulus in such a way that the vessel remains undamaged and, indeed, with the least possible disturbance to its endothelial lining. Adenosine diphosphate (ADP), which causes platelet aggregation *in vitro* and is apparently involved in aggregation caused by other naturally occurring

agents such as thrombin, is applied to the outside of small venules in the cheek pouch of anaesthetized hamsters by iontophoresis through micropipettes. By using highly radioactive ADP the rate at which it is released from the pipette can be determined (Begent & Born, 1970*b*). This provides an approximate value for the concentration of ADP immediately outside the vessel. The iontophoretic currents needed for the release of thrombogenic quantities of ADP are three or more orders of magnitude smaller than the electrical currents that have been used to induce platelet thrombi by direct vascular injury (Sawyer, Suckling & Wesolowski, 1960; French, MacFarlane & Sanders, 1964; Berman, 1969).

In earlier experiments we showed that the thrombogenic effect was specific to ADP and not shared by closely related nucleotides such as guanosine diphosphate which has similar chemical properties. We found that the platelet thrombi grew exponentially and demonstrated a dependence of the growth rate constant on the mean blood velocity. The small venules in which this was shown remained normal, both functionally and morphologically under the electronmicroscope, except for some loss of electron density in the cytoplasm of the endothelial cells.

Those observations raised several questions. First, it had to be established whether the circulating platelets were induced to adhere and to aggregate by the applied ADP or via a secondary process induced by the ADP in the vessel wall, e.g. in the endothelial cells. The endothelial abnormality was seen with iontophoretic currents of 300 nA. If platelet adhesion could be initiated with still smaller currents without any abnormality in the vessel wall demonstrable electronmicroscopically, it would be evidence for a direct effect of the applied ADP.

Furthermore, in moving from the pipette on the outside of the vessel into the lumen, ADP would presumably have to diffuse through intercellular junctions because, as a polyanion at physiological pH, ADP would be unable to pass through the endothelial cells themselves. If, therefore, it could be shown that the production of gaps in the endothelial lining had the effect of accelerating the thrombogenic action of ADP, it would provide more evidence for a direct action of ADP. Histamine and 5-hydroxytryptamine cause contraction of endothelial cells lining the venules in the cremaster muscle of the rat (Majno, Gilmore & Leventhal, 1967; Majno, Shea & Leventhal, 1969); histamine has a similar effect in venules of the hamster cheek pouch (Entrican, Simpson & Stalker, 1971). The effect of this contraction is to separate the endothelial cells so that gaps appear between them in which the basement membrane becomes exposed to the flowing blood. On the supposition that the appearance of such gaps should increase the rate of diffusion of externally applied ADP into the venules, we have examined the effect of histamine, which by itself has no

effect on platelets, on the initiation of thrombogenesis by iontophoretically applied ADP to venules in the cheek pouch of anaesthetized hamsters. We did not use 5-hydroxytryptamine because it has direct effects on platelets. Some of the results have been communicated to the Physiological Society (Begent & Born, 1972).

METHODS

Golden hamsters of either sex weighing about 100–160 g were anaesthetized with I.P. pentobarbitone (6 mg/100 g body wt.). The cheek pouch was everted and spread on a special Perspex stage (Begent & Born, 1970*a*). The top layer and fine connective tissue were removed and a Leitz Laborlux microscope (mostly with a × 20 objective and a × 10 eyepiece) was focused on the thin lower layer which was transilluminated from below with light from a xenon lamp. The preparation was bathed continuously in an aerated saline solution (composition in mM: NaCl 131, KCl 4·0, $CaCl_2$ 3·0, $MGSO_4$ 1·0, glucose 10 and Tris 5·0; pH 7·4), and maintained accurately at 37° C. The preparation remained in good condition, as assessed by the blood flow in the vessels and their reactivity as well as by the absence of oedema, for at least 2–3 hr.

A micropipette was filled with a 10 mM solution of ADP and micromanipulated closely against the outside of a small venule (diameter 40–70 μ). The reference electrode was immersed in the bathing solution. When a negative potential was applied to the micropipette to eject ADP, the diameter of the venule was unaltered. When the micropipette was applied to an arteriole of similar size it was constricted by iontophoretic current greater than 10 nA; this constricting effect was used as a test for the release of ADP from each of the pipettes used.

