PLATELET CLUMPING IN INJURED VESSELS

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ONE of the earliest accounts of thrombosis in living vessels was given by Wharton Jones (1851); while studying the inflammatory reaction in the web of a frog's foot, he observed the formation of white thrombi which subsequently embolised. Zahn (1875) repeated these observations and showed that formed blood elements adhered to the site of injury, and Bizzozero (1882) using the mesentery of a guinea pig or young rabbit, showed that the thrombi were formed from platelets which adhered to the injured vessel wall. These thrombi were then dislodged by the force of the blood stream, the process of formation and embolisation being repeated several times within the first fifteen minutes after the injury. Honour and Ross Russell (1962) examined this phenomenon in considerable detail, using arteries on the surface of the rabbit brain. They showed that thrombi could be produced in response to a variety of forms of injury, and that the white bodies were composed of closely packed but morphologically discrete platelets, no fibrin being detectable. They also showed that the thrombi were unaffected by heparin and by other agents known or thought to influence blood coagulation; by antihistamines, and by 5-Hydroxytryptamine (5-HT) or its antagonists.

In 1960 Hellem showed that a heat-stable, dialysable extract of human red cells could increase platelet adhesiveness *in vitro*, and he called the unknown active principle of his extracts " Factor R ". Gaarder, Jonsen, Laland, Hellem and Owren (1961) showed that the activity of the extracts was due to their adenosine diphosphate (ADP) content, and they also showed that ADP in very low concentrations, was capable of producing clumping of human platelets *in vitro*. Mitchell and Sharp (1963) investigated the effect of substances related to ADP, and of substances carried by the platelet. They confirmed that citrated human platelets were clumped by ADP at a concentration of 0·04 μg. per ml., and that adenosine triphosphate (ATP) was only slightly active, a concentration of 1·5 μg. per ml. being required. They also found that human platelets were clumped by 5-HT, and by adrenaline and nor-adrenaline at concentrations of 0·06 μg. per ml. Rabbit platelets behaved differently, being more sensitive to ATP than human platelets, a concentration of 0·22 μg. per ml. being sufficient to induce clumping. Rabbit platelets were insensitive to nor-adrenaline and adrenaline and showed a variable response to 5-HT, although their behaviour with ADP did not differ from that of human platelets, concentrations of 0·05 μg. per ml. producing clumping.

These observations led us to examine the effects of ADP, ATP and 5-HT on injured arteries on the surface of the rabbit brain, and this paper reports the results of these studies.

MATERIALS AND METHODS

Rabbits, anaesthetised with urethane, were used throughout, the cortex being prepared and the vessels observed microscopically as described by Honour and Ross Russell (1962).

In the observations reported here we have used two grades of arterial injury which we have designated as major and minor:

Major injury.—An artery 100–200 μ in diameter is firmly pinched with needle-pointed ophthalmic forceps. Such an injury produces haemorrhage and transient vasoconstriction. When the bleeding has stopped, usually within a few seconds, and the vessel has relaxed, white bodies form in a steady succession at the site of injury, and embolise. At such a site, the degree of damage appears to be such that it can maintain the production of thrombi spontaneously, for a period of several hours.

Minor injury.—If a cortical artery is gently gripped with the needle-point forceps, no bleeding occurs, and after the subsidence of slight transient vasoconstriction the vessel appears to be completely normal, and no thrombi are produced spontaneously. This minor injury to an artery, not in itself sufficient to produce a white body, does however enable certain substances applied to the vessel to initiate thrombus formation at the injury site.

After inflicting the injury, the cortex was bathed in warm 0·9 per cent saline; during applications of various test substances, the saline flow was stopped, and the excess lightly mopped off before applying the test material. The test solutions were then applied dropwise from a Pasteur pipette, 1 drop per second, until a definite white body was seen to be forming, when the application was stopped, the saline flow restored and the developing body observed until it embolised. More dilute solutions were then tested serially, until one was found which did not produce an effect. After a series of negative responses, due either to excessive dilution of the test substance or to the inherent inability of the substance to produce an effect, the site was retested with a solution, which in that particular preparation had produced a thrombus, to ensure that the site was still responsive.

Minor injuries were used to test substances for their activity in the production of thrombi, to determine the minimum concentration at which they acted, and to assess the effect of antagonists.

