Chronic Granulocytopenia in Childhood

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Among several chronic forms of granulocytopenia of obscure origin is an apparent entity observed chiefly if not exclusively in infants and young children. Although described some time ago by European investigators, notably Gasser, chronic benign granulocytopenia has received little or no attention in the American literature. Although apparently rare, the condition (hereafter abbreviated as C.G.) is of theoretical interest and, on the practical side, deserves recognition since it must be distinguished from granulocytopenic states of more serious import. The eminent chronicity and the rather benign course in the face of a depression of the circulating granulocyte count as profound as that of classical malignant agranulocytosis obviously reflect the special character of the disturbance. This report describes five cases and includes observations designed to explore the mechanism of the granulocytopenia and to relate the pathophysiology to the clinical course.

Case Material

The five cases were selected from a series of 42 infants and children studied in this laboratory over a period of 12 years who exhibited severe neutropenia due to causes other than leukemia, lymphoma, aplastic anemia, "hypersplenism" or any of the so-called collagen diseases. One other case of C.G. is not included here because of inadequate follow-up. Except for a classical example of cyclic neutropenia, the remaining cases were classified as "secondary" because they were attributed to known albeit diverse causes such as the administration of sulfonamides, chloramphenicol, ingestion of fuel oil, viral infections and, in one instance, overwhelming staphylococcal septicemia. Their salient features will be summarized below in order to illustrate the differences between the acute secondary granulocytopenias and C.G.

Clinical Features

The five patients with C.G. were infants of Caucasian ancestry ranging in age from 6 to 15 months at the time of diagnosis (table 1). The past history merged with that of the present illness in that there was a high incidence of infections dating in one instance as far back as the neonatal period but not recognized as being related to the granulocytopenic state until a more serious episode or the frequency of recurrences finally led to its discovery. In no case was the history suggestive of antecedent viral infections or allergies. One patient had earlier been found to be sensitive to penicillin which he had received in addition to sulfonamides and chloramphenicol several months prior to
### Table 1.—Summary of Data in Five Cases of Infantile Chronic Benign Granulocytopenia

<table>
<thead>
<tr>
<th>Case</th>
<th>Sex</th>
<th>Race</th>
<th>Age at Time of Diagnosis (mo.)</th>
<th>History Prior to Diagnosis</th>
<th>Clinical Course</th>
<th>Total Leukocytes/mm.(^2) Range</th>
<th>Total Leukocytes/mm.(^2) Average</th>
<th>Absolute PMN/mm.(^2) Range</th>
<th>Absolute PMN/mm.(^2) Average</th>
<th>Monocytes %</th>
<th>Duration (mo.)</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>W</td>
<td>15</td>
<td>history of impetigo, mouth ulcer, and abscess of vulva of 1 month duration</td>
<td>episodes of severe diarrheas, impetigo, paronychia, and upper respiratory infection in interval, doing well</td>
<td>4000–10,000</td>
<td>5000</td>
<td>100–2000</td>
<td>1000</td>
<td>1–10</td>
<td>42</td>
<td>recovered</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>W</td>
<td>6 1/2</td>
<td>abscess of right buttock of 1 week duration</td>
<td>episodes of abscesses and one episode of septicemia frequent ear infection</td>
<td>2500–8000</td>
<td>4000</td>
<td>0–400</td>
<td>250</td>
<td>6–31</td>
<td>38</td>
<td>recovered</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>W</td>
<td>6</td>
<td>frequent respiratory infection since birth</td>
<td>frequent “colds”</td>
<td>3000–8000</td>
<td>4000</td>
<td>0–500</td>
<td>200</td>
<td>2–13</td>
<td>27</td>
<td>recovered</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>W</td>
<td>10</td>
<td>series of “colds,” and mouth ulcers, of 3 months duration treated with penicillin, sulphanamides, and chloramphenicol</td>
<td>episodes of paronychia, cellulitis, mouth ulcers, and tonsillitis several attacks of otitis and lower respiratory infection</td>
<td>3000–8000</td>
<td>5000</td>
<td>100–2000</td>
<td>300</td>
<td>10–24</td>
<td>38</td>
<td>under observation</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>W</td>
<td>12</td>
<td>recurrent paronychia, and one episode of gingivitis</td>
<td>paronychia, and fever of unknown origin</td>
<td>2100–3000</td>
<td>2500</td>
<td>40–600</td>
<td>260</td>
<td>5–10</td>
<td>9</td>
<td>under observation</td>
</tr>
</tbody>
</table>
admission. Since the granulocytopenia subsequently persisted for almost 5 years without further exposure, drug sensitivity was not thought to have played any part in its etiology.

