ANALYTICAL REVIEW

Vascular Factors in the Pathogenesis of Hemorrhagic Syndromes

By Theodore H. Spaet, M.D.

In the past several decades considerable progress has been made in the understanding of those diseases characterized by excessive bleeding tendency. The advances in this field have come largely from the studies performed concerning the coagulation of the blood; and indeed, these investigations have been so fruitful that the trend of research has tended to drift away from the phenomenon of bleeding itself and the behavior of vessels at the actual site of their breakdown. Illuminating as the biochemical studies of clotting may be, there appears to be a limit to the phenomena seen in purpuric patients which can be explained in terms of derangements of the coagulation mechanism per se. In contrast, the relatively few studies done on alterations of the blood vessels in hemorrhagic diseases offer important supplementary data which may greatly enhance the understanding of these conditions. It is the purpose of the present review to organize the data on the role of the vessels in the pathogenesis of the bleeding diatheses, and to indicate that the actual phenomenon of purpura may depend largely upon alteration of vessels in such a way that they are unable to maintain the integrity of their walls.

Anatomical Considerations

No studies are available which clearly localize the site from which bleeding occurs in the hemorrhagic diatheses. However, it is generally conceded that the small vessels are involved; the term “capillary” has been loosely used to describe bleeding of this type. Accordingly, an account of small vessel anatomy is in order. The muscular endowment and contractile power of arterioles and venules has long been known; but the reactivity of the capillaries has been a matter of controversy which has only recently been clarified by the work of Chambers and Zweifach. On the basis of direct microscopy of the rat meso-appendix these authors have shown that the smallest vessels are mainly of two types: the A-V capillaries or metarterioles are contractile vessels with rudimentary muscle, which transmit blood more or less directly to the venous system; the true capillaries are simple endothelial tubes with no contractile ability, which branch from the metarterioles and have a sphincter at their point of origin. It is thus possible for blood to be shunted directly to the venous system by contraction of the “pre-capillary sphincter” or the flow can be directed into the true capillaries by relaxation of these structures. Normally an intermittent flow through the true capillaries is maintained. Recent studies

From the Ziskind Laboratories (Hematology Section) of the Joseph H. Pratt and New England Center Hospitals.
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have indicated that these same conditions hold true for the human capillary bed.  
Independent contractility of these true capillaries has been claimed by certain authors, and is attributed to the Rouget cells; but the direct application to them of vasomotor agents fails to elicit a response, and convincing evidence of capillary contractility has been obtained only with sub-mammalian species. Changes in capillary diameter probably can be attributed to passive responses caused by alterations in tone of the proximal vessels endowed with a muscular coat.

Structurally the capillary wall may be subdivided into a fibrillary basement membrane, endothelial cells which have the property of swelling when subjected to irritating stimuli and intercellular cement. This latter substance has been characterized by the studies of Chambers and Zweifach who showed that the material is highly susceptible to its physical and chemical environment. Reduction of calcium, lowering of the pH, inadequate colloids and the absence of particulate matter within the vessel all result in impaired integrity of the cement which is manifested by increased permeability, excessive stickiness to graphite, and as a final stage in deterioration, liability to the diapedesis of particulate matter. The cement appears to be elaborated only in the presence of ascorbic acid. These findings emphasize two points of importance in the understanding of the hemorrhagic diseases.

(1) Particulate matter would appear to have a function in maintaining vascular integrity in some nonspecific and mechanical manner. The platelets are located at the peripheral portion of the streamlined series of concentric cylinders which comprise the formed elements of blood in motion, as shown by the studies of Fihraeus, Knisely et al. and the Clarks. They are thus strategically located to perform the mechanical task of aiding vascular maintenance.

