Chromatin Clumping in Mature Leukocytes: A Hitherto Unrecognized Abnormality

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The relation of nuclear structural defects to abnormal proliferation of hematopoietic cells is not known. To date, the infrequent clinical occurrence of these disorders has not permitted acquisition of sufficient data to make any positive statements. In some cases, nuclear structural abnormalities have been observed in otherwise asymptomatic individuals. Nuclear anomalies, as described by Pelger and identified by Huet as an autosomal dominant trait, have been seen in apparently normal people. Nevertheless, the occurrence of similar bilobed nuclei in leukocytes of certain patients with leukemia brings up the unanswered question of whether Pelger-Huet changes may actually predispose to leukemia, or instead, may be the result of leukemic transformation. Cells with Pelger-Huet like nuclear abnormalities have made a temporary appearance in various disorders, including myxedema, influenza, and sulfasoxazole administration.

In 1964, Krill, Smith and Mauer, and Zuelzer independently described granulocytopenic syndromes in which the lobes of the nuclei appeared pyknotic and were connected by long, thin strands. Using DFP, Krill et al. described shortened granulocyte survival. Impaired granulocyte release from the marrow was observed which was temporarily overcome by infection. Zuelzer showed increased numbers of nonviable leukocytes in the bone marrow of his patients, decreased phagocytic activity of leukocytes in the peripheral blood, and inferior response to local irritants by the Rebuck skin window test. He showed that the impaired release of granulocytes could be temporarily overcome by the injection of bacterial endotoxin.

We have recently had the opportunity to study in two patients the production, structure and function of leukocytes with a bizarre nuclear anomaly.
characterized by clumping of chromatin, producing the appearance of nuclear fragmentation. There was also loss of segmentation which resembled somewhat the “homozygous” type of Pegler-Huet anomaly. The terminal event in one patient was characteristic for leukemic transformation. Both patients showed distinct differences from both the hereditary and acquired Pelger-Huet syndromes, as well as the other white cell abnormalities previously described.

MATERIALS AND METHODS

Clinical Data

Patient 1, E.O., was a 62-year-old Caucasian businessman who developed persistent pain in the right shoulder in 1961. This was treated with pain killers and traction, without improvement. A routine blood count at this time showed mild anemia (3.0 M. HBC/cmm), and leukopenia (3700 WBC/cmm). This count was done at another hospital and the slides were not available. However, the comment was made in the report that mature neutrophils had an “abnormal appearance.” He remained asymptomatic until June 1967, when the anemia became more severe and he began requiring frequent transfusions. At that time, a definite pancytopenia was present. His only symptoms were weakness and fatigue, and a distressing right shoulder pain. There was no weight loss. There was no history of exposure to toxins or drugs with the exception of insecticide sprays. Apparently, no irradiation was given for the shoulder pain. The only significant physical findings were fever, 99-100°F and hepatosplenomegaly. There was no lymphadenopathy.

A blood count in June 1967, showed Hgb 8.0 Gm., RBC 2.05 × 10⁶ per cubic millimeter, Hct 23.5 per cent, WBC 3050/cmm, platelets 66,000/cmm, and reticulocyte count 11 per cent. Differential count showed segmented forms 48 per cent, lymphocytes 51 per cent, and monocytes 1 per cent. There was essentially no change in the blood counts over the period of June 1967 to June 1968 except for recurrent anemia which necessitated transfusions. The reticulocyte counts dropped to below 1.0 per cent and stayed there.

Figure 1 is a photomicrograph of mature granulocytes taken from a Wright’s stained peripheral smear. The loss of segmentation and extensive clumping of chromatin gives a fragmented appearance to the cell nuclei. The bone marrow showed marked hypercellularity with a myeloid erythroid ratio of 4:1. In Figure 2, the “fragmentation” of nuclear chromatin can be seen in late normoblasts of the marrow as well as the more mature neutrophils. The abnormality was also seen in the mature eosinophils and basophils. Myeloblasts, progranulocytes, myelocytes, lymphocytes, plasma cells and megakaryocytes all had normal-appearing nuclei. The leukocyte alkaline phosphatase score was zero. There was positive staining of all granulocytes.

Peripheral blood smears from one brother, three children and five grandchildren were completely normal.