Histamine was applied iontophoretically (Duling, Berne & Born, 1968) with a positive potential through a second micropipette, the tip of which was micromanipulated to lie on the other side of the vessel opposite the ADP-filled pipette. Histamine was in the pipette at a concentration of 10 mM in distilled water and was released by a current of 300 nA for 2 min before and during the application of ADP. To check that histamine was released the pipette was positioned against an arteriole which constricted when the iontophoretic current was passed, in accordance with earlier results (Duling *et al.* 1968).

Bradykinin at a concentration of 10 or 1000 μg/ml. in 0·154 M saline was applied from a syringe through a fine (25 gauge) needle to the venule under observation from immediately above the ADP micropipette. The activity of this solution was established by showing that it caused dilatation of neighbouring arterioles.

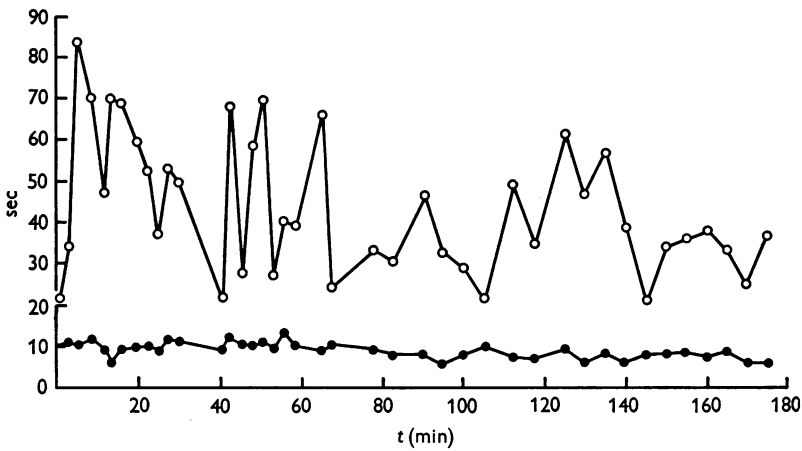
To quantitate the effects we measured the interval of time in seconds between switching on the iontophoretic current for ADP and seeing the first platelets adhere in the vessel opposite the tip of the micropipette. This measurement differs from that made earlier, i.e. the determination of the exponential growth rate of intravascular platelet aggregates, because we wished to time the earliest visible effect of the applied agent instead of the continuing aggregation process which may depend on a self-propagating chain reaction (see Born, 1965).

The *mean blood flow velocity* in the observed venules was measured by a technique already described (Begent & Born, 1970*a*) in which platelet aggregates are induced by iontophoretically applied ADP upstream in the venule and filmed when they embolize after the current is switched off.

RESULTS

Control experiments

First, a micropipette containing ADP was placed against a venule and the iontophoretic current of 300 nA was applied for short periods at frequent intervals to establish whether the response of the platelets remained constant or not. We measured both the time to first appearance of a white body as well as the time to the white body's embolization. Three experiments gave similar results. The time to the first appearance of white bodies was remarkably constant for up to 3 hr while the time to embolization was very variable (Text-fig. 1). We concluded that the experimental procedure, particularly the repeated iontophoretic application of ADP, did not alter the vessel wall appreciably and that the time to first appearance of adhering platelet aggregates was a valid measure of the effectiveness of the applied ADP.

