White Thrombo-embolism and Vascular Fragility in the Hamster Cheek Pouch after Anticoagulants

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PLATELET thrombo-embolism is receiving recognition as an important condition. It is, as yet, poorly understood and consequently difficult to diagnose. Lutz, Fulton and Akers have reported the presence of platelet thrombo-embolic conditions in hamsters after trauma, infection and malignant neoplasia and have related their results to various clinical manifestations and autopsy findings, characterized by platelet thrombi in practically all the peripheral blood vessels in 20 human cases. The significance of intravascular agglutination has been discussed by one of us in a recent review.

It is entirely logical that therapy with anticoagulants should be utilized in the treatment and prophylaxis of diseases associated with platelet thrombo-embolism. However, fundamental investigations pertaining to the effect of these materials on platelet thrombo-embolism have been limited for the most part to tests made in vitro on platelet adhesiveness. The testing of platelet adhesiveness in vitro may not necessarily give a true picture of platelet agglutinability as found in vivo. Thus the many reports of no change, or even a decrease, in platelet adhesiveness during anticoagulant therapy, are difficult to reconcile with the reported increase in platelet embolization under similar conditions.

The routine use of anticoagulants in patients and in animals should be undertaken with a critical consideration of the possibility of detrimental changes in the state of the small blood vessels and the characteristics of the blood flow. Evidence for such effects is present in the literature. As a result of heparin administration, platelet emboli of leukocytic size were seen in the living blood vessels of the cheek pouch of the hamster by Copley and by Lutz, Fulton and Akers. In hamsters treated with dicumarol for fourteen weeks, platelet thrombi and leukocytic coatings on the endothelium were observed and photographed by Lutz, Fulton and Akers. Zucker described the formation of platelet plugs as effective hemostatic agents in the mesoappendix of the rat treated with heparin and dicumarol. Jürgens reported that histamine and bacterial toxins caused agglutination of platelets in the mesenteric veins of the rabbit, even after anticoagulants. The formation of leukocytic coatings resulting from anticoagulant therapy has been described by Fleck and by Lutz, Fulton and Akers. Following intravenous injection of heparin in the rabbit, Essex and Graña observed leukocytes adhering to the endothelium and forming obstructive aggregates in the blood vessels within transparent ear chambers. Laufman,
Martin and Tanturri \(^{18}\) reported the intravascular agglutination of erythrocytes after vascular occlusion in dogs treated with heparin and dicumarol. Changes in vascular permeability in mice after dicumarol administration were reported by Kuschinsky and Ludewig. \(^{19}\) Chambers and Copley \(^{20}\) reported an increase in “vascular fragility” as indicated by petechial hemorrhage formation resulting from the application of various toxic agents to the nictitating membrane of the rabbit’s eye after heparin administration. No reports have been found in the literature on intravascular effects of two of the newer anticoagulants, Tromexan and phenylindanedione. It is evident that a more complete understanding of vascular effects of anticoagulants is necessary for their successful use in the control of thrombo-embolic disorders.

The proper choice of anticoagulant and the management of its administration should perhaps depend in part upon the effect on vascular fragility as well as on platelet and leukocytic adhesiveness. A re-emphasis has been placed by Copley \(^{21}\) on the distinction between “capillary” fragility, in the sense of “breaking strain” or rhexis, and resistance to diapedesis as determined by petechial formation. Furthermore, he points out that petechial formation is not necessarily an accurate indication of “capillary” fragility. Obviously, the production of petechial hemorrhage by toxic agents and negative pressures, when carried on without direct and continuous observation on transilluminated vessels, does not permit an accurate interpretation of the process involved, or even the determination of the actual vessels concerned. The assumption is sometimes made that petechial hemorrhages seen in the Rumpel-Leede test result from rupture of the capillary wall. \(^{22}\) There is reason to believe that the term “capillary” fragility may be a misnomer. Clark and Clark \(^{23}\) by direct observation of petechial formation in the tadpole tail and in the rabbit ear, find that erythrocytes pass through the endothelium without an actual break in the blood vessel wall. The term petechial formation should imply the production of minute hemorrhages by diapedesis of a limited number of erythrocytes, without loss of continuity of the vessel wall.