The family history was always non-contributory, and blood counts obtained in three instances on members of the immediate family were found to be normal.

Complaints and findings on admission were related to rather trivial infections of bacterial etiology, such as paronychiae, impetigo, gingivitis, ulcerations about the genitalia, and subcutaneous abscesses in various parts of the body. The respiratory tract was frequently involved and in one case was the sole site of infection. Hepatosplenomegaly was not present initially, but in Case 4 a moderate transient splenic enlargement was noted after 4 years. At times, slight regional lymphadenopathy was found in connection with localized inflammatory processes. Roentgenograms of the thorax obtained during respiratory illnesses showed only peribronchial and hilar infiltrations which disappeared as the episodes subsided. Intravenous pyelograms made in two cases showed no abnormalities.

The clinical course was characterized by continuing susceptibility to infections which generally remained localized and could be controlled by antibiotic therapy and suitable local measures. Only in one patient did generalized septicemia develop during the period of observation. One patient (Case 4) underwent laparotomy for recurrent intussusception of the large bowel during the 4th year, and later, because of fever and urinary frequency, was subjected to a cystoscopy which disclosed a low-grade cystitis and slight stenosis of the upper urethra. He tolerated both procedures well and made an uneventful recovery. In general the children appeared healthy during the intervals between infections, the profound granulocytopenia notwithstanding. The duration of the "free intervals" varied from a few weeks to many months. The response to treatment of infections was always satisfactory, but recurrences continued as long as the granulocytopenia persisted. Thus far, three of the patients have recovered, apparently spontaneously and with gradual return of a normal blood picture, after periods ranging from 27 to 38 months after diagnosis. Case 4 is still active 5 years after onset, and Case 5 has been under observation for 12 months thus far.

LABORATORY FINDINGS

Blood picture: The salient feature in every case was the severe depression of the circulating granulocyte count (Table 1). The total leukocyte counts were also as a rule depressed, but occasionally normal or even slightly elevated. At such times absolute lymphocytosis was present. Monocytosis, both absolute and relative, was the rule. The hemoglobin levels and the red cell and platelet counts were generally within the normal range.

The percentage count of granulocytes ranged from 0 to a maximum of 20. The absolute counts extended through a range from 50 to 1000 per cu. mm. and only rarely and for short periods of time exceeded 1500. Such granulocytes as were present were nearly all band forms. Fully developed polymorphonu-
Table 2.—Summary of Bone Marrow Findings

<table>
<thead>
<tr>
<th>Case</th>
<th>Total Cellularity of Bone Marrow</th>
<th>Myeloid Elements†</th>
<th>Erythroid Series</th>
<th>Lymphocytes</th>
<th>Myeloid/Erythroid Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Myeloblasts</td>
<td>Metamyelocytes, and band forms.</td>
<td>Erythrocytes</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Somewhat increased</td>
<td>4</td>
<td>17</td>
<td>17</td>
<td>33</td>
</tr>
<tr>
<td>2†</td>
<td>Normocellular</td>
<td>3</td>
<td>5</td>
<td>5</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>&quot;Diluted specimen&quot;</td>
<td>2.5</td>
<td>8</td>
<td>20</td>
<td>12.5</td>
</tr>
<tr>
<td>2</td>
<td>Normocellular</td>
<td>2</td>
<td>6</td>
<td>22</td>
<td>22</td>
</tr>
<tr>
<td>3</td>
<td>Slightly increased</td>
<td>2</td>
<td>7</td>
<td>19</td>
<td>21</td>
</tr>
<tr>
<td>4†</td>
<td>Moderately increased</td>
<td>2</td>
<td>9</td>
<td>10</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>Slightly increased</td>
<td>2</td>
<td>7</td>
<td>20</td>
<td>35</td>
</tr>
<tr>
<td>5</td>
<td>Moderately increased</td>
<td>3</td>
<td>5</td>
<td>3.5</td>
<td>15</td>
</tr>
</tbody>
</table>

*Five hundred cells counted.
†Eosinophils in all states comprised less than 5 per cent except in Case 4 which was 10 per cent of total nucleated cells.
‡Representative specimens obtained at different times in the course of the disease.