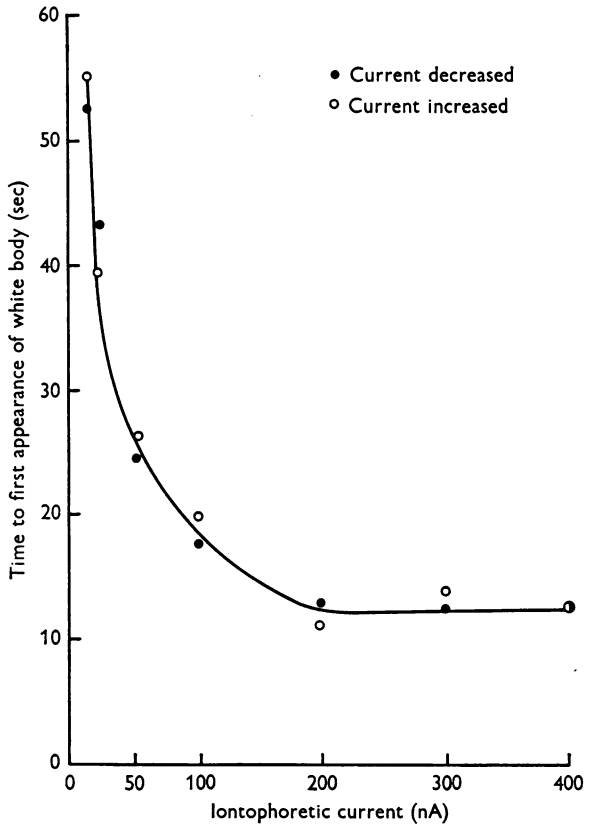


Text-fig. 1. Effect of repeated iontophoretic applications of ADP (10 mM in the pipette; 300 nA for 1 min periods) on the time interval (ordinate: sec) between switching on the iontophoretic current for ADP and seeing (*a*) the beginning of a white body (filled circles) and (*b*) embolization of the white body (open circles) plotted against time (abscissa: min). All measurements were made at the same site in one venule and each pair of points refers to one application of ADP.

Relation between the iontophoretic current for ADP and the time to first appearance of white bodies

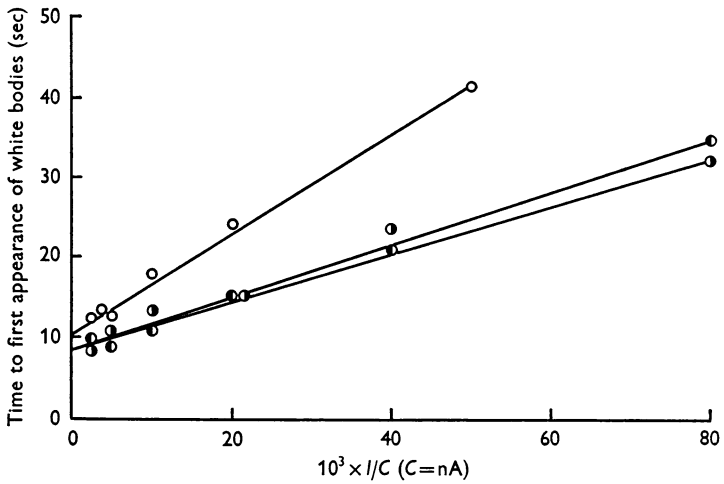
A micropipette containing ADP was positioned against a venule, an electrophoretic current of 400 nA passed and the time noted until the first appearance of a white body. With the pipette remaining in the same position this was repeated at 3 min intervals with decreasing current.

Text-fig. 2 shows that with currents smaller than 200 nA the time began to increase until, with currents of about 10 nA, no white body appeared however long the current was passed. With the pipette still in the same position the current was increased again and the time to first appearance

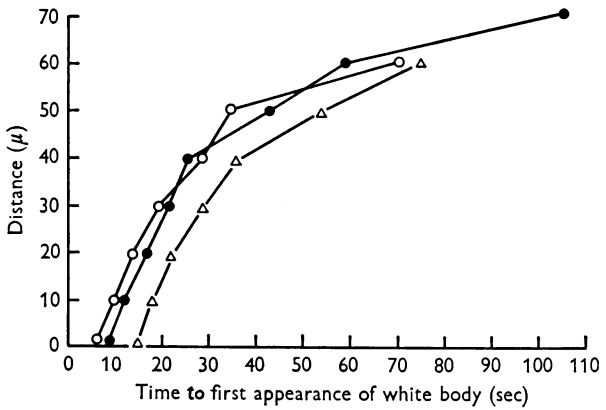


Text-fig. 2. Relation between iontophoretic current (nA) for ADP and time (sec) to the first appearance of white bodies. The current was first decreased from 400 nA in steps and a measurement made at each step; then the current was increased by the same steps and the measurements were repeated.

measured again until it reached a constant minimum. The results of three such experiments were similar. When the time to first appearance was plotted against the reciprocal of the current, straight lines were obtained (Text-fig. 3) which cut the Y axis at very similar points corresponding to minimum times of about 10 sec.



