Porphyirin Concentration and Myeloperoxydase Activity in the Tissues of the Shay Chloroma of the Rat

By Gabriel Kelényi, Julius Pongrätz, Stephen Orban and Georg Deak

Observations on human chloroleukemic tissue have shown that it contains porphyrin, which accounts for its red fluorescence in Wood's light and an enzyme, myeloperoxydase (or verdoperoxydase), to which the green color of the chloroleukemic tissue is due.

In recent years many chloroleukemic tumors have been described in animals where the tumor tissue also contained porphyrin and myeloperoxydase. A detailed study of the porphyrin and myeloperoxydase of chloroleukemic tissues in the rat has been made by Schultz et al. and in the mouse by Loeb et al. Their investigations corroborate the data obtained from human chloroleukemic tissues, and it now seems obvious that the chloroleukemic tissues of man as well as of animals contain porphyrin and myeloperoxydase.

In a previous study made on the Shay chioroma of the rat we observed that by varying the route of administration (subcutaneous, intraperitoneal and intravenous) and the age group of the inoculated animal (newborn, infantile and adult) various types of chloroleukemic and leukemic processes could be induced, which showed differences in their growth properties, their fluorescence in Wood's light (porphyrin) and in their green color (myeloperoxydase). Based on these differences the following types may be distinguished:

1. Regressive solitary subcutaneous chloromas,
2. progressive solitary subcutaneous chloromas,
3. disseminated chloromas, and a
4. leukemic form.

In table 1 the particulars of transplantation (site of inoculation and age of animals) by which these forms can be obtained, as well as the most characteristic features of these forms, are given.

In spite of their great differences in appearance, we suggested that these various forms were only variations of the same disease developing as a result of changes in the environmental conditions of the transferred cells.

In the present study the porphyrin concentration and myeloperoxydase activity of the tissues of these various forms of chloromatous and leukemic processes were determined by quantitative methods. A correlation between porphyrin concentration and myeloperoxydase activity was found to exist, viz. in chloroleukemic tissues with a slow rate of growth, high porphyrin concentration and low myeloperoxydase activity were observed, while in fast growing tissues the reverse was true.

From the Department of Pathological Anatomy, Medical University of Pécs, Hungary.
Submitted Mar. 17, 1961; accepted for publication July 20, 1961.
Table 1.—Particulars of Transplantation and Features of the Different Forms of the Shay Chloroleukaemia of the Rat

<table>
<thead>
<tr>
<th></th>
<th>Regressive solitary choroma</th>
<th>Progressive solitary choroma</th>
<th>Disseminated choroma</th>
<th>Leukemic form</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age of animal at inoculation</strong></td>
<td>Adult</td>
<td>Newborn</td>
<td>Newborn or infantile</td>
<td>Infantile</td>
</tr>
<tr>
<td><strong>Site of injection</strong></td>
<td>Subcutaneous</td>
<td>Subcutaneous</td>
<td>Intraperitoneal</td>
<td>Intravenous</td>
</tr>
<tr>
<td><strong>Survival time</strong></td>
<td>30-140 days</td>
<td>3-6 weeks</td>
<td>12-21 days</td>
<td></td>
</tr>
<tr>
<td><strong>Colour of the infiltrations</strong></td>
<td>Brownish-green, green</td>
<td>Greyish-green, green</td>
<td>Grey-yellowish, green-yellowish (lymph glands, bone marrow)</td>
<td></td>
</tr>
<tr>
<td><strong>Fluorescence of the infiltrations</strong></td>
<td>Very intense</td>
<td>Intense</td>
<td>Poor, patchy or intense, patchy</td>
<td>Nil, or poor, patchy</td>
</tr>
</tbody>
</table>

*In about 50% of the animals inoculated in the newborn age, in addition to the progressive solitary subcutaneous choromas, dissemination of the choromatous process occurs.

**Materials and Methods**

Methods of transplantation of the Shay chloroleukemia of the rat have been described in detail previously.