Major injuries, which were spontaneously producing white bodies, were used to determine the effect of various enzyme inhibitors, any effect produced by these substances being readily detected by a change in the size or rate of production of thrombi.

RESULTS

Effect of red cell extracts, adenosine phosphates, 5-HT, and nor-adrenaline

Major injuries.—The application of human and rabbit red cell extracts, prepared as described by Hellem (1960), and of adenosine monophosphate (AMP), ADP, ATP, 5-HT, and nor-adrenaline dissolved in 0·9 per cent saline, to arteries subjected to major injuries and actively producing white bodies spontaneously, did not modify the size or rate of production of these white bodies.

Minor injuries.—In arteries subjected to this degree of injury, no white bodies formed spontaneously, but the application of red cell extracts, or of ADP, ATP, or 5-HT dissolved in normal saline, produced white bodies, which continued to build up and embolize, so long as the solution was applied, and ceased as soon as the application of the activating substance was stopped. A series of observations was made using doubling dilutions of the active agents, to determine the lowest concentration necessary to produce white bodies, when applied to an artery with a minimal injury. Each substance was tested in this way on 8 rabbits, and after the end point had been determined, each site was checked with a higher concentration of the test substance to ascertain that the injury could still be activated. The results are shown in Table I. We also applied active substances to uninjured cortical arteries, injected them into the carotid artery on the

TABLE I.—*Lowest Concentrations of Various Agents, which, when Applied to the Outside of Minimally Injured Cortical Artery, would Produce White Bodies*

Substance	Range of lowest effective concentration (μg./ml.)	Mean (μg./ml.)
ADP . . .	0·25–16·0	3·0
ATP . . .	0·25–16·0	5·3
5-HT . . .	0·125–32·0	4·8
AMP . . .	Inactive	—
1-nor-adrenaline .	,,	—

side being observed, and into a marginal ear vein without observing white body formation in the cortical arteries.

These observations show that the agents which will clump rabbit platelets *in vitro*, as described by Mitchell and Sharp (1963), can also induce white body formation when applied to arteries with minor injuries on the surface of the rabbit brain. In the *in vitro* system, it had been noted that the time course of platelet clumping was slower with 5-HT than with ADP and ATP, and this is also true of the time course of white body formation ; ADP and ATP produce an effect within a minute of beginning the application, and if no white body has developed by this time, it will not do so subsequently. With 5-HT however, a large white body may begin to form several minutes after beginning the application.

Effect of 5-HT antagonists

Two substances were used (i) Ro-3-0837 (Roche Products), (ii) UML 491 (Sandoz). Minor injuries were inflicted on rabbit cortical arteries and their responsiveness to ADP, ATP and 5-HT determined. The two rabbits were then given 15 mg. and 60 mg. respectively of Ro-3-0837 intravenously (4·4 and 19·5 mg. per Kg.). Thereafter, applications of 5-HT to the previously responsive injury sites failed to produce white bodies, whereas ADP and ATP applied to the same sites produced white bodies at concentrations identical with those found to be effective before the 5-HT antagonist was given. Thus although the minor injury sites, in the presence of the antagonist, were completely unresponsive to 5-HT, their ability to respond to ADP and ATP was unimpaired.

As Ro-3-0837 affects platelet clumping *in vitro* in comparable concentrations to those likely to be attained in the two rabbits tested, it seemed possible that this was an effect on the circulating platelets and not on the minor injury site. Application of the antagonists locally in low concentration, however, to minor injury sites also abolished the response to 5-HT ; dropwise applications of Ro-3-0837 25 μg. per ml. or UML 491 1·0 μg. per ml. on to minor injuries made them unresponsive to 5-HT but did not modify the response to ADP or ATP applied topically to the injured arteries. It thus seems likely that the antagonists exert some local effect on the vessel wall and interfere with its response to 5-HT, in addition to whatever effect they are exerting on the whole mass of circulating platelets.

Parenteral and local administration of the 5-HT antagonists to rabbits with major injuries, where a steady stream of white bodies was forming spontaneously, did not modify their size or rate of production, suggesting that the major injury site is producing white bodies by a mechanism which does not involve clumping induced by 5-HT.