(2) Fragility and permeability appear to be distinctly independent properties of vessels. In the studies of Chambers and Zweifach, there was no diapedesis of particulate matter until severe destruction of the cement had developed. Similar findings were obtained by the Clarks in observations on the vessels of the rabbit's ear when irritants were applied to smaller vessels: edema and individual leukocyte diapedesis occurred well prior to the passage of erythrocytes. In contrast, Chambers and Zweifach were able to induce hemorrhage without edema formation by means of topical hyaluronidase. Landis, reviewing capillary permeability, has likewise dissociated fragility from permeability, and considered these phenomena as independent and frequently uncorrelated.

Whether there is normally a certain amount of erythrocyte diapedesis remains a moot point. Although such a phenomenon has not been reported by workers engaged in direct observations of vessels, Field and Drinker have found red blood cells to be present in lymph, and noted that an increase in venous pressure caused them to appear in greater numbers. It is of utmost importance to determine whether such diapedesis is physiologic, for certain theories explaining the phenomena of the hemorrhagic diatheses are based upon the assumption that the normal hemostatic mechanisms operate to prevent such spontaneous diapedesis from becoming excessive. According to such
concepts failure of this defense mechanism is responsible for purpura. As will be subsequently shown, such a view is not in conformity with the available data.

**Experimental Considerations**

Experimental studies on the pathogenesis of the hemorrhagic diseases may be approximated into two main groups: those dealing with failure of the hemostatic mechanisms with respect to the arrest of traumatic hemorrhage; and those which are concerned with the production of "spontaneous" hemorrhage and the factors leading to the formation of purpura, if that term be taken to include any sort of petechial or ecchymotic lesions.

The role of the vessels in arresting traumatic hemorrhage has been the subject of considerable controversy. MacFarlane\(^8\) typified the view that contraction of vessels serves as the major factor in initial hemostasis, and based his conclusions upon microscopic observations of the nail bed capillaries in man subjected to trauma by micro-needles. Disappearance of the vessels was interpreted as vasoconstriction, and was consistently seen in normal individuals. In contrast, patients with thrombocytopenic purpura failed to show loss of vessel visibility following injury, and this was interpreted as loss of vasomotor ability. Studies of this type are subject to the criticism that the human nail bed capillary wall is not visible through the intact skin, and conclusions as to its motion must be drawn with caution. The phenomenon of plasma skimming,\(^4\) in which fluid free of cells may traverse a vessel, can cause a vessel to become invisible when its visibility depends upon its cellular contents. Moreover, as has been mentioned, capillaries probably lack contractile power. That vasoconstriction is of considerable importance, at least in arterial injury, has been demonstrated by the studies of Chen and Tsai\(^9\) who showed that when arteries of the rabbit's ear, brain, or mesentery were severed, a profound vasoconstriction maintained hemostasis for as long as twenty minutes and until accessory reinforcement was supplied by coagulation. Quick has ably reviewed earlier studies which attempted to define the role of vasoconstriction in hemostasis.\(^2\)

Much of the confusion about the sequence of events in small vessel hemostasis has been dispelled by the studies of Zucker.\(^21\),\(^22\) Pictorial confirmation of her work has been supplied in the excellent motion pictures filmed by Shenker.\(^23\) Working with the rat meso-appendix preparation of Chambers and Zweifach,\(^24\) Zucker transected a variety of small vessels and observed the events leading to hemostasis under direct microscopic vision. Initial injury was often followed by a transient vasoconstriction in those vessels which were endowed with a muscular coat; but this vasoconstriction was not sufficient to produce complete hemostasis. Within minutes a platelet plug was seen to form within the injured vessel; it was not until this plug was complete that bleeding was arrested. A secondary vasoconstriction was then seen to occur which was of considerably greater duration, probably attributable to the liberation of specific vasoconstrictor substance from the agglutinated platelets. Such an agent was described by Janeway and others in 1918,\(^25\) and has been subsequently characterized in greater detail by other investigators.\(^26\) Of interest was the complete absence of fibrin within the severed vessel during this initial phase of hemo-
stasis. In animals rendered thrombocytopenic by means of anti-platelet serum, hemostasis failed to occur owing to faulty formation of the platelet plug incident to the thrombopenia. In animals treated with anticoagulants platelets failed to adhere to the vessel wall, and hemostasis was similarly deficient. An analogous sequence of events has been demonstrated in humans by Zucker who biopsied small skin incisions at spaced intervals of time and studied serial microscopic sections of these lesions.

