

## Serum Anti-Proteases in the Leukemias

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**D**URING THE PAST TEN YEARS considerable evidence has accumulated suggesting disturbances of protein metabolism in patients with neoplastic disease. Changes in serum albumin and globulin have been described by Huggins and others;<sup>1-4</sup> Greenstein has demonstrated interference with the synthesis of liver catalase;<sup>5, 6</sup> and quantitative differences in serum proteases and the corresponding anti-proteases have been reported in various pathologic states including malignancy.<sup>7-11</sup> Recently, the balance existing between two of the serum anti-proteases, anti-chymotrypsin and anti-rennin, has been correlated with tumor growth rate in uncomplicated cancer patients.<sup>11-12</sup>

The purpose of this paper is to present anti-enzyme studies in representative types of leukemia and to point out the similarity of the findings to those reported for neoplastic diseases in general. Because the subdivision of leukemia into acute, sub-acute and chronic varieties implies the relative activity of the disease, the association between the anti-enzyme patterns of the serum and the rapidity of abnormal cellular proliferation is even more readily apparent in leukemia than in carcinoma or sarcoma. Illustrative cases have been selected in which interference with the natural pattern of the disease due to treatment was minimal. The influence of specific therapy on the enzyme inhibitor balance in leukemia, and the use of such data in the clinical management of the patient will be reported in a subsequent paper.

### PROCEDURE

Quantitative determinations of anti-chymotrypsin and anti-rennin in the serum were made by methods described previously.<sup>10</sup> The range of normal values and the changes observed in cancer are summarized diagrammatically in figure 1. It will be noted that rapid tumor growth is usually associated with high anti-chymotrypsin and low anti-rennin values while the reverse is found in patients with inactive disease.

Diagnoses were confirmed by repeated bone marrow biopsy in all cases and at autopsy in four. Enzyme inhibitor patterns are shown during periods in which only supportive treatment was being given with the exception of one patient receiving maintenance doses of urethane. In order to facilitate comparisons, an attempt has been made to present the cases in order of decreasing activity of

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the leukemic process. Anti-enzyme titers are expressed on a scale which relates the height of the curves directly to the absolute concentration of enzyme inhibitor. Broad designations of cell type are employed in the figures. "Neutrophils" includes mature, juvenile and stab forms; "myelocytes" indicates the total of promyelocytes, myelocytes and metamyelocytes. All patients studied were adults and the data presented do not necessarily apply in childhood leukemias.

CASE REPORTS AND RESULTS

Case 1 (figure 2). A 40 year old white man admitted with the complaints of fever, weakness, bleeding gums and purpura of arms and legs of one week's duration. Small

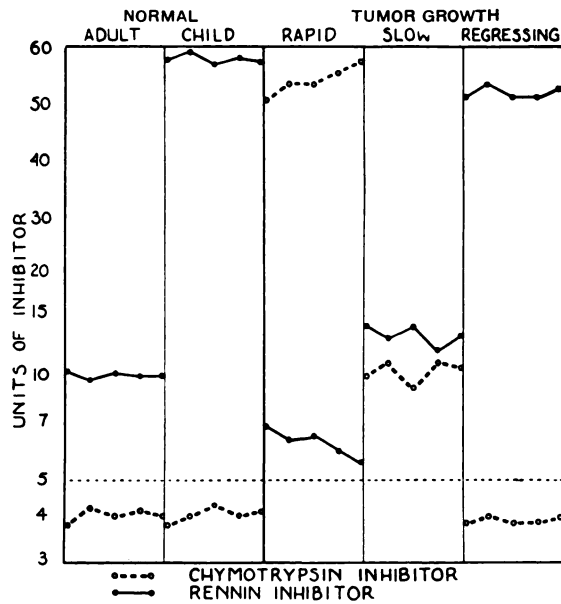


Fig. 1.—Enzyme inhibitor patterns in normal subjects and in cancer patients (diagrammatic).

posterior cervical nodes were palpable, the liver was slightly enlarged but the spleen could not be felt. The peripheral white count was 42,000 with 83 per cent blast forms. Sternal marrow revealed 95 per cent myeloblasts and 4 per cent promyelocytes. He deteriorated rapidly with continued fever, weight loss, anemia, pain in muscles, joints and epigastrium, bloody sputum and leukemic infiltration of lungs and peri-rectal areas. The patient died three months from the onset of symptoms.