Quantitative porphyrin determination: Known amounts, 1 to 20 Gm., of wet choromatous tissue were extracted with ethylacetate: acetic acid, 4:1, until they gave no red fluorescence in Wood's light. As a rule, three extractions were sufficient. The combined extracts were washed three times with 3 per cent sodium acetate solution. Then the ethylacetate containing the porphyrins was extracted with 1.5 N HCl solution, which was then neutralized, the porphyrins carried back to ethylacetate: acetic acid, 4:1, washed with 3 per cent sodium acetate and the porphyrins extracted with 1.5 N HCl. The extracts were allowed to stand at 4 C. overnight, filtered. A certain material which interferes with porphyrin fluorescence and is present in some of the extracts precipitates overnight and can be removed by filtration. The porphyrin concentration was determined by a Haveman-type fluorophotometer, using hematoporphyrin standards. No attempt was made to separate the various porphyrins. The amount of porphyrins present in the tissues was given as µg./100 Gm. wet tissue.

Because of the small amount of tissue available, determination of the porphyrin concentration in the leukemic forms was hardly possible. In those cases only the bone marrow, liver, spleen and occasionally the lymph glands were infiltrated with leukemic cells and no tumorous infiltrations whatever occurred. To obtain comparable data the infiltrated lymph glands, livers and spleens of the leukemic animals were extracted, and the values compared with those of normal animals. In a few cases the infiltrated bone marrows were pooled and extracted in the same way as described above.

Determination of myeloperoxidase activity: Myeloperoxidase activity was determined on tissue homogenates in 0.05 M tris-/hydroxymethyl/-aminomethane buffer at pH 7.0. The homogenates were prepared with a motor-driven all-glass Potter-type homogenizer. The determination of myeloperoxidase activity was performed by the method of Straus, but instead of a phosphate buffer at pH 6.6, a tris-/hydroxymethyl/-aminomethane buffer at pH 7.0 was used throughout the experiments. The modification, i.e. the use of tris-/hydroxymethyl/-aminomethane buffer instead of phosphate buffer, has the advantage that it extracts more myeloperoxidase (Loeb et al.) and, according to our observations, does not alter the reproducibility of the method. The determinations were carried out on 1 to 6 mg. of wet tissue.

In the method of Straus, dimethylparaphenylene-diamine is applied as hydrogen donor, and H₂O₂ as hydrogen acceptor. Activities are expressed as quinone-dimmonium red units/60 seconds/100 mg. wet tissue. The presence of oxidizing agents as well as the autooxidation of dimethylparaphenylenediamine were taken into account. The oxidation of dimethyl-
paraphenylenediamine by oxidizing agents was determined without the addition of H₂O₂ to the incubating medium. The autoxidation of dimethylparaphenylenediamine was determined in the presence of H₂O₂ but without tissue homogenate. Auto-oxidation was determined once in a series and the presence of oxidizing agents in each individual material. The values obtained for auto-oxidation and oxidizing agents were subtracted from those of myeloperoxydase activity.

According to our observations specimens taken from different parts of the solid subcutaneous tumors showed different degrees of activity. Therefore, in the case of such tumors, activity was always determined in three to four specimens. The values given represent means of those determinations.

Results

Porphyrin concentration was determined in 14 regressive, in 29 progressive solitary subcutaneous and in 12 disseminated chloromas. In the case of intravenously injected animals (leukemic form), the leukemic bone marrows were pooled from two groups of animals, consisting of 8 and 13 rats, respectively. Livers, spleens and a few infiltrated lymph glands were also extracted.

The activity of myeloperoxydase was determined in 12 regressive, in 23 progressive solitary subcutaneous, in 15 disseminated chloromas, and in 17 bone marrows, in 7 spleens, livers and lymph glands of animals with the leukemic form of the disease. Myeloperoxydase activities of only those organs which showed leukemic infiltrations microscopically were included. The leukemic bone marrows whose myeloperoxydase activity was determined consisted of about 98 to 100 per cent of leukemic cells and only in 0 to 2 per cent of other cells, mainly normoblasts.