Other suggestions as to the role of the vessels in hemostasis have included the concept of Roskam that the vascular endothelium somehow "opsonizes" platelets so that they become adhesive and readily form a hemostatic plug in response to injury. According to this author a prolonged bleeding time may represent a vascular defect in which "opsonization" becomes faulty. Chen and Tsai in the studies mentioned above have indicated that capillaries, and to a certain extent venules, seal immediately when injured by direct cohesion of their endothelial surfaces.

In addition to the role of the vessels in the normal and pathologic states with respect to hemostasis, their function in the production of spontaneous purpura has also been the subject of experimental investigation. The discovery by Cole in 1907 that platelets could serve as antigens in heterologous species to produce specific anti-platelet sera was soon translated into a reliable method for the induction of experimental purpura in animals. In 1915 Ledingham and Bedson produced anti-platelet sera against the cells of guinea pigs, rats and rabbits, and showed that such antisera were capable of producing thrombocytopenic purpura in the respective species. These findings have been repeatedly confirmed since then. Ledingham and Bedson further demonstrated that the purpura so induced depended upon two factors, namely, platelet and vascular. When previous splenectomy was performed upon guinea pigs, resulting in a thrombocytosis, anti-platelet serum then caused a drop in platelets to normal levels, and no purpura resulted. Such animals regained their susceptibility to the purpurigenic action of the serum when the initial postoperative platelet peak had disappeared.

A normal platelet level evidently was protective against the purpurigenic action of the serum. On the other hand, if platelets were profoundly reduced by means of the intravenous injection of agar such a thrombocytopenia was not in itself sufficient to induce hemorrhagic manifestations. When anti-erythrocyte serum was administered simultaneously with the agar, purpuric lesions were produced despite the failure of this latter serum to cause purpura when administered alone. In this case, normal vessels evidently were protective against the purpurigenic effects of thrombocytopenia. Other studies with purpura induced by anti-platelet serum have indicated that, as in acute thrombocytopenic purpura in man, changes in platelet count correlate poorly with alterations of the bleeding studies.

Elliott and Whipple working with rabbits showed that splenectomy performed after the administration of the anti-platelet serum reduced the thrombocytopenia, but did not protect against increased vascular fragility. These workers showed, moreover, that the increased vascular fragility outlasted the thrombocytopenia. Leonard and Falconer working with guinea pigs added the finding
that larger doses of the anti-platelet serum were needed to produce purpura than were required for the maximal thrombocytopenia. Of interest are the descriptions of pathologic findings in animals rendered purpuric by means of anti-platelet serum, for they correspond closely to those of human idiopathic thrombocytopenic purpura.31, 41

The vascular factors responsible for the development of experimental purpura have been most extensively studied by Ioskam41 in a series of brilliant investigations. He confirmed the studies of Ledingham and Bedson as to the inability of pure thrombocytopenia to produce purpura. By means of intravenous gelatin the platelet counts of dogs were reduced to as low as 35,500. This resulted in only slight prolongation of the bleeding time. He demonstrated, moreover, that in rabbits rendered purpuric with anti-platelet serum there was considerable variability of the bleeding time. Values ranged from near normal to severely prolonged at different sites. This phenomenon has a similar counterpart in thrombocytopenic humans. In cross transfusion studies Ioskam showed that the ears of normal rabbits perfused with the blood of rabbits treated with anti-platelet serum had relatively normal bleeding times despite the lack of platelets in the perfusing blood. In contrast, the animals given the serum had prolonged bleeding times in the ears perfused with normal blood despite the adequate platelets. Strong additional evidence for a vascular effect of the anti-platelet serum was its ability to produce local purpura without any alteration of the circulating platelet count when it was introduced intrapleurally. The local action of this serum had been previously noted by Lee and Robertson,33 and was subsequently confirmed by Tocantins.39