Text-fig. 3. Relation between the reciprocal of the iontophoretic current for ADP and the time (sec) to first appearance of white bodies, in two experiments. In one of the experiments, two micropipettes were used on the same venule; the tip diameter of one pipette was 4 μ (◐) and of the other 5 μ (◑).



Text-fig. 4. Distance (μ) from micropipette tip to the outside of the vessel wall plotted against the time (sec) to first appearance of white bodies in two venules, as follows:

Iontophoretic current for ADP (nA)	Venule number	Diameter (μ)	Micropipette number	Symbol
300	1	45	1	△
300	1	45	1	○
350	2	60	2	●

Relation between distance of micropipette tip from the vessel wall to the time of first appearance of white bodies

It was necessary to find out how critical the positioning of the micropipette was in relation to the venule walls in affecting the time to the first appearance of the white bodies, particularly for comparing the results of different experiments. For this purpose micropipettes filled with ADP were positioned as close as possible, i.e. less than 5 μ, to the outside of the venule wall and at increasing distances from it up to about 70 μ. At each position an iontophoretic current (300–350 nA) was passed which the previous experiments had shown to induce white bodies in the minimum time, and the time to first appearance of white bodies was recorded. Text-fig. 4 shows that the time to first appearance increased little and approximately linearly as the pipette tip was withdrawn about 20 μ from the venule; with greater distances the time increased progressively.

When the same micropipette was used in this way with different venules the two curves overlapped almost completely. However, when a different pipette was used on one of the same venules the form of the curve was similar but displaced a little to the right (Text-fig. 4). This suggested that the time to first appearance depended to some extent on the particular micropipette used, presumably because of differences in the rate of release of ADP (see Begent & Born, 1970*b*). It was concluded that small variations in positioning the micropipette tip against the venule had little effect on the time to first appearance of the white bodies. In all subsequent experiments the tip of the micropipette was placed within 5 μ of the outer wall of the venules.

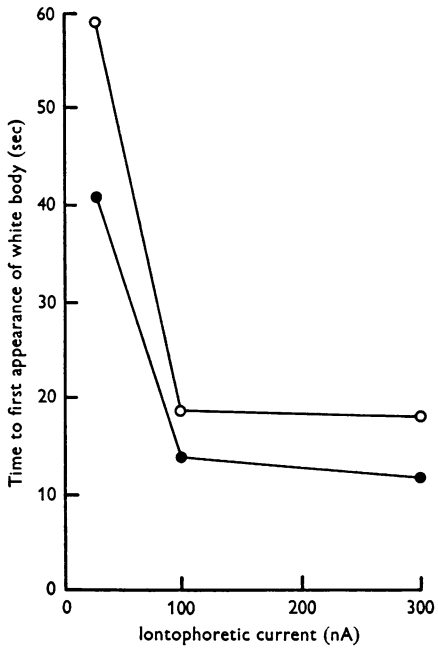
Effect of iontophoretically applied histamine on time to first appearance of white bodies

When histamine alone was applied for up to 3 min to venules as described under Methods, it had no effect either on the vessels or on the circulating platelets. To ensure that histamine was released the pipette was moved to a neighbouring arteriole which constricted.

The pipette was then moved back to a venule. Through another micropipette placed opposite the histamine pipette, ADP was applied at three different current strengths chosen to cover the range of times to first appearance of white bodies as shown in Text-fig. 2. Histamine was applied first for 2 min and then ADP was applied as well. Text-fig. 5 shows that the presence of histamine caused a decrease in the time to first appearance of white bodies at all three values of the iontophoretic current for ADP. This result was confirmed in another identical experiment as well as in five other experiments in which the iontophoretic currents for ADP were not varied;

the times decreased by up to 40 %. In three experiments histamine had no accelerating effect; in each of these the cheek pouch preparations had been in use for more than 2 hr and appeared inflamed so that the vessels had probably already been affected by the release of endogenous histamine.