clear leukocytes were virtually absent. Occasionally, acute increases in the granulocyte count were observed in the presence of deep-seated abscesses or at the time of surgical trauma, but even then the number of these cells remained well below the range considered normal for the age of the patients, and at no time did the response approach that expected in normal persons under similar circumstances. Moreover, not polymorphonuclear leukocytes but band forms, metamyelocytes and occasionally myelocytes accounted for these transient increases. A good illustration was provided by the findings in Case 4 at the time of cystoscopy. Immediately before, the total white blood count was 3400 with 6 per cent granulocytes. The next day the patient was febrile and the total count increased to 6200 with 88 per cent granulocytes of which all but 12 per cent were immature forms. By the next day the counts had returned to the baseline level.

Bone marrow: Bone marrow studies were performed at frequent although variable intervals in every case (table 2). Since the findings remained remarkably constant over long periods of time, only representative data are shown.

The over-all cellularity was normal or moderately increased. A variable degree of lymphocytosis was present and tended to persist even beyond infancy. The mass of erythroid precursors was assumed to be normal since the patients were able to maintain normal red blood counts and hemoglobin levels without undue reticulocytosis. Consequently, the relatively normal M:E ratios indicate that the total mass of granulocytic elements was likewise within normal limits.
The striking feature of every bone marrow was the virtual absence of fully mature, segmented neutrophilic leukocytes which normally account for approximately one-third of the granulocyte population, constituting the so-called storage pool. By contrast, the nearly mature cells, metamyelocytes and band forms were always plentiful (fig. 1) and in the latter the anlagen of future segments were frequently recognizable as short spurs. Earlier precursors were likewise abundant. The net effect of the deficit in polymorphonuclear leukocytes was thus a general "shift to the left" with a corresponding increase in the total number of immature cells but without major changes in the profile of the granulocytic cell renewal system. At times a further moderate "left shift" resulted from a relatively greater increase in the percentage of promyelocytes and myelocytes, constituting the "proliferative pool" of cells capable of mitotic division, in contrast to the "maturation pool" made up of the metamyelocytes and band forms. At other times the increase was greater in the latter categories so that a relative "right shift" was superimposed on the general increase in the number of immature granulocytes.

These relationships could be expressed as ratios between the size of the proliferative pool and that of the maturation pool. Allowing for wide variations, the average number of cells in each of the two pools normally approximates 20 per cent of the total marrow population, and the ratio approaches unity. In the present series, ratios ranging from 0.65 to 1.30 could be calculated from the data in table 2. In two cases the ratio was normal, indicating equal expansion of the two pools. In about half of the specimens the ratio was decreased, indicating a relative "right shift" due to expansion of the maturation pool, whereas in two cases ratios above unity reflected the secondary "left shift" due to a greater increase in the size of the proliferative pool.
Similar variations were noted in the relationship between the cytologic compartments within the proliferative pool. At times a further, tertiary "left shift" was evident in the relatively greater increase in the percentage of promyelocytes as compared to that of the myelocytes, whereas at other times the reverse was true. These secondary and tertiary trends, however, were inconstant and insignificant in comparison with the main features, to wit, severe depletion of the leukocyte storage pool accompanied by variable but generally moderate hyperplasia of the entire granulocytic cell renewal system including the compartments constituting the maturation pool. Thus, despite the extreme peripheral granulocytopenia, the bone marrow was evidently producing granulocytes at an increased rate and in an orderly fashion, and maturation was proceeding normally, at least through the stages represented by metamyelocytes and band forms.

Special Observations

Bone marrow mitoses: Mitotic indices were determined in three cases by counting all recognizable mitoses seen while enumerating 1000 cells in Wright-Giemsa preparations. This method is said to yield lower values than those obtained in Feulgen-stained squash preparations. The indices ranging from 11.0 to 18.0 were nevertheless substantially higher than those reported in normal adult males by Killmann and his associates who used the latter technic.

