The successful treatment of cancer and leukemia by agents which have hematopoietic effects depends upon a selective toxicity, whereby the tumor cell is inhibited rather than the normal cell. Further information is needed on the life of the leukocyte, particularly the generative cycle and the normal or abnormal physiologic rhythms.

The basic mechanism of the movement of leukocytes and the turnover of these cells are not completely defined. Leukocyte dynamics have been difficult to study because of the many factors that affect the level of these cells in the peripheral blood. Moreover, the peripheral blood cell counts do not always indicate the rate of hematopoiesis nor the size of the bone marrow reserve of the various cellular factors. By newer technics we are acquiring more insight into the dynamics of leukocyte movement in leukemia among various compartments.

Hydroxyurea (Hydrea®) is a compound currently employed in clinical trials in malignant disease.1,2 It has produced bone marrow suppression, megaloblastosis and antitumor effects in animals and man. This agent is able to control chronic and subacute myelogenous leukemia and has been effective in those patients refractive to busulfan. During the investigation of hydroxyurea in chronic myelogenous leukemia, unusual oscillations in the peripheral blood leukocyte count were observed which, on occasion, caused difficulty in the regulation of the dose of this antitumor agent.

Oscillation of the peripheral blood leukocyte count has been observed in healthy individuals presumably due to the activity of a homeostatic mechanism controlling granulopoiesis.3 The same effect has been reported in four patients with chronic granulocytic leukemia, suggesting that normal control of granulopoiesis is partially retained in some patients with this disease.4

The observation of a cyclic fluctuation of the leukocyte and platelet counts in patients with chronic myelogenous leukemia treated with hydroxyurea provides a further opportunity to study the generative cycle of the leukocyte and to investigate the mechanism of action of hydroxyurea.
MATERIALS AND METHODS

Twenty patients with chronic myelogenous leukemia were treated with hydroxyurea. Ten of these were refractory to previous treatment with busulfan and 10 were patients in whom the diagnosis had been determined recently. The diagnosis of chronic myelogenous leukemia was established by evaluation of the peripheral blood smear and bone marrow, and determination of the Philadelphia chromosome and leukocyte alkaline phosphatase. The dose of hydroxyurea was 50 mg./Kg./day in two divided doses until the leukocyte count was less than 10,000 per cu.mm., and then 20 mg./Kg./day with appropriate adjustments according to the weekly white blood count. The investigation has been conducted over a five-year period. All the blood counts were carried out by the same technician (Miss Kay Newton) utilizing the same standard method for the collection of blood samples and the determinations.

RESULTS

Twenty patients with chronic myelogenous leukemia were treated with hydroxyurea. In all of these the leukocyte count became normal, there was a decrease of thrombocytosis in all of the 11 patients with this phenomenon, anemia increased to over 12 Gm. per cent in 12 of 16 patients, splenomegaly decreased or disappeared in all the patients, and bone marrow improvement occurred in 17 patients examined. Megaloblastosis was noted in all marrow examinations.

The survival of the patients refractory to busulfan treated with hydroxyurea was comparable to the patients treated initially with hydroxyurea. Hydroxyurea did not prevent the development of hemolysis, myelofibrosis, or the acute phase of myelogenous leukemia. It has been concluded that hydroxyurea is as effective as busulfan in the treatment of chronic myelogenous leukemia; it is effective in those patients refractory to busulfan; it is rapid acting; and it is safe.

During the treatment of these patients it was noted on occasions that the leukocyte count appeared erratic with respect to the control produced by the hydroxyurea. Sudden increases or decreases of the count occurred without alteration of the dosage of hydroxyurea. There was no evidence that the patients were not taking the medication as instructed, and there appeared to be no factors present that would interfere with absorption of the agent.

With sharp changes in leukocyte count, a change in dosage of hydroxyurea resulted in further erratic behavior of the count. Since the effect of hydroxyurea is rapid and short, a significant decrease in dosage resulted in a quick increase in the leukocyte count and vice versa. It became evident that changes of dosage of hydroxyurea should not be done frequently, and when changed, time allowed for adjustment of the effect of this dosage alteration.

