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Diffuse Intravascular Clotting

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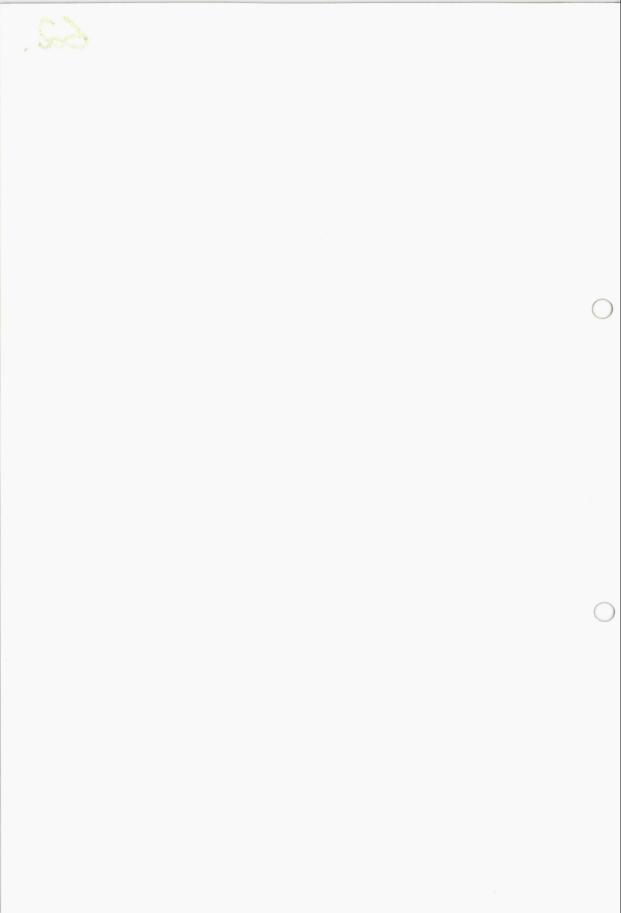**I. S. WRIGHT**
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172 Figures, 51 Tables

1. Continuous Hemostasis and Continuous Intravascular Clotting: Fact or Myth?

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Hemostasis stops bleeding, and it most frequently also prevents bleeding from minute vascular lesions. For example, when a hemophilic boy develops serious intracranial bleeding after a friendly pillow fight, it is reasonable to assume that his non-hemophilic friends had their vascular lesions repaired without clinical evidence of bleeding. Hemostasis, therefore, is probably active at all times at one or more places in the normal individual.

In addition, many investigators assume that hemostasis operates continuously on the entire vascular surface to maintain its structural integrity. The important point in this theory is not that hemostasis is active at all times in the normal organism, but that it is assumed to operate upon normal, uninjured endothelium.

Four years ago the relevant evidence was critically reviewed (63). Much of the evidence was then conflicting, and the theory could neither be accepted nor rejected. However, this theory is apparently so intellectually and teleologically satisfying that it is often referred to as an obvious truth (24, 78, 139).

This article continues the discussion from the previous review, trying to evaluate critically the arguments that are used to support the theory. It does not include the older information; its purpose is to present and discuss the evidence which has accumulated over the last four years. This new evidence has weakened the theory to the point where it is, in my opinion, no longer useful.

The material is arranged under the following headings:

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B. Turnover of clotting factors
C. Turnover of platelets
D. Coagulation products in normal blood
E. Endothelium and fibrin
F. Endothelium and platelets
G. Capillary permeability and hemostasis
H. Fibrinolysis.

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Clinical observations in bleeding disorders

Spontaneous bleeding should not occur from intact vessels, irrespective of platelet or clotting defects. Thus, if spontaneous bleeding does occur, there must be an underlying vascular lesion, and this lesion could conceivably result from a failure to maintain a »hemostatic coating« on the vessel walls. It is important, therefore, to examine the recent evidence bearing on this point.

Hereditary clotting defects

Clinical studies continue to emphasize that patients with severe clotting defects, even afibrinogenemia (56, 57), usually have little or no *spontaneous* bleeding. However, the tendency to bleed following trivial trauma does appear to vary from time to time in such patients. Since the hereditary clotting defects change little if at all in severity, Roskam (122, 123) suggested that these variations are due to independent vascular factors. In animal experiments with phenylindanedione, Jaques (73, 83) maintained the prothrombin time at values of 2—10 minutes (normal 12—15 seconds) for months without hemorrhage, but the animals bled to death if the anticoagulant was combined with stress in any form, probably because stress produced minute vascular damage. The nature of these vascular factors is still unknown, and Schulman (128), playing the role of the devil's advocate, challenged the entire concept, suggesting that bleeding could result from combined hemostatic defects not involving the vessels. Thus, more information is needed on the vessels at the site of bleeding, but it is still reasonable to conclude that uncomplicated severe clotting defects usually do not produce spontaneous bleeding.