Effect of enzyme inhibitors

If thrombus formation in an injured artery is the result of some specific change at the site of injury, it would appear that a minor injury provides an inadequate stimulus, and needs the addition of certain chemical substances to become effective. A major injury is an adequate stimulus, and can induce the specific change without any assistance. If the specific change responsible for white body formation depends on biochemical processes, then the action of enzyme inhibitors might help to elucidate its nature.

The substances tested were dissolved in 0·9 per cent saline, and applied dropwise at the rate of 1 ml. per min., for 10 min. except where otherwise stated, to the external surface of arteries with major injuries, which were producing white bodies spontaneously. The substance was then washed off with warm saline, and the usual saline application continued while the site was observed for some hours.

Sodium fluoro-acetate, at a concentration of 1 mg./ml., was applied to major injuries in three rabbits. For the first 15 min. after the beginning of the application the white bodies continued to appear, but they then diminished in size, fragmented during formation and production stopped entirely at 20 min. after the start of the application. The artery was observed for a further period of 2 hr. but no more white bodies were seen. It should be noted that at this concentration, (10mM), fluoroacetate does not affect the clumping of platelets *in vitro* (Mitchell and Sharp, 1963).

The effect of fluoroacetate on minor injuries was also studied. The end point for each clumping agent was determined, and fluoroacetate 1 mg. per ml. was then applied. The site was retested, 20 min. later, and the results are given in Table II, which shows that sodium fluoroacetate at that concentration did not prevent ADP, ATP and 5-HT from producing white bodies at a minor injury site.

TABLE II.—*Effect of Topically Applied Sodium Fluoroacetate on the Lowest Effective Concentrations of Clumping Agents at a Minor Injury Site*

Substance	Concentration (μg./ml.)	Vasoconstriction	White bodies
ADP	32	+	Large
	16	+	Small
ATP	64	+	Large
	16	+	Small
5-HT	64	None	Large
	16	,,	Small
Na fluoro-acetate 10 ml.	1 mg./ml.	Applied for 10 min.	Occasional Small
	Time interval before retesting—20 min.		
	(μg./ml.)		
ADP	16	+	Small
ATP	16	++	,,
5-HT	16	None	,,

Sodium mono-iodoacetate, 750 μg. per ml. (4mM) was applied to a cortical artery which had been subjected to a major injury, and which was spontaneously producing white bodies. The white bodies became smaller 20 min. after beginning

the application, tiny masses of platelets streaming off their surface as they were building up, and at 30 min. production stopped. The site remained quiescent for some 30 min., when tiny white bodies began to form and embolize, until at about 90 min. from the beginning of the application the injury site was behaving in the same way as in the control period.

In other experiments using sodium mono-iodoacetate at levels ranging from 750 μg. per ml. to 2 mg. per ml., similar results were obtained. In some experiments the production of white bodies did not stop completely, but the behaviour of the site always changed, the white bodies becoming tiny and embolising every few seconds. In every experiment a site treated with mono-iodoacetate retained its usual sensitivity to topically applied ADP and ATP.

Sodium azide.—Five minutes after beginning the application of sodium azide, 1 and 10 mg. per ml., to major injury sites, white body production ceased and did not begin again during a two hour observation period. Application of ADP to these quiescent sites produced white bodies for as long as the solution was applied.

Potassium cyanide, in concentrations of 4–1000 μg. per ml. produced intense vasoconstriction in all the cortical vessels, but did not affect the behaviour of major injury sites nor did it affect the ability of ADP, ATP and 5-HT to produce thrombi at a minor injury site, the end points for these substances before and after the cyanide application being identical.

2 : 4 *Dinitrophenol*, applied topically to major and minor injury sites in concentrations of 12·5–125 μg. per ml. was without effect on the production and size of white bodies at the major injury sites, and on the clumping efficiency of the three agents tested, as judged by their end point concentrations at a minor injury site.

Ability of clumping agents to reproduce effect of arterial injury in major injury sites blocked with enzyme inhibitors.—In the studies described with sodium fluoroacetate, sodium mono-iodoacetate and sodium azide we found that the external application of ADP, ATP, and 5-HT, to a major injury site whose spontaneous white body production had been stopped with fluoroacetate, fluoride, azide or iodoacetate, produced white bodies for as long as this application was continued. An application rate of 0·1–0·2 μg. per min. of ADP to one such site produced a succession of white bodies similar in size and timing to those occurring before the enzyme inhibitor had been applied.