The enzyme inhibitor pattern showed complete reversal of the normal balance between anti-chymotrypsin and anti-rennin with nearly maximum dissociation of the two factors. The anti-rennin titer continued to fall as the illness progressed and anti-chymotrypsin remained at approximately 30 units. Anti-rennin values under 7 units are usually an ominous sign when associated with high anti-chymotrypsin titers. The patient expired the day following the charted period. This enzyme inhibitor pattern is essentially the same as that found in fulminating malignant disease.

Case 2 (figure 2). A 52 year old white man who developed acute leukemia while hospitalized for treatment of a deformity resulting from traumatic fracture of the femur one year before. The onset was insidious with the appearance of left posterior cervical adenop-

athy unaccompanied by systemic symptoms or peripheral blood changes. A month later the patient began to run an afternoon temperature and complained of anorexia and low abdominal pain. At this time a mild anemia was present and the white count was 12,250 with 25 per cent unidentified blast forms. A diagnosis of acute monocytic leukemia was made by sternal biopsy. During the next two months the cervical nodes became larger and more discrete, the liver extended 8 cm. below the costal margin and the spleen tip became palpable. The total white count as well as the number of monoblasts rose steadily. Monocytes reached as high as 66 per cent of the total peripheral count on occasions. The patient died three and one-third months from the first symptoms.

The enzyme inhibitor patterns shown in the figure represent a four week period extending to within two weeks of death. Part of the earlier less active phase of the disease in

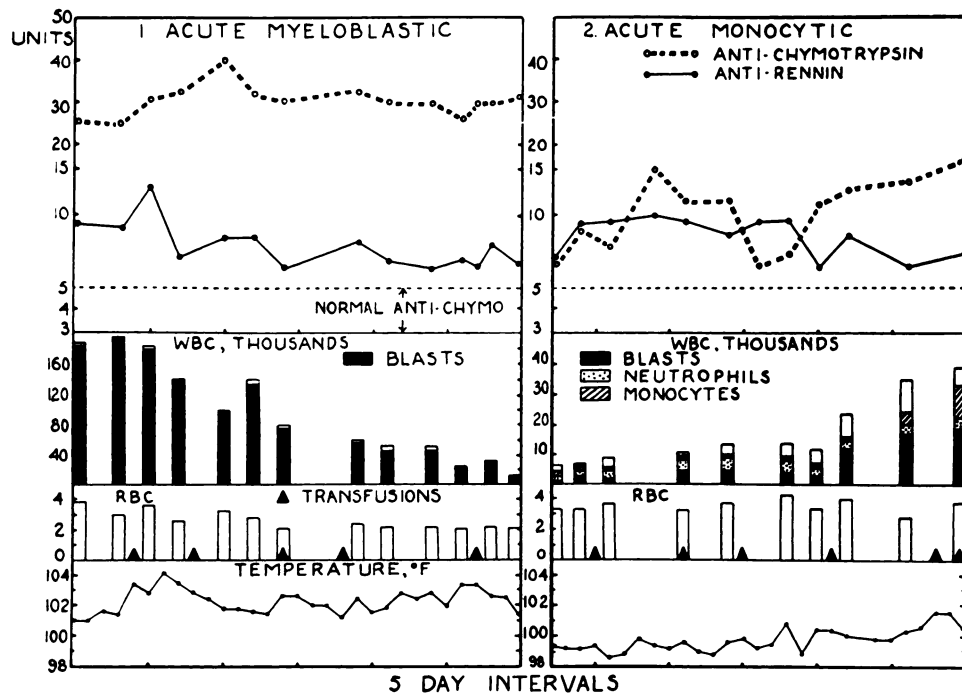


Fig. 2.—Enzyme inhibitor patterns in acute leukemias.

