A Clockface Chromatin Pattern in the Intermediate Megaloblast of Vitamin B\textsubscript{12} or Folate Deficiency

By LAWRENCE KASS

Recognition of the fully-developed megaloblast in deficiency of vitamin B\textsubscript{12} or folic acid is not difficult. In the case of the "intermediate megaloblast" or "megaloblastoid" cell, however, identification of megaloblastic changes often becomes more difficult, and more subjective.\textsuperscript{1} The present study attempts to reduce this subjectivity by demonstrating a unique clockface chromatin pattern along the nuclear membrane of fully-developed megaloblasts from patients with a deficiency of vitamin B\textsubscript{12}, folate, or both. A similar clockface chromatin pattern appeared in intermediate megaloblasts from patients with subsequently confirmed deficiencies of vitamin B\textsubscript{12} or folate. In this way, a "clockface sign" in an intermediate megaloblast might provide a morphologic clue to a deficiency of vitamin B\textsubscript{12} or folate.

\textbf{METHODS}

Sternal or iliac crest marrow was obtained by needle aspiration from twelve patients with suspected deficiencies of vitamin B\textsubscript{12} or folate. Simultaneous determinations of serum vitamin B\textsubscript{12} and folate were performed on each patient by Dr. J. Hines, Cleveland Metropolitan General Hospital. Control marrow samples were taken from three patients (J.W., C.N., A.T.) with nonhematologic conditions (arteriosclerosis, cerebellar degeneration, and traumatic hematuria) and normal hemoglobin. Control samples were also obtained from patients with refractory megaloblastic anemia (3), anemia due to uncomplicated iron deficiency (6), sideroachrestic anemia (4), chronic granulocytic leukemia (4), myelofibrosis (5), chronic lymphocytic leukemia (5), and erythroblastosis fetalis (2).

The specimens were stained with Wright's or tetrachrome stain and photographed with a Zeiss Planapo 100 \times 1.3 oil immersion lens, and a Kodak 99 Wratten green filter. This filter, which admits wave lengths of light from 520 m\textmu to 620 m\textmu, accentuates the appearance of nuclear chromatin and connections between chromatin strands.

\textbf{RESULTS}

In all cases of deficiency of vitamin B\textsubscript{12}, folate, or both (Table 1), the findings were identical. Fully-developed megaloblasts appeared as classically described.\textsuperscript{2,4} Chromatin strands were widely-separated and linked to neighboring strands by delicate threads. Especially prominent were chromatin particles which appeared to adhere to the interior of the nuclear membrane. The circumferential arrangement of these particles resembled minute markings on
Table 1.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Sex</th>
<th>Hemoglobin (Gm./100 ml.)</th>
<th>Serum Folate</th>
<th>Serum B12</th>
</tr>
</thead>
<tbody>
<tr>
<td>O.S.</td>
<td>27</td>
<td>M</td>
<td>6.0</td>
<td>0.1</td>
<td>470</td>
</tr>
<tr>
<td>W.N.</td>
<td>42</td>
<td>M</td>
<td>5.3</td>
<td>0.2</td>
<td>585</td>
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<tr>
<td>L.B.</td>
<td>39</td>
<td>F</td>
<td>2.0</td>
<td>0.2</td>
<td>830</td>
</tr>
<tr>
<td>J.T.</td>
<td>46</td>
<td>F</td>
<td>12.7</td>
<td>2.6</td>
<td>815</td>
</tr>
<tr>
<td>J.W.</td>
<td>34</td>
<td>F</td>
<td>4.05</td>
<td>0.4</td>
<td>100</td>
</tr>
<tr>
<td>I.R.</td>
<td>75</td>
<td>M</td>
<td>6.7</td>
<td>1.6</td>
<td>30</td>
</tr>
<tr>
<td>W.O'L.</td>
<td>49</td>
<td>M</td>
<td>10.45</td>
<td>2.8</td>
<td>105</td>
</tr>
<tr>
<td>L.B.</td>
<td>67</td>
<td>M</td>
<td>3.7</td>
<td>11.2</td>
<td>10</td>
</tr>
<tr>
<td>A.C.</td>
<td>80</td>
<td>F</td>
<td>8.02</td>
<td>29.0</td>
<td>15</td>
</tr>
<tr>
<td>B.T.</td>
<td>68</td>
<td>F</td>
<td>9.05</td>
<td>14.2</td>
<td>25</td>
</tr>
<tr>
<td>L.F.</td>
<td>43</td>
<td>F</td>
<td>5.4</td>
<td>7.2</td>
<td>50</td>
</tr>
<tr>
<td>A.B.</td>
<td>70</td>
<td>M</td>
<td>7.45</td>
<td>9.4</td>
<td>100</td>
</tr>
<tr>
<td>J.W. (normal)</td>
<td>31</td>
<td>M</td>
<td>15.6</td>
<td>8.8</td>
<td>945</td>
</tr>
<tr>
<td>C.N. (normal)</td>
<td>47</td>
<td>M</td>
<td>14.05</td>
<td>4.0</td>
<td>610</td>
</tr>
<tr>
<td>A.T. (normal)</td>
<td>78</td>
<td>M</td>
<td>13.0</td>
<td>6.6</td>
<td>430</td>
</tr>
</tbody>
</table>