The accelerating effect of histamine was reversible. This was observed in three experiments, one of which is shown in Text-fig. 6. After the iontophoretic current for histamine was switched off it took about 5 min before the time to first appearance of white bodies had increased to the initial control values.



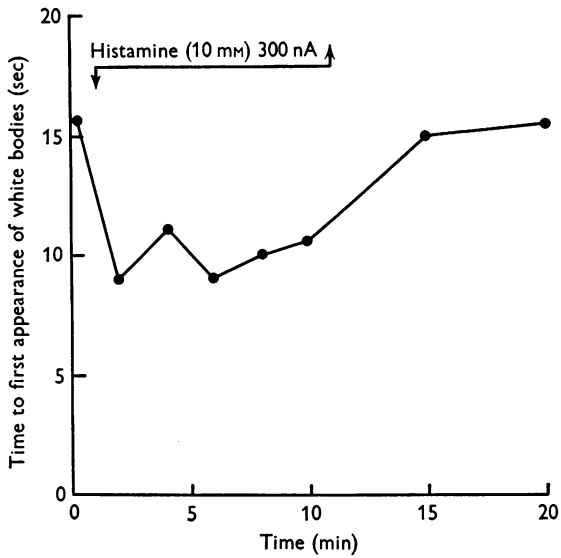
Text-fig. 5. Relation between iontophoretic current (nA) for ADP and time (sec) to the first appearance of white bodies in the absence (open circles) and presence (filled circles) of histamine (10 mM in the pipette) applied iontophoretically with a current of 300 nA.

Local blood flow during histamine application

The accelerating effect of histamine on white body formation might have been due to an increase in blood flow through the observed venule so that more platelets would have arrived opposite the ADP micropipette in unit time. To find out whether this was so, measurements were made in four experiments of the mean blood flow velocity through venules in the absence and presence of iontophoretically applied histamine. Table 1 shows that the mean blood flow velocity was not consistently altered by this local application of histamine.

Experiments with bradykinin

Bradykinin has an effect similar to that of histamine in widening intercellular junctions between endothelial cells of rat cremaster venules (Majno *et al.* 1969) but not in those of hamster cheek pouch (Entrican *et al.* 1971) although it causes vasodilatation and the formation of cytoplasmic spurs and vesicles. In agreement with this, bradykinin applied through a fine needle locally to the venule did not diminish the time to first appearance of white bodies induced by ADP.



Text-fig. 6. Effect of histamine (10 mM in the pipette), applied iontophoretically with a current of 300 nA between the arrows, on the time (sec) to first appearance of white bodies induced by ADP applied iontophoretically with a current of 100 nA.

TABLE 1. Effect of histamine on the mean blood flow velocity, as determined by the embolus technique, in four different venules

Venule diameter (μ)	Mean blood flow velocity (μ/sec)	
	Control	After histamine (500 nA for 2 min)
60	700	720
60	650	640
50	700	730
50	700	650

Microscopic appearance of venules

The separation of endothelial cells in venules can be demonstrated by the accumulation of fine carbon particles in the gaps some time after a suspension of the particles is injected into the circulation (see Florey, 1970). A hamster cheek pouch was prepared in the usual way and histamine was applied to a small venule with an iontophoretic current of 300 nA for 3 min. Then 0·1 ml. Pelican ink, which consists of a suspension of fine carbon particles, was injected into a heart chamber. Under the microscope the small vessels in the cheek pouch became temporarily dark grey as the carbon particles circulated through them. After a few min the greyness disappeared, leaving behind discrete patches of black particles along a limited stretch of venule wall just where the histamine had been applied (Pl. 1*A*).

Other cheek pouch preparations were observed by electron microscopy. After the experimental procedures the pouch was clamped at its pedicle, cut off and immersed in glutaraldehyde-osmium tetroxide fixative (1 part 2·5% glutaraldehyde in 0·1 M cacodylate buffer plus 2 parts 1% OsO_4 in 0·1 M cacodylate buffer) for 1 hr at 4° C. The tissue was then washed several times in chilled buffer solution and processed for transmission electron microscopy. On the electron micrographs the venules were normal and without endothelial gaps where no histamine had been applied, and remained so after the application of ADP. Where histamine had been applied endothelial cells were separated by gaps, several of which contained granulocytes (Pl. 1*B*).