which anti-rennin was in excess of anti-chymotrypsin is shown. Reversal of the anti-enzyme balance then occurred along with a rising white count and increasing blasts in the peripheral blood. Following a brief hematologic and clinical improvement which is reflected by temporary reduction in anti-chymotrypsin values, there was rapid acceleration of the disease. Serum anti-rennin which had been maintained at about 10 units fell abruptly. This, together with steadily increasing anti-chymotrypsin concentrations, produced the pattern of rapidly progressing neoplastic disease. At the same time, increased numbers of monocytes and monoblasts appeared in the circulation.

*Case 3* (figure 3). A 33 year old white man who entered the hospital complaining of increasing weakness and fever for two months. Positive findings included a few nontender cervical nodes, a firm spleen extending 8 cm. below the costal margin and several small leukemic skin infiltrations. His course was only slowly progressive and in spite of the sternal marrow diagnosis of acute myeloblastic leukemia he was considered clinically as

sub-acute. After four months in the hospital during which time he was ambulatory and cheerful he developed leukemic retinopathy, numerous purple leukemic skin infiltrations and finally died in an acute exacerbation of the disease with intraperitoneal hemorrhage six months from the onset of symptoms.

The enzyme inhibitor patterns in figure 3 are those obtained during the first months of hospitalization and are of interest in view of the clinical impression that this was a sub-acute leukemia at that time. The chymotrypsin inhibitor was much increased, varying between 10 and 20 units. However, in contrast to cases 1 and 2, it was accompanied by still greater anti-rennin levels. Although potential disease activity may be high, the ability of patients with elevated anti-rennin titers to hold the neoplastic process in check has been

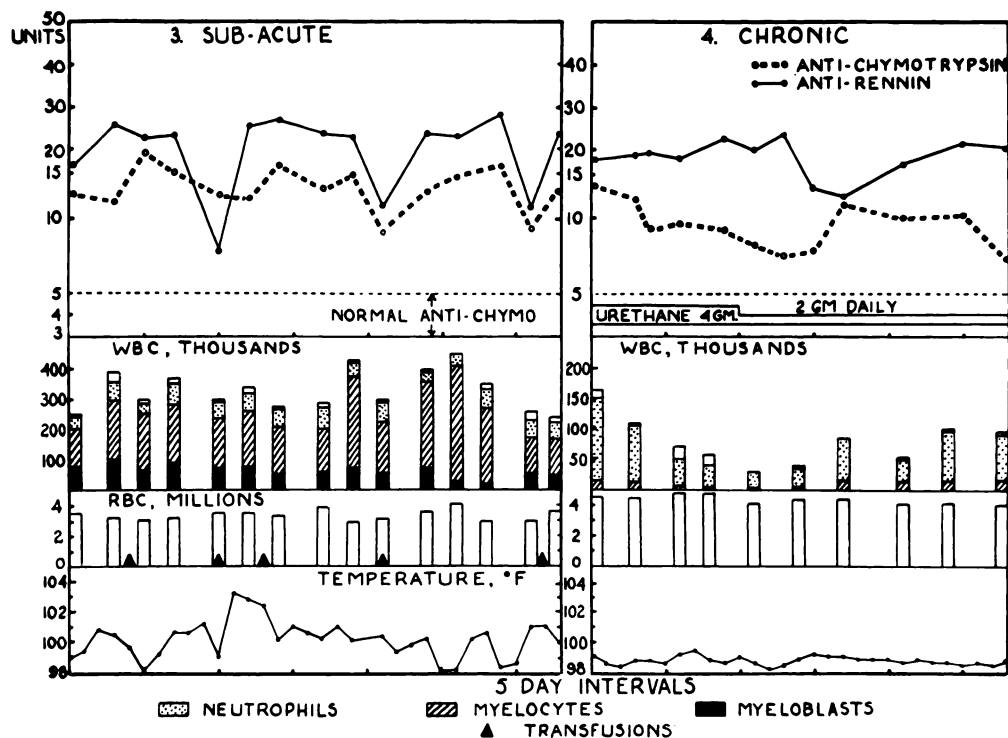


Fig. 3.—Enzyme inhibitor patterns in myelogenous leukemias.