No reference in the literature has been found to the use of a micro-electrode in the determination of vascular fragility. Such a method might be used to distinguish between fragility in the sense of Copley \(^{21}\) and “fragility” as determined by petechial formation resulting from other methods. Direct observation of transilluminated membranes would also permit the exact location of petechial formation resulting from toxic agents in the general circulation. Consequently, the cheek pouch of the hamster has been used for observing and cineraphography of the minute vessels during anticoagulant administration.

**Methods**

Hamsters of either sex, varying from 8 weeks to 1 year in age, and weighing 90 Gm. or more, were used in this study. The animals were anesthetized with a 6.5 per cent Nembutal solution (pentobarbital sodium, Abbott). Administration was by intraperitoneal injection of 0.15 cc. per 100 Gm. body weight as an initial dose with fortification by 0.05 cc. additional doses as needed.

The cheek pouch of the hamster was prepared for study of the small blood vessels without major operative procedure, according to the method described by Fulton, Jackson and Lutz. \(^{24}\) Direct observations and motion picture recordings were made, using equipment developed in this laboratory specifically for vascular studies. \(^{1}\)
WHITE THROMBO-EMBOLISM AND VASCULAR FRAGILITY

Whole blood coagulation time was determined by a modification of the Lee-White method, utilizing a volume of 0.15 cc. of whole blood in each tube. Prothrombin determinations were made, using an adaptation of the Rosenfield-Tuft method for estimation of prothrombin level from prothrombin time, as modified from the original method by Quick. Heparin activity was measured by the use of the De Takats heparin tolerance test. Blood for these determinations was obtained by intracardiac puncture directly through the thoracic wall.

Four anticoagulants were used in this investigation: heparin (sodium salt, Hynson, Westcott and Dunning), dicumarol (Abbott), Tromexan (ethyl biscoumacetate, Geigy) and phenylindanedione* (Danilone, Frosst). Dicumarol, Tromexan and phenylindanedione were given by intubation and the volume of fluid introduced was adjusted so that a single dose of the drug was contained in 0.75 cc. or less of fluid. Heparin was given by injection into the femoral vein.

Silver-glass micro-electrodes in series with an induction coil (Harvard Apparatus Company, Inc.) were used to produce minute vascular injuries. An electrode, 1 to 5 µ in diameter at the tip, was placed exactly on the point selected for testing, by means of an Emerson micromanipulator. An extremely brief series of tetanizing shocks of varying strengths was used throughout the present investigation. The key was closed momentarily by a tap with the index finger.

A semiquantitative measurement of vascular fragility was obtained by determining the threshold for hemorrhage at the tip of a micro-electrode in contact with the blood vessel wall. This method is proposed as a new procedure for investigation of vascular fragility. It is anticipated that the use of a precision stimulator in future work will improve the accuracy. The threshold for the formation of a platelet thrombus at the tip of a micro-electrode in contact with the wall of a blood vessel was used as an index of susceptibility to thrombo-embolism.

RESULTS AND IMPLICATIONS

Thrombo-embolic Susceptibility and Vascular Fragility in Normal Hamsters

In normal hamsters the blood vessels in the cheek pouches, transilluminated through either the double unoperated membrane or the single flap, are devoid of thrombo-embolic phenomena. The vascular pattern and characteristics of blood flow in the normal hamster have been described previously. Agglutinated platelets are absent and immobilized leukocytic pavements and leukocytic aggregates are lacking in the small blood vessels. Individual leukocytes may be detected moving slowly along the endothelium of many venules in normal cheek pouches. Significant differences in numbers of leukocytes and mobility are obvious when normal hamsters are compared with those infected with Staphylococcus aureus or bearing malignant neoplastic transplants.

In an attempt to determine the susceptibility of normal hamsters to platelet thrombo-embolism, arterioles and venules were stimulated with brief faradic current by placing a silver-glass micro-electrode directly on the wall of the selected vessel (fig. 1A). Stimulation of arterioles produced vasoconstriction, the degree depending upon the strength of the shocks. In all vessels in which the strength of stimulation was enough to invoke marked constriction, platelets subsequently accumulated at the stimulated point. Platelet accumulations in fast-flowing arterioles were continually washed away by the force of blood flow, without formation of an effective platelet thrombus.