It is still not known whether the clinical benefit of transfusions outlives the survival of the deficient clotting factor in the circulating blood. A study in three hemophiliacs suggested that monthly injections of purified factor VIII reduced the number and severity of bleeding episodes, but it was also found that traces of factor VIII could be demonstrated in the blood of the patients for almost a month (107).

Acquired clotting defects

Patients with acquired clotting defects may have severe spontaneous hemorrhages. Frequently the hemostatic defects are complex and involve coagulation, platelets, fibrinolysis and vessels. For example, patients with purpura fulminans have severe intravascular coagulation, thrombocytopenia, and probably vascular lesions with thrombosis and bleeding (66); many patients with intravascular coagulation also have active fibrinolysis which contributes to the bleeding (132).

For these reasons spontaneous bleeding in such patients cannot be attributed to a failure to maintain a normal »hemostatic coating« on the vessels.

Anticoagulant therapy

Anticoagulant therapy may be expected to increase bleeding from injured vessels, since large doses suppress the coagulation component of thrombosis and platelet plug formation (101). However, it does not provoke spontaneous bleeding (73). Dicumarol therapy may be an exception to this general rule, since large doses may have a toxic effect on the vessels (73).

Fibrinolysis

Fibrinolysis is discussed later.

Thrombocytopenia

The exact mechanism of bleeding in thrombocytopenia is still hotly debated. For many years Roskam has stressed that thrombocytopenia in itself does not produce bleeding; bleeding results only when vascular damage is superimposed upon the thrombocytopenia. He has recently summarized the arguments for this view (122, 123). What, then, is the nature of these vascular lesions?

According to one view, platelets and vascular endothelium have common antigens, and platelet antibodies would, therefore, also damage the vessels. Salmon (126) has recently restudied this problem. While platelets and endothelium do share antigens, e.g., those due to adsorbed plasma proteins, they do not share specific antigens. He, therefore, rejected the theory that platelet antibodies directly attack the endothelium.

Salmon's (124, 125, 126) extensive studies led him to propose another mechanism, viz., that bleeding results from the combined effect of thrombocytopenia and fibrinolysis. He found that thrombocytopenia induced by ^{32}P did not provoke bleeding, but it did so if the animal's fibrinolytic system was activated by streptokinase. He further showed that antiplatelet serum triggered fibrinolysis [it may possibly also trigger intravascular coagulation, see (121)], and that such serum did not provoke petechiae when fibrinolysis was blocked by epsilon aminocaproic acid. Clinically, he supported his theory by demonstrating active fibrinolysis in the blood from many patients with thrombocytopenia. This is impressive evidence, but more extensive clinical observations and clinical trials with epsilon aminocaproic acid are necessary before this theory can be finally evaluated. The nature of the vascular damage induced by fibrinolysis is also not known.

A third mechanism is suggested by Cochrane's studies (27). He demonstrated that antigen-antibody complexes and other macromolecules became attached to and damaged the vascular endothelium. Histamine might be an important mediator of such damage, since antigen-antibody reactions release histamine from platelets (54), and histamine damages the endothelium (85). If too few circulating platelets are available, petechial bleeding might result.

This information does still not settle the problem: would a patient with a »pure«, but severe thrombocytopenia bleed? Interesting as this question is, it may not be productive. The simple fact that patients may be thrombocytopenic for years with hardly any bleeding indicates that the normal maintenance of the vascular tree certainly does not require many platelets. However, when thrombocytopenia is combined with slight trauma, such as a tourniquet test, or with other hemostatic defects, such as anticoagulant therapy (73), fibrinolysis (126), or possibly vascular damage, severe bleeding may result.

Comments: Patients with hereditary clotting defects usually do not bleed spontaneously; the clinical evidence, therefore, does not support the concept that continuous coagulation is necessary to preserve the integrity of the vessels.

Patients with thrombocytopenia may have very little bleeding, suggesting that normal vessels can be maintained with few platelets. This need for platelets is increased by stasis, stress in every form, fever, slight trauma, etc. Therefore, platelets are necessary to maintain the vascular tree, but a young person in the »basal« state can do with few platelets; more — but still not very many — are required for ordinary life.

Turnover of clotting factors

Recent data confirm and extend previous findings [see (63)]. In Table 1 data obtained in man within the last four years have been compiled. These results are supported by careful animal studies (61, 89). However, more data are needed for factors V and XI; for factors VIII and IX isotope studies (9) do not agree with transfusion experiments and general clinical experience. Since transfusion experiments appear to be valid for fibrinogen and factor VII, they are usually also accepted for factors VIII and IX.