Other laboratory data included: negative direct Coombs’ antiglobulin test; ESR of 24 mm./hr. (Wintrobe); negative test for antinuclear factor; absence of hemoglobinuria or hemosiderinuria; serum haptoglobin, 56 mg.; serum bilirubin, 0.2 mg. direct and 0.7 total; serum iron, 172 µg. per cent, total iron binding capacity, 318 µg. per cent; serum uric acid, 5.9 mg. per cent; and, fecal urobilinogen, 121.4 mg./day.

Clinical Course

The patient was given blood transfusions in order to maintain his Hgb in the 8-9 Gm. range. By Spring 1968, he required two units of whole blood every two to three weeks, and had almost continuing symptoms of weakness and fatigue. Ferrokinetic study showed delayed plasma clearance of ⁵⁸Fe (T1/2=137 minutes) and a marked decrease in reappearance of ⁵⁸Fe in RBC’s (peak activity, 35% in 7 days). A RBC survival study done with the patient’s cells tagged with ⁵¹Cr showed a survival time of 19 days (normal range, 28 to 39 days). Differential counting over organ sites showed splenic sequestration of the labeled RBC’s with a spleen:precordial ratio of 2.1:1.0.
Fig. 1.—Patient 1 (E.O.). Representative mature neutrophils from peripheral blood, showing apparent nuclear "fragmentation." × 1600. Wright's stain.

In an effort to lessen the severity of his anemia and decrease his transfusion requirements, a splenectomy was performed on June 7, 1968. The spleen weighed 723 Gm. and showed marked myeloid metaplasia. Biopsy of liver and abdominal lymph nodes also showed hematopoietic foci. Table 1 shows the response of his hemogram in the postoperative period. There was an initial leukocytosis to 21,350/cmm with outpouring of mature cells, all with abnormal nuclei. In the next few days, he developed high fever (103°–104° F) with x-ray and clinical evidence of left subdiaphragmatic abscess and superimposed pneumonia. During this period, the leukocyte count continued to rise to a high of 52,000 and then dropped as he made a clinical response to antibiotic therapy and abscess drainage. At no time was there an increase in the number of immature neutrophils. The differential pattern was one of a leukemoid reaction, rather than a leukemic transformation or blast
Fig. 2.—Patient 1 (E.O.). Representative bone marrow fields showing apparent fragmentation of nuclei in mature neutrophils and normoblasts. Note absence of significant fragmentation in myelocytes. × 1000. Wright-Giemsa stain.

crisis. The platelet count remained low. Anemia was a continuing problem. There was no reticulocytosis and frequent transfusions were necessary to maintain his hematocrit.

On July 1, 1968, he was discharged. He subsequently developed recurrence of high fever with severe nausea, vomiting and rapid weight loss. On July 17, 1968, at the time of readmission, his Hgb was 9.6, Hct 28, Retics. 0.1 per cent, WBC 11,670 with 87 per cent neutrophils, 13 per cent lymphocytes and no immature cells. The platelet count was 18,000. The marrow showed a shift in the M:E ratio to 8:1 with no increase in per cent blasts or decrease in cellularity. The hemogram remained unchanged until 24 hours prior to death, except for decrease in hematocrit due to bleeding.
CIIROMATIN CLUMPING IN MATURE LEUKOCYTES

Table 1.—Postoperative Blood Counts on Patient 1

<table>
<thead>
<tr>
<th>Clinical Events</th>
<th>Admission</th>
<th>Splenectomy</th>
<th>Abscess Tapped</th>
<th>Discharge</th>
<th>Readmission</th>
</tr>
</thead>
<tbody>
<tr>
<td>Date</td>
<td>6/5/68</td>
<td>6/6/68</td>
<td>6/14/68</td>
<td>6/24/68</td>
<td>7/1/68</td>
</tr>
<tr>
<td>Hgb.</td>
<td>8.5</td>
<td>9.6</td>
<td>8.9</td>
<td>10.0</td>
<td>11.0</td>
</tr>
<tr>
<td>Hct.</td>
<td>25</td>
<td>28</td>
<td>27</td>
<td>29</td>
<td>33</td>
</tr>
<tr>
<td>Retics.</td>
<td>0.6</td>
<td>—</td>
<td>1.8</td>
<td>0.7</td>
<td>0.6</td>
</tr>
<tr>
<td>WBC</td>
<td>5580</td>
<td>21,350</td>
<td>52,000</td>
<td>12,000</td>
<td>6720</td>
</tr>
<tr>
<td>Neutro.</td>
<td>57</td>
<td>—</td>
<td>94</td>
<td>95</td>
<td>83</td>
</tr>
<tr>
<td>L</td>
<td>20</td>
<td>—</td>
<td>4</td>
<td>3</td>
<td>14</td>
</tr>
<tr>
<td>Metamyelo.</td>
<td>7</td>
<td>—</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Myelo.</td>
<td>7</td>
<td>—</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Progran.</td>
<td>2</td>
<td>—</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Blasts.</td>
<td>6</td>
<td>—</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Platelets</td>
<td>40,000</td>
<td>33,000</td>
<td>55,000</td>
<td>20,000</td>
<td>28,000</td>
</tr>
</tbody>
</table>

