HEMORRHAGE has been an important and ominous sign in victims of irradiation effects. Although its occurrence coincided with a period of intense thrombocytopenia, Allen and Jacobson in experimental observations in dogs observed a circulating heparin-like anticoagulant, and Allen concluded that it played an important role in this hemorrhagic state. The reports of Cronkite, Holden et al. and Jackson et al. indicated that the presence of a circulating anticoagulant was inconstant and they disclaimed any important role played by it. Their studies showed that with the fall in platelets there was an increase in the whole blood clotting time, slow to absent clot retraction, lysis of whole blood clots in some animals, an increase in the levels of fibrinogen from the eighth day on, and no change in the prothrombin time. Furthermore, studies by Cronkite et al., Woods et al. and Hjort, Perman and Cronkite clearly show that the hemorrhagic state in the experimental animal is related to thrombocytopenia and not to a circulating anticoagulant. No abnormality of the coagulation factors has as yet been reported. Cronkite, Jacobs and Schork, however, made the observation that the platelets in the degenerative phase of the thrombocytopenic period were less active in the utilization of prothrombin than the platelets in the regenerative phase. Jacobs, Cronkite and White observed that the evolution of SPCA (active factor VII) was impaired in the irradiated dog but that more than normal amounts were evolved when prothrombin conversion was accelerated by artificial means such as agitation, thrombin or thromboplastin. This effect in light of later developments in the field of coagulation can be interpreted as failure to generate a normal amount of the plasma thromboplastic material due to a lack of the contribution to this complex aggregate by the platelets. Holden et al. observed a decrease in the sedimentable thromboplastic material of plasma.

The opportunity to study the effects of total body irradiation in man became available when eight men were exposed to radiation as a result of the Y-12 accident at Oak Ridge, Tennessee, June 16, 1958. In three cases the calculated exposure was less than 100 r. The results of studies of the coagulation mechanism on the five who received high dose irradiation (cases A-E) are reported herewith.

Material and Methods

The five subjects were exposed to radiation on June 16, 1958. All were men, and the amount of radiation each received based on serum Na data was calculated to be as follows:
The studies were done on three occasions while the subjects were in the Medical Division of
the Oak Ridge Institute of Nuclear Studies, Oak Ridge, Tenn. The first, June 27, 1958, 11
days after exposure, was before the period of thrombocytopenia. The second, July 15, 1958,
29 days after exposure, was at the peak of the period of thrombocytopenia. The last study
on September 18,* 1958, the 94th day, was after complete recovery. The only bleeding
observed was petechiae and ecchymosis in Case A, petechiae in Case C, and slight gum
bleeding in Case E. The bleeding was observed shortly before the second study. The follow-
ing tests were done on each occasion:

Clotting time: (Lee-White)
Prothrombin time: (Quick)
Partial thromboplastin time:13 (0.1 ml. citrate plasma, 0.1 ml. of a 1:100 dilution of
lipid material after Bell and Alton14 diluted in saline, 0.1 ml. calcium chloride
0.03M.
Thromboplastin generation test:15 With platelets adjusted to a count of 300,000
per mm.3
Fibrinolysis: A mixture of 0.5 ml. of the euglobulin fraction of the plasma to be tested,
0.01 ml. of commercial (Upjohn) thrombin (previously freed of material precipitable
at pH 5.2, then itself precipitated with acetone and redissolved in water), and 0.5
ml. of human fibrinogen prepared by the freeze-thaw method was observed for the
time of lysis of the fibrin clot.
Fibrinogen: Clottable tyrosine.

Because of slightly short partial thromboplastin times at the first study, specific assays
of various clotting factors were done on the second and third studies. They were as follows:

Plasma and serum prothrombin.16
Factor VII and X combined test.17
Factor X.18
Factor V:19 Corrective effect of the test plasma diluted 1:10 in oxalate saline on the
prothrombin time of aged plasma.

