Evidence for a Vessel Wall Defect in Immuno-thrombocytopenic Hamsters

By Rajendra G. Desai and George P. Fulton

In thrombocytopenic states the bleeding defect is generally attributed to the platelets, although in recent years another factor, vessel wall defect, has been postulated as playing an important part in its pathogenesis. If a vessel wall defect were present in thrombocytopenic purpura, direct evidence might be expected to come from in vivo observations of the blood vessels and microcirculation in transparent membranes.

In the present investigation, we have produced experimental immuno-thrombocytopenic purpura in the Syrian hamster (Mesocricetus auratus) in order to evaluate various factors (coagulation and vascular) responsible for alterations in hemostasis. In vivo tests have been applied to the cheek pouch to determine the susceptibility to petechial formation and vascular fragility.

Materials and Methods

Hamsters of either sex, varying from 5 to 10 weeks of age and weighing 75 to 100 Gm., were used. The animals were anesthetized with Nembutal (pentobarbital sodium, Abbott), given intraperitoneally, 10 mg./100 Gm. body weight, with fortification by 3 mg. increments as needed. The cheek pouch of the hamster (fig. 1A) was prepared for observation of the blood vessels according to the method of Fulton and Lutz.

Cinephotomicrographic records of vascular changes were made by technics previously described. Hematologic studies in vivo have been recorded on motion picture film by this technic under magnifications from 200 to 1800 x.

Hematologic procedures.—Hemoglobin, red cells, white cells and differential counts were made by routine, standard technics. The platelet counts and reticulocyte counts were performed by the method of Dameshek. Bleeding time determinations were made by making an incision 1 mm. long and 1 mm. deep on the tail skin, and blood was blotted at regular intervals on a filter paper. The normal bleeding time was 109 ± 19 seconds in hamsters of both sexes weighing 75 to 100 Gm.

Coagulation times were performed by a modified Lee-White method. The values of 143 ± 50 seconds were obtained in control hamsters. The prothrombin times were 10.5 ± 0.2 seconds. The clot retraction, fibrinolysis, and bone marrow studies were done by routine technics.

Production of immuno-thrombocytopenic purpura.—Hamsters of both sexes weighing 150 ± 29 Gm. were used as sources for pooling blood for obtaining platelets, with the use of siliconized glassware and disodium EDTA in isotonic saline as anticoagulant. Plate-
Fig. 1.—Experimental purpura in hamster. A, Cheek pouch everted and spread for transillumination of the microcirculation. B, Petechiae in the cheek pouch at 24 hours after anti-platelet serum. C, Postmortem appearance of viscera in thrombocytopenic purpura showing hemorrhage. D, Microelectrode test showing hemorrhage at low voltage in thrombocytopenia, indicating a vessel wall defect (× 200; reduced).

In viva tests for vascular fragility.—The moccasin venom test, negative pressure test,12 and microelectrode test13 were used.

RESULTS

Hematologic Findings

Controls.—Sixty-nine hamsters of either sex weighing between 75 to 100 Gm. were used for complete blood determinations. The values did not deviate markedly from those reported by other workers.14-18 The bone marrow findings were similar to those reported by Sherman.17

Immuno-thrombocytopenic hamsters.—Thrombocytopenic purpura was produced in hamsters by injection of 1 to 2 ml. of anti-platelet serum intravenously. Depending on the dose, spontaneous petechiae and ecchymoses were observed in the cheek pouch (fig. 1B), and in almost all the organs of the
Table 1.—Hematologic Values in Hamsters at 24 Hours after Antiplatelet Serum (2.0 ml. Intravenous)

<table>
<thead>
<tr>
<th>Type of determination</th>
<th>Values before APS (11 hamsters)</th>
<th>Values at 24 hours after APS</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC ((\times 10^6/cu.mm.))</td>
<td>7.1 ± 1.7</td>
<td>4.1 ± 1.1</td>
</tr>
<tr>
<td>WBC ((\times 10^9/cu.mm.))</td>
<td>5.24 ± 1.20</td>
<td>6.12 ± 1.3</td>
</tr>
<tr>
<td>Platelets ((\times 10^5/cu.mm.))</td>
<td>9.02 ± 0.05</td>
<td>0.67 ± 0.32</td>
</tr>
<tr>
<td>Hemoglobin (Gm. (%))</td>
<td>15.5 ± 4.1</td>
<td>8.2 ± 2.3</td>
</tr>
<tr>
<td>Reticulocytes ((%))</td>
<td>2.5 ± 1.2</td>
<td>6.8 ± 1.35</td>
</tr>
<tr>
<td>Polymorphs ((%))</td>
<td>20.0 ± 6.0</td>
<td>27.0 ± 11.0</td>
</tr>
<tr>
<td>Lymphocytes ((%))</td>
<td>61.0 ± 7.5</td>
<td>59.0 ± 14.5</td>
</tr>
<tr>
<td>Monocytes ((%))</td>
<td>3.0 ± 0.5</td>
<td>2.0 ± 1.2</td>
</tr>
<tr>
<td>Eosinophils ((%))</td>
<td>1.8 ± 0.2</td>
<td>2.5 ± 0.5</td>
</tr>
</tbody>
</table>