No new site of infection could be found. He showed no response to reintroduction of antibiotic therapy. Abdominal distention appeared with hematemesis and guaiac positive stools. On August 5, 1968, he developed gross hematuria and melana, and began requiring constant nasogastric suction. Prednisone therapy was begun at 60 mg./day with no effect. The platelet count remained low (4000 to 10,000) and bleeding continued. There was a terminal rise in white count to a high of 86,000 on the day he expired. The differential count showed 95 per cent mature neutrophils all with the nuclear abnormality. There was no increase in immature forms.

Postmortem examination showed diffuse mucosal hemorrhage in the stomach, small intestine, colon and urinary tract. Submucosal bleeding had produced an infarction of a segment of small intestine. There were also bilateral subdural hematomas, a left periaortic hematoma and hemorrhage into the prostate and kidneys. There was surgical absence of the spleen with a L subphrenic hematoma. The kidneys revealed acute tubular necrosis and acute pyelonephritis. There was a resolving empyema of the left pleural cavity and mycotic ulcers in the esophagus and stomach. The liver and lymph nodes showed no infiltration of marrow elements at the time of death.

Patient 2, a 73-year-old woman, was seen in May 1968 because of weakness, easy fatigability, and exertional dyspnea for two or three months. Her appetite was poor, and she lost six pounds during the month. There was no history of prior hematologic abnormality. On physical examination she exhibited pallor and weighed 90 pounds. The thyroid gland was enlarged and multinodular. There was a grade 3/6 systolic ejection murmur at the aortic area. There was no lymphadenopathy, hepatosplenomegaly, or purpura.

Laboratory evaluation revealed the following data: Hgb 8.0 Gm. per cent, Hct 24 per cent, RBC 2.79/mm.3, MCV 86 μ3, MCH 29μgm., MCHC 33 per cent. Reticulocyte count was 0.8 per cent, WBC count 6310/mm.3, stabs 7 per cent, segs 50 per cent, lymphs 21 per cent, monos 22 per cent, platelets 67,000. Serum iron was 106 μg. per cent, iron binding capacity 294 μg. per cent, direct Coombs' antiglobulin test negative, serum hemoglobin 8.84 mg. per cent, and total serum bilirubin 0.7 mg. per cent. Routine urinalysis 15–25 WBC/s/HPF, and many bacteria seen in the sediment.

Protein bound iodine was 11.5 and 12.9 μg. per cent. Twenty-four-hour radioactive iodine uptake by the thyroid was 45 per cent (normal 10–35%). Activity of the isotope was localized to the right lobe of the thyroid gland. Because of hyperthyroidism due to a toxic nodular goiter, on May 17, 1968, she was given 20 mCi of 131I. Subsequent tests of thyroid function in the next eight months were normal.

On May 23, 1968, bone marrow aspiration was performed. The marrow was hypercellular and megakaryocytes were decreased. There was an increase in early granulocytic forms with a myeloid erythroid ratio of 6:1. Clumping of the nuclear chromatin was noted in many band and segmented neutrophils in the peripheral blood and marrow (Figs. 3 and 4). The marrow showed granulocytic hyperplasia with clumping of chromatin in mature
Fig. 3.—(Upper) Patient 2 (L.H.). Peripheral blood neutrophil showing apparent nuclear fragmentation. Early myelocyte within nuclear anomaly. × 1600. (Lower) Patient 2 (L.H.). Peripheral blood during blast crisis. Most cells are blast, but in center of field is mature neutrophil with nuclear fragmentation. × 1000. Wright's stain.

Granulocytes; early white and red cell precursors showed a normal nuclear appearance.

On May 27, 1968, a ferrokinetic study showed that plasma clearance of $^{59}$Fe, expressed as T½, was 110 minutes and RBC utilization was 75 per cent at day 7.