There is still room for larger and more detailed studies, but it is established that the clotting factors do turn over faster than most other proteins. For instance, the T/2 is 21 days for albumin (145) and about 7.5 days for transferrin (75).

Is this rapid turnover of clotting proteins due to a continuous coagulation, as is so often assumed? This would be a tempting explanation for factors I, II, V and VIII, which are consumed during coagulation *in vitro* and which are also

known to be consumed during experimental intravascular coagulation (114, 116). Previously, we believed that this theory could not explain the rapid *in vivo* turnover of those clotting factors which are not consumed during coagulation *in vitro* (63). This objection is no longer valid, since infusion of thromboplastin accelerates the disappearance of factors VII, IX and X (116), probably because these factors become sufficiently altered during coagulation to be removed by cellular mechanisms.

Nevertheless, there is new and convincing evidence that this rapid normal turnover of fibrinogen, at least, is *not* due to continuous coagulation. Thus, the

Table 1. *Turnover of some clotting factors in man: recent investigations.*
The half-life (T/2) is the time it takes to decrease the plasma concentration of a factor by 50%.
It is determined graphically, assuming that the relevant part of the curve reflects an exponential function.

Factor	T/2	Experimental subjects	References
Fibrinogen	1.5—3.0 days 3.8—6.1 days 4.5 days 2—5 days 4.8 days	Normals (isotope) Normals (isotope) Normals (isotope) Patient (trans.) Patient (trans.)	8 20 59 56 57
Prothrombin	3 days	Patient (trans.)	19
Factor V	12—15 hours 36 hours	Patient (trans.) Patient (trans.)	22 147
Factor VII	5 hours	Patient (trans.)	88
Factor VIII	9—18 hours 15—17 hours 2.9 days	Patient (trans.) Patient (trans.) Normals (isotope)	19 2 9
Factor IX	18—30 hours 28—40 hours 24 hours 8.4 days	Patient (trans.) Patient (trans.) Patient (trans.) Normals (isotope)	19 82 91 9
Factor X	2 days	Patient (trans.)	19
Factor XI	Some hours		Reliable data not available
Factor XII	52 hours	Patient (trans.)	143
Factor XIII	4—5 days 3—4 days	Patient (trans.) Patient (trans.)	70 41

turnover of fibrinogen was found to be normal in patients with hemophilia (59, 117, 152), with von Willebrand's disease, and with hypoproconvertinemia (152). It was also normal in dicumarolized dogs (8, 81) and men (8), in heparinized dogs (81), and in irradiated rats (129). — It must also be noted, however, that normal turnover rates have been found in postoperative patients (8) and in febrile rabbits (118). In patients with coronary disease or with lipemia, fibrinogen turnover has been reported to be either normal (20) or decreased (59).

If fibrinogen were catabolized mainly through coagulation the fibrinogen level might be expected to rise during short-term anticoagulant therapy. This does not happen (43).

Finally, it should be mentioned that the fibrinolytic system is also not responsible for the normal disappearance of fibrinogen, since administration of epsilon aminocaproic acid to normal animals did not prolong its turnover rate (17, 52, 104).

Comments: These new data agree with those previously reviewed (63). They do not rule out the possibility that a *small* and probably variable fraction of the clotting factors disappears from the circulation as a result of clotting secondary to repair of the minor vascular injuries of normal life, but they indicate that the major fraction of these factors is *not* catabolized through intravascular clotting. The rapid turnover of clotting factors should therefore not be used as an argument for a continuous coagulation *in vivo*.

Turnover of platelets

Investigators still agree that platelets normally survive for about 10 days in man (100, 113). However, they also still disagree about the mechanism of platelet removal. The »straight-liners« claim that intrinsic factors determine platelet survival and that platelets leave the circulation as they reach their predetermined age (113). The »exponentialists« insist that platelet survival is determined by extrinsic factors which make the platelets leave the circulation in a random fashion (5, 6).

At present it seems reasonable to combine these theories (36, 42, 100): platelets do have a finite life span, but this may be cut short by extrinsic factors. Such factors appear all-important in the experimental dog, leaving virtually no platelets to die of old age (6). In other species, including man, knowledge is incomplete, but several factors, such as age (4), disease, diet, smoking, and drugs may shorten platelet survival (100). Atherosclerosis may (4, 100) or may not (113) have the same effect.