The experimental production of hemorrhagic states in animals by agents other than those which depress the platelets has received less consideration, although several noteworthy studies have been published. Bingham and co-workers50 in observing the effects of dicumarol intoxication upon dogs noted that a single massive dose of this drug caused a profound hypoprothrombinemia, but did not result in a tendency to bleed. In contrast, repeated but somewhat smaller doses produced widespread hemorrhages involving the smaller vessels although the prothrombin content of the blood was not as depressed as that seen in the single dose studies. In some instances histologic changes could be demonstrated in the vessels. These consisted of endothelial swelling, vacuolization and proliferation, and necrotic changes of the media with rupture of elastic fibers. Acute overdosage with phenylindandione likewise does not produce a hemorrhagic syndrome despite marked hypoprothrombinemia.52 Such studies indicate that hypoprothrombinemia as such is not sufficient to initiate a hemorrhagic diathesis, but that a vascular factor is also needed. Whether dicumarol is directly toxic to the vessels or whether the lack of prothrombin incident to its administration produces a deficiency state in the vascular wall is at present a matter of speculation. Heparin is similarly unassociated with a hemorrhagic effect even when uncoagulable blood is produced in acute experiments,53 although the danger of producing hemorrhage with long term therapy is well known. More direct evidence in favor of a noxious effect by heparin on blood vessels has been provided by the studies of Chambers and Copley54 who showed that this drug administered intravenously to rabbits caused a reduced threshold
to the hemorrhagic effect of local irritants applied to the nictitating membrane of the eye.

On the basis of the studies cited above it would appear that the two major manifestations of the hemorrhagic diseases, impaired hemostasis and purpura, have separate although related pathogeneses. Platelets seem to represent the keystone in the hemostatic arch, their agglutination and adhesion to a vascular rent causing the primary arrest of bleeding. Subsequent reinforcement and permanence is supplied by the fibrin clot, the formation of which is dependent upon the various factors involved in blood coagulation. The role of vasoconstriction is probably of secondary importance. Thus, the failure of the hemostatic mechanism in the hemorrhagic diseases seems to be directly related to disorders involving platelets and coagulation factors whereas the blood vessels themselves play a minor role. In direct contrast, purpura and spontaneous hemorrhage probably stem from vascular disturbances. This is evident from the convincing studies which have consistently demonstrated that in the acute situation the platelets could be maximally depressed or the blood could be rendered incoagulable without the appearance of hemorrhagic manifestations. The addition of a vascular component under these circumstances resulted in purpura.

It therefore remains to elucidate the nature of the vascular defect which is responsible for the appearance of purpura. Histologic studies have been conflicting and have not served to clarify the nature of the vascular lesion. Characteristically the smaller blood vessels appear completely normal in the hemorrhagic diseases, and no bleeding points or breaks in continuity can be found at the sites of hemorrhage. Microscopic examination of vessels in vivo have been little more revealing. In rats rendered purpuric by means of anti-platelet serum or by topical application of moccasin venom the vessels of the mesoappendix showed the greatest vulnerability at the location of junctions and branching points, both on the arteriolar and venous sides. In these studies there was no visible evidence of vascular alteration, no demonstrable change of vasomotility, and in the serum-treated animals none of the criteria for damage as outlined by the Clarks. Evidently the changes which occur in the vessels represent functional disturbances not demonstrable by the usual technics.