In June 1968, she received three units of red cell mass and her hematocrit remained around 30 per cent for the next three months. In December, her hemoglobin was 8.2 Gm./100 ml., hematocrit 20 per cent, retics. 4.6 per cent, WBC count 3800/cmm, stabs 4 per cent, segs 42 per cent, lymphs 44 per cent, monos 10 per cent, and platelets 56,000/cmm.
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Fig. 4.—Patient 2 (L.H.). Bone marrow during blast transformation. Note nuclear clumping in mature neutrophils and normoblasts. × 1000. Wright-Giemsa stain.

No new medications were given. Her clinical and laboratory status remained unchanged until June 1969, when she developed pleuritis and bronchitis, which improved following treatment with tetracycline and an expectorant. Her hematocrit remained around 22 per cent, her WBC count ranged from 3300 to 11,700, and her platelet count remained low. On several occasions early granulocytic forms, including a rare blast, were seen in the peripheral smear. Clumped nuclear chromatin continued to be seen in the more mature granulocytes.

She was readmitted to the hospital in July 1969 for persistent lethargy and pleuritic chest pain. Physical exam revealed pallor, scattered small purpuric spots over the extremities, and rales over both lung bases. The liver and spleen were not palpable. A single 2-cm. lymph node was palpable in the left axilla. She weighed 106 pounds.

Laboratory data: Hemoglobin 9.3 Gm./100 ml., hematocrit 29, RBC count 2.86 M/cmm, reticulocyte count 6.6 per cent, WBC count 12,700/cmm, stabs 17 per cent, segs 17, lymphs 32, monos 31, metamyelocytes 1, myelocytes 2, with occasional abnormal nuclei, platelets 75,800/cmm, serum haptoglobin 101 mg. per cent hemoglobin-bound (normal 40–150), Coombs' test weakly positive, sugar water test negative, and leucocyte alkaline phosphatase score 0. Bone marrow examination revealed hypercellularity with markedly decreased megakaryocytes. Granulocytic hyperplasia was noted with the differential count showing 13 per cent myeloblasts and 15 per cent progranulocytes. Many stabs and segmented neutrophils showed a plump single or double lobed nucleus with clumping of the nuclear chromatin. Similar clumping was seen in late erythrocytic precursors. Plasma cells and eosinophils were reduced. Stainable iron was decreased.

A 51chromium RBC survival study revealed a T1/2 of 13 days (normal 25–35 days) with a spleen to precordium ratio of 2.25 to 1. A 59Fe ferrokinetic study showed rapid plasma clearance with T1/2 of 31 minutes and a maximum RBC utilization of 70 per cent at 7 days. She was discharged from the hospital after having been transfused.

Her condition was unchanged until November 1969 when she noted increased weakness, nausea, vomiting and painful areas of indurated reddened skin over both posterior thighs. Her WBC count rose to 57,000 with 45 per cent atypical monocytes, 3 per cent metamyelocytes, 7 per cent myelocytes, and approximately 25 per cent blast forms. Her hematocrit fell to 19 per cent and she developed anterior chest pain and breathlessness. Bone marrow
examination revealed a markedly hypercellular marrow with no megakaryocytes. The predominant cell was a very immature cell which appeared to be a blast with reticulum cell characteristics. Erythroid precursors were scarce. Clumping and smudging of the nuclear chromatin was seen in many cells. She was hospitalized again and received two units of red cell mass with amelioration of her chest pain. Additional laboratory studies: leukocyte alkaline phosphatase score, 5 units (control 201 units). Treatment with Prednisone 60 mg. daily, Allopurinol 100 mg. three times daily, and Vincristine, 1 mg. intravenously per week was begun.

Her WBC count continued to rise, reaching a peak of 460,000. 6-mercaptopurine, 150 mg. daily, was added to the regimen and shortly before her death, her WBC count was 240,000.

Her condition worsened with increasing weakness, dyspnea, cough, fever, anorexia and nausea. She gradually lost consciousness and expired on November 26, 1969.

Postmortem examination revealed acute fibrinous pericarditis, bilateral pneumonia, and widespread leukemic infiltrates in kidneys, liver and lungs.

Response of Peripheral Blood Lymphocytes to Phytohemagglutinin (PHA)

Suspensions of $6 \times 10^6$ white cells obtained from the peripheral blood were incubated at $37\,^\circ C$ in stationary culture for 72 hours with 0.2 ml. phytohemagglutinin (PHA), 0.2 mg. penicillin, streptomycin solution, 7.5 ml. TC medium 199, and 2.5 ml. autologous plasma. The per cent blast transformation of lymphocytes and accumulation of mitotic figures was determined two hours after addition of 0.2 ml. of colchicine to a 70-hour preparation. The number of mitotic figures/1000 nuclei and per cent blast transformation was then determined by differential counting of 4000 cells following treatment with hypotonic solutions and stained with Wright-Giemsa or Orcein stains.