Factor VIII: A modification of the method of Langdell, Wagner and Brinkhaus13 was
used. An artificial substrate similar to that described by Didisheim20 was used in
place of hemophilic plasma, and consisted of human oxalated plasma freed of fac-
tors VIII and V by aging and of thromboplastic debris by filtration through char-
coal.17 Factor V was restored by adding adsorbed beef serum. The test was done
as follows: Citrated plasma was adsorbed with a suspension of aluminum hydroxide.
To 0.1 ml. of the substrate was added 0.1 ml. of a dilution of the adsorbed test
plasma in a 1:50 dilution of adsorbed beef serum (supply of factor V). This mix-
ture was incubated at 37 C. for 5 minutes and then the clotting time was measured
after addition of 0.1 ml. of calcium chloride 0.03M. A dilution curve for normal
plasma was constructed by plotting the clotting time against concentration using a
logarithmic scale and the 1:10 dilution as 100 per cent, 1:20 as 50 per cent, 1:40
as 25 per cent, etc. The test plasmas were then assayed in the same way at dilutions
of 1:10 and 1:40. The activity in per cent of normal was then read off the dilution
curve. The activity at a 1:40 dilution was multiplied by four. This assay is specific
and quantitative for factor VIII as shown by assays done on patients with various

*This date was in error in our original report.11
coagulation defects and preparations of purified factor VIII. The details are to be reported elsewhere. Blood samples were taken directly from an Arquad coated needle into test tubes. Citrated samples taken in silicone coated tubes were used for partial thromboplastin times, platelets, assay for specific factors, and the plasma for the thromboplastin generation test. Oxalate and uncoated tubes were used for prothrombin times. For reference values two normal subjects supplied plasmas which were treated in a manner identical to that of the cases. The same two normals were used for the first two studies, but because of unavailability one was substituted by a third at the last study. Dilutions of material in the tests for levels of factors was increased from the usual 1:10 to 1:40 to pick up instances where there were supernormal values of the coagulation factors. The average of the two normals was taken as 100 per cent.

RESULTS

No indication of deficiency in coagulation factors was observed at any of the studies (table 1) with the exception of a slightly abnormal thromboplastin generation on two subjects. This was believed to be due to difficulty in harvesting platelets because the defect was corrected by the addition of normal platelets or the platelets from one of the other subjects. The serum prothrombins tended to be slightly abnormal on the second and third studies. The degree of difference in all cases was not sufficient to be clearly abnormal.

It was noted that the partial thromboplastin times were slightly shorter at the time of both the first and the second studies. Since it was considered possible that there might be an excess of a clotting factor to cause this, specific assays were done at the second and third studies.

The results (table 2) indicated somewhat elevated levels of factor V in four of five patients and distinctly high levels of factor VIII in all patients at the time of the second study which was at the peak of the thrombocytopenia. At the third study, when bone marrow recovery had taken place, the values had returned to normal levels. In one instance (patient E) the level of factor VIII dropped to slightly below the normal range of 68-165 per cent given for this test. It is doubtful that any significance can be placed on this isolated finding. The levels of fibrinogen were elevated at the first study.

It seemed reasonable to suspect a relationship between low platelets and supernormal values of factors V and VIII. Consequently, the levels of these

<table>
<thead>
<tr>
<th>Material date</th>
<th>Partial thromboplastin time</th>
<th>Prothrombin time sec</th>
<th>Clotting time min</th>
<th>Thromboplastin generation test (thromboplastin time) sec</th>
<th>Factor VIII (FVIII) %</th>
<th>Factor VIII (FVIII) %</th>
<th>Factor VII</th>
<th>Factor X</th>
</tr>
</thead>
<tbody>
<tr>
<td>June 27</td>
<td>93</td>
<td>13.9</td>
<td>10</td>
<td>8 &lt;60</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>July 16</td>
<td>70</td>
<td>13.2</td>
<td>11</td>
<td>9.2 &lt;60</td>
<td>22</td>
<td>122</td>
<td>106</td>
<td>109</td>
</tr>
<tr>
<td>Sept. 22</td>
<td>117</td>
<td>11.9</td>
<td>11</td>
<td>10.5 &lt;60</td>
<td>90</td>
<td>108</td>
<td>89</td>
<td>81</td>
</tr>
<tr>
<td>Range of normal values— all dates</td>
<td>95–120</td>
<td>11.6–14.5</td>
<td>6–18</td>
<td>8.2–11</td>
<td>&lt;60</td>
<td>5–20</td>
<td>94–106</td>
<td>91–116</td>
</tr>
</tbody>
</table>
COAGULATION FACTORS IN IRRADIATION