The results we have obtained with these enzyme poisons suggest that the phenomenon first observed by Wharton Jones (1851) of white body production in injured arteries, may be related to release from the damaged tissues in the artery wall of substances, similar in properties to ADP and ATP, which instruct the passing platelets to adhere to each other and to the vessel wall at the point of injury. 5-HT does not seem to be implicated in this phenomenon because we were unable to modify white body production at a major injury site by powerful 5-HT antagonists.

Effect of other substances

Toluidine blue.—Mitchell and Sharp (1963) showed that this strongly basic dye inhibited ADP-induced platelet clumping *in vitro*. We therefore inflicted minor injuries on cortical arteries and determined the lowest effective concentrations of the 3 clumping agents which, when applied to the site, produced white bodies. Toluidine blue, 1 per cent in 0·9 per cent saline, was then injected into

a marginal ear vein, 2 ml. every 5 min., and the injury site retested between each injection. The concentrations of the 3 substances needed to produce platelet masses in the artery were unaltered ; the bodies which formed were no longer white, but were blue. The dye must be selectively concentrated on the platelets, but if the animal is tested repeatedly with white body producing agents after a single dye injection the " blue bodies " form for 1–3 min., subsequent masses being uncoloured, so the initial preferential attachment to the platelets, must then be capable of being reversed.

Reserpine, administered to an animal for a period reduces the concentration of 5-HT in platelets. As it seemed possible that an alteration in the chemical composition of circulating platelets might affect their behaviour in an injured vessel, we gave 15 mg. reserpine (Serpasil-Ciba) intravenously to a rabbit, and 24 hr. later, when the platelet 5-HT content would be very low or nil (Kuntzman, Udenfriend, Tomich, Brodie and Shore, 1956), we inflicted a minor injury on a cortical artery. Applications of ADP, ATP, and 5-HT were then made and the lowest concentration of these which produced white bodies was determined. No control observations were of course possible, but the values obtained fell within the range found in untreated rabbits.

Nialamide (*Pfizer*), which is a mono-amine oxidase inhibitor, was tested because Shimamoto and Fujita (1961) had claimed that it prevented platelet build-up in injured central ear arteries in the rabbit. The minor injury end-points for the three clumping agents, in rabbits treated with Nialamide, 40 mg. per day by mouth, for 2 days, fell within the range observed on untreated rabbits.

Thrombin.—Human thrombin (Fibrindex) applied to a minor injury site produced vasoconstriction and white bodies, down to a concentration of 12·5 N.I.H. units per ml. Bovine thrombin (Maw) showed no activity.

When the human thrombin solution was boiled for 5 min., and retested on the minor injury site, its white body producing activity was unimpaired, but this solution was no longer capable of clotting citrated plasma. Preparations designated as " thrombin " contain many materials other than the specific enzyme which catalyses fibrin formation, and it seems likely that the white body producing activity of this batch of human thrombin was not due to its high molecular weight coagulant protein factor.

Vasoconstriction in cerebral cortical arteries

If a solution which is active in producing both vasoconstriction and white bodies is applied to an artery with a minor injury, within a few seconds the segments on either side of the injury contract, leaving the point of injury, which does not contract so actively, standing out like a bead on a string, for the lumen in the adjacent segments may be reduced almost to a thread. The vasoconstriction passes off within a few seconds and the white body then begins to form.

ADP, ATP, many of the tissue extracts and a number of the other materials tested, caused vasoconstriction in injured cortical vessels. Some of these agents also produced white bodies, and it seemed possible at first that vasoactivity and white body producing activity ran in parallel. However, 5-HT, which readily produces thrombi in injured arteries, never produced vasoconstriction in our brain preparations, and conversely, histamine acid phosphate produced vasoconstriction at a concentration of 0·25 μg. per ml. but never produced thrombi, even when concentrations 200 times greater than this were used.

We have observed that blood flowing from a major injury site, where free external bleeding occurs initially, will produce intense vasoconstriction where it flows over adjacent vessels ; if this stream of blood is deflected over a vessel with a minor injury, a white body will form in the minimally injured artery and embolize, this process being repeated for as long as the blood flow over the site persists.