In normal hamsters relatively strong faradic shocks were required to produce

* Kindly supplied by the C. E. Frosst Company, Montreal, Canada.
platelet accumulations in venules, even those carrying slow-flowing blood. Repeated stimulation at a single point sometimes produced a platelet thrombus, which increased in size and formed platelet coatings on the endothelium, extending downstream and partially filling the vessel. At times the vessels became

![Image of microphotomicrographs](image_url)

**Fig. 1.** — Enlargements from cinephotomicrographic sequences demonstrating an increase in fragility of venules after heparin, determined by threshold for hemorrhage at tip of microelectrode in contact with vessel wall. Magnification X 200.


D, E and F—Heparinized hamster. In D, small bubble (b) indicates weak stimulus. Bleeding (h) in E and F indicates increased fragility. In F, hemostatic platelet plug (p) forms at point of stimulation and platelet coatings (e) line endothelium.

completely occluded by the platelet masses. In some instances the platelet plug appeared as a loose porous mass, allowing a few erythrocytes to channel through; in others, the platelet plug was entirely effective, causing complete hemostasis. Constriction of venules after faradic stimulation was not observed, presumably
because the selected venules were nonmuscular. It was difficult to produce platelet thrombi in venules carrying a fast-moving stream of erythrocytes (fig. 1 A, B and C), since the platelets were dislodged from the point of injury as fast as they became attached.

Bleeding from venules was difficult to produce in the normal preparation (fig. 1 A, B and C) even with very strong and repeated faradic stimulation. When a hemorrhage was produced, the visual field was obscured almost immediately by extravasated erythrocytes. After the injured area was carefully cleared of extravasated blood, a platelet plug was found at the point of rupture. This plug was effective in stopping the hemorrhage. Stimulation at the point of attachment of a hemostatic plug usually resulted in voluminous hemorrhage.

Vascular Effects of Heparin

Heparin administered intravenously, 1.5 mg. per 100 Gm. of body weight, was tolerated well by the hamster and produced an extensive hypocoagulability of whole blood. Ten and 20 minute samples of blood were made incoagulable for 24 hours by this amount of heparin, when tested by the De Takats method for heparin tolerance. The 30 minute sample had a coagulation time of 12½ hours, and the 80 minute sample a coagulation time of 80 minutes. All experimental work involving the determination of the effect of heparin was carried out in the period of hypocoagulability within 80 minutes after injection of the drug.

Before injection of heparin all cheek pouch preparations were carefully surveyed for platelet thrombi, emboli and for leukocytic adherence to vascular walls. Heparin was given only to hamsters showing no platelet agglutination in the vessels of the cheek pouch. Small platelet emboli were seen in the arterioles and small venules immediately after the injection of heparin. This confirms the observations of Copley, Copley and Lalich, Fleck, and Lutz, Fulton and Akers. In addition, large platelet emboli were seen with the binocular dissecting microscope at 15X in vessels 90 to 150 μ in diameter. Occasionally platelet thrombi were found after heparin.

Leukocytic coatings as reported by Fleck and Lutz, Fulton and Akers were found composed of layers of leukocytes several cells in thickness (fig. 2A). The coatings were restricted to the venules. Leukocytic pavements, one cell thick, were found partially occluding small venules 5 minutes after injection of heparin. Leukocytic thrombosis was evident initially on the venous side of the circulation and, within 30 minutes after injection, the thrombosis extended to the fast-flowing arterioles. After 80 minutes leukocytes were found sticking to the walls of all vessels, and many of the small arterioles were completely occluded.

Brief faradic stimulation of the arteriolar wall of the heparinized hamster produced a vasoconstriction followed by adherence of platelets at the stimulated point to an extent greater than in the normal preparation. The platelet thrombi formed quickly and occluded the vessels. Three to 5 minutes after stimulation, erythrocytes were seen channeling through the masses of platelet thrombi, eventually washing away some thrombi, platelet by platelet, or forcing other thrombi to become detached as emboli. The walls of the arterioles were as resistant to rupture by electrical stimulation as were those of the untreated animal.

The wall of the venule was definitely more fragile in the heparinized hamster
than in the controls. Moderately strong shocks applied to the wall of a venule caused an immediate rupture, with a resulting hemorrhage quickly obscuring the visual field. Shocks of much weaker strength caused a minute hemorrhage which was contained within the perivascular sheath. Hemostasis resulted from the resistance of the perivascular sheath. The minute platelet thrombus which formed at the point of injury never attained sufficient size to stop the blood flow and did not form emboli. Hemorrhage that extended into the surrounding connective tissue beyond the perivascular sheath (fig. 1 D and E) was stopped, at least in part, by the formation of an effective platelet plug at the site of the rupture (fig. 1 F). A platelet plug sometimes built up until it partially occluded the injured vessel, and in some instances gave off platelet emboli.

