Radiation-induced Hemorrhagic Diathesis in Dogs Unassociated with Thrombocytopenia: Association with an Intravascular Protein-Polysaccharide Particle

By H. S. Winchell, A. C. Anderson and Myron Polycove

THROMBOCYTOPENIA appears to be the cause of the hemorrhagic diathesis following exposure to radiation. Consistent, significant defects in other classical parameters concerned with hemostasis have not been demonstrated following irradiation.1,2 This relation between thrombocytopenia and radiation-induced bleeding is consistent when the whole body is exposed to x- or y-rays. However, correlation between platelet levels and bleeding is less convincing when other radiation modalities and exposures are used.

In a previous paper we described a technic using internally administered Y90 chelated with diethylene-triamine penta-acetic acid (DTPA) for "somewhat selective" irradiation of lymphatic structures.3 This procedure results in relatively little decrease of platelets; yet we have seen severe post-irradiation hemorrhagic diatheses in dogs following its use. This abnormal bleeding occurred at times when the circulating platelet concentrations were adequate. Lack of close correlation between radiation bleeding and thrombocytopenia led us to investigate our material for the intravascular substance seen by Andersen following total body x-irradiation in the dog.4-6 This substance is characterized histochemically as a protein-polysaccharide complex (intravascular polysaccharide substance "IVS"). Andersen noted particles of this substance within small blood vessels, frequently occluding the lumen, and associated with extravascular red blood cells in the surrounding tissues. These findings suggested an intimate association between hemorrhage into tissues and the presence of large globules of this material in small vessels.

This paper presents data correlating bleeding induced by Y90-DTPA radiation, circulating thrombocyte concentration and the histologic finding of the "IVS" of Andersen.

MATERIAL AND METHODS

Purebred beagle dogs between the ages of 6 months and 2 years were used. The Y90-DTPA was administered in a manner described previously.3 (The relative dosimetry to various organs using this procedure can be found in this same reference.) Quantities of stable Yttrium comparable to those given these animals is not associated with a bleeding tendency.

Platelet counts were performed in duplicate on venous blood samples using phase-contrast microscopy. Tissues, fixed in Bouin's solution, were stained with hematoxylin and

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RADIATION-INDUCED HEMORRHAGIC DIATHESIS

Fig. 1.—Relation between platelet count and time of death in dogs given $\gamma^{90}$DTPA. The crosses indicate time of death and are connected by a dashed line to the corresponding preterminal platelet count. Where cross appears next to platelet count the animal expired on the same day as the platelet count was obtained.

cosin, Pollak's trichrome, and methylene blue 0. The degree of petechial hemorrhage was estimated at the gross postmortem examination on the basis of the number of petechial or purpuric lesions present in the gastrointestinal mucosa, pleura, lungs, heart or kidneys. The platelet counts listed are the last made before the death of the animal. The "IVS" is easily recognizable as eosinophilic intravascular globules in tissues fixed in Bouin's solution and stained with Pollak's trichrome. The quantity of IVS present was evaluated independently by two observers. If, upon initial examination, only one or two particles of IVS material were noted in all of the sections, the animal was scored as 1+. If IVS could readily be recognized in the tissues, but a large fraction of the small vessels were free of this material, then the animal was scored as 2+. If most of the small vessels observed in the tissue contained IVS, then the animal was scored as 3+ or 4+ depending on the amount of the material. An animal was considered to have unequivocally significant quantities of IVS if scored 2+ or greater.