Effect of antihistamines on vasoconstriction.—The lowest concentration of the various substances which produced vasoconstriction was first determined on a minor injury site. An antihistamine, promethazine hydrochloride, was then given intravenously in a dose of 2·5 mg. and the tests were repeated. The results are shown in Table III and indicate that the antihistamine is effective against vasoconstrictor substances, but where these substances also produce white bodies, the latter effect is unimpaired. The effect of the antihistamine injection on vasoconstriction persisted for more than 2 hr.

TABLE III.—*Effect of Antihistamines on Vasoconstriction Produced by Various Agents at a Minor Injury Site*

Substance	Concentration	Vasoconstriction	White bodies
Platelet extract	1/256	+	+
	1/512	+	None
	1/1024	Doubtful	,,
	1/2048	None	,,
Histamine acid phosphate	1 μg./ml.	+	,,
	0·5 μg./ml.	+	,,
	0·25 μg./ml.	+	,,
Promethazine hydrochloride		2·5 mg. i.v. very slowly	
Platelet extract	1/1	None	++
	1/128	,,	+
	1/256	,,	+
Red cell extract	1/1	,,	++
		90 min. interval	
Histamine acid phosphate	100 μg./ml.	None	None
Platelet extract	1/1	,,	++

At the end of these observations, cortical veins were injured and the flowing blood was allowed to pass over an adjacent artery on which a minor injury had been inflicted. Definite vasoconstriction occurred, despite the effect of the antihistamine, and white bodies were produced at the site of injury for as long as flowing blood passed over it. As the site did not respond to topically applied histamine, blood flowing from an injured vessel must contain a vasoconstrictor substance other than histamine.

Constrictor substances in blood.—Samples of blood withdrawn from rabbit ear veins by needle puncture, and human blood samples obtained by finger-prick were active in producing both vasoconstriction and white bodies when applied to arteries with minor injuries. Blood taken direct from the lumen of a large vessel (through a polythene cannula passed down the common carotid artery) into silicone treated syringes failed to produce thrombi, although it did produce intense vasoconstriction.

In a series of experiments designed to study the properties of shed blood in more detail, one rabbit was used as a donor, and blood was withdrawn from an indwelling polythene cannula inserted down the carotid artery into the aorta. A

second animal with a minor injury to a cortical artery was used as a test animal and arterial blood (i) diluted with an equal volume of normal saline, (ii) taken into a syringe wetted with heparin (10,000 u/ml.), (iii) mixed with 3·8 per cent trisodium citrate 0·5 ml. to 2 ml. of blood, was applied to the injury site before and after intravenous promethazine had been given ; the results are shown in Table IV. The Table shows that an antihistamine completely abolishes the vaso-

TABLE IV.—*Effect of Various Blood Samples on A Minor Injury Site*

Substance	Vasoconstriction	White bodies
Rabbit red cell extract	+	+
Blood, arterial	Intense	None
Blood + heparin	,,	,,
Blood + 3·8 per cent citrate	Less intense	,,
Rabbit red cell extract	Intense	+
Promethazine hydrochloride	2·5 mg. i.v.	
Blood, arterial	Slight	None
Promethazine hydrochloride	1·25 mg. i.v.	
Blood, arterial	None	None

constriction which must therefore be due to some histamine-like substance present, or formed in shed blood. This vasoconstrictor substance is not responsible for the production of white bodies for the results presented so far show that these two activities can operate independently of each other.

The difference between the activity of blood taken by needle puncture or by finger-prick, and blood taken directly from the lumen by a cannula, led us to consider the possible role of the pain producing substance (P.P.S.) which is a polypeptide present in contact-activated plasma (Armstrong, Jepson, Keele and Stewart, 1957) but not in fresh plasma.