observed repeatedly in these enzyme studies. A curious discovery in this case was the cyclic fall in serum anti-rennin at ten day intervals. The patient experienced lassitude and malaise on these occasions which was clinically obvious but which could not be correlated with any objective findings. Eventually, the anti-rennin titer fell and did not rise again; the enzyme inhibitor pattern thus became that of an acute process, and at the same time the patient entered the accelerated phase of his disease.

Case 4 (figure 3). This patient, a white man 45 years of age, sought medical attention because of persistent low grade evening fever, profuse sweating and 20 pounds weigh loss in the preceding four months. His skin was sallow and moist, several small cervical and inguinal lymph glands were palpable and the spleen extended to the iliac crest. The initial red count was 3,400,000, the white count 254,000 with immature cells of the neutrophil series but no blasts. Shortly after admission his white count rose to 840,000 with 3-5 per cent myeloblasts, and he was placed on urethane to which he responded well. He is still living, twelve

months after the onset of symptoms, his spleen has regressed, he has gained 22 lbs. and carries on his usual activities. There was no specific therapy except urethane which he still receives in maintenance doses. On the basis of laboratory and clinical data this case is considered as a relatively active form of chronic myelogenous leukemia.

The enzyme inhibitor pattern is intermediate between the sub-acute and chronic leukemias. Anti-rennin is in excess of anti-chymotrypsin as is characteristic of the less active neoplastic diseases, but anti-chymotrypsin tends to be greater than 10 units. It is apparent that without therapy this case might easily enter a more acute phase and this actually occurred before urethane was given. The enzyme inhibitor pattern shown in figure 3 is complicated by the favorable effects of treatment. A steady decrease in anti-chymotrypsin

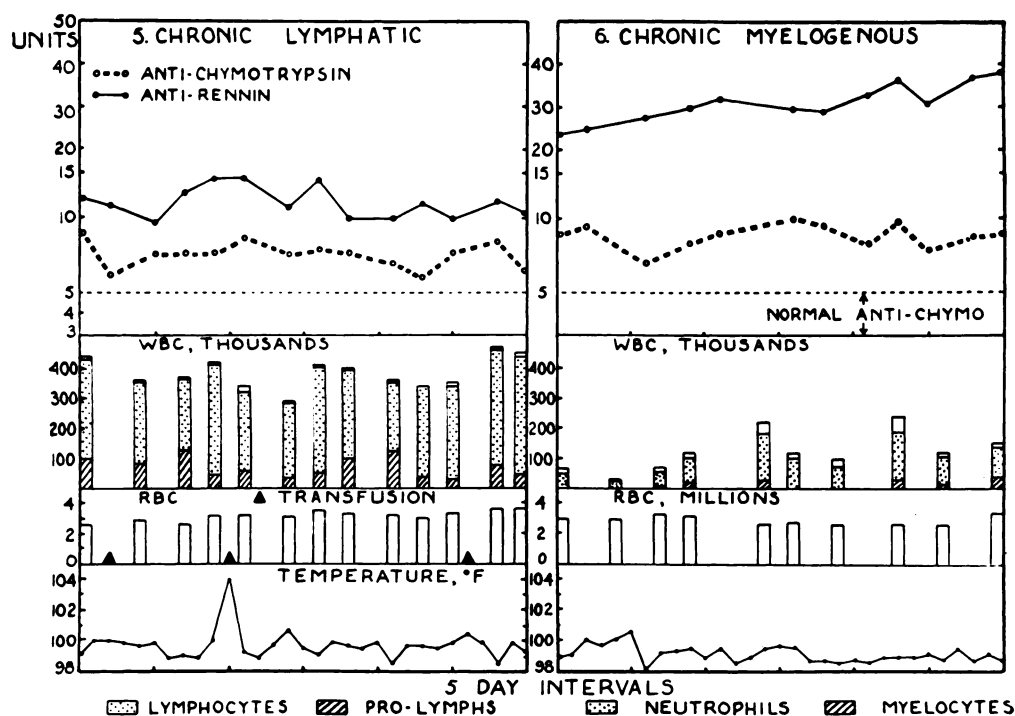


Fig. 4.—Enzyme inhibitor patterns in chronic leukemias.