Thrombin clumps platelets (133, 134), and large doses irreversibly destroy them *in vivo* (7). Therefore, all — or at least some — platelets could be destroyed in the circulation by coagulation (6, 100). This hypothesis requires proof that anticoagulants and clotting defects prolong platelet life span in young, healthy, non-stressed individuals. Unfortunately, the evidence on this important point is still only suggestive. Large doses of *heparin* significantly prolonged platelet life span in dogs (6, 100, 102) and in patients, most of whom had vascular disease (99); small doses had little or no effect (98, 99), and excessive doses actually shortened the life span due to bleeding (100). Large doses of *dicumarol* or *warfarin* also prolonged platelet life span in dogs (6, 8). In man, small doses of dicumarol, if anything, shortened the life span, while large doses prolonged it significantly (94, 95). Large doses of *phenylindanedione* have been reported both to prolong the platelet life span in man (4) and to have no effect (113). Hemophiliacs had normal (113) or high normal (100) values, while hemophilic dogs had a prolonged platelet life span (100).

In man, large doses of dicumarol changed the disappearance curve for labeled platelets from an exponential function to a straight line (4, 8), suggesting that clotting is the most important random removal mechanism. Nevertheless, dicumarol increased the T/2 only from 2.86 to 4.13 days (95). In dogs, anticoagulants slowed the disappearance rate, but the curve was still exponential, suggesting that other extrinsic removal mechanisms also operate in this species (6). These observations, especially in the dog, are compatible with widespread coagulation in the normal organism, capable of removing platelets from the circulation. However, further studies are still needed on the effect of anticoagulants on platelet survival in young, healthy, non-stressed dogs, rabbits and humans.

Two points should be stressed before these findings are interpreted. First, they do not necessarily indicate a continuous coagulation throughout the vascular tree; multiple discrete foci of coagulation would give the same result. Platelet aggregates could constitute such foci. If the general clotting potential is normal (136) or high (26, 109), sufficient thrombin may form in such aggregates to make them irreversible; if clotting is blocked, the aggregates break up and are washed away (62, 96, 101). Secondly, such widespread clotting may not at all be »favorable«. It should be emphasized that platelets do not depend on clotting to perform »anti-petechial« function, since large doses of anticoagulants very rarely produce petechiae (63). It is possible, therefore, that significant clotting, except in the platelet plugs which seal larger vascular lesions, is pathological. One can visualize a spectrum of ever increasing degrees of coagulation, from tiny foci of coagulation in the non-stressed organism to frank intravascular coagulation in certain pathological states, such as the generalized Shwartzman reaction [see

(65)]. If this is true, the experimental dog may not always be in a »physiological state« with regard to coagulation, and results should be interpreted with caution.

Coagulation is not the only extrinsic mechanism which can remove platelets from the circulation. Adherance and aggregation do not require clotting [see (60, 101, 138)], and there is evidence that drugs, such as sulphinpyrazone, may prolong platelet survival by reducing platelet adhesiveness (100).

If platelets continuously maintain the endothelium, thrombocytopenic individuals should have a »platelet-starved endothelium« which would devour transfused platelets. This concept is supported by the fact that washed intact platelets immediately stop thrombocytopenic bleeding (64). Therefore, platelet survival in thrombocytopenia due to decreased production should differ from the normal pattern, and perhaps in two ways. First, the initial recovery of transfused platelets should be decreased. This has not yet been adequately demonstrated; in fact, Ebbe et al. (42) found a normal recovery in rabbits made thrombocytopenic with radioactive phosphorus. Secondly, platelet survival should be reduced even though the thrombocytopenia stems from decreased production. The evidence on this point is still scant. Platelet survival is reported normal in previously non-transfused patients with thrombocytopenia due to failure of production (28, 105). On the other hand, platelet survival in thrombocytopenic patients in general is proportional to the pre-transfusion platelet level (100, 105). This shorter survival in more severely thrombocytopenic patients may be due to a greater »platelet debt«, but it may, of course, also be due to a more active pathological removal mechanism.

Comments. All these findings may be explained by proposing three removal mechanisms in the normal individual. First, some platelets are removed for the »anti-petechial« function (see later). This fraction is probably not very large, since patients with thrombocytopenia due to a failure of production may have a normal platelet life span and yet no purpura (28, 105). Secondly, some platelets are used to form irreversible platelet plugs which stop bleeding from the somewhat larger but still minor vascular injuries incident to normal life. The initial phase of platelet plug formation does not require coagulation, but coagulation makes the plugs irreversible and also increases their size (60, 101, 138). This function probably is increased in patients with vascular disease and thrombus formation. It may become greatly increased in abnormal »hypercoagulable« states, even to the point where platelets clump in the flowing blood. In normal unstressed man, this function is vital, but it does probably not use up very many platelets, since severe hemophiliacs may have long periods with no bleeding. Thirdly, those platelets which are left over from these two mechanisms are removed by senescence.

Coagulation products in normal blood

Clotting intermediates prior to thrombin

Coagulation can be described as a series of successive activations of clotting factors (38, 84), but none of these intermediates have been demonstrated in normal blood. This is not surprising, since they probably disappear quickly, either through inactivation in the blood or through clearance by the reticulo-endothelial system (12).