Comparison of serum, contact-activated plasma, and fresh plasma.—A minor injury site was first tested with rabbit red cell extract to determine that it was capable of producing both white bodies and vasoconstriction. Rabbit serum, fresh heparinized and citrated plasma, and plasma activated by being kept in contact with a glass surface for from 3 min. up to 60 min., were then applied to the site. Then, after intravenous promethazine had been given, the applications were repeated. The results are given in Table V, and show that there is a vaso-

TABLE V.—*Effect of Serum and of Plasma Prepared in Various Ways, on a Minor Injury Site*

Substance	Vasoconstriction	White bodies
Rabbit red cell extract 1/1	Marked	++
Rabbit plasma, heparinised, non-activated	V. slight	None
Rabbit plasma, citrated, non-activated	None	,,
Rabbit plasma, heparinised, activated by glass contact	,,	,,
Rabbit plasma, citrated, activated by glass	,,	,,
Rabbit serum 1/1	Gross	,,
Rabbit serum 1/8	Slight	,,
Promethazine hydrochloride	1 ml. i.v. 2·5 mg.	
Rabbit serum 1/4	None	None
Rabbit red cell extract 1/1	,,	++

constrictor substance in rabbit serum which is blocked by antihistamines. It is separate and distinct from the agents which produce platelet thrombi, and also

from the pain producing substance which develops in glass contacted plasma, for such plasma shows neither vasoactivity, nor white body promotion. It is also interesting to note that the vasoactivity of serum is usually ascribed to 5-HT derived from platelet breakdown. The rabbit brain vessels used in our studies did not constrict with 5-HT so there is clearly a vasoactive substance present in serum other than 5-HT, and our experiments show that this substance resembles histamine.

Many attempts have been made to isolate and identify the P.P.S. (Keele, 1960); its properties have been shown to have many features in common with bradykinin (Lewis, 1961), but its final identification is not yet certain. It seemed worthwhile to study the effect of bradykinin on injured cortical arteries and various concentrations of synthetic bradykinin (BRS 640 Sandoz) were applied to cortical arteries with minor injuries. Apart from slight dilatation of the vessel when the highest concentration used was applied (10 μg. per ml.) no changes were observed, and no thrombi were produced. Bradykinin applied to major injuries did not affect the size or rate of production of the white bodies which were forming spontaneously.

DISCUSSION

The part played by platelet deposition in the arrest of haemorrhage and in the formation of intravascular thrombi is now well established (Poole and French, 1961). The main contribution of the work described here is that by using a replicable stimulus in conjunction with some substances which have been shown to clump platelets *in vitro*, an experimental analysis of some of the factors concerned with platelet adhesiveness and thrombus formation in injured vessels has been made possible.

First we have shown that the three substances which clump rabbit platelets *in vitro* (Mitchell and Sharp, 1963) produce intra-arterial platelet masses when they are applied to the outside of a minimally injured artery. There are three mechanisms by which this effect could arise (Fig. 1) :—(i) Diffusion. The injury breaches the vessel wall and allows the active agents to diffuse in and affect the passing platelets. (ii) Summation. The injury is already producing subliminal amounts of some white-body producing substance and in conjunction with the externally applied agents an effective concentration is attained. (iii). Transformation. The externally applied agent is used by the vessel wall for the production of another substance which is the active agent in white body formation. Our failure to influence the behaviour of a minor injury site toward ADP and ATP with enzyme poisons, suggests that their action is related to diffusion or summation, rather than transformation. 5-HT however, was no longer able to produce white bodies at a minor injury site to which 5-HT antagonists had been applied, suggesting that the vessel wall plays a more active part in the phenomena observed with this agent. As 5-HT antagonists do not however influence spontaneous white body formation at a major injury site, 5-HT is unlikely to be playing a significant role in the development of the embolizing platelet masses which form in response to a marked injury.

Second, by using enzyme poisons we have been able to prevent an artery, to which a major injury has been inflicted, from forming white bodies. As far as we are aware, in none of the previous studies which have followed from Wharton Jones' observations in 1851, including the detailed assessment of anticoagulants

and anti-inflammatory agents reported by Honour and Ross Russell (1962), has this proved possible. Fig. 2, based on Dixon and Webb (1958) and Baldwin (1952), shows the effects of various substances which stopped white body production. Fluoroacetate blocks the tricarboxylic acid cycle by forming fluorocitrate, which interferes with the enzyme aconitase (Peters, 1953) and brings the cycle to a halt. Fluoride poisons the enzyme enolase and prevents synthesis of phosphopyruvic acid. Monoiodoacetic acid poisons triosephosphate dehydrogenase, but this action is a non-specific one, for other enzymes which are dependent on sulphydryl groups such as succinic dehydrogenase are also affected. All these