Table 2.—Individual Values of Factors V, VIII, and Fibrinogen

<table>
<thead>
<tr>
<th>Date</th>
<th>Factor V % normal*</th>
<th>Factor VIII % normal</th>
<th>Fibrinogen mg. %</th>
<th>Platelet Count x 10⁷/mm.³</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case A</td>
<td>1.54</td>
<td>120</td>
<td>234</td>
<td>88</td>
</tr>
<tr>
<td>Case B</td>
<td>115</td>
<td>106</td>
<td>228</td>
<td>106</td>
</tr>
<tr>
<td>Case C</td>
<td>1.54</td>
<td>128</td>
<td>214</td>
<td>121</td>
</tr>
<tr>
<td>Case D</td>
<td>137</td>
<td>97</td>
<td>220</td>
<td>146</td>
</tr>
<tr>
<td>Case E</td>
<td>1.50</td>
<td>127</td>
<td>214</td>
<td>52</td>
</tr>
<tr>
<td>Normal 1</td>
<td>1.94</td>
<td>-</td>
<td>108</td>
<td>-</td>
</tr>
<tr>
<td>Normal 2</td>
<td>96</td>
<td>109</td>
<td>109</td>
<td>92</td>
</tr>
<tr>
<td>Normal 3</td>
<td>-</td>
<td>64</td>
<td>-</td>
<td>112</td>
</tr>
</tbody>
</table>

*The factor V levels were adjusted so that the normals averaged 100% and are slightly different from that of the original report.
†Platelet counts were supplied by the Medical Division, Oak Ridge Institute for Nuclear Studies, and done by the method of Brecher and Cronkite.

Two factors were measured in eight cases of thrombocytopenia due to idiopathic or secondary thrombocytopenia. The results are shown in table 3. The levels of factor VIII were increased in all and were over 300 per cent in three patients. The levels of factor V were elevated in only two cases. The level of fibrinogen was elevated in one case.

The explanation of the supernormal values of factors V and VIII would therefore seem related to the low level of platelets rather than to any direct effects of the radiation. One obviously possible mechanism would be the well known capacity for the platelets to adsorb these two clotting factors. If a given amount of total circulating factor V and VIII is adsorbed on the platelets, then when normal plasma is harvested by centrifugation the adsorbed portion will sediment with the platelets and will be unavailable to the assay in the supernatant plasma. If the platelets were deficient, however, there would be less adsorbed and more would remain in the supernatant plasma.

To test this hypothesis platelet rich or platelet poor hemophilic plasma was mixed equal parts with thrombocytopenic plasma, incubated for 10 minutes at room temperature, centrifuged at 3,000 rpm for 30 minutes and then assayed for factor VIII. Another sample of mixtures was incubated 6 hours at 4°C., centrifuged and assayed. There was no significant decrease in the level of factor VIII when the high factor VIII ITP plasma was allowed to come into contact with the hemophilic platelets (table 4).

Another possibility would be that a decrease in the turnover of clotting factors might allow for a buildup of levels of factor V and VIII. To test this hypothesis, levels of factor VIII were assayed in patients receiving oral coumarin anticoagulants on a long term basis. The results, shown in table 5, indicate that in these subjects the level of factor VIII is likewise although less remarkably elevated.