Case 1. This 45-year-old man during the early months of his initial hydroxyurea therapy had variation in the leukocyte count (Fig. 1). As the count reached 160,000 per cu.mm., the dose of hydroxyurea was increased. This was followed by a drop of the leukocyte count to normal, and hence the dose of hydroxyurea was lowered. Immediately the leukocyte count increased to high levels, the hydroxyurea was increased and the count went to below normal levels. On a fixed dose of hydroxyurea the leukocyte count continued to fluctuate, but gradually settled to more normal values. Six months later this patient was studied in greater detail. The rhythmic oscillations were again noted with leukocyte counts sometimes ap-
I
2 3 4 5 6
Time (in months)

Fig. 1.—Case 1. Oscillations of leukocyte count duration administration of hydroxyurea.

proaching 90,000 per cu.mm. (Fig. 2). Two separate, slow undulations were noted during this interval of study.

Case 2. This 45-year-old female had a cyclic variation of the leukocyte count during the early phase of hydroxyurea therapy. (Fig. 3). Subsequently, her family physician adjusted the dose each week, and although there were suggestions of oscillations at low values, the data cannot be interpreted because of the constantly changing doses. The time period for two cycles appeared to be about 35–40 days per cycle.

Case 3. In this 42-year-old female, oscillations of the leukocyte counts were apparent at an early phase of treatment (Fig. 4). Subsequently, the counts were not normal, but the insufficient number of determinations prevents clear delineation of oscillations of the leukocyte count. Erratic counts were observed at infrequent intervals. A second peak at 17 months suggests a second biorhythm in process.

Case 4. This 30-year-old male had definite cyclic variations of the leukocyte count over three years of observation (Fig. 5). Moreover, this individual demonstrated another undulation of the leukocyte count with peaks at the ninth and 31st months. This phenomenon of
Case 1. Oscillations of leukocytes and platelets still occurring one year after onset of hydroxyurea therapy.

undulation was true of both the high and low values of the individual cyclic oscillations occurring at more frequent intervals.

Case 5. This 43-year-old male demonstrated the same slow undulation of the leukocyte count over an 18-month interval in addition to the more frequent cyclic oscillations (Fig. 6). The peaks of the slow undulations appeared at about a 12-month interval, while the cyclic oscillations approximated 40 days. With an increase of hydroxyurea dosage at the 18th month, the leukocyte count fell below the normal values and no oscillations were apparent. After an additional year of therapy another undulating rise was again noted, and as the leukocyte count increased, the cyclic oscillations were again apparent.

The platelet counts also demonstrated cyclic oscillations in all the studied patients. The first patient during his initial study period at a constant dose level of hydroxyurea demonstrated platelet count oscillations with the same periodicity as the leukocyte count (Fig. 2). In patients four and five the cycle of the patients also was in the same phase and periodicity as that of the leukocyte count. The slow undulations that occurred with the leukocytes may be noted in the platelets (Fig. 2). Changes in spleen size did not correlate with the variations of leukocyte or platelet counts.
Fig. 3.—Case 2. Cyclic variations of leukocyte count during administration of a constant dose of hydroxyurea.

Fig. 4.—Case 3. Cyclic variations of leukocytes and suggestion of an underlying undulation at 17 months.
Fig. 5.—Case 4. Undulation of leukocyte count at nine and 31 months with superimposed cyclic oscillations.

Fig. 6.—Suppression of cyclic oscillations and undulation with increase in dose of hydroxyurea.

No cyclic variations were noted in the hemoglobin or reticulocyte count. Differential counts of the leukocytes revealed that the cyclic changes were of the granulocytic series.
CYCLIC LEUKOCYTE OSCILLATIONS

DISCUSSION

Variations of the peripheral blood leukocyte count have been observed in healthy individuals. Garrey and Bryan showed that the neutrophil count varies within narrow limits characteristic of that individual, and these changes occur in the absence of exercise, pain, emotion, or other factors known to alter the leukocyte count. Fluctuations in leukocyte counts under normal conditions in female subjects have been associated with variations in the levels of endogenous hormones. Morley showed that a neutrophil cycle existed in eight of 11 normal males with a period of 14-23 days. The peak-to-trough amplitudes of oscillation were 1000-1500 neutrophils per cu.mm. He contended that a similar cycle might exist in normal females but would be obscured by the more obvious neutrophil cycle associated with the menstrual cycle.

Oscillation of the neutrophil count occurs in cyclical neutropenia, a disease characterized by episodes of neutropenia of 14 to 30 days at intervals of 20 to 21 days. Morley suggests that the periodicity in cyclical neutropenia may be due to an exaggeration of the normal neutrophil cycle.

In a study of four patients with chronic granulocytic leukemia, Morley, Baikie and Galton detected an oscillation of leukocytes in which the periods of oscillation were greater than those observed in normal subjects. They suggested this might be evidence that normal control of granulopoiesis is partly retained in at least some patients with this leukemia.