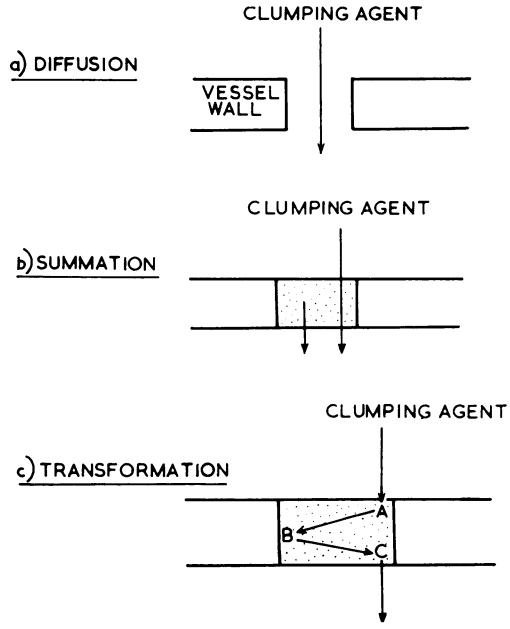

FIG. 1.—The three ways in which externally applied clumping agents might induce platelet thrombus formation in a minimally injured artery.

materials stop white body formation in injured arteries, which suggests that the injury site is releasing some material whose production is prevented by the enzyme poisons. A major injury site whose white body production has been stopped with these agents, can be made to form platelet masses again by the external application of ADP or ATP in low concentrations, (0·1 μg. per min. of ADP reproduces the original, pre-inhibitor conditions), and it seems possible that ADP, ATP, or related substances, released from the injury site, could be responsible for the platelet thrombus formation in injured arteries.

Mitchell and Sharp (1963) showed that significant amounts of platelet clumping substance could be extracted from artery wall, but in normal tissues, ADP and ATP take part in energy transfer reactions, without necessarily changing their overall concentrations. There is however evidence that injured tissues handle nucleosides in a way which differs from that found in normal tissues. Billings and Maegraith (1938) measured the concentration of adenosine-like substances

(" A-substance ") in blood, and in view of Hellem's observations on the localisation of the platelet clumping agent, " Factor R " in red cells, it should be noted that they could not detect any " A-substance " in whole blood, plasma, or red cells suspended in saline, but when the red cells were lysed they found 40–200 μg. ml. in the extract. Billings and Maegraith measured the level of this substance in blood returning from a limb before, during and after a period of ischaemia produced by arterial occlusion. They found that while the muscle was suffering ischaemic injury, the " A-substance " level in the returning blood doubled, and they also showed that these substances were present in higher concentrations in damaged muscle than in normal muscle. Bollman and Flock (1944) also studying

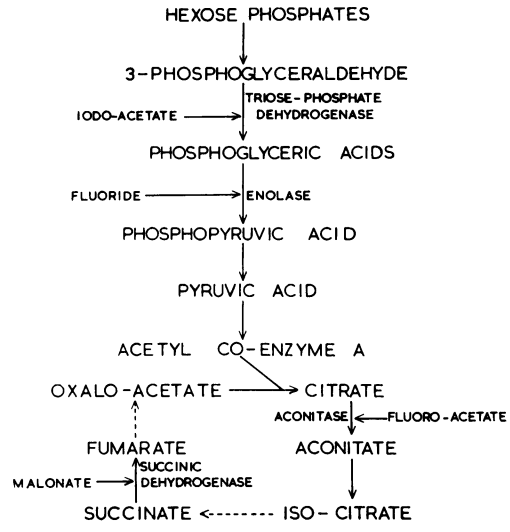

FIG. 2.—Mode of action of enzyme inhibitors, shown on a simplified diagram of intermediate cell metabolism.