DISCUSSION

The results confirm that the iontophoretic application of ADP to the outside of small venules causes the growth of platelet aggregates inside the vessel opposite the pipette tip and show, in addition, that this process can be accelerated by the simultaneous iontophoretic application of histamine. The effect of ADP could be due to an action on the vessel wall or on the platelets circulating through the vessel or on both. At first sight it would seem improbable that an agent such as ADP diffusing through the vessel wall into the flowing blood would attain a sufficient concentration to act on the platelets directly as it does *in vitro* (Born, 1970). It seems, however, that under the conditions of our experiment the diffusion profile of ADP in the blood stream should permit a considerable proportion of platelets to be affected directly (P. D. Richardson, personal communication).

It remained possible that the applied ADP also affected the endothelium of the venules so as to make it more adhesive to colliding platelets. Such an abnormality might be expected to show itself in an increase in the

time between stopping the iontophoretic current and the subsequent embolization of the white bodies. However, when the application of ADP was repeated at the same site at frequent intervals for up to 3 hr, no such effect was observed except, perhaps, in the first few minutes. This suggests that the applied ADP did not increase the adhesiveness of the endothelium towards circulating platelets in any permanent way, as happens when endothelium is damaged. The observations fail to support the proposition that transient intravascular aggregation of platelets damages the endothelium to which they adhere (Jorgensen, Rowsell, Hovig, Glynn & Mustard, 1967; Hovig, 1970). The only evidence of an abnormality in the walls of venules was some loss of electron density in the endothelial cytoplasm with iontophoretic currents of 300 nA or more (Begent & Born, 1970*a*). Still smaller currents (down to 10 nA) cause platelets to adhere to endothelium in which the cells do not show any abnormality electronmicroscopically.

There was an inverse relation between the iontophoretic current for ADP and the time to the first appearance of adhering white bodies. Plots of this time against the reciprocal of the current gave straight lines which cut the *Y* axis at about 10 sec. This minimum time was presumably made up of the time which ADP took to diffuse through the venule wall before reaching a sufficient concentration in the blood stream to affect the platelets, plus the time taken by the platelets to react which is very short, i.e. less than 1 sec, as measured *in vitro* by the velocity of the initial changes in morphology (Born, 1970). Most of the minimum time was presumably taken up by the diffusion of ADP through the vessel wall. To what extent the different constituents of the wall hinder the free diffusion of ADP is unknown. It seemed likely that the highest barrier would be endothelium in which the only diffusion paths for a highly charged molecule like ADP were presumably the narrow intercellular junctions. These junctions were widened by iontophoretically applied histamine so that intravascularly injected carbon particles accumulated in the venule walls close to the tip of the histamine pipette. Histamine markedly accelerated the effect of externally applied ADP on circulating platelets. This is evidence in support of the conclusions that the applied ADP was directly responsible for the white body formation and that the endothelium was the main barrier to the diffusion of ADP. Supporting evidence was the absence of an accelerating effect of bradykinin similar to that of histamine in venules known not to be affected by bradykinin (Entrican *et al.* 1971).

If this interpretation is correct, the reversibility of the accelerating effect of histamine on white body formation suggests that contracted endothelial cells are capable of complete relaxation to their original form so that the intercellular junctions become as narrow as before. Under the con-

ditions of these experiments, relaxation took about 5 min. This observation gives an indication of the time course of endothelial cell relaxation.

The micro-iontophoretic technique was introduced to make it possible to quantitate reactions to agents acting on smooth muscle of individual micro-vessels (Duling *et al.* 1968). The results reported in this paper suggest that the technique also permits quantitative observations on reactions of micro-vascular endothelium.

D.E.S. thanks the National Institutes of Health of the United States for a Fellowship.

REFERENCES

BANG, N. U., HEIDENREICH, R. O. & MATSUDA, M. (1970). Plasma protein requirements for human platelet aggregation. In *Platelet Adhesion and Aggregation in Thrombosis: Countermeasures*, ed. MAMMEN, E. F., ANDERSON, G. F. & BARNHART, M.I. Stuttgart: Schattauer.

