It has long been postulated that fibrinolysis plays an important role in hemorrhagic disorders. Although it is rather recent when the mechanism of fibrinolysis has been elucidated to some extent, there have been various papers to prove that plasminogen proactivator in blood converts into activator, which then transforms plasminogen to plasmin, and this plasmin acts on fibrin to cause fibrinolysis.

There seems to be little doubt that this serial sequence of fibrinolysis is closely related to hemorrhagic diatheses, although the exact relationship still remains unclarified. Therefore it seems to be of help to investigate the attitude of plasminogen proactivator in various hemorrhagic disorders in order to evaluate the importance of the role of this mechanism as a contributing factor in hemorrhagic disposition.

Since Takada et al. have been successful in fractionating plasminogen proactivators in human plasma by gel filtration through Sephadex G-200 in 1964, their method encouraged the research in this field as an important tool, and Yamamoto et al. already found some evidence of abnormal value of plasminogen proactivator in infantile eczema.

In this research we attempted to investigate the attitude of plasminogen proactivator in children with hemorrhagic disorders such as leukemia, aplastic anemia, together with the difference of its chromatographic pattern between normal and these pathological groups, and further to find their variation in terms of their ages.
Blood was obtained from 15 cases of hemorrhagic group and 18 cases of control group. The former consists of 11 cases of acute leukemia and two cases of aplastic anemia ranging 3 to 15 years and one each adult case of aplastic anemia and leukemia. The latter comprises 15 cases of non-hemorrhagic children such as cleft palate, benign obesity, dermatitis and bronchitis ranging 3 to 15 years, and three cases of healthy adults.

Double oxalated blood was centrifuged and plasma was separated and stored frozen at -15°C until ready to further analysis.

Gel filtration method was essentially the same as reported by Takada et al.7) The dimension of column was 2×50cm, and Sephadex G-200 manufactured by AB Pharmacia, Uppsala was used. 0.1 M tris-HCl buffer (pH 8.0) in 0.2 M NaCl was used as eluent. 0.1 ml of tris-buffer was added to 0.5 ml of thawed plasma, and 1.0 ml of this mixture was flowed through Sephadex G-200 column, then the column was eluted with above tris-buffer and this elute was collected in approximately 30 test tubes, each containing 3.5 ml using automatic fraction collector.

Proactivator: The clot lysis test5) was performed as follows. 0.9 ml of the effluent was mixed with 0.1 ml of 500 u/ml streptokinase (Varidase, Lederle Lab. A.C.C.), then it was incubated at 37°C for 10 minutes. The lysing system was prepared by mixing 0.2 ml of this activated solution with 0.4 ml of 1/15 M phosphate saline buffer solution (pH 7.4), 0.05 ml of 100 u/ml thrombin solution (Mochida Co., Tokyo) and 0.3 ml of 0.33% fibrinogen solution (bovine fraction I, Armor Lab.). The time required for the complete lysis of the formed clot was measured in minute at 37°C. The amount of proactivator was defined as follows: (the reciprocal of lysis time) × 10³ of this lysing system was called the proactivator units in 1 ml of the original solution, therefore total proactivator units can be expressed by multiplying with the volume of each effluent fraction.

Total proactivator units = (Proactivator in 1 ml) × (each effluent volume).

Plasminogen: Plasminogen value was determined by heated fibrin plate, which was made by heating standard fibrin plate3 at 85°C for 30 minutes. Standard fibrin plate was prepared by mixing 8 ml of 0.4% fibrinogen solution and 4 ml of 10 u/ml thrombin solution. To measure plasminogen, 0.03 ml of activated solution described above was placed on heated fibrin plate and dissolved areas were measured after incubation at 37°C for 18 hours.

Protein: Determination of protein in each tube was made by a modified Folin’s method.4)
RESULTS AND DISCUSSION

1) Total plasminogen proactivator unit.

The results on the values in 11 cases of hemorrhagic children and 2 adult cases are shown in Fig. 1, together with the values in 15 cases of nonhemorrhagic children and 3 cases of normal adults.

![Graph showing total plasminogen proactivator units in hemorrhagic group and control group.](image)

It can be mentioned that total units in the group with hemorrhagic disorders are generally higher than those in non-hemorrhagic cases.

As previously reported\(^2\)\(^6\)\(^9\) that, total values of plasminogen proactivator in normal newborn infant are considerably low, while they increase rapidly and reach to the lower limit of adult value at the age of about 6 month. Since our subjects in this report are children from 3 years up to 15 years and adult, no correlation between age and total units of plasminogen proactivator has been observed in both control group and the group with hemorrhagic disorders.

2) A and B fractions of plasminogen proactivator.

As Takada *et al.*\(^9\) previously reported, two peaks of plasminogen proactivator in normal infant were detected by this Sephadex gel filtration method. We name a peak which comes out first, plasminogen proactivator A, and its molecular weight is assumed to be more than 200,000. The second peak, we name plasminogen proactivator B, is assumed to be around 150,000 in molecular...
weight. We abbreviate plasminogen proactivator A and B as PPA and PPB respectively. These two peaks are demonstrated in the blood of normal infant except that of newborn period, especially up to around 7 day old and also except the blood in the umbilical cord. The latter exception has been proved in this research.

The most common pattern observed in normal human adult is remarkably different from this double peak type. In this type we observe only one peak (PPB) as shown in Fig. 2. In normal children beyond infantile period, we find

![Fig. 2 Normal pattern of plasminogen proactivator in human subject except of early infant.](image1)

![Fig. 3 Pattern of plasminogen proactivator in the case of acute myelocytic leukemia: 7 year old girl. Both PPA and PPB exist in this case.](image2)
only this single peak type.

Fig. 3 shows the case of acute myelocytic leukemia in a 7 year old girl, in which both PPA and PPB are clearly observed.