ischaemic muscle showed that during the period of injury, creatine phosphate and ATP were degraded to inorganic phosphate ; if the blood flow was restored these substances were resynthesised. Macfarlane and Spooner (1946) showed that after 5 hr. of ischaemia, the pyro-phosphate content of the muscle had fallen from 0·36 to 0·04 mg. per g. due to dephosphorylation of ATP. It does therefore look as though injured tissue may be dealing with substances like ADP and ATP in an abnormal fashion. Little detailed work on the biochemistry of injured tissues has so far been undertaken, and the relevance of these observations to our finding that white body production in an injured artery can be stopped with enzyme poisons must remain uncertain until the platelet clumping substance released by the injury site can be collected and identified. We have studied blood and saline which has traversed an injured artery but have not been able to demonstrate a platelet clumping substance ; this is hardly surprising as a concentration which is adequate at the point of release, will be substantially reduced by admixture with blood which has not passed over the injured segment, and the clumping agent may also be rapidly broken down by enzyme systems in the blood. The fact that the platelet masses only continue to grow while they are attached to

the injury site may support this concept, for when they embolise they do not get bigger but may begin to fragment which suggests that some property of the site, or of its immediate surroundings, rather than a generalised change in the blood streaming past the site, is the key factor in the build-up of platelet thrombi.

The third point of interest, is that whereas blood flowing from an injured vessel (on the cortex, in the ear, or the human finger) produces white bodies when applied to a minor injury site, blood taken cleanly from the lumen of an artery does not. All types of shed blood, and serum were however vasoactive and it has been assumed that the vasoconstrictor principle in serum is 5-HT derived from platelet breakdown (Page, 1958). 5-HT did not produce vasoconstriction when applied to injured brain arteries, so some other substance must also be present, and as the vasoactivity could be abolished by antihistamines it thus seems likely that the vasoconstrictor material is histamine or a closely related substance.

Although the relevance of our observations to haemostasis and thrombosis is of course unknown, there are three situations in which the " white body " phenomenon may be of importance. (1) In the thrombosis which arises in mechanically injured arteries. This condition is particularly common in the internal carotid artery after injury to the head and neck or after tonsillectomy (Hockaday, 1959 ; Gleave, Hughes and Brownell, unpublished). (2) In the thrombus formation which occurs in vessels traversing inflamed areas. (3) In transient ischaemic attacks. In patients whose episodes have involved vision, white bodies similar in appearance to those we have been producing in the rabbit cortical vessels have been seen traversing the retinal arteries (Fisher, 1959 ; Ross Russell, 1961), and these bodies have been shown to consist of closely packed platelets (McBrien, Bradley and Ashton, 1963).

SUMMARY

The effect of human and rabbit red cell extracts, ADP, ATP and 5-HT on injured rabbit cerebral cortical arteries has been studied. Applied to minimally injured arteries, which do not form platelet thrombi spontaneously, they give rise to platelet masses at the injury site.

5-HT antagonists stop the production of white bodies by locally applied 5-HT without impairing the ability of the minor injury site to respond to red cell extracts, ADP or ATP. Spontaneous white body production at a major injury site is not affected by 5-HT antagonists, suggesting that the mechanisms concerned in spontaneous white body formation do not depend on 5-HT.

Certain enzyme inhibitors stop the formation of white bodies at major injury sites. With azide, fluoride, and fluoroacetate this action was irreversible ; with monoiodoacetic acid the activity returned, while cyanide and 2 : 4 dinitrophenol did not affect thrombus formation. When spontaneous activity had been abolished with enzyme poisons, the sites remained responsive to the clumping agents, and at minor injury sites the lowest effective concentrations of the agents were unchanged after enzyme inhibitors had been applied.

The vasoconstrictor activity of many of the white body producing materials and of serum, was abolished by antihistamines. Fresh and glass contacted plasma showed no vasoactivity and produced no white bodies. 5-HT does not cause constriction when applied to injured cerebral vessels on the surface of the rabbit brain so a histamine-like vasoconstrictor substance must be present in serum.

Blood flowing from an injured vessel over a minor injury site, produced white bodies at this second site, for as long as the blood flow persisted. Blood taken by vessel puncture and by finger-prick also showed this property, while arterial blood taken direct from the circulation by a polythene cannula did not.

Our studies suggest that the phenomenon of white body production in injured arteries may be related to the release from the damaged artery wall of substances with similar properties to ADP and ATP. The presence of these substances in high concentration at the injury site, causes the platelets to clump together and to adhere to the vessel wall at the site of injury.

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