Thrombin

Previously, we have rejected the claim that thrombin can be demonstrated in normal blood (63). New evidence has not appeared on this point. In spontaneous or experimental »hypercoagulable« — i.e., abnormal — states, however, there is some evidence of thrombin in blood (140).

Fibrinopeptides

Thrombin splits off two pairs of peptides from each molecule of fibrinogen. These peptides might be present in the blood, and this seems all the more reasonable since peptide B has a physiological effect, sensitizing smooth muscle tissue to stimulation, e.g., by bradykinin (78). However, these peptides have not been demonstrated in normal blood (78, 130).

Fibrin intermediates

When fibrinogen is exposed to thrombin, peptide A is released at a much higher initial speed than peptide B. Intermediate »fibrins« may therefore form, viz., fibrinogen which has not yet lost all of the four peptides. Shainoff and Page (130, 131) described such an intermediate in rabbit plasma and called it cryo-profibrin. Cryoprofibrin precipitated in the cold and was clottable by thrombin (in contrast to the fibrin split products). Large amounts were found in the plasma from endotoxin treated rabbits, but normal rabbit plasma also contained significant amounts. They concluded that this was evidence of *thrombin* formation in normal blood, but also evidence against *fibrin* formation, since the amount of cryoprofibrin was much too small to allow its precipitation as fibrin in blood.

When thrombin splits off the four peptides from the fibrinogen molecule, four free glycine-groups appear. Abildgaard (3) therefore studied normal and pathological human plasma samples for the presence of free glycine-groups. Normal plasma did not contain such groups. However, plasma from a patient with a severe hypercoagulable state (55) formed a precipitate in the cold, and in this plasma about 3% of the potentially available glycine-groups were free. Abild-

gaard concluded that there is no evidence of fibrin intermediates in normal human plasma and that only trace amounts are found in hypercoagulable states. These findings in man appear to differ somewhat from the findings of Shainoff and Page (130) in the rabbit. Further studies are therefore needed on this point including studies on the clearance of such fibrin intermediates from blood.

Cryofibrin may be related to the »heparin-precipitable fraction« which has been demonstrated in normal human plasma (137). However, the role of clotting in the development of this protein is still not known.

Comments: Normal blood probably does not contain coagulation products indicative of continuous clotting, although further evidence should be collected on the fibrin intermediates. In abnormal hypercoagulable states, however, fibrin intermediates may be found.

Endothelium and fibrin

Several workers have suggested that vessels are lined with a layer of fibrin [see (63)]. Copley (29, 30, 31) has developed this concept in detail. He postulates that there is a fine hemostatic balance in the nearly stationary layer of plasma along the endothelium, so geared that it maintains an »endoendothelial fibrin film (EEFF) of submicroscopic dimensions«. It extends around the endothelial cells and continues in an exoendothelial sheath of fibrin (EXES) between the endothelial cells and the basal membrane. Lysis of this layer increases capillary fragility.

The endothelium is probably negatively charged (127), as is fibrin at physiological pH. Copley (30) found that a fibrin surface decreased the apparent viscosity of blood, thereby aiding the circulation. He also claimed that fibrin has anticoagulant properties which maintain the fluidity of blood (32). If so, it is difficult to understand why coagulation would go on in this layer. Furthermore, this observation has been challenged by Dalin and Lewis (35) who found that fibrin, like glass, promotes coagulation.

Although there have been earlier claims that fibrin could be demonstrated on normal endothelium by the electron microscope [see (63)], recent studies do not even mention this possibility (21, 85). If there is such a layer, it has not been demonstrated by the electron microscope.

Several workers have claimed to demonstrate a layer of fibrin (or fibrinogen) on normal endothelium by immunofluorescent techniques (31, 110), but there are also negative reports (see 63). These observations can hardly be taken to prove that a fibrin film has actually been deposited on the endothelium; a plasma layer would probably produce similar effects. Fibrin has also been reported beneath the surface of the intima, especially in older people (150).

Clinical support for the fibrin film has come from an interesting patient with congenital hypofibrinogenemia who developed massive arterial thrombosis, supposedly due to a lack of this film (86). However, the histological finding was panarteritis, and patients with afibrinogenemia are not particularly prone to thrombosis.

A final set of arguments for the fibrin film theory stems from studies on the clotting and fibrinolytic properties of the vascular wall. Four different activities must be considered. Thromboplastic activity can be extracted from the inner layers; much less from the adventitia (15). In addition, the vessel wall contains a so-called vasculokinase which clots fibrinogen in the absence of thrombin (97). Fibrinolytic activators are found in the adventitia (15), but also in the inner layers, especially of veins (142, 146). Finally, a heparin-like substance can be extracted from the vessels (39). It is difficult to relate these findings to the *intact* vessel wall, but it seems fair to conclude that injured vascular tissue can release thromboplastic activity, whereas fibrinolytic activators may be released from normal endothelium (142, 146).