Clinico-Pathologic Considerations

In 1896 Unna attempted to define the vascular lesion in human purpura. Based upon histologic studies of autopsy material from a diverse variety of hemorrhagic diseases he concluded that all cutaneous hemorrhages could be traced to an actual break in vessel continuity. It is now generally conceded that there is no constant histologic evidence of altered vessel morphology; previously described vascular rents probably represent artifacts which can be found commonly in normal vessels. Even the hemorrhagic nature of certain purpuric lesions has been questioned. Peck, Rosenthal and Erf performed cutaneous biopsies on the petechial lesions of both the spontaneous variety and those produced by negative pressure methods. Histologic studies revealed considerably less extravasated blood than would have been anticipated from the gross appearance of the lesions, but sufficient vasodilatation was present to account for their size. These workers further observed that petechiae disap-
peared merely by fading, in contrast to the typical sequence of color changes which was seen in the case of extravasated blood. Friedenwald on the basis of histologic studies of punctate retinal petechiae concluded that these lesions consisted of capillary aneurysms incompletely lined with endothelium. Ballantyne and Loewenstein presented convincing histologic evidence that the “hemorrhages” in diabetic retinopathy were frequently globular masses of red blood cells lined with a complete endothelial membrane. Tocantins and Stewart observed aneurysmal dilatations of veins in dogs with thrombocytopenic purpura induced by anti-platelet serum. Klemperer expressed the view that petechiae may be maximally dilated capillaries. Most recently Orbison has demonstrated capillary and arteriolar aneurysms in thrombotic thrombocytopenic purpura. These aneurysms were most numerous at vascular junctions. Marshaling evidence in favor of the view that petechiae are true hemorrhages, Humble described studies of human cutaneous capillaries as seen by capillary microscopy during performance of the Rumpel-Leede test. Patients with various types of increased capillary fragility were investigated by this method, and it was concluded that although the means of evolution of the petechiae differed among the various conditions, petechiae consisted universally of extravasated blood. The petechiae seen in the meso-appendix of thrombocytopenic rats show no evidence of aneurysm formation, although Krogh has described the course of events in the hemorrhage induced by inflammation as follows: “In capillaries which have gone into stasis the wall of the vessel may slowly bulge out in one or (generally) more places, and suddenly it is found that the corpuscles contained in such a varicose dilatation are outside the vessel. The most interesting point is that the integrity of the wall seems in all cases to be restored very rapidly.” Aneurysm formation as a stage in the development of hemorrhagic lesions would be a phenomenon of the greatest interest, and is worthy of further study.

The lack of histo-pathologic evidence notwithstanding, the presence of vascular damage in those hemorrhagic diseases in which purpura is a feature, seems to be fairly certain. Although attempts have been made to assign hemorrhagic levels below which the platelets and the coagulation factors must fall to induce purpura, such levels do not withstand critical analysis. On the contrary, the correlation between such depletions and bleeding manifestations is rather low. Spence and Roskam have emphasized such discrepancies in the case of thrombocytopenic purpuras, a most dramatic example of which is the response to splenectomy where cessation of bleeding manifestations may occur prior to any change in the blood platelet count. Likewise, profound defects of the coagulation mechanisms may be present without associated purpuric manifestations. Single massive doses of dicumarol inadvertently administered to patients have produced blood that is virtually incoagulable without the appearance of bleeding; patients with congenital afibrinogenemia and hemophiliacs rarely display hemorrhagic manifestations unless injured, although both these conditions are characterized by faulty blood coagulation. On the other hand, purely vascular disorders may result in spontaneous purpura in the presence of completely normal coagulability of the blood and platelets, as is the case in senile purpura, Henoch-Schonlein’s purpura and “pseudohemo-
philia." Such clinical findings are in keeping with the experimental studies on animals, both of which appear to indicate that spontaneous purpura is determined by the presence of some vascular defect.