Petechial hemorrhages appeared 30 minutes after injection of heparin. They formed at the capillary-venous junctions and along the post-capillary venules. Microscopic examination of these areas revealed extravasation of intact erythrocytes, but no platelet thrombosis in the involved vessels and no stasis. Although the flow of venous blood was slow, erythrocytes did not adhere to the endothelium or to the leukocytic pavements. The fact that vascular fragility is increased by heparin administration suggests, as previously proposed by Copley, that the hyperheparinemia reported after radiation22 may produce the hemorrhagic effects of radiation sickness by weakening the blood vessel walls. The increased vascular fragility affords a possible explanation for petechial hemorrhages reported by clinicians in thrombocytopenic purpura, especially in hepatic disease in which a circulating anticoagulant (heparin-like) has been demonstrated.22

Endothelial damage might be caused by the direct action of heparin or by the indirect effect of white thrombosis as proposed by Fleck.14 The appearance of petechial hemorrhages in the absence of white thrombosis is evidence for a direct effect of heparin on the vascular wall as opposed to an indirect effect dependent upon thrombotic occlusion. It is possible that the extended bleeding time of a blood vessel, ruptured by electrical stimulation of the wall, is the result of a thrombocytopenia resulting from platelet agglutination produced by heparin. It is equally possible that the extended bleeding time may be accounted for by an attenuation of the perivascular connective tissue sheath.

Vascular Effects of Dicumarol

Fifty hamsters were fed daily doses of dicumarol varying from 12.5 mg. per Kg. of body weight for periods as long as seven months, to 250.0 mg. per Kg. for ten days. The results have been summarized in table 1. Examinations were made after the third day of drug administration, daily from the third to the tenth day, at the end of fourteen weeks, and after seven months. Abnormalities were found in the prothrombin time of only 2 hamsters: 1 receiving 25.0 mg. per Kg. for eight days had a prothrombin time of 42 seconds; and a second receiving 37.5 mg. per Kg. for four days had blood plasma which would not coagulate (table 1). Both hamsters died but the cause of death cannot necessarily be attributed to dicumarol. Intrapericardial bleeding as a result of the heart puncture complicated by hypoprothrombinemia, may be the explanation, since autopsy revealed blood in the pericardium. In the absence of prothrombin time extension,
absorption of dicumarol was indicated by prolongation of the bleeding time and by white thrombo-embolism. The lack of effect of dicumarol on the plasma prothrombin level may indicate that the hamster is extremely resistant to this drug. Boyd and Warner\textsuperscript{33} reported that rats escape from the effect of dicumarol in a few days. Zucker\textsuperscript{34} could not reduce the prothrombin level in rats below 20 per cent of normal except by using a vitamin K diet. Marple and Wright\textsuperscript{34} stated that horses do not contract the "sweet clover disease". Smith\textsuperscript{37} reported a marked variation in resistance of rabbits to dicumarol and proposed that a genetic factor might explain the differences.

Preliminary results of the plasma level determination of dicumarol in the hamster indicate absorption of not more than 10 per cent of that administered, depending upon the time elapsed between administration and removal of blood.