and slight rise in anti-rennin paralleled the fall in leukocytes to 30,000. On reducing the dosage of urethane there was temporary disturbance of the anti-enzyme balance after which the patient became stabilized with a white count of 100,000.

*Case 5* (figure 4). A 61 year old white man was alert, ambulatory and cooperative when admitted. There were no particular complaints except for low-grade fever. The patient had been aware of his diagnosis of chronic lymphatic leukemia for five years and requested therapy to reduce his white count which he felt should be kept under 200,000. The liver and spleen were not palpable. Other than a few small inguinal glands and an emaciated appearance there were no positive findings. Under observation and supportive therapy he followed a constant course.

The enzyme inhibitor pattern exhibits two features characteristic of slowly growing neoplasms. Anti-chymotrypsin although above the normal range is less than 10 units and anti-rennin is well in excess thereby maintaining the normal balance between the two factors.

*Case 6* (figure 4). This patient had been diagnosed as chronic myelogenous leukemia three years before the present admission. No therapy had been given during this period. His chief complaints were referable to a complicating arteriosclerotic obliteration of some of the smaller vessels in the extremities. He was under weight and moderately anemic. The liver and spleen were enlarged 8 cm. and 6 cm. respectively below the costal margin. No lymphadenopathy was found. The white count was 186,000, platelets 800,000. Ten millicuries of radiophosphorus were given six weeks before the charted period.

The enzyme inhibitor pattern in this extremely chronic leukemia is the opposite of that described in Case 1. Serum anti-rennin is present in high concentration and far in excess of anti-chymotrypsin. With this "reserve" the possibility of reversal of the enzyme inhibitor balance in the near future would appear to be rather remote. This pattern differs from that found in totally quiescent or regressing neoplasms only in the increase of chymotrypsin inhibitor beyond the normal range.

#### DISCUSSION

Whether or not one considers leukemia as a type of malignant neoplasm, it is of practical interest that the serum anti-proteases are similarly related to disease activity in both. The enzyme inhibitor pattern in acute leukemia with a high percentage of primitive cells in the blood and marrow is indistinguishable from that found in rapidly growing anaplastic carcinoma. Conversely, the anti-enzyme balance in chronic leukemia closely resembles that seen in slowly growing well differentiated tumors. The data presented emphasize the importance of considering both enzyme inhibitors in interpreting results, since opinions based on a study of one are supplemented and modified by the other. The anti-enzyme patterns appear to be correlated with maturity of cell type and rate of progress of the disease. They do not bear any general relation to total red, white, or platelet counts or to temperature. In individual cases however, fluctuations in leukemic activity as recorded in the white and differential counts are closely paralleled or preceded by corresponding changes in anti-chymotrypsin and anti-rennin concentrations of the serum.

The fundamental significance of these changes in the proteolytic enzyme systems of serum is not yet apparent and the relationship to neoplastic diseases remains on a purely empiric basis. It would seem that a study of the mechanisms involved offers a promising approach to the problem of malignant growth. In spite of our lack of knowledge concerning the anti-proteases, the determination of enzyme inhibitor patterns in leukemia and cancer patients has been of practical value in recording disease activity and evaluating therapeutic agents.

#### SUMMARY

Quantitative changes in two serum anti-proteases in several types of adult leukemia are described. High anti-chymotrypsin and low anti-rennin concentrations were observed in the acute leukemias while the reverse was found in chronic forms of the disease. The relationship between the enzyme inhibitors and disease activity in leukemia is similar to that reported in cancer patients.

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