In a system, such as the bone marrow, characterized by continuous proliferation as well as differentiation of cells, the mitotic index does not necessarily reflect the degree of mitotic activity. This depends on unknown variables such as the duration of mitosis and the length of time the cells spend in the marrow. As yet no direct evidence for the occurrence of significant variations in the duration of mitosis under natural conditions has been presented, and there is no basis for the assumption that it was prolonged in the cases at hand. The possibility of a shortening of the transit time of granulocytes through the cytologic compartments of the series cannot be excluded. Thus the absence of mature forms could be the result of an increased exit rate from the bone marrow. However, in view of the presence of normal or increased numbers of metamyelocytes and band forms, it seemed unlikely that this mechanism was the primary cause of the elevation of the mitotic indices. Further complexities arose because of the high percentages of lymphoid cells in most of the specimens, for large numbers of such cells would tend to depress the mitotic index of the myeloid population proper. Nothing is known regarding the transit time of lymphocytes in the bone marrow. The interpretation of the mitotic indices is thus at best speculative. The assumption that they reflect accelerated rates of mitotic activity is based on the collateral evidence for increased granulocyte production.

For a more detailed picture, 100 consecutive mitotic figures were counted in each of four specimens (table 3) and the cells were identified as far as possible by size and staining properties. No attempt was made to distinguish
between myeloblasts and lymphoblasts in mitosis, but since the percentage of such immature cells in interphase was not significantly elevated, this source of error was negligible. The cells were classified in three categories corresponding to levels of differentiation in each series. The well known difficulties of assigning an exact maturation grade to a given cell was recognized, but the distinction between cells of the myeloid and those of the erythroid series presented no problems. In the series of bone marrows of normal adults studied by Killmann et al. the ratio of granulocyte to erythroid mitoses was of the order of 30:70. In the present series this ratio was consistently elevated, and in one instance actually reversed (Case 5, table 3). On the assumption that erythropoiesis was normal, the high ratio reflected an increase in granulocyte mitoses which in turn was due predominantly to cell divisions at the promyelocyte level.

Maturation studies in vitro: The preceding observations seemed to indicate that in C.G. the proliferative activity of the granulocytic cell renewal system is increased while maturation proceeds in a normal fashion, at least to the stage of neutrophilic band forms. It remained to investigate the possibility of an "arrest" of the final stage of maturation.

A direct approach to the problem was suggested by the unique circumstance that the marrow in C.G. contains an abundance of nearly mature, but almost no fully mature segmented granulocytes. Their appearance in serial short-term cultures of explanted fragments would thus constitute prima facie evidence for the ability of younger forms to complete their maturation, at least in vitro, and incidentally add to the scanty knowledge concerning these events. In normal bone marrow cultures the distinction between polymorphonuclear cells already abundantly present in the initial explant and those formed by maturation of precursors during incubation would be impossible, at least without DNA labeling. The shifting relationships between the various cell compartments with unpredictable rates of proliferation, differentiation and degeneration, as well as the tendency of late forms to migrate into the medium, would preclude even semiquantitative analysis. C.G., on the other hand, constitutes an experiment of nature suitable for such a study.

Bone marrow particles from one of the patients (Case 4) were placed in fresh compatible heparinized donor plasma, transferred onto coverslips and incubated at 37 C. in individual miniature Petri dishes containing amounts of Eagle's tissue culture medium barely sufficient to cover the specimens. After 2 hours and at intervals up to 72 hours, the coverslips were removed,
Fig. 2.—Bone marrow culture (Case 4). Representative field after 20 hours incubation, showing abundant polymorphonuclear leukocytes.

blotted to remove excess fluid and, after spreading the particles in the usual manner, were stained with Wright-Giemsa. Suitable specimens were obtained up to 48 hours.

The differential count on a pre-incubation particle showed 9.8 per cent metamyelocytes, 16.4 per cent band forms and less than 1 per cent polymorphonuclear leukocytes. After incubation for more than 4 hours, all cultures showed considerable numbers of typical mature polymorphonuclear neutrophilic leukocytes with from two to four segments separated by filamentous connections (fig. 2). Both these cells and band forms had migrated extensively into the periphery of the particles. For this reason alone, no differential counts of consecutive specimens were obtained. The ratio of fully mature to young forms increased sharply with the length of incubation up to 8 to 12 hours, at which time about one-third of the nuclei had become filamentous. Although such a ratio could have no absolute meaning, it was nevertheless sufficient to show that under in vitro conditions, the last stage of granulocyte maturation was unimpaired.