DISCUSSION

Under the circumstances of this study there was no evidence of any defect in the clotting mechanism other than thrombocytopenia after the exposure to total body irradiation. First, it is possible that at this level of radiation there is no effect, but at a higher dose there could be. Actually no serious hemorrhagic state was observed in these cases. Secondly, it is possible that in the
Table 3.—Factor V, VIII and Fibrinogen Levels in Cases of Idiopathic and Secondary Thrombocytopenia

<table>
<thead>
<tr>
<th>Case</th>
<th>Platelets x $10^9$/mm.³</th>
<th>Factor V % normal</th>
<th>Factor VIII % normal</th>
<th>Fibrinogen mg. %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>15</td>
<td>172</td>
<td>400</td>
<td>245</td>
</tr>
<tr>
<td>2</td>
<td>21</td>
<td>81</td>
<td>400</td>
<td>382</td>
</tr>
<tr>
<td>3</td>
<td>35</td>
<td>80</td>
<td>200</td>
<td>176</td>
</tr>
<tr>
<td>4</td>
<td>50</td>
<td>150</td>
<td>150</td>
<td>394</td>
</tr>
<tr>
<td>5</td>
<td>51</td>
<td>124</td>
<td>160</td>
<td>240</td>
</tr>
<tr>
<td>6</td>
<td>9</td>
<td>100</td>
<td>150</td>
<td>346</td>
</tr>
<tr>
<td>7</td>
<td>25</td>
<td>108</td>
<td>314</td>
<td>600</td>
</tr>
<tr>
<td>8</td>
<td>–</td>
<td>–</td>
<td>167</td>
<td>–</td>
</tr>
</tbody>
</table>

Table 4.—Lack of Removal of Factor VIII from Idiopathic Thrombocytopenic Purpura (ITP) Plasma by Hemophilic Platelets—The Mixtures Were Incubated, Centrifuged and Then Assayed

<table>
<thead>
<tr>
<th>Factor VIII assay</th>
<th>Mixtures of plasma</th>
<th>Incubated 10 min. at 20 C.</th>
<th>Incubated 6 hrs. at 4 C.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Platelet-rich hemophilic + platelet-poor ITP</td>
<td>96%</td>
<td>100%</td>
</tr>
<tr>
<td></td>
<td>Platelet-poor hemophilic + platelet-poor ITP</td>
<td>104%</td>
<td>110%</td>
</tr>
</tbody>
</table>

Table 5.—Prothrombin and Factor VIII Levels in 7 Patients on Long Term Oral Coumarin Anticoagulation

<table>
<thead>
<tr>
<th>Case</th>
<th>Prothrombin Concentration% normal</th>
<th>Factor VIII % normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7.5</td>
<td>180</td>
</tr>
<tr>
<td>2</td>
<td>15.5</td>
<td>184</td>
</tr>
<tr>
<td>3</td>
<td>17.5</td>
<td>132</td>
</tr>
<tr>
<td>4</td>
<td>23</td>
<td>154</td>
</tr>
<tr>
<td>5</td>
<td>27</td>
<td>181</td>
</tr>
<tr>
<td>6</td>
<td>29</td>
<td>172</td>
</tr>
<tr>
<td>7</td>
<td>30</td>
<td>118</td>
</tr>
</tbody>
</table>

The observation of increased levels of fibrinogen, factor VIII and factor V are of some interest and deserve speculation as to their etiology. The increased level of fibrinogen happened before the fall in platelets, and would seem to be due to a mechanism different from the increased levels of factors V and VIII. Increased levels of fibrinogen were not seen in thrombocytopenia due to causes other than irradiation. Therefore, the most satisfactory explanation would seem to be a direct effect of radiation similar to increased fibrinogen levels seen in inflammatory disorders. The increased levels of factors V and VIII, on the other hand, bear a relation to the development of thrombocytopenia rather than to the direct effect of the irradiation, since increased levels were seen in thrombocytopenia without irradiation. The possibility of decreased adsorption by platelets has been considered, but no support could
COAGULATION FACTORS IN IRRADIATION