RESULTS

Figure 1 demonstrates the pattern of platelet-count changes following irradiation. The point marked with a cross connected with a dotted line to the symbol of the particular animal signifies the time of death following the last platelet count of that animal. In the cases in which the animal died on the same day that the last platelet count was obtained, the cross is placed adjacent to the symbol for that animal. No fall in platelet count was seen in the three animals that died less than 5 days following irradiation. Of the remain-
Table 1.—Relation of Preterminal Platelet Count, “IVS,” and Severity of the Hemorrhagic Diathesis Following Y\(^{99}\)-DTPA Irradiation

<table>
<thead>
<tr>
<th>Dog No.</th>
<th>Dose Y(^{99}) mc./lb. Body Weight</th>
<th>Time of Death Following Irradiation</th>
<th>Estimation of Amount of “IVS” in Tissues</th>
<th>Preterminal Platelet Count Z/mm.(^3) of Blood</th>
<th>Estimated Severity of Petechial Bleeding</th>
<th>Gastrointestinal Bleeding</th>
<th>Other Manifestations of Bleeding</th>
</tr>
</thead>
<tbody>
<tr>
<td>2A</td>
<td>19.24</td>
<td>14 days</td>
<td>+++</td>
<td>38,000</td>
<td>+++</td>
<td>melena in bowel at postmortem</td>
<td>hemorrhage into lungs, medias-</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>bloody diarrhea from 5th–24th</td>
<td>tinum, G.I. tract and bladder</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>day after irradiation</td>
<td>submucosal bleeding in bladder</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>bloody diarrhea from 5th–12th</td>
<td>and G.I. tract; hemosiderin in</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>day after irradiation</td>
<td>lymph nodes indicating previous</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>hemorrhage into lungs and G.I.</td>
</tr>
<tr>
<td>David</td>
<td>13.50</td>
<td>12 days</td>
<td>+++</td>
<td>600,000</td>
<td>+++</td>
<td></td>
<td>tract</td>
</tr>
<tr>
<td>Chain*</td>
<td>9.87</td>
<td>14 days</td>
<td>+++</td>
<td>140,000</td>
<td>+++</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4-G*</td>
<td>8.40</td>
<td>12 days</td>
<td>+++</td>
<td>330,000</td>
<td>+++</td>
<td>melena in bowel at postmortem</td>
<td>hemorrhage into lungs, G.I. mu-</td>
</tr>
<tr>
<td>R-14</td>
<td>19.51</td>
<td>12 days</td>
<td>++</td>
<td>161,000</td>
<td>+++</td>
<td>Red blood in bowel at postmortem</td>
<td>hemorrhage into wall of cecum</td>
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<td></td>
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<td>and into mesenteric nodes</td>
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<td></td>
<td></td>
<td>hemorrhage into pleura, perito-</td>
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<td>neum, G.I. tract, lymph nodes,</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td>kidneys and bladder</td>
</tr>
<tr>
<td>234655</td>
<td>22.60</td>
<td>4 days</td>
<td>++</td>
<td>315,000</td>
<td>++</td>
<td>bloody diarrhea on day of death</td>
<td>hemorrhage into G.I. tract, kid-</td>
</tr>
<tr>
<td>82</td>
<td>16.09</td>
<td>5 days</td>
<td>++</td>
<td>340,000</td>
<td>+</td>
<td></td>
<td>neys and bladder</td>
</tr>
<tr>
<td>M-8</td>
<td>15.73</td>
<td>4 days</td>
<td>++</td>
<td>475,000</td>
<td>+</td>
<td></td>
<td>petechiae found in lungs, G.I.</td>
</tr>
<tr>
<td>Brutus</td>
<td>8.91</td>
<td>12 days</td>
<td>++</td>
<td>420,000</td>
<td>+++</td>
<td></td>
<td>tract and bladder</td>
</tr>
</tbody>
</table>

*Dogs Chain and 4-G had attempted autologous bone marrow infusions following irradiation.*
ing seven animals, all died more than 10 days after irradiation: one had an elevation in platelets above control values; two had levels essentially the same as the control values; one had a platelet count on the day of death of 140,000; and two had preterminal platelet counts of 38,000 and 45,000. The remaining animal had a platelet count of 161,000 6 days prior to death. Table 1 summarizes the results of preterminal platelet counts and correlates this with the postmortem evidences of a hemorrhagic diathesis and the histologic finding of the polysaccharide particles (IVS) of Andersen. Petechial hemorrhage is evaluated separately from gross hemorrhage such as massive G.I. bleeding. All animals had evidence of a hemorrhagic diathesis, which, in eight instances, significantly contributed to the death of the dog. All animals had significant amounts of IVS (see figure 2 for appearance of IVS in vessels). None of the animals had severe thrombocytopenia preterminally. The two dogs not showing a hemorrhagic diathesis (No. 82; No. M-8) died of the radiation-produced gastrointestinal syndrome, and in these the few petechiae seen may have been in part agonal.