### Table 1—Vascular Effects of Dicumarol in the Hamster

<table>
<thead>
<tr>
<th>Dose in mg per Kg Duration</th>
<th>Number of Hamsters</th>
<th>Platelet Thrombosis</th>
<th>Thrombosis Emboli</th>
<th>Increased Adhesive Leukocytic-</th>
<th>Leukocytic-</th>
<th>Aneurysmal Constrictions</th>
<th>Bleeding Time</th>
<th>Whole Blood Coagulation Time</th>
<th>Prothrombin Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>12.5 mg. 3 to 5 days</td>
<td>8</td>
<td>none</td>
<td>none</td>
<td>+</td>
<td>in all</td>
<td>+</td>
<td>Min.</td>
<td>Min.</td>
<td>10.2-10.8</td>
</tr>
<tr>
<td>12.5 mg. 8 to 10 days</td>
<td>9</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>in all</td>
<td>+</td>
<td>Min.</td>
<td>Min.</td>
<td>10.0-10.8</td>
</tr>
<tr>
<td>12.5 mg. 14 weeks</td>
<td>6</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>in all</td>
<td>none</td>
<td>Min.</td>
<td>Min.</td>
<td>10.0-10.8</td>
</tr>
<tr>
<td>12.5 mg. 7 months</td>
<td>6</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>in all</td>
<td>none</td>
<td>Min.</td>
<td>Min.</td>
<td>10.6-11.4</td>
</tr>
<tr>
<td>25.0 mg. 4 to 10 days</td>
<td>6</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>in all</td>
<td>none</td>
<td>Min.</td>
<td>Min.</td>
<td>10.0-11.4</td>
</tr>
<tr>
<td>25.0 mg. 8 days</td>
<td>1</td>
<td>Deceased</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>37.5 mg. 8 to 10 days</td>
<td>4</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>in all</td>
<td>none</td>
<td>Min.</td>
<td>Min.</td>
<td>10.4-11.2</td>
</tr>
<tr>
<td>37.5 mg. 4 days</td>
<td>1</td>
<td>Deceased</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>75.0 mg. 6 to 10 days</td>
<td>3</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>in all</td>
<td>none</td>
<td>Min.</td>
<td>Min.</td>
<td>10.2-11.0</td>
</tr>
<tr>
<td>150 mg. 6 to 10 days</td>
<td>3</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>in all</td>
<td>none</td>
<td>Min.</td>
<td>Min.</td>
<td>10.4-10.8</td>
</tr>
<tr>
<td>250 mg. 6 to 10 days</td>
<td>3</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>in all</td>
<td>none</td>
<td>Min.</td>
<td>Min.</td>
<td>10.6-10.8</td>
</tr>
</tbody>
</table>
samples. This is in marked contrast with the nearly complete absorption reported in man receiving 100 to 200 mg. daily or approximately 4.0 mg. per Kg.\textsuperscript{34} Absolute values for plasma dicumarol in the hamster varied from 23.0 to 67.0 μg. per ml. of plasma at 4 hours after administration as determined spectrophotometrically using the method described by Shapiro and Weiner.\textsuperscript{35} According to Shapiro and Weiner\textsuperscript{35} as little as 5 to 10 μg. of dicumarol per ml. of plasma will extend the prothrombin time in man. This suggests that the prothrombin formative process in the liver of the hamster may be resistant to dicumarol. The possibility of dicumarol-antagonistic substances in the hamster, such as vitamin K, should be investigated because of the fact that large daily doses of dicumarol administered intravenously and by cardiac puncture do not alter the prothrombin time significantly.\textsuperscript{27} These findings will be reported fully elsewhere.

Vascular observations in hamsters receiving 12.5 mg. of dicumarol per Kg. were similar to those reported by Lutz, Fulton and Akers.\textsuperscript{1} No platelet thrombi and no platelet emboli were found, either spontaneously (table 1) or after trauma produced by vascular occlusion. However, as the dosage was increased or the time of observation extended, or both, several additional phenomena were observed. Instances of arterial spasm were observed in all groups. Spontaneous platelet emboli were seen in all groups after the sixth day. Platelet thrombi occurred after the eighth day in hamsters receiving 12.5 mg. per Kg. and sooner after large doses. Leukocytic pavements were found in the hamsters on large doses of dicumarol (37.5 mg. to 250.0 mg. per Kg.). The dosage of dicumarol had little or no effect on whole blood coagulation time beyond the initial extension in time from 3 minutes or less to 7 produced by the minimal dose used (12.5 mg. per Kg.).

Brief electrical stimulation of the walls of the arterioles produced results similar to those obtained in hamsters treated with heparin. The vessels were not as easily ruptured as in the heparinized animals. However, the vascular endothelium of dicumarolized hamsters was much more susceptible to injury than the endothelium of normal hamsters, since the weakest possible stimulus produced platelet agglutination. When shocks were applied of sufficient strength, bleeding was extensive and became arrested only after a large platelet plug had formed at the point of rupture. The plug formed in the dicumarolized hamster was not as firm as in either the normal or the heparinized animal, and was easily washed off by the force of the blood stream.