Normal values

Serum folate (L. casei): 4-18 nanograms/ml.

the face of a clock. These small chromatin masses often appeared like round “hillocks,” with convex surfaces toward the center of the nucleus (Fig. 1). Particles of chromatin attached to narrow stalks or “pedicles” also appeared frequently (Fig. 1). Both the “hillock” and “pedicle” configurations were usually not connected to other chromatin. When they were joined to other strands, it was often by a thin filament of chromatin. These changes were observed in 50–70 per cent of 500 red cell precursors examined in each marrow. The clockface chromatin pattern appeared most prominent in basophilic megaloblasts and polychromatophilic megaloblasts. A clockface chromatin pattern did not appear in giant stab forms or metamyelocytes when these were present. Chromatin of both the earliest and latest megaloblast precursors did not appear significantly different from the corresponding normoblast precursors.

Bone marrows from two patients (B.T. and J.T.) contained mostly intermediate megaloblasts. These cells appeared as described by others. They were smaller than fully-developed megaloblasts and their chromatin strands appeared to be less widely-separated. In both cases, 30–50 per cent of the intermediate megaloblasts exhibited clockface chromatin patterns (Figs. 2, 3). Results of serum vitamin B12 and folate assays subsequently confirmed a deficiency in each instance (Table 1).

Developing erythroblasts from three patients with refractory megaloblastic anemia (Fig. 4) showed widely-separated chromatin strands and increased nuclear size. In general, these cells appeared very similar to the fully-developed megaloblasts of vitamin B12 or folate deficiency. However, only 2–3 per cent of erythroblasts from patients with refractory megaloblastic anemia exhibited a clockface chromatin pattern.
In three patients without anemia and with normal serum vitamin B₁₂ and folate levels (Table 1), the chromatin of normal-appearing red cell precursors from basophilic erythroblast to polychromatophilic normoblast was arranged in large clumps around the interior of the nuclear membrane (Fig. 5). Each clump appeared to radiate toward the center of the nucleus, and was almost always joined to neighboring clumps of chromatin by thick, rope-like strands. Isolated, unconnected chromatin clumps or delicately-connected chromatin clumps were infrequent. Red cell precursors from six patients with anemia due to uncomplicated iron deficiency (Fig. 6) were often smaller than red

Fig. 5.—Patient J.W. (normal) In this basophilic erythroblast, the chromatin adjacent to the nuclear membrane is arranged in large radial clumps. They are connected to each other by heavy strands of chromatin. (× 1800)

Fig. 6.—Uncomplicated iron deficiency anemia. The chromatin pattern of this basophilic erythroblast does not appear significantly different from the normal in Figure 5. (× 1800)
Fig. 7.—Sideroachrestic anemia, basophilic erythroblast. Several vacuoles are present in the cytoplasm. (x 1800)

Fig. 8.—Chronic granulocytic leukemia, basophilic erythroblast. (x 1800)

Fig. 9.—Myelofibrosis, basophilic erythroblast. (x 1800)

cell precursors from normal patients, but the chromatin patterns of both cell types were indistinguishable. Developing erythroblasts from patients with sideroachrestic anemia (Fig. 7), chronic granulocytic leukemia (Fig. 8), myelofibrosis (Fig. 9), chronic lymphocytic leukemia, and erythroblastosis fetalis did not differ significantly in appearance from the normal.

Discussion

It is unlikely that the “clockface sign” is an artifact of fixation and staining, since photomicrographs of supravitally-stained living megaloblasts viewed under the phase contrast microscope exhibit clockface chromatin patterns. Normal living erythroblasts observed under identical conditions lack the “clockface sign.” At present it is not possible to say whether the “clockface sign” antedates hypersegmentation of neutrophils, since hypersegmentation was seen in all patients studied, regardless of the degree of their anemia or their serum vitamin B₁₂ or folate levels. Attempts to correlate the initial appearance of the “clockface sign” with specific serum levels of vitamin B₁₂ and folate are currently in progress.