body (fig. 1C). Hemorrhagic fluid effusions were common in the peritoneal cavity. Bleeding was observed in gums and mucous membranes, and blood was usually seen in the urine and feces. Hematologic changes after injection of APS are recorded in table 1. The red cell and hemoglobin values dropped, and platelet counts were reduced to levels below 100,000 cu.mm. The white cells were not affected. The bone marrow was hypocellular to normocellular and showed rounded, small, immature-looking megakaryocytes.

Coagulation Factors and Vascular Fragility

Controls.—Coagulation times in 15 normal hamsters averaged 143 ± 50 secs., comparable to values reported by Akers,\textsuperscript{10} Bleeding times in 36 hamsters averaged 109 ± 19 sec. The prothrombin times in 6 normal hamsters were 10.5 ± 0.2 secs. Clot retraction tests showed 0.26 ± 0.11 ml. of serum resulting after an hour interval. The fibrinolysis test revealed no abnormal findings. The susceptibility to petechial formation was determined as a possible index of the degree of fragility of blood vessels. In 11 normal hamsters, the negative pressure test applied to the cheek pouch produced 0 to 4 petechiae at one hour when the suction was applied for 1 minute at 20 cm. of mercury. These values have been reported previously.

The microelectrode test for vascular fragility was used to stimulate walls of arterioles and venules of 25 to 160 \(m\) in diameter. The vessels were arbitrarily graded into three sizes: small (30 ± 5 \(m\)), medium (75 ± 7 \(m\)) and large (150 ± 10 \(m\)). Capillaries were also tested. The microelectrode was tested initially at low voltages to determine the “bubble threshold” (usually 1.5 to 3 volts). The threshold for constriction of arterioles was 4 to 9 volts, and that of venules 9 to 18 volts. Not all venules constricted because of irregular distribution of neuromuscular elements on the venous side of the circulation,\textsuperscript{20} The voltage was increased until a thrombus formed at the site of stimulation (thrombus threshold). Platelet thromboses occurred at voltages between 4 to 20, persisting in venules for a longer time than in arterioles. The voltages were further increased to determine the threshold for rupture of the vessel wall. For the most part, the vessel wall remained intact at all voltages. In 4 of 63 vessels tested, the wall was broken and im-
Immediately sealed at both ends. This phenomenon was termed "electro-coagulation."

**Immuno-thrombocytopenic purpura.**—Various tests for hemorrhagic diathesis were performed in hamsters given anti-platelet serum, and abnormalities were seen in the bleeding time, clot retraction and fragility of the vessel wall. The bleeding times (table 2) were increased to 240 sec., as compared with 109 ± 19 sec. in normal hamsters. Clot retraction determinations showed poor retraction.

In hamsters with gross manifestations of purpura, the susceptibility to petechial formation as a possible measure of vascular fragility was determined by the snake venom test. Profuse formation of petechiae occurred in 30 minutes to one hour after injection of moccasin venom between the layers of the cheek pouch.

In addition, microelectrodes were used as a fragility test (table 3), at 4 hours and 24 hours in hamsters previously injected with 2.0 ml. of anti-platelet serum. At 4 hours, 10 hamsters were studied and a total of 43 venules and 25 arterioles were tested. The thresholds for constriction and for thrombus formation were comparable to the controls. At 24 hours, pronounced effects were seen. The cheek pouch, skin and mucous membranes all exhibited spontaneous petechiae and ecchymoses. Bleeding was seen in almost all the organs of the body. White cells were rarely seen adhering to the endothelium. Thrombosis was not observed except for a brief accumulation of a few platelets in 4 animals with voltages near the fragility threshold. These observations were correlated with the lowered platelet counts. The fragility threshold of the blood vessels was altered significantly. Vessel rupture and bleeding were demonstrable after single shock stimulation with 60 to 150 volts (fig. 1D). As many as 30 per cent of the venules tested in a group of 47 broke at voltages below 150. The arterioles were fragile also, and 8 became hemorrhagic from single shocks of less than 150 volts. No differences were detected between the breaking tendencies of arterioles and venules.

