Effects of Total Body X-Irradiation on Blood Coagulation in the Rat

By STANTON H. COHN, M.S.

THE HEMORRHAGIC SYNDROME is known to be a major cause of the high mortality among animals exposed to large doses of ionizing radiation.1-5 Because of the complexity of this hemorrhagic response, no single mechanism which can account for all the symptoms encountered has been isolated. However, Cronkite, in a recent review, indicated that the majority of the explanations for this phenomenon could be organized around one of the following two principles: (1) hemorrhage results from damage to the vascular system, and (2) hemorrhage occurs as a result of a defective blood clotting mechanism, usually ascribed to thrombocytopenia.

This paper is concerned with an investigation of the latter principle. A study was made of blood coagulation in order to determine whether a defect existed in the blood of irradiated animals, and if so, what the characteristics of this defect were and what its relationship was to hemorrhage.

PROCEDURE

The experiments reported in this paper were specifically designed to study post-irradiation blood coagulation. A number of tests were used to study the coagulation process both prior to and at various time intervals following irradiation. The principal test that was employed is based on the measurement of the electrical resistance of a sample of blood. This determination was made throughout the entire period of clot formation and retraction. The instrument used to measure the electrical resistance was developed by Rosenthal and Tobias.6 It provides continuous, quantitative information on blood coagulation, heretofore unavailable.

Other tests of coagulation employed in this study were the Lee-White7 clotting time and the heparin clotting time. The former indicates the in vitro clotting time of blood, while the latter measures the influence of a fixed amount of heparin on this clotting time.

Another group of measurements made on the blood include red cell count, white cell count and differential and platelet count. An attempt was made to correlate the level of these blood constituents with coagulability as measured by the clotting tests and the electrical resistance test.

The data were also taken to provide standardized quantitative norms for the

From the Biological and Medical Sciences Division, U. S. Naval Radiological Defense Laboratory, San Francisco, Calif.

The author wishes to thank Bryant Pickering for performing the blood cell counts for these studies.

The opinions or assertions contained herein are the private ones of the author and are not to be construed as official or reflecting the views of the Navy Department or the Naval Service at large.

Submitted July 6, 1951; accepted for publication October 1, 1951.
clotting reaction for use in the evaluation of the effect of various therapeutic agents on the hemorrhagic response.

EXPERIMENTAL

Female rats of the Sprague-Dawley strain, weighing 180 to 200 Gm., were used. Forty-three rats were given 400 r total body x-irradiation, administered in a single dose. Two of these rats subsequently died (v.i.). Thirty-five animals were used as controls, and received no irradiation. The x-ray dosage factors were as follows: 250 KVP; 15 ma; 0.5 mm. Cu and 1 mm. Al; HVL 1.5 mm. Cu; dose rate 25 r/min.; target-to-skin distance 40 inches.

The responses of the rats were studied at intervals during the 28 day period following the irradiation. Blood samples were obtained, under Nembutal anesthesia, by cardiac puncture after opening of the chest cavity. The blood was carefully drawn to avoid contamination with tissue juice and exposure to air. The sample was discarded when a clean puncture was not obtained. In addition, the first and last cubic centimeters of blood in the syringe were not used for these tests. A 6 cc. blood sample was sufficient for all the tests performed.

Blood Cell Studies

Red and white cell counts, differential counts, and platelet counts were performed in accordance with standard laboratory procedures. Rees-Eckers diluting fluid and red cell pipets were used in obtaining the platelet count.

Gross Hemorrhage

All the animals were observed for external evidences of bleeding throughout the experiment. Gross hemorrhage was studied at postmortem in the following organs: stomach, caecum and large intestine, small intestine, lungs, lymph nodes, bladder, kidney and adrenals. The hemorrhage was graded semi-quantitatively as follows:

<table>
<thead>
<tr>
<th>Degree of Hemorrhage</th>
<th>Units of Hemorrhage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slight Hemorrhage</td>
<td>1</td>
</tr>
<tr>
<td>Moderate Hemorrhage</td>
<td>2</td>
</tr>
<tr>
<td>Marked Hemorrhage</td>
<td>3</td>
</tr>
</tbody>
</table>

The total score of each animal (in arbitrary units of hemorrhage) is the sum of the scores for the individual organs examined.