In an attempt to demonstrate a possible humoral inhibitory factor, bone marrow particles freshly obtained from the same patient were incubated in parallel sets of Petri dishes containing 50 per cent tissue culture medium and 50 per cent of normal plasma and of the patient’s plasma respectively. No differences between the two series of cultures could be demonstrated. Again mature polymorphonuclear leukocytes were found in abundance following incubation.
Response to pyrogens: Bacterial endotoxins administered to normal persons cause an increase in the number of segmented granulocytes in the circulation to approximately twice the baseline level. The response has been shown to be a measure of the marrow granulocyte reserve.\(^7\) As mentioned, several patients had shown transient leukocytosis associated with a "left shift" during natural febrile reactions following surgical trauma or acute infection. To test the response to pyrogens under controlled conditions, 0.05 mg. of Pyrexal,\(^\circ\) a lipopolysaccharide derived from Salmonella abortus, was administered to one patient (Case 4). The response (table 4) followed exactly the pattern of the reaction to spontaneous febrile episodes in the same patient. A second child received 0.3 mg. of Pirumen,\(^1\) another less potent pyrogen prepared from Pseudomonas. Evidently the dose was inadequate as neither the patient nor a control case reacted with fever or leukocytosis. Unfortunately the experiment could not be repeated.

Response to adrenal hormones: Epinephrine is said to effect a redistribution of granulocytes in the blood by causing a shift from the "marginal" to the "circulating" pool.\(^3\) The epinephrine test was administered to three of the patients, with inconsistent results. In Case 1 a short-lived but substantial response was obtained with elevation of the total leukocyte count from a baseline level of 8400 to 18,000 90 minutes after the injection of 0.5 ml. of a 1:1000 solution, and a corresponding increase of granulocytes from 500 to 9500. Unfortunately in this case, studied in 1951, no record of the character of the granulocytes appearing during the test was kept. In Case 2 the response was less striking but still significant. The total granulocytes rose from 200 to 2700 2 hours following the injection of epinephrine. In Case 4 the number of granulocytes did not change during the test period.

One patient received a daily dose of 40 mg. of prednisone (2 mg./Kg.) for 6 weeks without changes in the composition of the bone marrow.

Response to local stimuli: Following the technic of Re buck,\(^9\) two patients (Cases 4 and 5) were studied for response to superficial irritants. On abrasions made on the forearm a drop of typhoid vaccine or purified tuberculin protein was placed and the denuded areas were covered with Wright's stain and examined for cellular composition of the exudate. Normal controls showed an exudate rich in polymorphonuclear leukocytes during the early hours of the

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\(\circ\)Obtained from A. Wander, Berne, Switzerland.
\(\dag\)Obtained from Flint Eaton Co., Morton Grove, Ill.

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**Table 4.—Results of Pyrexal Test in Case 4**

<table>
<thead>
<tr>
<th>Time</th>
<th>WBC</th>
<th>PMN</th>
<th>“Stab”</th>
<th>Myelocytes and Meta-</th>
<th>Eosino-</th>
<th>Baso-</th>
<th>Mono-</th>
<th>Lympho-</th>
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<tbody>
<tr>
<td>Preinjection</td>
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<td>2100</td>
<td>1</td>
<td>1 0 5 53</td>
<td>0 0 2 4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Injection</td>
<td>9:45</td>
<td>-</td>
<td>-</td>
<td>- - - - - - - - -</td>
<td>- - - -</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Postinjection</td>
<td>9:54 pm</td>
<td>2500</td>
<td>0</td>
<td>5 0 3 8 4</td>
<td>0 8 2 9</td>
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<tr>
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<td>1 0 4 1 9 5</td>
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<td>2</td>
<td>22 4 6 3 2 61</td>
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<td>46 1 2 1 7 42</td>
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<td>35 0 3 0 9 52</td>
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</table>
test, followed by a predominantly monocytic exudate (fig. 3). In the two patients with C.G., the exudate was virtually acellular during the first few hours (fig. 4). Later, moderate numbers of monocytes appeared (fig. 5). In Case 4 no granulocytes were seen at any time. In Case 5 an occasional band form was noted.

In contrast to the agranulocytic response to such superficial stimuli, cytologic examination of pus from a deep-seated abscess obtained in Case 2 showed abundant granulocytes. Marked degenerative changes made it impossible to distinguish between band forms and segmented granulocytes.