**Table 2.**—*Effect of Antiplatelet Serum in Hamsters at 24 Hours after Intravenous Injection of 2.0 ml. (11 Animals)*

<table>
<thead>
<tr>
<th>Platelet x 10^9/cu.mm. Before</th>
<th>Platelet x 10^9/cu.mm. After</th>
<th>Bleeding time (min.)</th>
<th>Spontaneous petechiae (hours)</th>
<th>Death interval after injection (hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td>980-1230</td>
<td>20-108</td>
<td>&gt;240</td>
<td>3-33</td>
<td>24-48</td>
</tr>
</tbody>
</table>

**Table 3.**—*Microelectrode Test in Experimental Purpura (A.P.S., 2.0 ml., I.V.)*

<table>
<thead>
<tr>
<th>Experimental procedure</th>
<th>No. of animals</th>
<th>No. of vessels</th>
<th>Constriction threshold (volts)</th>
<th>Thrombus threshold (volts)</th>
<th>Frailty</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls (young, old)</td>
<td>62</td>
<td>362</td>
<td>4-13</td>
<td>7.5-30</td>
<td>No bleeding</td>
</tr>
<tr>
<td>A.P.S. 4 Hr.</td>
<td>19</td>
<td>97</td>
<td>4-13</td>
<td>7.5-30</td>
<td>No bleeding</td>
</tr>
<tr>
<td>A.P.S. 24 Hr.</td>
<td>27</td>
<td>162</td>
<td>5-19.5</td>
<td>Negative</td>
<td>Bleeding 43.9%</td>
</tr>
<tr>
<td>A.P.S. 4th Day</td>
<td>14</td>
<td>47</td>
<td>10-30</td>
<td>Negative</td>
<td>Bleeding 29.0%</td>
</tr>
</tbody>
</table>
Since the anti-platelet serum was prepared from rabbit blood by repeated injections of hamster platelets, control experiments with normal rabbit serum were necessary. No abnormalities were found in the hemostatic mechanism.

**DISCUSSION**

Since the elements of blood are transported in a closed system of vessels, an effective but invisible exchange occurs constantly between the intravascular and extravascular fluids. Under normal conditions the formed elements do not pass through the walls of the blood vessels. In the absence of frank bleeding from ruptures of the vessel wall, egression of the formed elements would require passage through the interendothelial cement and the perivascular sheath resulting from a defect in either or both. The occurrence of a disturbance in the coagulation mechanism does not necessarily produce spontaneous bleeding. Furthermore, it is evident from the clinical literature that the platelets may be maximally depressed without the appearance of hemorrhagic lesions. For this reason, a third factor, damage to the vessel wall, has been postulated as important in the pathogenesis of hemostasis.

**CONCLUSIONS AND SUMMARY**

Experimental purpura was produced in the hamster by administration of anti-platelet serum obtained from rabbits previously injected with hamster platelets. Spontaneous petechiae and generalized bleeding were observed. The derangement in the hemostatic mechanism has been analyzed by study of the changes in blood, bone marrow and vessel walls. The platelet count in peripheral blood fell from $9.02 \pm 0.85 \times 10^5$ to $0.66 \pm 0.32 \times 10^5$ at 24 hours after 2.0 ml. intravenous injection of antiplatelet serum. The red cell and hemoglobin values dropped to 50 per cent before death related to generalized bleeding occurred. Significant changes were seen in the megakaryocytes of the bone marrow. The bleeding time and clot retraction were extended. Evidence for a defect in the vessel wall has been shown by the micro-electrode, moccasin snake venom and negative pressure tests. The cause of bleeding has been postulated as a double defect resulting from a decrease of platelets in the circulation and an alteration in the integrity of the vessel wall or perivascular supporting sheath.

**SUMMARIO IN INTERLINGUA**

Purpura experimental esseva inducite in hamsters per le injection de sero anti-plachettal que esseva obtenite ab conilios pretractate con injectiones de plachettas de hamster. Esseva notate le formation spontanee de petechias e de sanguination generalisate. Le disturbance del mechanismo hemostatico esseva analysat per studios del alterationes in le sanguine, le medulla, e le parietes vascular. Le numeration plachettal in sanguine peripheric descendeva ab $9.02 \pm 0.85 \times 10^5$ usque a $0.66 \pm 0.32 \times 10^5$ al fin de 24 horas post le injection intravenose de 2.0 ml de sero antiplachettal. Le valores erythrocytic e hemoglobinico descendeva per 50 pro cento ante le occurrentia del morte in association con hemorrhagia generalisate. Significative alterationes esseva
vidite in le megacaryocytos del medulla ossee. Le tempore de sanguination e le retraction del coagulo esseva prolongate. Indicationes de un defecto in le parietes vascular esseva obtenite per medio del microelectrodo, del test a veneno de agkistrodon, e del test a pression negative. Es postulate que le causa del sanguination esseva un duple defecto resultante ab un reduction de plachettas in le circulation e un alteration del integritate del pariete vascular o del supportante vaina perivascular.

REFERENCES

Evidence for a Vessel Wall Defect in Immuno-thrombocytopenic Hamsters

RAJENDRA G. DESAI and GEORGE P. FULTON