Blood Coagulation Tests

Clotting time (modification of Lee-White test). A 1 cc. sample of blood was placed in a test tube (13 x 100 mm.), and the tube was inverted at one minute intervals until a solid clot formed. The time elapsed between the appearance of blood in the syringe and the formation of a solid clot is taken as the clotting time.

Heparin clotting time. A 1 cc. sample of blood was added to 0.1 cc. heparin (0.004 mg. heparin in saline) in a 13 x 100 mm. test tube. The tube was inverted at one minute intervals until a solid clot formed. The heparin clotting time is taken as the interval of time between the appearance of blood in the syringe and the formation of a solid clot. All measurements for both clotting tests were made in a constant temperature bath set at 37 C.

Electrical Resistance Measurement

Electrical resistance of the blood during clotting was measured on a 1.5 cc. sample. The specific resistance was then calculated from the cell constants. The details of this technic have been described by Rosenthal. From the curve of electrical resistance as a function of time, the following information can be obtained: (1) the initial blood resistance, before
clotting occurs; (2) the period of initial resistance increase; (3) the resistance at the end of the initial increase in resistance; (4) the time elapsed until clot retraction begins (the beginning of clot retraction is taken to be indicated by a sharp inflection point on the electrical resistance curve); (5) the resistance at the beginning of clot retraction; (6) the rate of clot retraction, obtained by measuring the rate of change of blood resistance during the phase of clot retraction.

RESULTS

Blood Cell Study

The results of the blood cell investigation are recorded in table 1. Below is a summary of the hematologic changes in the irradiated animals.

Red Blood Cells. The erythrocyte count dropped gradually to a minimum value of 65 per cent of the control mean by the twenty-eighth day (table 1).

<table>
<thead>
<tr>
<th>Day after irradiation</th>
<th>No. of rats</th>
<th>RBC × 10⁶ per cu. mm.</th>
<th>Leukocytes</th>
<th>Platelets × 10⁴ per cu. mm.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Control</td>
<td>Irradiated</td>
<td>Control</td>
</tr>
<tr>
<td>1</td>
<td>3</td>
<td>8.04</td>
<td>7.88</td>
<td>9,350</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>6.54</td>
<td>6.18</td>
<td>8,310</td>
</tr>
<tr>
<td>6</td>
<td>3</td>
<td>6.63</td>
<td>6.53</td>
<td>8,200</td>
</tr>
<tr>
<td>9</td>
<td>4</td>
<td>7.58</td>
<td>6.93</td>
<td>8,880</td>
</tr>
<tr>
<td>13</td>
<td>16</td>
<td>8.83</td>
<td>6.20</td>
<td>10,939</td>
</tr>
<tr>
<td>16</td>
<td>20</td>
<td>6.93</td>
<td>5.29</td>
<td>8,248</td>
</tr>
<tr>
<td>20</td>
<td>28</td>
<td>6.64</td>
<td>5.52</td>
<td>9,270</td>
</tr>
<tr>
<td>28</td>
<td>3</td>
<td>8.57</td>
<td>4.85</td>
<td>8,643</td>
</tr>
</tbody>
</table>

Lymphocytes. The lymphocytes declined to 3 per cent of the control mean at 24 hours, and then increased to 8 per cent by the sixth day. The level then decreased slightly until the sixteenth day, after which a rapid recovery ensued, and the lymphocytes approached the normal value by the twenty-eighth day.

Heterophils. Heterophils, after showing an initial increase of 50 per cent of the control value at 24 hours, fell rapidly to 1 per cent by the sixth day. After another rise at the ninth day, the heterophil level again fell to a minimum of 1 per cent of the control mean, at which value it remained until the twentieth day. The level then rose rapidly, and reached the normal range by the twenty-eighth day.