**Immunologic studies:** Leukocyte antibodies have been incriminated in several leukopenic states. The findings in C.G. are compatible with depletion
of the marrow leukocyte storage pool due to rapid peripheral destruction of granulocytes, conceivably as the result of autoimmune processes. Case 4 and Case 5 were studied repeatedly for the presence of leukocyte antibodies with consistently negative results, both when the patients' own and when normal donor leukocytes were used in the tests. The demonstration of leukoagglutinins by the method of Dausset et al., which gave positive results in two control cases of patients exhibiting severe febrile reactions after numerous blood transfusions, was unsuccessful. The theoretically more sensitive mixed agglutination reaction was then tried, using an adaptation of the technic described by Chalmers et al.11

Leukocyte suspensions were obtained by sedimentation of the whole blood to which a 50:50 mixture of 5 per cent EDTA and 10 per cent gum acacia had been added. After removal of plasma and refrigeration overnight, the cellular elements were washed repeatedly in the same mixture and centrifuged. Contaminating erythrocytes were removed by one or more 30-second exposures to hypotonic NaCl, and the final yield of leukocytes was washed and suspended in a 1 per cent solution of EDTA in saline. The suspensions were incubated with a 1:32 dilution of a potent antihuman globulin serum of rabbit origin and washed 5 times in saline-EDTA. The clumps of leukocytes were thoroughly dispersed and a 0.5 per cent suspension of group 0 Rh+ cells sensitized with an incomplete anti-Rh serum and washed thoroughly in saline was added. The mixture of anti-globulin-treated leukocytes and sensitized red cells was centrifuged lightly and transferred to glass slides with an opsonic pipet, sealed under a coverslip with paraffin and examined microscopically. With each experiment controls were run involving unsensitized leukocytes, normal sensitized leukocytes, and unsensitized erythrocytes, respectively. This procedure constituted the direct mixed antiglobulin test. For the indirect test, normal and patients' own leukocytes prepared as above were incubated with the patients' own sera prior to being exposed to the antiglobulin reagent, and with added suitable controls the test was carried out in the same manner as in the direct method.

No mixed agglutination was observed in the direct tests with either the patients' cells or those from the control cases with known leukocyte isoagglutinins. With the indirect method, mixed agglutination was obtained with the positive control sera. Normal control sera and the sera from both patients gave negative results.

Ancillary studies: Serum protein electrophoresis on paper and on starch gel was carried out in four cases. No consistent pattern was obtained. One patient had an essentially normal pattern, two showed a slight increase and one a slight decrease in gamma globulin. In this latter case the anti-A isoagglutinin titer was 1:32.

The direct antiglobulin test with the erythrocytes of the patients was negative in the three instances in which it was performed. The heterophil agglutination test was negative in all five cases, as were the serologic tests for syphilis. Liver function tests obtained in four cases gave normal results.

Discussion

The first case of C.G. seems to have been reported by Hotz in 1941.12 His patient was a twin girl with increased susceptibility to infection since the age of 2 weeks. Neutropenia was first discovered 4 months after birth and
persisted until the age of 2\(\frac{1}{2}\) years. Between 1942 and 1949 Tobler and Buser-Pluso,\(^13\) Salmonsen,\(^14\) and Ström\(^15\) each reported a single case. In 1952 Vrtilek,\(^16\) Gasser,\(^1\) and Vahlquist and Anjou\(^17\) reported each a small series adding up to 14 cases. Two of Vrtilek’s cases cannot be accepted as true examples of C.G. since one of the patients died with the picture of panmyelophthisis while the other had a short period of neutropenia following the administration of pyramidon and was probably a case of drug-induced agranulocytosis. The single case of McLean in 1957\(^18\) brings the total number of acceptable examples up to this time to 17. Since then, no further reports appeared but the condition may not be as rare as the paucity of articles would seem to indicate.

The distinction from the common hematologic disorders often associated with persistent neutropenia, such as aplastic anemia, leukemia and “hypersplenism,” presents no problem. The duration alone suffices to separate C.G. from neonatal neutropenia, recently described and attributed to the transient effect of maternal leukocyte isoantibodies.\(^19\)\(^,\)\(^20\) Infantile genetic agranulocytosis is a syndrome characterized by familial incidence, early onset and a high mortality.\(^21\)\(^,\)\(^22\) In the families studied by Kostmann,\(^21\) most of the patients died in less than 2 months and few lived beyond the age of 6 months. Cyclic neutropenia occurs in infancy but is readily distinguished by its periodicity.