It should also be pointed out that platelets are clumped by very small concentrations of thrombin (134). An endothelial layer of fibrin might therefore be expected to contain platelets, but platelets are not found on the normal endothelium (see next section).

Comments: A fibrin film cannot be demonstrated on normal endothelium with the electron microscope. However, injured endothelium is probably capable of triggering coagulation, and fibrin may then form as part of the hemostatic plug. Under pathological conditions, a more solid layer of fibrin may also be deposited in the vessels, e.g., in the kidney during the generalized Shwartzman reaction (see 66).

Endothelium and platelets

Clinically, there can be no doubt that platelets have an »anti-petechial« or »endothelium-supporting« function, since platelet transfusions immediately stop bleeding and normalize capillary fragility in a severely thrombocytopenic patient. However, so far it has proved extremely difficult — in fact, impossible — to demonstrate a diffuse deposition of platelets in or on normal intact endothelium.

The electron microscope shows that the »cement lines« of the endothelium do not correspond to any anatomical structure (49). The endothelial cells are extremely close to each other, the intercellular distance being less than 100 Å (44, 85). In normal intact endothelium the electron microscope does not reveal platelets or platelet fragments (51, 85). However, endothelial cells react to stress or injury by allowing intercellular gaps to form, sometimes reaching through

the basement membrane. Platelets are often found in such gaps, e.g., following mechanical injury (51, 87), heat or chemical injury (87, 92), histamine and serotonin (85), or antigen-antibody reactions (93). Following injury, platelets have also been demonstrated in the cytoplasm of the endothelial cells (87). Similar findings were reported in thrombocytopenic patients who had been transfused with platelet concentrates 4—16 hours old (74). In these patients an area was first infiltrated with local anesthesia. Then, a fairly deep cut was made and allowed to bleed for 20 minutes before the wound was excised. These vessels could therefore hardly be considered normal.

These studies were all concerned with depositions of platelets on defects so small that they could perhaps be classified as »jobs for a single platelet«. However, accumulations of platelets in numbers sufficient to form microthrombi have been demonstrated on the aortic endothelium from normal swine (53). These microthrombi were distributed around vessel orifices and bifurcations only; therefore, they could not be considered essential to the integrity of the intima, but rather as forerunners of atherosclerosis. These thrombi started in the neighborhood of the cell margins (101). The fate of such platelet deposits is not known. Larger platelet aggregates become incorporated in the vascular wall and covered by endothelium (58).

Using radioactively labeled platelets, Cronkite et al. (34) could not demonstrate uptake in the endothelium of normal rats, but thrombocytopenic rats appeared to concentrate radioactivity in the endothelium. For technical reasons they did not consider their observations conclusive evidence for a platelet-endothelium interaction. In similar experiments Mustard et al. (102) found radioactivity in the endothelium, suggesting accretion of platelet material on the normal endothelium. However, these experiments are complex and probably more difficult to interpret than the electron micrographs.

Immunological methods might also be applied to this problem, partly by investigating whether platelets and endothelium have common antigens, and partly by studying the endothelium with fluorescent anti-platelet serum. The first approach led Salmon (126) to conclude that platelets and endothelium did not share specific antigens. To my knowledge, there are no studies based on the second approach.

Comments: These observations are all compatible with the view that platelets are not incorporated into the normal, intact intima. However, platelets are easily trapped in the gaps between endothelial cells which stem from minute injury. They accumulate in greater numbers at sites of more definite injury. The important conclusion is that some degree of injury is necessary before the platelets stick; there is no evidence of a diffuse deposition of platelets along uninjured endothelium.

The mechanism of platelet adhesion and aggregation has recently been reviewed (60, 101, 138). Platelets also have phagocytic capacity (37), and it is interesting to speculate that phagocytosis and hemostasis might invoke related mechanisms.

Capillary permeability and hemostasis

If continuous hemostasis maintains the vascular wall, hemostatic defects might increase capillary permeability. This simple concept is difficult to investigate because direct capillary damage by the experimental procedure is so hard to exclude. Hyman et al. (69) discussed this problem in an extensive study on rabbits treated with dicumarol and antiplatelet-serum. Jaques (73) concluded from his observations in rats that dicumarol probably had a direct toxic effect on the vessels.

We have previously reviewed the evidence (63), and there is not much to add. Oiwin et al. (111) found similar disappearance rates for Evans blue in normal dogs, in dogs treated with a small dose of heparin, and in dogs whose fibrinolytic system had been activated by nicotinic acid. They considered this evidence against a fibrin film on the intima. In contrast, continuous administration of large doses of heparin, after a delay of some hours, increased the output of red cells in the lymph (100, 151). Whether this was caused by a disappearance of a fibrin film or by a failure to repair vascular damages resulting from the procedure is difficult to know. Heparin certainly does not produce petechial bleeding in patients.