In table 2 the results of the porphyrin and myeloperoxydase determinations are given. Between the various forms of chloromatous and leukemic processes there are appreciable differences in porphyrin concentration and myeloperoxydase activity, and it seems that these differences are related to the rate of growth of the chloromatous and leukemic tissue. In the slow-growing subcutaneous chloromas high porphyrin concentration and low myeloperoxydase activity were observed, whereas in the fast-growing leukemic tissue the activity of myeloperoxydase was high, and porphyrin was present only in traces. Expressed another way, in the tissues with higher myeloperoxydase activity, the porphyrin concentration was lower, while in those with lower myeloperoxydase activity it was higher.

Discussion

If the findings of Schultz et al.⁷ are compared with ours it may be said that the porphyrin values are of the same order of magnitude. The values of myeloperoxydase activity can not be compared, since Shultz et al. and Loeb et al.³ used methods different from ours. However, it should be mentioned that Loeb et al. examined myeloperoxydase activity in regressive chloromas and found, as we did, values lower than in tumors growing progressively.

The interpretation of the reverse relationship of myeloperoxydase activity and porphyrin concentration is not easy. It is not clear whether the free porphyrin in the chloroleukemic tissue represents a material not utilized for the heme groups of myeloperoxydase or one not related to the synthesis of myeloperoxydase. The recent findings of Vannotti et al.¹² may support the former
Table 2.—Porphyrin and Myeloperoxidase Values of Chloromatous and Leukemic Tissues

<table>
<thead>
<tr>
<th>Porphyrin (µg./100 Gm. wet tissue)</th>
<th>Myeloperoxidase activity (units/100 mg. wet tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>No. tumors examined</strong></td>
<td><strong>Mean ± standard error (range)</strong></td>
</tr>
<tr>
<td>(1) Regressive solitary subcutaneous chloromas</td>
<td>14</td>
</tr>
<tr>
<td>(2) Progressive solitary subcutaneous chloromas</td>
<td>29</td>
</tr>
<tr>
<td>(3) Disseminated chloromas</td>
<td>12</td>
</tr>
<tr>
<td>(4) Leukemic form Bone marrow, liver and spleen (pooled from 8 and 13 rats)</td>
<td>17</td>
</tr>
<tr>
<td>— Lymph gland (pooled from 8 and 13 rats)</td>
<td>7</td>
</tr>
<tr>
<td>—</td>
<td>7</td>
</tr>
<tr>
<td>—</td>
<td>7</td>
</tr>
</tbody>
</table>

*Myeloperoxidase activity of normal adult (150-250 Gm. body weight) rat bone marrow: 188 units/100 mg. wet tissue and of normal infantile (20-50 Gm. body weight) rat bone marrow: 76 units/100 mg. wet tissue.

†Normal rat livers and lymph glands show no measurable myeloperoxidase activity.
‡Myeloperoxidase activity of normal (infantile or adult) rat spleen: 7.9 units/100 mg. wet tissue.

hypothesis. These authors observed that mature granulocytes of the rat were unable to synthesize porphyrin from the precursor material, δ-aminolevulinic acid. And since these cells of the rat do not exhibit significant myeloperoxidase activity according to histochemical observations, it may be suggested that the lack of ability of porphyrin biosynthesis is responsible for the lack of myeloperoxidase activity within these cells.

According to Schultz and Schwartz, it may be that in the chloromatous tissue free porphyrin is built in the enzyme myeloperoxidase. If it is assumed that the porphyrins in the immature myeloid cells of chloroleukemic tissues are units necessary for building up myeloperoxidase, our findings can be interpreted in the following way: the cells of slowly growing chloroma (regressive or progressive) with a low myeloperoxidase activity do not utilize the whole amount of synthesized porphyrins, whereas all porphyrin is utilized by the fast growing leukemic tissues.