Spontaneous platelet thromboembolism in the dicumarolized hamster may be due to the increase in susceptibility of the endothelium to injury and not necessarily to a direct effect of dicumarol on the platelets per se. This might account for the discrepancy between the decrease in platelet adhesiveness determined in vitro using blood from dicumarolized rabbits\textsuperscript{4} and the increase in platelet thrombo-embolism in vivo as indicated by observations of the transilluminated hamster cheek pouch. Furthermore, it is possible that the blood samples removed for the determinations of platelet adhesiveness may have lost many of the more adhesive platelets, which have adhered to the endothelium. The leukocytic pavements which were seen consistently throughout these experiments may be the cause of the increase in permeability of the blood vessels, which Kuschinsky and Ludewig\textsuperscript{49} reported to be independent of the prothrombin level.
Vascular Responses to Tromexan

Tromexan administered to hamsters produced results that were quite similar to those with dicumarol. The prothrombin time was not extended significantly. Bleeding times and whole blood coagulation times were extended only slightly. Spontaneous platelet thrombi, platelet emboli and leukocytic coatings (fig. 2 B) were found in all the treated animals. However, leukocytic emboli were not found, and arteriolar spasms were not seen.

A total of 14 hamsters was treated with either 150 to 400 mg. of Tromexan per Kg. of body weight in single doses or initial doses of 300 mg. per Kg. with 150 mg. per Kg. supplements at 24 hour intervals. Pathologic changes have been reported in the liver and kidney of mice after daily doses of 100 mg. per Kg. of Tromexan for a period of thirty days. Hamsters treated with a single dose of 300 mg. per Kg. showed erythrocytes and pus cells in the urine, indicating renal damage. The effects were not fatal since several animals were kept on Tromexan for ten days without death. The urine of animals treated with dicumarol did not show evidence of hematuria. Single doses of 525 mg. per Kg. were lethal for the hamster.

The blood vessels in all hamsters treated with Tromexan were relatively susceptible to the formation of thrombi by experimentally produced injury at focal points. A microprobe rubbed on the outer surface of a venule caused platelets to accumulate at that point, but did not produce extensive thrombosis. The act of crushing a venule with a microneedle produced an extensive platelet thrombus. Massive crushing resulted in complete stasis due to the formation of many white thrombi. Faradic stimulation, definitely weaker than that required for normal preparations, produced extensive bleeding from venules and escape of the extravasated blood from the perivascular sheath. Emboli frequently formed from the intravascular portion of the hemostatic plug.

Petechiae developed around small venous vessels at points of junction during the time the cheek pouch was exposed. Microscopic examination failed to show sticking of erythrocytes or of platelets to the intravascular wall at the petechiae. Hamsters treated with Tromexan appeared to be more susceptible to petechial formation than dicumarolized hamsters.
Vascular Effects of Phenylindanedione

Phenylindanedione, an antivitamin K substance, is a true hemorrhagic agent for the hamster. An initial dose of 250 mg. per Kg. with 150 mg. per Kg. supplements at 24 hour intervals for four days produced an extension of the bleeding time, lengthening of the whole blood coagulation time, and considerable elevation of the prothrombin time, with a reduction in the prothrombin level of 9 to 25 per cent. A total of 10 hamsters were used in this group.

The vascular effects of phenylindanedione were almost exactly the same as those produced by large doses of Tromexan and dicumarol. Platelet emboli and thrombi were formed extensively, and leukocytic pavements were found in most of the small venules. Platelet coatings in small venules were of much greater magnitude than those found in hamsters treated with heparin, dicumarol or Tromexan, and were comparable with those found in hamsters infected with S. aureus.1

Electrical stimulation of arteriolar walls caused nonuniform constriction, giving the arterioles a sausage-like appearance. Platelet thrombi, sometimes occluding, were produced in arterioles by strong stimulation. Stimulation of venules produced platelet thrombi, which formed extensive coatings adjacent to the point of injury without occluding the vessels. The thrombi thus formed increased in size and produced large platelet emboli. Leukocytes became attached to the platelet thrombi forming mixed thrombi. Strong stimulation of brief duration applied directly to the walls of venules caused rupturing with extensive hemorrhage, which was not limited to the perivascular connective tissue sheath. Hemorrhages were arrested by the formation of large platelet plugs on the endothelial surface. By using a microneedle as a probe, gentle pressure on the plug at the point of injury again caused bleeding. Erythrocytes at the point of injury could be dislodged easily, indicating that hemostasis was not the result of a fibrin clot.

