The Prothrombin Concentration in the Plasma of Normal and Leukemic Rats*

Ernest Sturm

(From the Laboratories of the Rockefeller Institute for Medical Research, New York, N. Y.)

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It was noted in a previous investigation of a transmissible lymphoid leukemia of the rat (2) that animals in the late stage of the disease frequently show active bleeding from the nose and conjunctivae. Autopsy of these animals reveals enormous enlargement of the liver due to leukemic infiltration and extensive hemorrhages in the lungs, liver, testes, subcutaneous tissue, and in the abdominal and femoral muscles. Other rats dying with the disease in an equally fulminating form show no evidence at autopsy of a hemorrhagic diathesis and relatively little leukemic infiltration of the liver.

A series of experiments has been carried out to determine whether variations in plasma prothrombin level might be the controlling factor in the difference in hemorrhagic tendency. Quick's method (3, 4) for prothrombin time determination was utilized in this study.

METHODS

Young rats of the Wistar strain were injected intraperitoneally with 0.2 cc. of a suspension of leukemic cells. Between the seventh and ninth day following injection there appeared clinical manifestations of the disease: namely, generalized lymphadenopathy, hemorrhages from the nose and conjunctivae, and elevation of the white blood count. When the disease had reached an advanced stage, 2.7 cc. of blood were drawn from the heart into a syringe containing 0.3 cc. of 0.1 M sodium oxalate solution. The red blood cells were removed by centrifugation, and 0.1 cc. of the supernatant clear plasma was placed in a test tube. To this plasma were added 0.1 cc. thromboplastin solution and 0.1 cc. of 0.025 M calcium chloride solution. The tube was gently shaken, placed in a water bath at 38°C, and the clotting time recorded with a stop watch.

The thromboplastin solution was prepared by the emulsification of 0.3 gm. of dehydrated rabbit brain in 4.9 cc. physiological salt solution and 0.1 cc. of 0.1 M sodium oxalate. The mixture was incubated at 45°C for 10 minutes and then centrifuged for 3 minutes to remove the larger particles. The milky supernatant fluid was used in the majority of the tests, although a commercial product was substituted in a few instances.

Experiment 1.—The prothrombin time of plasma from 12 rats with well established leukemia and 20 normal rats of the same age and strain was estimated by Quick's method. The average prothrombin time for the leukemic plasma was 31 seconds, while that for the normal plasma was 25.8 seconds. The difference was not considered significant.

Since all the leukemic rats of the group above showed extensive hemorrhages and prolonged clotting time, it seemed possible that the hemorrhagic diathesis was caused by a reduction in thromboplastin. Actually there is some evidence of thromboplastin deficiency, since the blood platelets are usually reduced in the terminal stage of the disease. In view of the notable infiltration of the liver and bone marrow by the tumor cells, it is surprising, however, that the animals should appear to have almost the normal amount of prothrombin. The prothrombin concentration might be considerably below normal and yet enough above a critical level to yield a relatively normal prothrombin time. A phenomenon of this type would be demonstrable by dilution of the plasma.

Experiment 2.—In the course of a year, an estimate was made of the prothrombin time of whole plasma and of plasma diluted with physiological saline from 86 rats with advanced leukemia. The animals were divided into two groups: the first included those without gross hemorrhages; and the second, animals with extensive infiltration of the liver and multiple hemorrhages. As a control, the same test was made on samples of plasma from 48 normal rats of the same strain, age, and average weight. Tests were also made on the plasma of 14 rats with large lymphosarcomas that had metastasized to regional lymph nodes. These tumors developed as a result of inoculation of leukemic cells subcutaneously in the groin, a method that produces local tumors without generalized leukemia, liver damage, or hemorrhagic tendency. The percentage of prothrombin was calculated as follows:

\[
\text{Normal animal prothrombin time} \times 100 \div \text{Test animal prothrombin time}
\]
TABLE 1: Results of the Quick Test for Prothrombin Applied to the Plasma of Normal and Leukemic Rats

<table>
<thead>
<tr>
<th>Source of Plasma</th>
<th>Number of tests</th>
<th>Average prothrombin in plasma, percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal rats</td>
<td>48</td>
<td>100.0 68.4 57.7</td>
</tr>
<tr>
<td>Rats with lymphosarcoma</td>
<td>14</td>
<td>92.0 63.4 59.0</td>
</tr>
<tr>
<td>Leukemic rats. No hemorrhage</td>
<td>37</td>
<td>83.8 42.6 24.7</td>
</tr>
<tr>
<td>Leukemic rats. Liver involvement. Extensive hemorrhage</td>
<td>49</td>
<td>76.4 29.2 18.5</td>
</tr>
</tbody>
</table>

The average figures for the four groups are presented in Table 1 and are shown graphically in Fig. 1.

The average prothrombin time for rats with liver damage and extensive hemorrhages does not include the figures for 10 animals whose plasma failed to clot under 180 seconds when diluted 1:1. The prothrombin time for the plasma of several of these animals ranged from 240 to 600 seconds.

SUMMARY

Whole plasma from leukemic rats with pronounced liver involvement and hemorrhagic tendency shows little difference in prothrombin time from normal plasma. A large deviation from normal is evident when the leukemic plasma is diluted 1:1 and 1:2 with saline and compared with normal plasma similarly diluted. The results indicate that a plasma prothrombin deficiency exists in a transmissible rat leukemia associated with extensive leukemic infiltration of the liver and spontaneous hemorrhages. The deficiency is not demonstrated in whole plasma by means of the Quick test, but is clearly apparent when the plasma is diluted.

The present observations are in accordance with those of Kark and Lozner (1), who found that dilution of human plasma may bring out evidences of prothrombin deficiency not demonstrable when tests are made on whole plasma.

REFERENCES

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