Marrow from patients with deficiency of vitamin B₁₂ or folate contained a preponderance of megaloblasts exhibiting clockface chromatin patterns. Although many red cell precursors from patients with refractory megaloblastic anemias appeared strikingly similar to these classic megaloblasts, only 2–3 per cent of them showed clockface chromatin patterns. In contrast, 30–50 per cent of “intermediate megaloblasts” from subsequently documented cases of deficiency of vitamin B₁₂ or folate did exhibit clockface chromatin patterns. These observations suggest that when 30 per cent or more megaloblasts exhibit a clockface chromatin pattern, one may more strongly suspect a deficiency of vitamin B₁₂ or folate rather than one of the other varieties of anemia in which “megaloblastoid” cells can sometimes be found.

The pathogenesis of the “clockface sign” is unknown. However, certain observations in normal cells suggest, by analogy, that both the “clockface sign” and the chromosomal abnormalities seen in deficiency of vitamin B₁₂ or fo-
late\textsuperscript{9}-\textsuperscript{11} might reflect an abnormality of megaloblast histone. Under the electron microscope, preparations of nucleohistones appear as a delicate meshwork of anastomosing fibrils.\textsuperscript{12} Isolated DNA strands do not show such interconnections and aggregations unless histone is added.\textsuperscript{13} Chicken erythrocyte histone contains a characteristic band in starch gel electrophoresis while lacking a component present in spleen and liver histone, suggesting that erythrocyte histone may be unique.\textsuperscript{14} Also, calf thymus lymphocyte nuclei depleted of lysine-rich histone lose the clumped appearance of their chromatin. The resulting loose chromatin can be reconverted to dense chromatin clumps by adding lysine-rich histone.\textsuperscript{15}

By analogy, one might postulate that an abnormal histone in the megaloblast of vitamin B\textsubscript{12} or folate deficiency could cause its chromatin to appear loose or widely-separated. Faulty histone crosslinkages between DNA strands could encourage the formation of isolated "hillocks" of chromatin adherent to the nuclear membrane in a clockface pattern. These clumps of chromatin may fail to unite with chromosomes, disappear during mitosis along with the nuclear membrane, and cause distorted, broken or missing chromosomes\textsuperscript{9}-\textsuperscript{11} to appear.

**SUMMARY**

In the fully-developed megaloblast of vitamin B\textsubscript{12} or folate deficiency, unique alterations occur in the chromatin adherent to the nuclear membrane. This chromatin is often tenuously connected to or separated from other chromatin, and gives the nucleus a clockface appearance. A clockface chromatin pattern appears only rarely in "megaloblastoid" erythroblasts from cases of refractory megaloblastic anemia. Normal erythroblasts and developing erythroblasts from a variety of anemias do not exhibit this chromatin pattern. Although the pathogenesis of the "clockface sign" is unknown, alterations in megaloblast histone might cause both the clockface chromatin pattern and subsequent chromosomal abnormalities. When the "clockface sign" appears in an intermediate megaloblast, it may provide a morphological clue to a deficiency of vitamin B\textsubscript{12} or folate.

**SUMMARIO IN INTERLINGUA**

In le plenemente disveloppate megaloblasto ab subjectos con carentia de vitamina B\textsubscript{12} o de folato, alterationes de character unic occurre in le chromatina adherente al membrana nucleari. Iste chromatina es frequentemente separate o non plus que laxemente connectite con altere chromatina e produce in le nucleo le apparentia de un disco a cifras de horologio. Iste configuration appareva solo rarmente in erythroblastos "megaloblastoide" ab casos de refractori anemia megaloblastic. Erythroblastos normal e erythroblastos in stato de disveloppamento ab un variatate de anemias non exhibeva iste configuration del chromatina. Ben que le pathogenese del "signo del horologio" non es cognoscite, alterationes in le histona megaloblastic es possibilemente su causa e etiam le causa de subsequente anormalitates chromosomal. Quando le "signo del horologio" apare in un megaloblasto intermedie, illos representa forsan un constatation morphologic que pote suggerer le diagnose de carentia de vitamina B\textsubscript{12} o de folato.

**ACKNOWLEDGMENTS**

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REFERENCES


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