Platelets. The platelet count showed no significant fall until after the third day following irradiation. (The statistical analysis of these data is presented in Table 1.) A minimum value was obtained at sixteen days, after which time the platelet count rose, reaching the normal range by the twenty-eighth day (see fig. 3).
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Gross Hemorrhage

Under “Evidence of Hemorrhage,” table 2, are listed the number of irradiated animals exhibiting external hemorrhage, and semi-quantitative evaluations of internal hemorrhage as seen at postmortem. The results indicate that the internal hemorrhage increased slightly in severity in the first nine days, and reached the highest degree by the sixteenth day. After this time the degree of the hemorrhage dropped to 50 per cent of the maximum value (by the twentieth day), remaining

<table>
<thead>
<tr>
<th>Day after irradiation</th>
<th>No. of rats</th>
<th>Clotting time (min.)</th>
<th>Heparin clotting time (min.)</th>
<th>Evidence of hemorrhage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Irradiated</td>
<td>Control</td>
<td>Irradiated</td>
</tr>
<tr>
<td>1</td>
<td>3</td>
<td>6</td>
<td>2.3</td>
<td>2.8</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>6</td>
<td>2.8</td>
<td>2.6</td>
</tr>
<tr>
<td>6</td>
<td>3</td>
<td>3</td>
<td>3.7</td>
<td>3.7</td>
</tr>
<tr>
<td>9</td>
<td>4</td>
<td>5</td>
<td>2.5</td>
<td>2.3</td>
</tr>
<tr>
<td>13</td>
<td>6</td>
<td>4</td>
<td>2.0</td>
<td>4.0</td>
</tr>
<tr>
<td>16</td>
<td>6</td>
<td>4</td>
<td>2.8</td>
<td>4.5</td>
</tr>
<tr>
<td>20</td>
<td>6</td>
<td>3</td>
<td>2.7</td>
<td>3.3</td>
</tr>
<tr>
<td>28</td>
<td>3</td>
<td>3</td>
<td>4.7</td>
<td>3.7</td>
</tr>
</tbody>
</table>

\( X \) 2.8 14.3

Fig. 1.—Electrical resistance of blood clotting in the rat.
at this level at the twenty-eighth day. In all animals examined, the hemorrhage was found to be mainly in the lungs, stomach, intestines and lymph nodes.

Only one of the irradiated animals showed external signs of hemorrhage. It was found in a moribund state on the sixteenth day, exhibiting gross hemorrhage from the nose. Two other animals died on the twentieth day, exhibiting some internal hemorrhage. The cause of death was not ascertained.

![Graph](image)

**Fig. 2.**—Measurements obtained from the electrical resistance curves of clotting.

**Clotting Tests**

Table 2 summarizes the data obtained from the various blood clotting tests.

**Clotting time.** The clotting time, as obtained by the Lee-White method, showed no significant variation from the normal range during the twenty-eight days. The control values ranged from 2 to 5 minutes, with a mean of 2.8 minutes. The highest mean value of post-irradiation clotting times was 4.5 minutes, and the readings ranged from 2 to 6 minutes. It must be pointed out that this test does not lend itself to a precise measurement, inasmuch as the end point is not well defined. Further, because the clotting time of the rat is so brief, the percentage error of the clotting time measurement by this technic may be very high.
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**Fig. 3.**—Clot retraction rate and blood platelet level.