Chromosome Studies

Squash preparation for chromosomal study were prepared in our laboratory from peripheral blood at the time of our mitotic index studies. Karyotyping was done by Dr. Patau at the University of Wisconsin in Madison, Wisconsin.

Chromosome preparations were made in two ways: two hours after the addition of colchicine to freshly aspirated marrow; and after a 24-hour period of in vitro incubation with colchicine added in the final two hours.

DNA and RNA Synthesis in Marrow Culture

In vitro incorporation of tritiated substrates into bone marrow was studied by the method previously published by Pisciotta and Kaldahl. Fresh heparinized marrow was incubated at $37\,^\circ C$ in a rotating action water bath over a five-hour period with 5 mc. each of tritiated thymidine ($^3$H-TdR), deoxyuridine-6 ($^3$H-UdR-6), and uridine ($^3$H-UR-5) (specific activity of each: 1.5 c/mMole). Smears were prepared from the buffy coat at hourly intervals. The smears were allowed to remain in contact with film emulsion in the dark for seven days, then developed, fixed and stained. The per cent labeling of granulocyte precursors and normoblasts was determined by differential counting.

Motility Studies

Sealed cover glass preparations were made from freshly drawn peripheral blood and were examined with the Leitz-Heinecke phase contrast microscope. No stimulating substance was added to the preparations.

Mitotic Index

The mitotic index was determined in regular marrow smears by counting the number of mitotic figures in 1000 consecutive granulocyte precursors. A mitotic figure was assigned to the granulocytic series if specific granules were seen in the cytoplasm.
Electron Microscopy

Bone marrow and peripheral blood specimens were heparinized and immediately centrifuged (4°C) for five minutes at 2000 rpm. Small fragments of buffy coat were fixed in 1 per cent osmium tetroxide buffered with 0.1 M sodium phosphate buffer at pH 7.4. Specimens were dehydrated in graded alcohols and embedded in Maraglas. Thin sections were cut with diamond knives and collected on uncoated copper grids. They were stained with concentrated aqueous uranyl acetate and lead citrate, and examined with RCA EMU-3F electron microscope.

Phagocytic Index

To evaluate phagocytic activity, the method of Hunt was used with *Staphylococcus aureus* as the organism rather than *Brucella*. Five ml. of blood from our patient and a normal control were added to 0.2 cc. of 20 per cent sodium citrate each. One cubic centimeter of citrated blood was mixed with 1 cc. of *Staphylococcus aureus* saline suspension equivalent in turbidity to 500 parts per million of kaolin in water and incubated for thirty minutes at 37°C. Direct smears were then made from the sedimented cells from each specimen and stained with Wright’s stain. The number of bacteria phagocytized per 100 neutrophils was counted and the results reported in number of bacteria/cell.

Response to Local Irritants

The skin window technique of Rebuck was used to determine whether an exudative response could be elicited. A 5-mm.² area of skin was denuded and intermediate strength PPD applied. Cover slip preparations of the exudate were examined at intervals up to 24 hours.

RESULTS

PHA Studies

The peripheral blood lymphocytes from Patient 1 proved to be responsive to PHA. In 72 hours, blast transformation was found to be 55 per cent and 32 mitotic figures per 1000 nuclei accumulated 24 hours after the addition of colchicine. Both values agreed with the normal range obtained in our laboratory for his age group.

![Graph](image)

Fig. 5.—Incorporation of ³H-Thymidine into granulocyte precursors of two patients with nuclear fragmentation.
Chromosome Studies

The chromosome preparations made from peripheral lymphocytes from both patients showed a normal karyotype. The marrow preparations that underwent in vitro incubation for 24 hours showed no mitotic figures. Marrow preparations made two hours after it was aspirated showed a few rudimentary mitotic figures; none were suitable for karyotyping.