BEGENT, N. A. & BORN, G. V. R. (1970*a*). Growth rate *in vivo* of platelet thrombi, produced by iontophoresis of ADP, as a function of mean blood flow velocity. *Nature, Lond.* **227**, 926–930.

BEGENT, N. A. & BORN, G. V. R. (1970*b*). Determination of the iontophoretic release of adenosine diphosphate from micropipettes. *Br. J. Pharmac.* **40**, 592*P*.

BEGENT, N. A. & BORN, G. V. R. (1972). Effect of endothelial contraction on the initiation of platelet thrombi in apparently normal venules. *J. Physiol.* **222**, 138–139 *P*.

BERMAN, H. L. (1969). Studies of thromboembolism and related phenomena in the microvascular system. In *Thrombosis*, pp. 534–546, ed. SHERRY, S., BRINKHOUSE, K. M., GENTON, E. & STENGLE, J. M. Washington: Nat. Acad. Sci.

BORN, G. V. R. (1965). Platelets in thrombogenesis: mechanism and inhibition of platelet aggregation. *Ann. R. Coll. Surg.* **36**, 200–206.

BORN, G. V. R. (1970). Observations on the change in shape of blood platelets brought about by adenosine diphosphate. *J. Physiol.* **209**, 487–511.

BORN, G. V. R. (1971). Functional Physiology of Blood Platelets. In *Human Blood Coagulation, Haemostasis and Thrombosis*, ed. BIGGS, R. Oxford: Blackwell.

BORN, G. V. R. & CROSS, M. J. (1964). Effects of inorganic ions and of plasma proteins on the aggregation of blood platelets by adenosine diphosphate. *J. Physiol.* **170**, 397–414.

CROSS, M. J. (1964). Effect of fibrinogen on the aggregation of platelets by adenosine diphosphate. *Thromb. Diath. Haem.* **12**, 524–527.

DULING, B. R., BERNE, R. M. & BORN, G. V. R. (1968). Microiontophoretic application of vasoactive agents to the microcirculation of the hamster cheek pouch. *Microvascular Res.* **1**, 158–173.

ENTRICAN, J. H., SIMPSON, J. G. & STALKER, A. L. (1971). The effects of histamine and bradykinin on the post-capillary venule. *J. Path. Bact.* **104**, p. iii.

FLOREY, H. W. (1970). Inflammation. In *General Pathology*, ch. 3, pp. 40–123, ed. FLOREY, LORD. London: Lloyd-Luke.

FRENCH, J. E., MACFARLANE, R. G. & SANDERS, A. G. (1964). The structure of haemostatic plugs and experimental thrombi in small arteries. *Br. J. exp. Path.* **45**, 467–474.

HOVIG, T. (1970). The role of platelets in thrombosis. *Thromb. Diath. Haem.* suppl. **42**, 137–153.

JORGENSEN, L., ROWSELL, H. C., HOVIC, T., GLYNN, M. F. & MUSTARD, J. F. (1964). Adenosine-induced platelet aggregation and myocardial infarction in swine. *Lab. Invest* **17**, 616–644.

MAJNO, G., GILMORE, V. & LEVENTHAL, M. (1967). On the mechanism of vascular leakage caused by histamine-type mediators. A microscopic study *in vivo*. *Circulation Res.* **21**, 833–847.

MAJNO, M., SHEA, S. M. & LEVENTHAL, M. (1969). Endothelial contraction as a mechanism of action of histamine-type mediators. An electron microscopic study. *J. cell Biol.* **42**, 647–672.