**Table 3.**—Electrical Resistance Measurements of Blood Coagulation

<table>
<thead>
<tr>
<th>Day of</th>
<th>Initial blood</th>
<th>Period of</th>
<th>Resistance</th>
<th>Time until</th>
<th>Resistance</th>
<th>Clot retraction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>rate</td>
<td>resistance</td>
<td>resistance</td>
<td>at beginning</td>
<td>at end of</td>
<td>rate</td>
</tr>
<tr>
<td></td>
<td>(ohm-cm.)</td>
<td>(ohm-cm.)</td>
<td>(ohm-cm.)</td>
<td>resistance</td>
<td>resistance</td>
<td>(ohm-cm./min.)</td>
</tr>
<tr>
<td>Control</td>
<td>177 160</td>
<td>4.33 5.0</td>
<td>241 217</td>
<td>13.0 14.8</td>
<td>244 209</td>
<td>22.6 0.5 0.01</td>
</tr>
<tr>
<td>Irradiated</td>
<td>168 151</td>
<td>4.50 3.8</td>
<td>230 178</td>
<td>12.0 23.2</td>
<td>246 192</td>
<td>19.8 7.9 +</td>
</tr>
<tr>
<td>Control</td>
<td>159 146</td>
<td>4.00 3.5</td>
<td>221 160</td>
<td>9.8 39.5</td>
<td>216 163</td>
<td>13.6 3.0 0.01 +</td>
</tr>
<tr>
<td>Irradiated</td>
<td>177 148</td>
<td>4.83</td>
<td>261 149</td>
<td>10.2 &gt;40</td>
<td>287 149</td>
<td>21.0 0.1 0.01 +</td>
</tr>
<tr>
<td>Control</td>
<td>167 121</td>
<td>3.25 0</td>
<td>203 121</td>
<td>&gt;40</td>
<td>199 121</td>
<td>16.0 0.4 6.6 &lt;0.01 +</td>
</tr>
<tr>
<td>Irradiated</td>
<td>166 109</td>
<td>4.67 1.1</td>
<td>230 121</td>
<td>9.7 &gt;40</td>
<td>247 120</td>
<td>18.0 0 +</td>
</tr>
<tr>
<td>Control</td>
<td>175 148</td>
<td>5.00 4.6</td>
<td>244 176</td>
<td>9.7 11.6</td>
<td>263 182</td>
<td>20.1 2.7 5.2 &lt;0.01 +</td>
</tr>
<tr>
<td>Irradiated</td>
<td>192 165</td>
<td>5.00 6.7</td>
<td>260 252</td>
<td>12.5 12.3</td>
<td>255 253</td>
<td>13.2 12.8 0.1 &gt;0.01 0</td>
</tr>
</tbody>
</table>

X... 173 ±4.44  ±1.52 ±35.2 ±1.35 ±41.2 ±4.95
σ... ±12.4 ±1.9 ±35.2 ±1.35 ±41.2 ±4.95
Heparin clotting time. The heparin clotting time showed no significant increase over the control mean value until the sixth day, when a very rapid increase took place. A maximum value of more than twenty-five times the control mean was obtained about the sixteenth day. The values then fell rapidly, reaching the normal range about the twentieth day. They remained in this normal range until the conclusion of the experiment at the twenty-eighth day.

**Electrical Resistance Measurements**

The typical electrical resistance curves of blood clotting in the rat before and after irradiation are shown in figure 1. The control curve labeled (A) represents the mean curve obtained from twenty normal rats.

The mean curve obtained three days after 400 r x-irradiation is labeled (B). The curve as a whole has been shifted, indicating a lowered resistance. The plateau between the initial resistance increase and the onset of clot retraction has been lengthened, and the slope of clot retraction decreased. Curve (C), representing data taken sixteen days after irradiation, shows that the resistance has fallen still further, and after a slight initial resistance increase, the curve becomes a straight line with zero slope.

As mentioned previously, one can obtain from the electrical resistance curve six different quantities (see table 3). The changes in the values of these quantities after irradiation are presented graphically in figures 2 and 3.

**Discussion**

The hemorrhagic syndrome in rats following their exposure to x-irradiation closely resembles that of thrombocytopenic purpura, with petechial hemorrhages throughout the body, thrombocytopenia, decreased clot retraction, and normal (Lee-White) clotting time.

While the Lee-White clotting test revealed no abnormality in the blood clotting response of the irradiated animals, an impairment of the blood coagulation in the rat following 400 r x-irradiation was observed. This defect in coagulation was quantitatively reflected by an increased heparin clotting time, and by alterations in the blood resistance measurements (overall lowered resistance, increased time until the beginning of clot retraction, and decreased clot retraction rate). A decreased platelet level was associated with these changes.

An explanation for this anomaly of normal Lee-White clotting time in the presence of impaired coagulation is presented by Quick. He found that while coagulation time is generally normal in thrombocytopenic purpura, there is a defect in the clotting mechanism, as denoted by a drop in the amount of prothrombin converted to thrombin. Quick has found that in the clotting process, the rate of conversion of prothrombin is related to the number of platelets present. However, he suggests that the presence of even a minute number of platelets can allow the production of enough thrombin in a few minutes to cause sufficient clotting for a normal Lee-White clotting time. Thus, a definite coagulation defect can be masked by a normal clotting time.