DNA and RNA Synthesis in Marrow Cultures

The pattern of incorporation of tritiated nucleotides into marrow cells was similar in both patients. Figure 5 shows in vitro incorporation of $^3$H-TdR into granulocyte precursors. Labeling index of granulocyte precursors and normoblasts was comparable to normals in the first hour of incubation. However, the labeling index for the remaining four hours did not increase as it did in the normals. Similar results were obtained using $^3$H-deoxyribouridine-6; avid incorporation occurred in the first hour, but there was no increase in labeling in-
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Table 3.—Bacterial Killing Effect of Leukocytes from Patient 2

<table>
<thead>
<tr>
<th>W.B.C.</th>
<th>Opsonin</th>
<th>% Bacterial Survival/120 Minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient</td>
<td>Control</td>
<td>2.9</td>
</tr>
<tr>
<td>Patient</td>
<td>Patient</td>
<td>1.1</td>
</tr>
<tr>
<td>Control</td>
<td>Control</td>
<td>3.2</td>
</tr>
<tr>
<td>Control</td>
<td>Patient</td>
<td>9.2</td>
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<tr>
<td>No Cells</td>
<td>Control</td>
<td>123.1</td>
</tr>
<tr>
<td>No Cells</td>
<td>Patient</td>
<td>87.4</td>
</tr>
</tbody>
</table>

dex with further time in culture. Figure 6 illustrates that at each time point, incorporation of $^3$H-Uridine-5 into granulocyte precursors was slightly less than that of normals in both patients. Incorporation of $^3$H-TdR, UdR-6 and UR-5 into normoblasts was similar to that obtained with granulocyte precursors.

**Mitotic Index (Table 2)**

The mitotic index was determined in regular marrow smears. In Patient 1 (E.O.), three mitoses were found per 1000 consecutive granulocyte precursors. In Patient 2 (L.H.), two mitoses were found in two marrows during the time preceding leukemic transformation. When the marrow had become completely transformed to blasts, the mitotic index in L.H. was 4/1000. Mitotic indices in normal subjects ranged between 4 and 10 mitoses. It was apparent that each patient showed mitotic activity less than normals.

**Motility Studies**

Both patients’ mature neutrophils showed granular movement and ameboid motility comparable to normal controls. The nuclear abnormality could be readily recognized with phase contrast.

**Phagocytic Index**

In a single determination, on Patient 1, leukocytes phagocytized nine bacteria/cell as compared to 14.6 bacteria/cell in the control. Although this value was less than that of the normal, it was evident that phagocytic property was present. No studies of bacterial killing were done on Patient 1.

Bacterial killing studies were performed on Patient 2 by Dr. Gilbert Thatcher. Data in Table 3 show that leukocytes and serum obtained from the patient had bacterial killing properties similar to that of normals.

**Response to Local Irritants**

In a normal control, cellular exudate composed of almost entirely mature neutrophils appeared within the first hour. By 12 hours, about half of the cells were neutrophils, and by eighteen hours, monocytes and lymphocytes predominated. On two occasions early in our study, each of the two patients could not produce cellular exudate at the site of trauma. During the leukocytosis in the immediate post-splenectomy period in Patient 1, the Reubuck skin window was repeated. The exudate remained acellular for eighteen hours with a small number of degenerating polymorphonuclear leukocytes appearing between 18 and 24 hours.
Electron Microscopy

**Myelocytes** (Fig. 7). Myelocytes were recognized by the development of specific cytoplasmic granules, namely neutrophilic, eosinophilic, or basophilic. Numerous free ribosomes and profiles of endoplasmic reticulum, predominantly the rough surfaced form, appeared throughout the cytoplasm. Mitochondria were few. The nucleus was round and showed slight chromatin condensation along the nuclear membrane, not unlike that seen in normals. These electron dense areas are referred to as heterochromatin. The remaining, lightly stained nuclear mass is known as euchromatin. Heterochromatin was distributed as small blocks predominantly along the periphery between numerous strands of euchromatin which extended to the nuclear membrane, opposite the nuclear pores. These pores were not observed in opposition to heterochromatin. The centrosome commonly observed in the perinuclear region contained a pair of centrioles which were arranged with their long axis perpendicular to each other. The centrioles were surrounded by well developed Golgi complex. The latter consisted of flattened cisternae, appearing in parallel array, and numerous small vesicles. In some instances the cisternae were dilated and contained ill-defined aggregates of electron dense material. In close relation to the Golgi cisternae, there were discrete vesicles, each with a central core of various density. These structures were considered to be neutrophilic granules in different stages of development.

**Metamyelocytes and Band Cells.** These two forms are arbitrarily staged by the development of nuclear indentation without specific cytoplasmic changes. Metamyelocytes showed distinct nuclear indentation, which continued to progress in more mature forms (Figs. 8 and 9). At the same time, the chromatin condensation became more apparent and nucleoli were not seen. Nuclear pores were fewer than in myelocytes. Of particular interest was the distribution of heterochromatin in band cells. It appeared as well delineated, electron dense areas separated from each other by intervening euchromatin, thus giving the appearance of “nuclear fragmentation.”