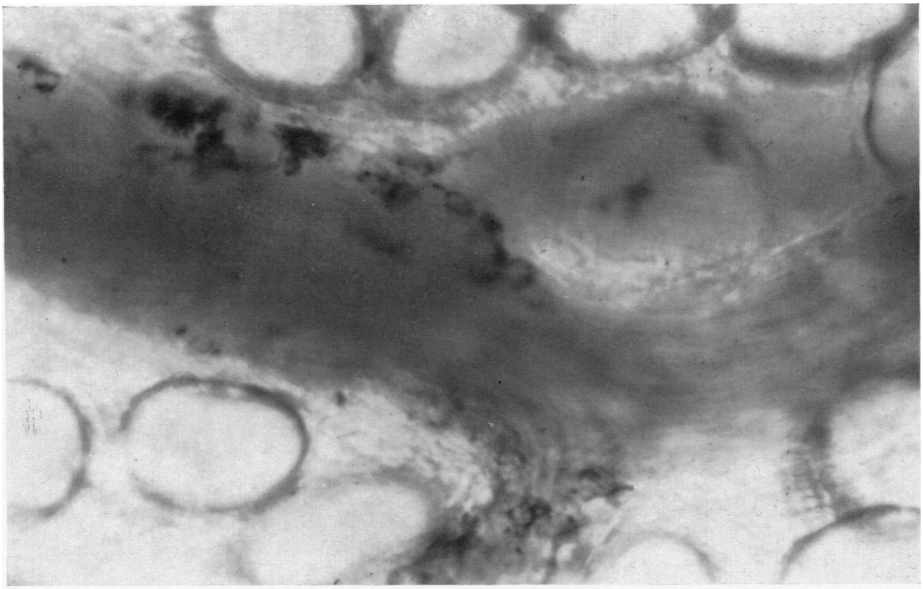
MUSTARD, J. F. & PACKMAN, M. (1970). Factors influencing platelet function: adhesion, release and aggregation. *Pharmac. Rev.* **22**, 97–187.

SAWYER, P. N., SUCKLING, E. E. & WESOLOWSKI, S. A. (1960). Effect of small electric currents on intravascular thrombosis in the visualized rat mesentery. *Am. J. Physiol.* **198**, 1006–1010.

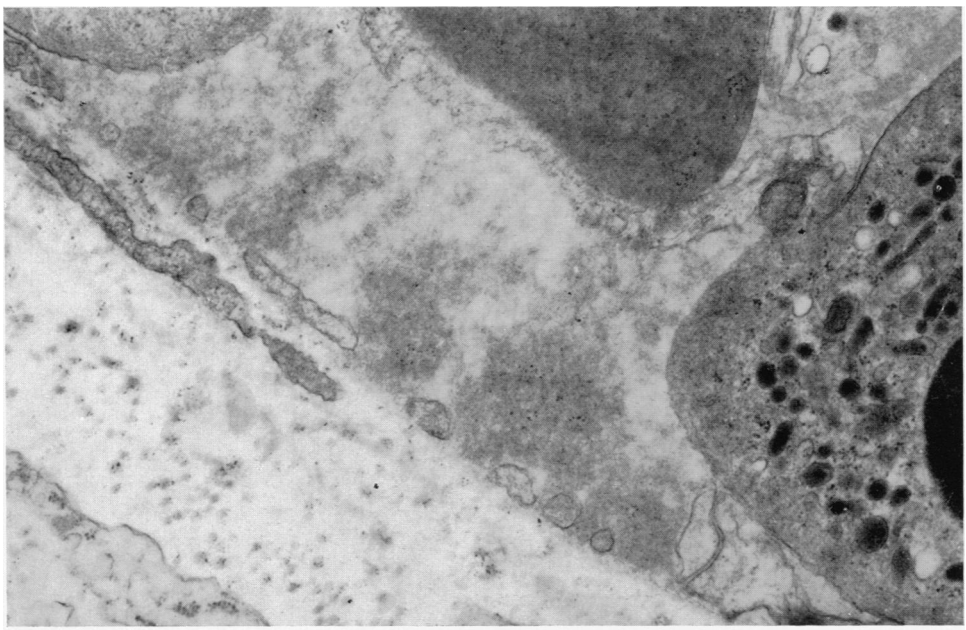
EXPLANATION OF PLATE

A, venule (45 μ in diameter) with discrete accumulations of carbon particles near a micropipette from which histamine had been released iontophoretically for 3 min; × 600.

B, electronmicrograph of part of a venule to which histamine had been applied iontophoretically, showing gaps in the endothelial lining; × 36,000. The endothelial cytoplasm is the thin strip of interrupted tissue running across the picture from top left to bottom right and the vessel lumen is above it with parts of an erythrocyte and a granulocyte.



A



B