The effect of heparin on the clotting time of blood has been studied by a number of investigators. The basis for the heparin clotting time test used in this
experiment is an outgrowth of the work of De Takats, who measured the effect on the clotting time of heparin injected in vivo, and of Waugh and Ruddick, who made the same measurements with heparin added to blood in vitro. Jaques and Ricker and Best and Jaques made further observations on the relation of heparin dosage and clotting time.

Attempts to correlate platelet level with various components of the coagulation process have been made. Quick et al. studied the effect of heparin on platelets in vivo. Rosenthal has discussed the significance of the correlation between heparin clotting time and platelet level. Conley, Hartmen and Lalley observed that normal human plasma could be made more sensitive to the effects of added heparin by removing most of the platelets by centrifugation. Allen offers an explanation for the increased sensitivity of blood to heparin in thrombocytopenia, in terms of heparin inactivation by platelets.

Several other investigators have noted the fact that clot retraction is quantitatively and qualitatively related to the platelet level. The data in this experiment confirm these observations, and, most strikingly, they indicate a correlation between the level of the platelets and the rate of clot retraction. The variation of these values with time is very similar. The platelet level and the clot retraction rate reached a minimum value at the same time and began to recover at the same time (compare curves of fig. 3). It was also noted that clot retraction in the rat ceased to occur when the platelet level dropped below a value of 38,000 per cu. mm. It is interesting to note that Fonio reports the cessation of clot retraction in humans at a platelet level of 36,000 per cu. mm.

However, a significant change in the clot retraction rate was observed as early as one to three days following irradiation, while the initial decrease in platelet level occurred in the period from three to six days following irradiation. Inasmuch as the retraction rate decreased before any significant change was measured in the platelet count, it cannot be concluded from these data that the decrease in the clot retraction rate is entirely a result of the lowering of the platelet level.

The greatest defect in clotting occurred at sixteen days, as denoted by the maximum deviations from normal in the clot retraction rate, platelet level, heparin clotting time, and time until the beginning of clot retraction. These measurements correlate with the degree of hemorrhage, as the degree of internal hemorrhage also reached its maximum value at sixteen days.

The decreased level of platelets in blood following exposure of the rat to radiation appears to be a major factor in defective clotting, and thus an important factor in post-irradiation hemorrhage. That platelet level may be an important factor in the hemorrhagic syndrome has been postulated by Quick, who states that, "since the speed of prothrombin conversion determines hemostatic effectiveness, it is obvious why a diminution of platelets becomes a significant factor in producing a hemorrhagic tendency."

Because of the complex nature of the clotting process, a complete explanation for the hemorrhagic syndrome cannot be made solely in terms of defective clotting and lowered platelet level. Nevertheless, by means of this approach, it may be possible to clarify an important portion of the post-irradiation hemorrhagic syndrome.
STANTON H. COHN

SUMMARY

1. The hemorrhagic syndrome following exposure of rats to 400 r x-irradiation was studied. A blood coagulation defect was found in the irradiated animals.

2. This coagulation defect is quantitatively reflected in a marked increase in partial thromboplastin time, and in alterations in the blood resistance measurements: lowered resistance, increased time until the beginning of clot retraction, and decreased clot retraction rate.

3. The decreased level of platelets in blood following exposure of the rat to radiation appears to be a major factor in defective clotting.

4. The post-irradiation hemorrhage appears to be correlated with the defective blood coagulation. At sixteen days after irradiation the degree of internal hemorrhage has reached its maximum value. Maximum deviations are also noted in clot retraction rate, time until inception of clot retraction, platelet level and heparin clotting time.

5. The heparin clotting time measurements and the electrical resistance measurements presented in this paper provide quantitative baselines for the coagulation reactions before and after 400 r x-irradiation. Such criteria make possible the evaluation of various therapeutic agents in terms of their effect on coagulation.

REFERENCES


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