Cytoplasmic changes were characterized by an increase of neutrophilic granules and other cytoplasmic organelles, such as endoplasmic reticulum, mitochondria, and free ribosomes. Golgi complex was not as prominent as in myelocytes. Cytoplasmic glycogen, not observed in earlier forms, was demonstrated in band cells. It appeared in the form of discrete particles throughout cytoplasm.

**Polymorphonuclear Neutrophil.** This cell differed from younger forms by its smaller size, more condensed cytoplasm and specifically by multilobulated nucleus (Fig. 10). The nuclear lobes were connected by long narrow filaments which consisted of heterochromatin surrounded by nuclear membranes (Fig. 11). The “nuclear fragmentation” observed in band cells was even more conspicuous; blocks of heterochromatin occupied large areas of nucleoplasm, and only narrow strands of euchromatin were present. This chromatin pattern differed from that of control cells which showed a much smaller quantity of heterochromatin (Fig. 12). Concentration of cytoplasmic glycogen was elevated; however, the number of other cytoplasmic organelles, except for granules, was
Fig. 7.—Patient 1 (E.O.). Myelocyte. Cytoplasm contains azurophilic (ag) and neutrophilic (ng) granules; Golgi complex (GC); nucleus (N). × 24,000.
Fig. 8.—Patient 1 (E.O.). Metamyelocytes. Cytoplasm contains also azurophilic (ag) and neutrophilic (ng) granules. Nucleus (N) is indented; Golgi complex (GC) with centriole (arrow). × 24,000.
Fig. 9.—Patient 1 (E.O.). Band cell. Composition of cytoplasmic organelles is almost identical with that of metamyelocytes. Blocks of heterochromatin (BH) appear at periphery, producing “nuclear segmentation.” × 30,000.
Fig. 10.—Patient 1 (E.O.). Neutrophilic granulocyte. Both nuclear lobes show large blocks of heterochromatin (BH). No specific cytoplasmic changes are observed. × 21,000.

decreased. Golgi complex was small and the formation of new granules was not evident. Electron microscopy of mature neutrophils from Patient 2 showed a similar abnormality (Fig. 13).

**Discussion**

Several features suggest the possibility of “atypical” chronic granulocytic leukemia in our patients. Immature myeloid cells persisted in the peripheral blood throughout the course, even in the absence of infection or toxic state. The bone marrows were consistently hypercellular with myeloid elements predominating (M:E ratio of 4:1 initially, later rising to 8:1). There was myeloid metaplasia of the spleen, liver and lymph nodes at the time of splenectomy.
Neutrophil alkaline phosphatase staining was absent. We were unable to
demonstrate a Philadelphia (Ph') chromosome in our patients' marrows, be-
cause we were not successful in developing a satisfactory chromosome prepara-
tion in vitro.

The nuclear abnormality seen in the mature granulocytes is different from
those previously described in the literature, despite a resemblance to the Pelger
anomaly. The clumping and loss of segmentation is more striking than gen-
erally seen in the Pelger cells and the defect is present not only in neutrophils
but also late normoblasts, basophils and eosinophils. The apparent fragmenta-
tion of nuclear chromatin, as visualized by conventional microscopy may be
comparable to the appearance of a collection of pomegranate seeds.

Electron microscopy confirmed that morphological changes in nuclear chro-
matin occur during the later stages of cell differentiation. In immature cells, the
nuclear matrix consists mainly of euchromatin; only small aggregates of
heterochromatin are visible along the nuclear membrane. During successive
stages of maturation, however, the ratio of these two forms of chromatin are
reversed. It is considered that cells richly endowed with euchromatin are
metabolically more active than those possessing large masses of hetero-
chromatin.14

Electron microscopy of myeloid series revealed normal maturation process
of granules and other cytoplasmic organelles, as it has been described previ-
ously.12-14 However, the appearance of chromatin patterns differed. Band
Fig. 12.—Normal segmented neutrophilic granulocyte. Heterochromatin (H) is condensed along the nuclear membrane, in contrast to Fig. 10, where it occupies large areas of nuclear matrix. × 12,000.
Fig. 13.—Patient 2 (L.H.). Band cell. Nucleus shows large masses of heterochromatin, most of which appears at periphery. × 21,000.
cells and mature granulocytes showed changes which were referred to as "nuclear fragmentation." The intact nuclear membrane demonstrated that the nucleus was not fragmented as suggested by conventional microscopy. Instead, it appears that these nuclear changes were related to increased amounts of heterochromatin.