**Discussion**

All animals presented had evidence of radiation-induced hemorrhagic diathesis but none had severe preterminal thrombocytopenia. In four of the animals presented in this paper, platelet counts were obtained on the date of death and in all of these the platelet levels were in excess of 140,000. Of the remaining six animals, two had platelet counts 1 day, two had counts 2 days, and one had counts 3 days prior to their demise. These animals could conceivably have had precipitous drops in their platelet counts immediately before death; which, however, would not be expected for most of them from the pattern of their platelet count changes before this time. Such an acute thrombocytopenia has been produced in dogs by plasmaphoresis by Craddock et al.\(^7\) and by use of ion exchange resins by Winchell et al.,\(^8\) but they did not find evidence of an accompanying hemorrhagic diathesis. Therefore, if a thrombocytopenia had developed in some of these animals after the preterminal platelet count was obtained, this acute thrombocytopenia in itself could not explain the hemorrhagic diathesis seen. The possibility that the circulating platelets might be morphologically distinct but nonetheless have a radiation-induced functional defect was not evaluated in these experiments.

The intravascular polysaccharide of Andersen was a constant finding in association with the bleeding diathesis in these animals. Its significance is obscure. In staining characteristics it is similar to the polysaccharide seen in the small vessel lesions of thrombotic thrombocytopenic purpura as described by Moore and Schoenberg.\(^9\) Moore felt that the polysaccharide in T.T.P. was a manifestation of vascular damage and represented aberrancies in the production of the chondroitin sulfuric-acid ground substance in the vascular wall. An analogous situation may exist following radiation exposure in which the vasculature is damaged by radiation events with resultant production of abnormal polysaccharide material. This suggests that the intravascular polysaccharide substance may be a manifestation of direct damage to the vasculature.
Fig. 2.—Kidneys containing globules of IVS in dogs 2A (left) and 224685 (right).
In these dogs no attempt was made to study the kinetics of platelet production or destruction. Platelet transfusions were not given to study their effect on bleeding. The possibility that the circulating platelets might be morphologically distinct but nonetheless have a radiation-induced functional defect was not evaluated. From the data presented, the only warranted conclusion is that peripheral blood platelet concentration does not correlate with bleeding manifestations following a certain form of radiation exposure in the dog.

CONCLUSIONS

1. Significant thrombocytopenia is not present preterminally in dogs given appropriate doses of Y⁰⁰-DTPA irradiation. Nevertheless, these animals have a severe hemorrhagic diathesis at postmortem examination.

2. Dogs systemically irradiated with Y⁰⁰-DTPA, and having a terminal hemorrhagic diathesis, demonstrate an intravascular polysaccharide substance at postmortem examination. This polysaccharide has staining characteristics similar to those first reported by Andersen in dogs systemically irradiated with x-rays, and more recently observed in the lesions of thrombotic thrombocytopenic purpura.
SUMMARIO IN INTERLINGUA

1. Nulle significative grados de thrombocytopenia es presente preterminalmente in canes tractate con appropriate doses de irradiation ab Y\(^{90}\) chelatione con acido diethyleno-triamino-penta-acetic (ADTP). Tamen, in tal animales, sever diathese hemorrhagic es constatabile in le examine post morte.

2. Canes con diathese hemorrhagic terminal post irradiation ab Y\(^{90}\)-ADTP monstra in le examine post morte le presentia intravascular de un substantia polysaccharidic. Iste polysaccharida ha caracteristicas tincturatori simile a illos primo reportate per Andersen como occurrente in canes post generalisate roentgeno-irradiation e observate plus recentemente in lesions de thrombotic thrombocytopenic.

REFERENCES


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