The periods of oscillation in the four patients studied by Morley et al. were 50-55, 30, 40 and 100-120 days. The leukocyte counts appeared to oscillate spontaneously with no antileukemic therapy. Moreover, a cyclic fluctuation was superimposed on the leukocyte fall due to busulfan therapy. In these studies, in two of the patients the platelet count also was found to oscillate with the same periodicity. In one of these the oscillations were in the same phase as the leukocyte count, but this was not demonstrated in the other patients. There was no obvious periodicity of the reticulocytes.

The current report describes five patients in whom cyclic oscillations of the leukocyte count were observed during hydroxyurea therapy. The cyclic periods usually ranged from 30 to 50 days, but with considerable similarity in each individual. Wide ranges of counts occurred in the peak-to-trough values of single cycles; a normal count to 170,000 per cu.mm. in one patient.

A second phenomenon was the cyclic undulation of the leukocyte count with heights of the undulating peaks occurring at approximately 9, 11, 14 and 20-month intervals. The short cyclic oscillations were superimposed on these more slowly undulating variations.

Rhythmic variations of the platelet counts were also observed in all of the patients, but in only three was the data sufficient to demonstrate that the period and phase of the cycle were the same as that of the leukocyte count. No cyclic oscillations were noted in the hemoglobin or reticulocyte counts.

These clinical studies of rhythmic oscillations of leukocyte counts in normal healthy individuals and patients with chronic myelogenous leukemia provide more data on the life cycle of the leukocyte. Such information is critical to the understanding and treatment of neoplasms, especially of the myeloproliferative
types. In a study of the etiology of leukemia, Schoyer postulated that cells have a natural tendency to growth which normally is restrained by inhibitory substances which may act on a well-defined part of the nucleus referred to as "receiving genes." An autonomous neoplasm would result from damage to the receiving gene, whereas a conditioned tumor such as chronic myelogenous leukemia could result from a deficiency of the inhibitory substance. Other studies by Perry have suggested that leukocyte dynamics are different from the normal situation.7

The most likely cause of the neutrophil cycle lies in the nature of granulocytopoiesis. Cronkite and Fliedner emphasized that in the consideration of granulocytopoiesis one must consider each phase of a cell proliferation system that is in a steady-state equilibrium with fluxes superimposed upon the steady state equilibrium by physiologic influxes into the blood and effluxes from the blood.8 Granulocytopoiesis is concerned with proliferation within the stem cell pool, effluxes from this pool into the differential proliferating pool, time parameters of the generation cycle in each compartment of the preceding pools, the ultimate efflux from the proliferative pool into the maturation pool, and the influx ultimately into the peripheral pool. They contend that the long-term control of granulocytopoiesis must reside with differentiation of stem cells into the granulopoietic pathway. They postulated that perhaps the "steady state" and demand induction of stem cells into the granulopoietic pathway is in part or wholly regulated by some feedback loop. Morley implied the existence of a negative feedback circuit acting on the marrow at the level of the proliferative granulocyte pool and at an early stage as that of the stem cell.9 The long-term variations he noted in neutrophil counts of healthy individuals were thought to explain the periodicity occurring in cyclical neutropenia, but with wider implications in regard to the control of granulopoiesis. Morley suggested that the oscillations of neutrophil count occur because granulocyte production by the bone marrow is not constant but cyclical, being controlled by a negative feedback circuit in which the presence of a time delay results in oscillation.4

The presence of periodicity in the four patients with chronic myelogenous leukemia of Morley et al. and the five patients reported here suggest that a normal control mechanism is still partly retained in some patients with chronic myelogenous leukemia and that proliferation of the leukemic cells is not completely unrestrained. Both reports observed oscillating platelet counts with similar periodicity and phases as the leukocyte count. This might be in keeping with a concept that the feedback stimulus of chronic myelogenous leukemia controls proliferation of a precursor common to the granulocytic and megakaryocytic series.