Further data confirmed that abnormal bone marrow function resulted in decreased delivery of erythrocytes to peripheral blood. The delayed clearance of $^{59}$Fe from the blood and diminished incorporation into erythrocytes in Patient 1 indicates a defect in erythropoiesis. While the low white count is probably on the same basis, our data do not permit us to explain the mechanism of leukopenia fully. It seems more likely that diminished white cell production was taking place, instead of accelerated peripheral destruction of white cells. Radioautographic studies in vitro indicated a proliferative defect in development. Avid DNA synthesis was indicated at the beginning of marrow culture, as manifested by labeling index well within normal limits. Despite this, there was no increase in labeling index as cultures proceeded in both patients. The reason for this is not entirely clear, but a number of factors must be considered in interpreting the significance of increasing labeling index in vitro. As new cells go into "S" phase in vitro they incorporate $^{3}H$-TdR. In some cases, granulocytes may have incorporated a quantity of $^{3}H$-TdR too minute to permit radioautographic recognition. As these cells continue to incorporate $^{3}H$-TdR during incubation, they may take up sufficient isotope to exceed the threshold to permit development of a radioautograph, and a further increase in labeling index. A significant factor that contributes to the increment in labeling index is cell division; as cells divide, they distribute radioactive material between each of the daughter cells with increases in labeling index.

It would appear from our data that our patients' precursor cells were actively engaged in DNA synthesis, but could not divide because of a defect localized somewhere between the G-2 and M phase of the cell cycle. Our data, while not conclusive, are consistent with the following speculative concept. As the cells did not divide, they may have continued to synthesize and accumulate DNA; then they proceeded to differentiate. This resulted in the development of a mature series of cells with more than the usual quantity of DNA per cell, resulting in the strange clumped appearance of nuclear chromatin.

The similar defect observed in both normoblast and granulocyte populations suggests the presence of a common stem cell. A similar precedent for this idea occurs in those cases of chronic granulocytic leukemia in which the Philadelphia chromosome is present in both erythrocytic and granulocytic precursors. As in CGL, the defective here is apparently limited to granulocyte and erythrocyte precursors; peripheral lymphocytes had a normal appearing nucleus, responded normally to stimulation with PHA, developed normal mitotic activity and 'blast transformation, and showed a normal complement of chromosomes.

Some evidence also showed that the product of these strange marrow cells was fundamentally abnormal. The erythrocytes, labeled with $^{51}$Cr, had a
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diminished life span but the marrow was unable to keep pace with accelerated destruction.

Impairment of function in peripheral leukocytes was shown by the extremely poor response to trauma in the Reubuck skin window and the slightly decreased phagocytic capacity, but bacterial killing by leukocytes of Patient 2 was well within normal limits. Although Patient 1 produced good leukocytosis in response to stress, he was unable to cope with infection and subsequently developed a post-operative abscess which led to his demise. The development of terminal blast crisis in Patient 2 suggests that this nuclear anomaly may be a preleukemic lesion.

SUMMARY

A 62-year-old man and a 73-year-old woman were found to have a nuclear abnormality characterized by exaggerated clumping of the chromatin in the mature granulocytic and erythroid cells. The mature granulocytes showed loss of normal segmentation. The abnormality was not noted in other family members and appeared to be related to an atypical leukemic process. The nuclear abnormality was shown to be associated with a defect in cellular production within the marrow. Granulocyte precursors and normoblasts incorporated \(^{3}\)H-TdR but did not divide. Electron microscopy suggested the distribution of more than normal quantities of heterochromatin to mature leukocytes. Ferrokinetic studies disclosed ineffective erythropoiesis. The sharing of this abnormality by the myeloid and erythroid cells suggests a common stem cell origin.

A functional defect of phagocytosis and cellular aggregation was demonstrated in the neutrophils, but bacterial killing was not affected. The nuclear anomaly described herein may be a pre-leukemic abnormality.

Since this paper was submitted for publication, we have treated two more patients with an identical nuclear defect; an 82-year-old woman and a 79-year-old man. The woman succumbed of acute leukemic transformation, while the man is still alive.

REFERENCES


Chromatin Clumping in Mature Leukocytes: A Hitherto Unrecognized Abnormality

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