be established for this hypothesis. It is still possible that this could be the mechanism in spite of the failure to demonstrate adsorption of factor VIII on hemophilic platelets. It could be that there was still enough factor VIII in the hemophilic to saturate his platelets, thereby preventing any further adsorption, although not enough to maintain normal blood levels. More attractive and with foundation in experiments of others would be the hypothesis that with thrombocytopenia there is less than normal intravascular coagulation, and consequently, less utilization of factors V and VIII. Penick, Roberts, Webster, and Brinkhous21 have shown in dogs that the intravenous administration of thromboplastic materials lowers the circulating levels of factor VIII. They postulate that small quantities of thrombin destroy factor VIII. If it is the normally circulating infinitesimal amounts of thrombin which govern the level of circulating factor VIII, then it is quite conceivable that a decrease in the rate of normal formation of thrombin by lowering of the level of platelets would allow the level of factor VIII to rise. This theory is supported by finding increased levels of factor VIII in patients on anticoagulants.

Finally, it should be pointed out that Penick, Cronkite, Godwin, and Brinkhous24 studied the levels of factor VIII in irradiated dogs but reported no increase. Since they were attempting to see if lymph tissue was the source of factor VIII, they were looking for decreases in the levels. It is possible that the design of the tests would not pick up increased levels without using diluted samples, and it is felt that this is most likely the cause of the discrepancy between their results and those reported here.

SUMMARY

Investigation of the clotting factors in five humans accidentally exposed to total body irradiation disclosed no deficiencies. There was no evidence of a circulating anticoagulant in any case. Moderate fibrinolysis was seen in two instances in the same individual but was not evident in the others. At the eleventh day there was uniformly a moderate increase in the levels of fibrinogen. At the twenty-ninth day there was observed an increase in the levels of factors V and VIII coincidental with thrombocytopenia. This change reversed itself with recovery. A similar increase in the levels of factor VIII and less so of factor V was seen in cases of idiopathic thrombocytopenic purpura and secondary thrombocytopenia. An increase in level of factor VIII was observed in patients on oral coumarin anticoagulation. It is concluded that the supernormal levels of factors V and VIII are secondary to lowered thrombin levels in the circulating plasma as a result of the thrombocytopenia and not due directly to the effect of irradiation.

SUMMARY IN INTERLINGUA

Le investigation del factores coagulatori in cinque subjectos human qui habeva accidentalmente essite exponse a irradiation del corpore total revelava nulle deficientias. Indicationes del presentia de un anticoagulante in le circulation mancava in omne le casos. Moderate grados de fibrinolyse esseva
notate in duo occasiones in le mesme subjecto; nulle tal esseva evidentce in le alteres. Le dece-prime die, moderate augmentos in le nivellos de fibrinogeno esseva uniformemente presente. Le vinti-none die, augmentos esseva observate in le concentrationes de factor V e factor VIII. Iste phenomeno coincidava con le declaration de thrombocytopenia. Le alteration de factores V e VIII e le thrombocytopenia dispareva con le restablimento del patientes. Un simile augmento del concentrationes de factor VIII e —minus pronunciatemente— de factor V esseva vidite in casos de idiopathic purpura thrombocytopenic e de thrombocytopenia secundari. Un augmento in le nivellos de factor VIII esseva observate in patiente sub tractamento con coumarina oral. Es concludite que le concentrationes supranormal de factor V e factor VIII es secundari a reduce niveles de thrombina in le plasma circulante. Illos essera assi un resultado del thrombocytopenia e non un effecto immediate del irradiation.

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We wish to thank the ORINS Hospital Staff, Dr. Gould Andrews and Dr. Marshall Brucer for their kind cooperation and for the opportunity to study these cases. Also, Dr. Leonard Hamilton for reviewing, and to Miss Violet Peters for preparation of the manuscript.

REFERENCES

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Blood Coagulation Factors in Total Body Irradiation

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