Morley attempted to explain the longer periods of oscillations in the patients with chronic myelogenous leukemia than those found in normal individuals. Although some of the increase was thought to be due to slow clearance of leukemic cells from the blood, most of the delay was postulated to be the result of prolonged retention of granulocytes in the proliferative marrow pool.4 Perry has suggested that in leukemic relapse there may be a disturbance in a "release mechanism" controlling the entry of leukocytes into the blood.10
has postulated a sequestration of cells in the tissues with recycling back into the blood.\textsuperscript{9}

According to Frenkel a repetitive quantitative periodic rhythm of DNA synthesis and cell division does appear to characterize normal human cells of certain tissues and at least some neoplasms appear to retain a similar oscillatory behavior.\textsuperscript{10} Assessing the kinetics of myeloid cells in chronic myelogenous leukemia appears to be complicated by the fact that they are apparently synthesizing DNA and undergoing cell division in the blood and tissues as well as the marrow.\textsuperscript{9} Hydroxyurea inhibits the synthesis of DNA with the production of megaloblastosis in the bone marrow.\textsuperscript{1,11} This agent in some manner may alter a phase of cell development, encouraging synchronous growth, or at least allowing an accentuation of the normal oscillations of leukocytes and platelets.

The observation of a slow undulation of the leukocyte count over many months demonstrates another biorhythm that exists in granulocytopoiesis. Such changes in chronic myelogenous leukemia may be accentuations of changes that are present in the normal, but are not apparent.

Logical models for granulocytopoiesis are emerging from experiments in animals and man. As patterns in periodicity and phase of normal and abnormal granulocytopoiesis are characterized, we establish a rationale for an attack on the defects in the control mechanisms for the production of leukemic states of granulopoiesis. Drug and dose schedules of antitumor agents assume critical importance should the rhythmic patterns observed relate to the drug sensitivity of either normal or neoplastic cells. Chronopharmacology may more accurately develop the use of antitumor agents by its investigation of drug effects on biologic characteristics as a function of biologic timing.\textsuperscript{12}

The unusual oscillations in the peripheral blood leukocyte count during hydroxyurea therapy of chronic myelogenous leukemia may account for the difficulty of dosage regulation of this antileukemic agent encountered by some investigators. The recognition of the oscillations and their significance may aid in the selection of the proper dosage schedule for hydroxyurea.

By “microscopic” procedures of numerical analyses of one or more rhythms, these findings can be exploited for a more efficient chemotherapy and more effective control of the leukemia.

\textbf{SUMMARY}

Cyclic fluctuations of the leukocyte and platelet counts were observed in five patients with chronic myelogenous leukemia during treatment with hydroxyurea. The cyclic periods ranged from 30 to 50 days, were similar in each individual, but the peak-to-trough values of single cycles varied in magnitude. A second biorhythm was a cyclic undulation of the leukocyte count with heights of the peaks at nine-to-20-month intervals. Rhythmic variations of the platelet counts were also observed; the period and phase of the cycle being the same as that of the leukocyte cycle. No cyclic oscillations were noted in the hemoglobin or reticulocyte counts. The recognition of the phenomenon may aid in the use of hydroxyurea or other chemotherapeutic agents in the
control of myelogenous leukemia. Chronopharmacology may more accurately
develop the use of antitumor agents by its investigation of drug effects on
biologic characteristics as a function of biologic timing.

ACKNOWLEDGMENTS

The author gratefully acknowledges the contributions of Drs. Dorothy Sundberg and
Richard Brunning in interpretation of the bone marrow examinations.

REFERENCES

1. Kennedy, B. J., and Yarbro, J. W.: Metabolic and therapeutic effects of hy-
1966.
4. Morley, A. A., Baikie, A. G., and Gal-
ton, D. A. G.: Cyclic leucocytosis as evi-
dence for retention of normal homeostatis
control in chronic granulocytic leukemia.
6. Schoyer, N. H. D.: The aetiology of
leukemias. Illustrating an alternative concept
of aetiology of malignancy in general. Lan-
7. Perry, S.: Leukocyte kinetics in leuk-
emia. In Zarafonetis, C. J. D. (Ed.): Proceed-
ings International Conference on Leukemia–Lymphoma. Philadelphia, Lea
and Febiger, 1968, pp. 229–244.
9. Athens, J. W., Bishop, C. R., and Cart-
wright, G. E.: The kinetics of neutrophilic
granulocytes in chronic myelocytic leukemia
—a review. In Zarafonetis, C. J. D. (Ed.):
10. Frenkel, E. P.: Implications of cir-
cadian factors in leukemia–lymphoma. In
Zarafonetis, C. J. D. (Ed.): Proceedings
International Conference on Leukemia–
Lymphoma. Philadelphia, Lea and Febiger,
1968, pp. 271–279.
11. Yarbro, J. W., Kennedy, B. J., and
Barnum, C. P.: Hydroxyurea inhibition of
Cyclic Leukocyte Oscillations in Chronic Myelogenous Leukemia During Hydroxyurea Therapy

B. J. KENNEDY