Fig. 4 shows the case of aplastic anemia of 4 year old girl, congenitaly

![Graph showing pattern of plasminogen proactivator in congenital aplastic anemia](image)

**Fig. 4** Pattern of plasminogen proactivator in the case of congenital aplastic anemia: 4 year old girl. PPA exceeds PPB in height.

![Graph showing plasminogen proactivator A and B in hemorrhagic group](image)

**Fig. 5** Plasminogen proactivator A and B in hemorrhagic group.

![Graph showing plasminogen proactivator A and B in hemorrhagic group](image)

**Fig. 6** Plasminogen proactivator A and B in hemorrhagic group.
missing radial bones on both sides, which is also termed Fanconi's type. This is the only case in our experiment in which the peak of PPA exceeds that of PPB in height. This is a remarkable difference from normal controls. It is also noted that the plasminogen always exists in PPB fraction but not in PPA fraction even in pathological group. Our results on 11 cases of acute leukemia and two cases of aplastic anemia, ranging 3 to 15 years, together with two cases of leukemia and aplastic anemia in adult, and 15 cases of control ranging 3 to 11 years are summarized in Fig. 5 and Fig. 6 respectively. In pathological group, PPA was readily demonstrable, ranging 27.7 to 422.1 with exceptions of two adult cases, #12, #15, having no PPA (Fig. 5). On the contrary, 11 out of 15 control cases did show no demonstrable PPA, in the remaining four cases, #2, 9, 13, 15, only low values of 92, 27, 28, 72 were observed respectively (Fig. 6).

3) PPA ratio \[\text{PPA}/(\text{PPA}+\text{PPB}) \times 100\] %.

Since the total amount of plasminogen proactivator increases as age increases up to 6 month, when this value reaches to that of adult, the ratio of PPA rather than its actual amount seems to be preferable to designate the characteristic feature in normal situation as schematically illustrated later (Fig. 7). Using this ratio, it can be clearly shown that the increase of PPA in infant period other than newborn period really remarkable. However this ratio de-
Table 1

Clinical findings and results of peripheral blood and bone marrow examination in the leukemia of children. Underlined cases were diagnosed as “Remission.” Bottom line indicates the criteria, which the diagnosis of remission were based on.

<table>
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<th>No.</th>
<th>Case</th>
<th>Peripheral blood</th>
<th>Bone marrow</th>
<th></th>
<th>Pyrexia</th>
<th>Hemorrhage</th>
<th>Splenomegaly</th>
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<td></td>
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<td>Hb g/dl</td>
<td>Granulocyte</td>
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<td>Blast cell + Lymphocyte</td>
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Criteria for Remission

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<th>Hb g/dl</th>
<th>Granulocyte</th>
<th>Platelet</th>
<th>Blast cell</th>
<th>Blast cell + Lymphocyte</th>
<th>Pyrexia</th>
<th>Hemorrhage</th>
<th>Splenomegaly</th>
<th>Hepatomegaly</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;11.0</td>
<td>&gt;1500</td>
<td>&gt;70.0</td>
<td>&lt;5</td>
<td>&lt;10</td>
<td>&lt;30</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

creases as age increases further until it becomes extremely small in adult.

PPA ratio in pathological group seems to show the similar tendency, although the actual ratio is generally much higher than that of the corresponding age group in normal subject. PPA ratios in rabbit and rat, are also shown in the same figure.

Table 1 shows the results of routine blood examination and physical findings in leukemia around or at the time of PPA measurements. As shown in this table, #1, 3, 9 and 10 were considered to be at the stage of remission in the course of leukemia, according to the criteria for remission based mainly on findings in peripheral blood, bone marrow, and clinical signs. It is rather obvious that PPA ratio in these four patients approaches to the normal at the time of remission.

Finally we summarized Takada's results and ours schematically basing on the average values and upper and lower limits of PPA ratio in several age groups (Fig. 8).
As one can see, human adults and children over one year, and animals seem to be the extreme on the opposite direction. It is of interest to point out that the patterns in newborn period blood and cord blood resemble to that of adult, while the cause for this similarity remains unexplained. PPA ratio in the infant around 6 months approaches closest to that of animals.

In hemorrhagic children, as illustrated with open rhombus their figures shift to the right side from normal position. This means that they tend to turn back to the pattern of the more younger ones in these pathological situations. In the stage of remission, they again shift back to the left and stay away from more primitive pattern.

We would rather think that this high PPA ratio in hemorrhagic diathesis is one of the sequence of pathological process, than to think that this high PPA ratio itself is exerting any unfavorable effect on the subject.

Anyhow it is interesting that the undifferentiatedness of children can be proved in this regard even in normal subjects, and this undifferentiatedness shows more immaturity when children have hemorrhagic tendency.

We have reported here the characteristic feature of proactivator pattern in pathological children, which we found while we were checking the activity of proactivators in these cases.
PLASMINOGEN PROACTIVATOR IN HEMORRHAGIC DISORDERS

SUMMARY

1) The total plasminogen proactivator units in 15 cases of hemorrhagic group showed higher value than those in 18 cases of control group.

2) The deviation of proactivator pattern in hemorrhagic children from the normal pattern has been observed chromatographically.

3) When PPA in pathological group is expressed as PPA ratio, this value approaches to the normal as age increases.

4) In the remission stage of leukemia these ratios approached to the normal.

From our results, the undifferentiatedness of children apparently can be demonstrated even in fibrinolytic system.

The authors wish to express heartly thanks to Prof. Shosuke Okamoto, Kobe University and Assia. Prof. Utako Okamoto, Keio University for their kind advice and constructive discussion, and we are grateful to Dr. Akikazu Takada and Yumiko Takada, Keio University for their cooperation.

REFERENCES