Its course and the consistent findings in the bone marrow suggest that C.G. is a true entity distinct from classical agranulocytosis and from the reversible acute granulocytopenias which constituted the majority of the 35 cases referred to earlier as “secondary.” In this form the bone marrow generally showed a conspicuous increase in the M:E ratio associated with an extreme “left shift” in the granulocytic series. This picture, in short, was that often referred to as “maturation arrest.” This term has been justly criticized but may serve to indicate a situation in which early precursors multiply at an increased rate but are only reproducing equally immature cells. The renewal of more highly differentiated cells is almost totally in abeyance and the marrow granulocyte reserve becomes depleted to the vanishing point.

By contrast, in C.G. the processes of proliferation, differentiation and maturation, at least through the penultimate stage, were manifestly intact. The “shift to the left” was minor and chiefly the result of the absence of mature polymorphonuclear leukocytes and band forms. The marrow granulocyte reserve was reduced to the size of the maturation pool but by no means totally depleted.

These findings conform to the earlier descriptions and explain the paradox of the chronic benign course despite the prevailing depression of the circulating granulocytes. The bone marrow can produce and mobilize immature but functional cells, released chiefly when special demands arise, as with infection. Otherwise a sort of “steady state” seems to prevail in which the needs of the body can be met by minimal numbers of granulocytes and by monocytes, but which permits bacterial invasion much more readily than in normal persons. Thus, minor local insults which would ordinarily cause migration of granulocytes from the blood to the site of injury but are not of sufficient...
magnitudes to stimulate the bone marrow, produce an acellular or monocytic exudate. More serious infections call forth the release of such granulocytes as are available in the marrow, as shown by the character of the exudate in the abscess in Case 2. Severe systemic stimuli such as generalized infections, major surgical trauma and, experimentally, the intravenous injection of a bacterial endotoxin, result in the maximal leukocytosis of which these patients are capable. The availability of this mechanism affords a measure of protection which is lacking when not only the storage pool but also the maturation pool is depleted as in true agranulocytosis.

The nature of the primary disturbance remains obscure. Since the proliferative functions of the granulocytic cell renewal system are being carried out adequately and the release mechanism is evidently intact, only two basic possibilities remain: A disturbance in the last stage of maturation, or excessive destruction of mature leukocytes within the bone marrow or elsewhere.

The segmentation of the granulocyte nucleus is regarded as a function of age. According to classical theory, the number of segments increases as constrictions develop consecutively along the band-shaped nucleus, so that the oldest cells have the greatest number of lobes. Rohr assumes that segmentation can begin at different levels of differentiation and that the number of segments is predetermined by the stage of development of the precursor cell. The more primitive precursors would require the longest time to mature and develop the largest number of segments whereas the progeny of highly differentiated cells mature rapidly with minimal segmentation.

Gasser analyzed the number of segment anlagen of the band forms in the bone marrow and blood in C.G. and found a normal frequency distribution. Unable to explain these findings by a progressive increase in lobulation with age, he concluded that they supported Rohr's hypothesis and interpreted the lack of segmented granulocytes in C.G. as a maturation arrest at the penultimate stage. It would seem, however, precisely because the number of segment anlagen is normal, that the relationship between numbers of segments and age of the cells is not germane to the problem at hand. The question is rather why, or whether in fact, band forms with any given number of segment anlagen fail to develop into mature leukocytes with the corresponding number of segments, i.e., whether the process of constriction between potential segments is abolished.

The assumption of a last-stage maturation defect is not supported by the in vitro studies. The presence of many leukocytes with fully segmented nuclei after a few hours of incubation of bone marrow containing initially no cells more mature than band forms indicates that under these conditions maturation can be completed. Failure of inhibition by the patients' plasma in vitro further militates against in vivo inhibition of segmentation.

By a process of elimination, one is led to consider increased removal from the circulation and perhaps destruction of leukocytes. While direct evidence is lacking, the findings are compatible with this view. The composition of the bone marrow can plausibly be interpreted as a chronic state of depletion of mature leukocytes with a compensatory increase in the number of immature granulocytes of all classes, in analogy to the erythroid hyperplasia of
hemolytic anemias. The presence of numerous metamyelocytes and band forms, the absence of major shifts to the left among earlier precursors and the slight degree of over-all hyperplasia suggest that the drain on the granulocyte cell renewal system is far from maximal. An increased rate of removal of leukocytes from the circulation can thus only be moderate, sufficient to produce neutropenia but compatible with the maintenance of a "steady state."