Plasma from all hamsters treated with phenylindanedione had a red tinge, possibly the result of the indicator reaction of phenylindanedione in an alkaline medium3 rather than hemolysis. The unconcentrated urine was also definitely red. Microscopic examination revealed a severe hematuria of approximatey 200 erythrocytes per low power field (100×). The erythrocytes in the urine showed no evidence of either crenation or hemolysis. The red color in the urine may be due partly to the excretion of phenylindanedione, as reported previously in man.2

DISCUSSION

The results show clearly that heparin, dicumarol, Tromexan and phenylindanedione do not prevent, but actually enhance, the formation of hemostatic platelet plugs at points of blood vessel rupture. Since the blood is made hypocoagulable by these substances, a distinction must be recognized between the effects of anticoagulants on agglutinability of the formed elements (platelets and leukocytes) and coagulation involving fibrin formation. Our work indicates that bleeding from ruptured small blood vessels during anticoagulant administration is controlled in part by platelet plug formation. The significance of an enhanced platelet agglutinability produced by anticoagulants is therefore ap-
parent in view of the marked hypocagulability and the increased fragility produced by these substances.

The increased platelet adhesiveness resulting from anticoagulants may be advantageous in controlling bleeding, but deleterious under certain conditions such as hypertension, heart disease or increased general susceptibility to white thrombo-embolism (thrombocytic aeroangiothrombosis). The importance of white thrombo-embolism resulting from anticoagulant therapy requires further investigation. It is possible that anticoagulants are contraindicated at times on the basis of enhanced white thrombo-embolism, even though red clots may be prevented from forming or propagating. Further work is in progress in our laboratory on the effects of repeated and prolonged production of white thrombo-embolism in the hamster and the significance of this phenomenon. Investigations are in progress on the role of white thrombo-embolism in red clot formation.

It is obvious that the findings in the hamster may not necessarily apply to man. In fact, significant differences have been reported in this paper with respect to the effect of dicumarol. Furthermore, the micro-electrode test for vascular fragility has been applied only to the hamster cheek pouch. Adequate tests for altered vascular fragility in man are lacking. The negative and also positive pressure tests measure susceptibility to petechial formation, which is not necessarily the same phenomenon as fragility.

A kodachrome motion picture film (375 feet, 16 mm., silent with titles) entitled “White thrombo-embolism and vascular fragility in the hamster cheek pouch after anticoagulants” has been prepared illustrating the results reported in this paper.40

Summary

1. Heparin, dicumarol, Tromexan and phenylindanedione did not prevent, but actually enhanced, the formation of platelet plugs at the site of hemorrhages produced at the tip of a stimulating microelectrode in contact with the walls of small blood vessels in the hamster cheek pouch. Furthermore, these anticoagulants per se produced an increased adhesiveness of platelets and leukocytes to the endothelium of small venules. In particular, heparin produced platelet embolism immediately after administration. The circulating emboli varied in size from that of leukocytes to cylindrical masses which filled the lumina of vessels measuring up to 75 \( \mu \) in diameter. Platelet embolism was not found during the first five days of administration of dicumarol, but appeared subsequently.

2. The significance of increased platelet agglutinability during the hypocoagulable state induced by anticoagulants and the need for a critical distinction between agglutinability of the formed elements and coagulation involving fibrin are discussed.

3. An increase in the fragility of the walls of venules followed the administration of heparin, dicumarol, Tromexan and phenylindanedione. A brief series of relatively weak faradic shocks from a micro-electrode produced a hemorrhage. Shocks of much greater strength applied to venules of normal untreated hamsters produced no visible effect. This method is proposed as a new semiquantitative procedure for investigation of vascular fragility in accessible membrane
preparations. It may possibly distinguish between fragility in the sense of rhexis or breaking of the wall and petechial formation such as that produced by the application of negative pressure and perhaps erroneously referred to in the literature as “fragility.”

4. Petechial hemorrhages appeared at the points of junction of the postcapillary venules after anticoagulant administration, but not on the capillaries. Spontaneous ruptures were not found in capillaries. No evidence was found for an increase in capillary fragility. Consequently, the term “vascular fragility” is proposed to replace “capillary” fragility.

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White Thrombo-embolism and Vascular Fragility in the Hamster Cheek Pouch after Anticoagulants

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