The concept that purpura depends upon vascular factors received support from the field of therapeutics, inasmuch as certain agents have been found to alleviate bleeding manifestations in some of the hemorrhagic diatheses secondary to platelet and coagulation defects without affecting the primary process. Roskam reported that a platelet extract was able to cause increased vascular resistance in experimental purpura without alteration in the platelet level, and blood transfusion has produced a temporary alleviation in clinical thrombocytopenic purpura in the absence of a platelet elevation. In 1945 Ungar noted a shortened bleeding time in rats and guinea pigs subjected to stress. This effect appeared to be at least partially mediated through the pituitary-adrenal axis, and seemed to depend upon the liberation by the spleen of a specific humoral agent. A splenic extract was prepared from many species of animals which had the ability to shorten the bleeding time, increase vascular resistance, and to inhibit the liberation of histamine from injured cells. This substance was named “splenin.” Moolten described a compound of similar properties which appeared to be steroid in nature and was designated “thrombocytosin.” It was found in a variety of organs, and was said to have the ability to increase platelet adhesiveness, and to produce partial and temporary clinical remission in patients with thrombocytopenic purpura without affecting their platelet levels.

ACTH and cortisone appear to have an effect upon hemorrhagic manifestations in thrombocytopenic purpura independent of the effect in raising platelet levels, according to the recent reports of Robson and Duthie and of others. Significant reduction in the bleeding manifestations of the disease may be produced together with decreased vascular fragility, either without any demonstrable effect upon the blood platelet level, or well prior to the subsequent increase in platelets. Additional data of this type are provided by the finding that ascorbic acid tends to reduce the hemorrhagic effect of dicumarol at given prothrombin levels. Thus, the therapeutic effect of certain agents in the hemorrhagic diseases appears to depend upon their ability to reinforce the vessels and re-establish their integrity, lending further support to the concept that it is the vascular damage which is mainly responsible for the appearance of purpura.

DISCUSSION

The experimental and clinical data cited favor the theory that the appearance of purpura is largely dependent upon the development of vascular damage. In general it would appear that purpuric manifestations will not become manifest in a disorder of the hemostatic factors of the blood unless such vascular damage is present. Such a view must be reconciled with the common clinical observation that a profound drop in platelets or a severe depression of one of the coagulation factors is most frequently associated with the appearance of a hemorrhagic diathesis. These contrasting findings suggest that there is an intimate relationship between the integrity of the blood coagulation system and the integrity of the blood vessel wall. Although no hint has been provided as yet concerning the nature of this relationship, it appears possible that in the turn-
over of coagulation factors and platelets, some substance (or substances) are elaborated which contribute to the normal function of the vascular endothelium or cement. According to such an hypothesis, depletion of one of the circulating coagulation factors would lead to a corresponding depletion of the vascular factor after a certain "latent period" or lag. Purpura would appear only after depletion of the vascular factor. The hypothesis could be extended to explain the beneficial effects of ACTH, "splenin," and "thrombocytosin," and of the other agents which have an independent effect upon the vascular integrity. These various materials might shift the reaction "coagulation factors—vascular factor" to the right by means of an enzyme-like effect either directly or through an intermediary substance. On the other hand vascular purpuras could be explained by a shift of the reaction to the left or an inhibition of it by other hypothetical agents. These concepts are admittedly highly speculative, but they have the virtue of being consistent with the paradoxical findings seen in the disorders of hemostasis, such as faulty coagulability of the blood without the appearance of purpura, and purpura with complete normality of the entire coagulation system. They are, moreover, consistent with the beneficial effects of agents which do not affect the coagulation factors, and the characteristic lag between the production of a coagulation defect and the appearance of purpura. Experimental verification of the theory outlined must certainly be produced, however, before it can receive serious consideration.

Meanwhile, the strong suggestion that vascular lesions are of utmost importance in the production of the purpuras enlarges the therapeutic horizon in these disorders, for agents may be sought which will aid vascular integrity as well as those to correct the primary defect.

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VASCULAR FACTORS IN HEMORRHAGIC SYNDROMES


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THEODORE H. SPAET