Comments: Our previous conclusions still seem valid: animal experiments often strongly suggest a relation between capillary permeability and hemostasis, but, however impressive, they must be confirmed in patients with severe hereditary clotting defects and in patients with thrombocytopenia due to a failure of production before a final evaluation can be made.

Fibrinolysis

Continuous coagulation requires as its counterpart a balancing, continuous fibrinolysis. It is therefore necessary to examine the studies on fibrinolysis relevant to this problem.

Bleeding caused by fibrinolysis

If the integrity of the vessels depends upon a continuous deposition of fibrin, excessive fibrinolysis should produce spontaneous hemorrhages. Patients with spontaneous abnormal fibrinolysis may bleed quite severely, but they usually

bleed from vessels damaged by disease, injury or operation. Furthermore, these patients frequently have evidence of intravascular coagulation (132) and — paradoxically — of clotting defects stemming from abnormal coagulation and fibrinolysis. Therefore, these clinical situations are extremely complex, and bleeding cannot simply be explained by digestion of a normal layer of fibrin on the vascular wall.

Fibrinolytic agents have been widely used for therapeutic purposes. The situation in these patients may appear less complex than in patients with spontaneous fibrinolysis, but it is still too complex for clear-cut analysis. Many patients also receive anticoagulant therapy, and — more important — they develop serious hemostatic defects due to depletion of fibrinogen and other clotting factors, and to the generation of fibrin split products. These products interfere with the polymerization of fibrin (11, 47), with clot structure (18), with the enzymatic effect of thrombin (77), and with the hemostatic functions of the platelets (76, 77). Therefore, these patients have severe fibrinolysis, a serious clotting defect and defective formation of platelet plugs. In spite of all these defects, bleeding is not very common, and severe bleeding is nearly always local, originating from injured (venipuncture sites) or from diseased vessels (cerebral bleeding) (40, 48, 112, 144).

Thus, fibrinolysis does not produce generalized bleeding, and the clinical evidence does not support the theory that fibrin is a normal and essential part of the vascular wall.

Inhibitors of fibrinolysis

Powerful inhibitors of the fibrinolytic system are now available, and four sets of observations with such agents are relevant.

First, do fibrinolytic inhibitors decrease bleeding in hemophiliacs? Following up the original claim by Boudreaux and Frampton (23) that peanuts decrease the bleeding tendency in hemophilia, Astrup et al. (13) demonstrated a protease inhibitor in peanuts and interpreted the clinical effect in terms of the »dynamic balance between fibrin deposition and fibrin resolution«. These findings have been supported (14, 33), but not subjected to statistical trial. A powerful fibrinolytic inhibitor, epsilon aminocaproic acid (EACA), has also been reported to reduce the bleeding tendency in hemophilia (1, 119), but a statistical trial gave negative results (115). It has been argued that an effect can be expected only in milder cases (14, 33), and it is therefore possible that too severe bleeders were selected for this trial. Further studies are thus required to settle this problem, but it should be pointed out that a beneficial clinical effect could reasonably be explained by better *local* hemostasis: the weak hemostatic plugs in a hemophiliac

might have a better chance of »surviving« if they are protected from fibrinolysis. Such evidence should therefore not be used to support a general and continuous hemostatic balance throughout the circulation.

Secondly, do fibrinolytic inhibitors promote thrombosis? After a detailed review, Sweeney (141) concludes that no unequivocal case of thrombosis secondary to EACA-treatment has been reported. However, clinical situations are often difficult to interpret, and Nordöy's (108) animal experiments are therefore important. He found that EACA did increase the incidence of thrombosis in chemically traumatized veins. Since coagulation is an essential step in the development of firmly anchored, irreversible platelet thrombi (see above), this effect was to be expected. Again, however, the effect can be explained on a *local* level without invoking alterations in the general hemostatic balance.

Thirdly, do fibrinolytic inhibitors slow down the normally rapid turnover of clotting factors? Data are only available for fibrinogen, whose turnover was not altered by EACA (17, 52, 80, 104).

Fourthly, do fibrinolytic inhibitors promote a general deposition of fibrin on the vascular wall? Salmon (125) gave daily injections of EACA to rats and studied tissue sections with immunofluorescent techniques. Control rats had no fibrin on the endothelium, but treated rats had fibrin (or fibrinogen) on the intima of arteries in kidney sections. To my knowledge, these important observations have still not been confirmed and extended to other species. — It is of some relevance to this problem that EACA delayed removal of subcutaneous clots in normal rats (103) and enhanced accumulation of fibrinogen in abscess areas (104) and tumors (17). Thus, fibrinolysis certainly is active in removing extravascular fibrin deposits.