Remaining to be considered are possible causes of increased removal of granulocytes. Autoimmune processes have been incriminated in many neutropenias,\textsuperscript{25-27} including cases with at least a similarity to C.G.\textsuperscript{28} The significance of leukoagglutinins in these conditions is questionable. In our two cases of C.G. studied for anti-leukocyte antibodies, the evidence was negative. The failure of response to corticosteroids, while not conclusive, militates against an immune process too subtle to be recognized by immunologic technics. Gasser noted slight phagocytosis of neutrophilic leukocytes by monocytes within the bone marrow in five of seven cases of C.G. but attributed little significance to this finding. In the present series phagocytosis could not be shown at all, nor was there evidence for a "hypersplenic" mechanism.

A disturbance in the physiologic mechanisms governing the distribution of leukocytosis might be involved, but these mechanisms are too poorly understood for meaningful speculation. Much of the granulocyte population of the blood, probably about 50 per cent, is known to be sequestered in capillaries, but this "marginal pool" is normally in dynamic equilibrium with the freely circulating cells.\textsuperscript{8} The results of the epinephrine test, although inconsistent, suggest that sequestration may play a contributory role in C.G., possibly by enhancing the removal of leukocytes from the circulation, but abnormal distribution within the vascular bed cannot be the major reason for the granulopenia. A true state of depletion exists as shown by the absence of mature leukocytes from the marrow, the character of the response to pyrogens and the absence of granulocytes in exudates produced by superficial irritants.

There is a constant and apparently irreversible removal of granulocytes from the blood and the lung plays an important role in this process.\textsuperscript{29,30} No association between leukopenic states and pulmonary disease has been reported, and no evidence of abnormal pulmonary function was found in the patients with C.G.

There is no basis for assuming a disturbance in the as yet hypothetical regulatory mechanisms involving the central nervous system or hormonal control. It is of interest that usually leukopenia as well as neutropenia was present so that the number of circulating lymphocytes was likewise decreased although at times absolute lymphocytosis did occur. In general, it may be said that the findings in the blood in C.G. represent an exaggeration of the normal infantile tendency toward relative lymphocytosis associated with relative granulopenia. The reason for this age-determined behavior has never been clearly established. It may be significant that C.G. is a disorder of early life. The same mechanisms, whatever their nature, which manifest themselves in the normal infant may be operating in an exaggerated fashion, or the development of normal countermechanisms may be in abeyance.
CHRONIC GRANULOCYTOPENIA IN CHILDHOOD

Summary

Observations in five cases of chronic benign granulocytopenia are reported. The disorder appears to be a true entity occurring in early life. Despite severe depression of the circulating granulocyte count, the course is benign because a reserve of immature granulocytes is available in the bone marrow. Depletion of the leukocyte storage pool by increased peripheral destruction, possibly enhanced by sequestration, rather than a maturation arrest at the penultimate stage is the probable mechanism. This process appears to be of moderate intensity, permitting the maintenance of a “steady state” of low efficiency, resulting in increased susceptibility but not total lack of resistance to infection. The disorder represents a natural model of a special disturbance in leukokinetics. Its ultimate cause remains unknown but is probably related to age-determined factors.

Summary in Interlingua

Es reportate observationes in cinque casos de chronic granulocytopenia benigne. Le disordine pare esser un ver entitate que occurre in subjectos de basse etate. In despecto de un depression sever del numeration de granulocytos del circulation, le curso del morbo es benigne, viste que un reserva de immatur granulocytos es disponibile in le medulla ossee. Le mechanismo probabile es depletion del magasinate thesauro de leucocytos per un intensificate destruction peripheric, possibilemente promovite per sequestration, e non un arresto del processo de maturation in su penultime stadio. Il pare que iste processo es de intensitate moderate, lo que permette le matenentia de un “stato stabile” de basse efficacia, con le resultato de un augmentate susceptibilitate pro infectiones sed non de un complete absentia de resistentia contra illos. Le disordine representa un modello natural de un disturbance particular in le leucocinetica. Su ultime causa remane occulte, sed illo es probablemente relationate con factores determinate per le etate.

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Chronic Granulocytopenia in Childhood

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