However, there are also objections to this hypothesis. (1) It is not quite sure that free porphyrins in their oxidized state can be utilized for myeloperoxidase synthesis. It may be that for the building up of heme molecules, porphyrin can be utilized only in the reduced state (porphyrinogen). Furthermore, there is no evidence that in the living cells there are compounds with a reducing power strong enough to turn porphyrins into porphyrinogens.
It is well known that myeloperoxidase is of green color.\textsuperscript{2,7-9} It is difficult to understand why the tissues with a vivid green color, such as subcutaneous chloromas, have lower activity of myeloperoxidase than the leukemic cells of the bone marrow and especially the cells of the normal rat bone marrow which is not green at all. If green color indicates tissue of high myeloperoxidase activity, one would expect subcutaneous chloromas to exhibit the highest activity and the leukemic bone marrows, with a greyish-yellow color, the lowest. However, the reverse is true.

Thus, at the present state of affairs, it is not possible to determine the functional significance of the reverse proportion of porphyrins and myeloperoxidase in chloroleukemic tissues. There is, however, little evidence on the functional role of porphyrins and myeloperoxidase in the chloromatous tissues. It might be also possible that bone marrow and chloroma peroxidase are of different nature.

Finally it is worth mentioning that according to Schultz et al.\textsuperscript{10} the porphyrins and myeloperoxidase in chloroleukemic tissues are in a bound state and cannot be separated from each other by repeated chromatography, electrophoresis on paper, dialysis or repeated precipitation with ammonium sulphate. It is possible that in this state the enzyme is not fully active as it may be inhibited by the porphyrin. This hypothesis, however, requires experimental confirmation.

**Summary**

Previous investigations have shown that when cells of the Shay chloroleukemia of the rat are inoculated into animals of different age subcutaneously, intraperitoneally or intravenously, various forms of chloromatous and leukemic processes develop.

In the present studies, the porphyrin concentration and the myeloperoxidase activity of these various forms were studied by quantitative methods.

It was found that the porphyrin concentration and the myeloperoxidase activity of the tissues show an inverse relationship; i.e., in tissues with high myeloperoxidase activity, porphyrin concentration was low and in those with low myeloperoxidase activity, the porphyrin concentration was high.

Possible causes of the reverse relationship of the two components of the chloroleukemic tissues are discussed.

**Summario in Interlingua**

Previe investigationes ha monstrate que quando cellulas de chloroleucemia Shay del ratto es incoluate subcutaneae-, intraperitoneae-, o intravenoemente in animales de differente etates, varie formas de processo chloromatose e leucemic se disveloppa.

In le hic-reportate studios, le concentration de porphyrina e le activitate de myeloperoxydase in ille varie formas esseva studiate per methodos quantitative.

Esseva trovate que le concentration de porphyrina e le activitate de myeloperoxydase del tissus es in relation inverse, i.e., in tissus con alte activitate de myeloperoxydase le concentration de porphyrina esseva basse, e in tissus con basse activitate de myeloperoxydase le concentration de porphyrina esseva alte.
Es discutite le possibile causas de iste inverse relation inter le duo componentes del tissus chloroleucemic.

REFERENCES


Gabriel Kelényi, M.D., Assistant Professor in Pathology, Department of Pathological Anatomy, Medical University, Pécs, Hungary.

Julius Pongrätz, M.D., Research Assistant, Department of Pathological Anatomy, Medical University, Pécs, Hungary.

Stephen Orbán, M.D., Research Assistant, Department of Pathological Anatomy, Medical University, Pécs, Hungary.

Georg Deák, Senior Medical Student, Department of Pathological Anatomy, Medical University, Pécs, Hungary.
Porphyrin Concentration and Myeloperoxidase Activity in the Tissues of the Shay Chloroma of the Rat

GABRIEL KELÉNYI, JULIUS PONGRATZ, STEPHEN ORBAN and GEORG DEAK