Evidence of active fibrinolysis in normal blood

When normal human blood clots in a tube, the clot does not lyse. Nevertheless, such blood contains weak fibrinolytic activity which can be demonstrated by appropriate *in vitro* tests, and this activity is greatly increased by various forms of stress (see 45, 50, 135). Recent techniques have extended these *in vitro* observations to *in vivo* conditions. When fibrinogen or fibrin is lysed, several split products are formed, and these products may be identified by sensitive immunological techniques. Several investigators have demonstrated such products in blood from patients with pathological fibrinolysis (46, 106), and they may also be found in normal blood (126), at least from some individuals (106).

The fibrinolytic activity in normal blood probably originates, at least in part, from an activator released by vascular endothelium (25, 142, 146), especially

from the veins (25, 142). Stasis (16) and vasoactive drugs (67) enhance the release of this activator.

The most reasonable explanation for these findings is that normal blood contains weak fibrinolytic activity, too weak to attack circulating proteins, including fibrinogen. Fibrin deposits, however, which are washed by circulating blood may specifically absorb and concentrate fibrinolytic activity in amounts sufficient for lysis (135). This concept explains present findings, but, unfortunately, it applies both to a continuous layer of fibrin on the vessel walls and — equally well — to discrete depositions of fibrin at sites of vascular damage. Therefore, these observations cannot be used to argue for or against a continuous coagulation.

Platelets and fibrinolysis

If platelets are continuously deposited on the endothelium, how are they ultimately removed? The theory of a hemostatic balance between deposition and resolution tacitly assumes, I believe, that platelets are removed by fibrinolysis. However, this point is by no means clear.

Platelets carry proactivator activity which probably is absorbed from the plasma (79) and released during viscous metamorphosis (120). Platelets also have anti-plasmin activity (10), although the amount may be small (148). It is not possible to predict the net effect of these opposing factors. Although plasmin *is* capable of lysing platelets after they have undergone viscous metamorphosis (71, 72), it is evident from *in vitro* experiments that platelet thrombi lyse more slowly than fibrin thrombi (71, 90, 149). *In vivo* observations confirm that the platelet head is relatively resistant to lysis (68); in fact, it is usually incorporated into the endothelium and slowly converted into an atheromatous plaque (58). There are several possible explanations for these observations: structural differences between the platelet thrombus and a fibrin clot, anti-plasmin and lipids in the platelets, serotonin adsorbed to the platelet surface, etc. Much more detailed knowledge is required before this point can be settled, but, at present, it is reasonable to conclude that platelet thrombi are not easily lysed by fibrinolytic mechanisms.

Comments: Normal blood is capable of dissolving fibrin. This is compatible with the theory of continuous coagulation, but by no means proves it. The finding of split products in blood can be accounted for by either continuous intravascular coagulation or by discrete foci of coagulation. At least two findings argue against continuous clotting: that marked experimental or therapeutic fibrinolysis does not necessarily provoke spontaneous bleeding, and that epsilon aminocaproic acid usually does not provoke thrombosis.

Conclusions

The theory of a continuous hemostatic balance is very attractive at first sight, and it has been useful in stimulating much experimentation and discussion. However, the evidence against this hypothesis continues to grow. Thus, serious clotting defects usually do not result in spontaneous bleeding; anticoagulant therapy does not slow the turnover rate of clotting factors; intermediates of clotting can not be demonstrated in normal blood, and fibrin can not be demonstrated on normal endothelium by electron microscopy.

The evidence of a continuous participation of platelets in hemostasis seems better founded, since severe thrombocytopenia increases capillary fragility and also is frequently associated with spontaneous bleeding. However, platelets have not been demonstrated attached to normal endothelium. All available evidence supports the view that platelets *repair* vascular damage, rather than *maintain* an endothelial surface which is already intact.

Much, of course, depends on what is meant by the word *»normal«*. Platelets and coagulation actively maintain the integrity of »normal« vessels, but only at sites on the walls of these normal vessels that have become *abnormal*. There must be some local trigger for hemostatic action; the evidence does not support the concept that the hemostatic mechanism operates continuously over the entire endothelial surface.

The hemostatic mechanism appears to be designed for *local* action. Platelets stick only to an abnormal surface. Coagulation also tends to become localized, for plasma strongly inhibits thrombin but effectively promotes polymerization of fibrin. Similarly, plasminogen activator must be adsorbed on to a clot to achieve an effective concentration; in plasma, it is quickly inhibited. Thus, the very nature of the hemostatic mechanism seems to argue against a diffuse and continuous hemostasis on normal endothelium.

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