Studies on the Etiology of the Elevated Serum Isomerase in Chronic Myelocytic Leukemia

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The serum activity of phosphohexose isomerase, the enzyme facilitating the conversion of glucose-6-phosphate to fructose-6-phosphate, has recently come into use as an index of tumor growth and metastatic disease. Bodansky was able to demonstrate increased serum isomerase activity in metastatic carcinoma of breast and prostate. Subsequently, we reported increased serum isomerase activity in chronic myelocytic leukemia. In contrast to this, the serum of patients with chronic lymphocytic leukemia had values within the normal range. At that time, it was postulated that the increased isomerase might arise from either increased formation by, or release from, cells of the granulocytic series. We have since attempted to elucidate the source and mechanism of the raised serum isomerase in the chronic myelocytic leukemia group. This was done by following the serum isomerase levels over long periods of time in cases of chronic myelocytic leukemia, by estimating the enzyme activity in separated leukocytes, and by following the enzyme activity in dogs with induced leukocytosis and leukopenia.

Materials and Methods

1. Phosphohexose isomerase activity was determined according to the method of Bodansky, and the results expressed in the same unit of activity.

2. Separation of leukocytes: 3 ml. of blood were taken into 1 ml. of a 3.5 per cent isotonic solution of polyvinylpyrrolidone. This was incubated at 37°C for 2 hours, following which the supernatant was removed and allowed to sediment for a further hour. The supernatant was again removed, centrifuged, and the leukocytes washed with 0.85 per cent saline, resuspended and washed twice more in a similar fashion. The leukocytes were made up to a volume of 0.5 ml. with saline, and total and differential leukocyte counts were performed. The cell suspension was made up to a volume of 2 ml. with distilled water and was ground in a mortar to rupture the cell membrane and facilitate enzyme release. This was made up to a final volume of 4 ml. with distilled water, centrifuged, and the supernatant used in the same manner as serum for enzyme assay.

3. Six dogs, each weighing between 5 and 7 kg., were injected intravenously with 4 mg. of Pseudomonas polysaccharide (Pirmen) and their total leukocyte count, differential count, and serum isomerase estimated at 2 to 12 hour intervals. Another series of seven dogs was injected intravenously with methyl bis ß-chloroethylamine (HN2) in a dose range of 0.1 mg. to 0.7 mg. per Kg. as one injection and similar studies performed at intervals over a four to six day period.

Results

1. Ten patients with chronic myelocytic leukemia under treatment with radiation or 1:4 dimethanesulphonoxylbutane (Myleran) were followed over a

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period of 10 months or more. In seven cases the variations in serum isomerase closely paralleled the total leukocyte count and total granulocyte count. An example of this is shown in figure 1. The serum isomerase reflected the granulocyte count rather than the clinical findings and general condition of the patient. In three patients with terminal acute myeloblastic relapse including periosteal and bone involvement (one with pathological fractures), the total leukocyte counts remained below 30,000 per cu. mm. and the isomerase remained below 40 units. This would indicate that bone erosion per se is not the reason for the elevation of serum isomerase activity in this group, although this possibility was mentioned previously.4

2. Ten samples of leukocytes from patients with chronic lymphocytic leukemia and 23 leukocyte preparations from cases of chronic myelocytic leukemia were assayed. The first group all had leukocyte suspensions containing more than 85 per cent mature lymphocytes; the second group had leukocyte suspensions containing more than 85 per cent of cells of the granulocytic series in various stages of maturation. The results were expressed as:

\[
\text{isomerase units} \times 10^3
\]

The mean value obtained for the lymphocyte suspensions was 0.57 (S.D. ±0.33). The values obtained for the granulocyte preparations gave a mean value of 1.96 (S.D. ±1.1). The difference between these groups is highly significant with a P value of less than .001.

3. The average phosphohexose isomerase activity in the serum of 14 dogs was 25 units, with a range of 9 to 45 and a standard deviation of ±11. In the group of six dogs receiving 4 mg. of Piromen, four developed a leukocytosis of 30,000 WBC per cu. mm. or more. The leukocytosis was of the granulocytic series, the

![Graph](image-url)

**Fig. 1.**—The total leukocyte count and serum phosphohexose isomerase in a patient with chronic myelocytic leukemia treated with Myleran.
Fig. 2.—The leukocyte counts and serum isomerase in 2 dogs injected intravenously with 4 μg. of Piromen.

Fig. 3.—The leukocyte counts and serum isomerase in 2 dogs injected intravenously with 0.4 mg./Kg. of HN₂.
absolute lymphocyte count remaining essentially unchanged in all animals. There was no consistent pattern in the serum isomerase. In three dogs, the isomerase rose to levels above what we considered the upper limit of normal in dogs (over 45 units). These dogs had serum enzyme levels of 70, 86, and 115 units. The peaks occurred at 10, 24, and 48 hours at a time when the leukocytosis had been present for 2 to 24 hours. In one dog no leukocytosis resulted and no rise in serum isomerase took place. Two dogs developed leukocytosis with no increase in the serum isomerase. Representative curves are shown in figure 2.

4. Seven dogs received 0.4 mg. to 0.8 mg. per Kg. of HN₂ intravenously. The total leukocyte count in all the dogs fell within 48 hours. The lymphocytes were particularly labile and reached low levels or disappeared completely from the peripheral blood in 24 to 48 hours. The granulocytes reached levels less than 50 per cent of the initial values in 48 hours. As the dogs developed leukopenia, the isomerase showed a consistent pattern—a sharp increase occurring within 10 hours of injection, decreasing to normal in 10 to 30 hours, again increasing to a maximum in 30 to 70 hours, and returning again to normal. Representative curves from two dogs are shown in figure 3. The rise in serum isomerase was proportional to the leukopenia. In two other dogs with leukocytosis of infection (28,000 and 30,000 WBC per cu. mm.) given tetracycline, no rise in serum isomerase accompanied the fall in leukocytes to normal values which took place within 24 hours.

DISCUSSION

We previously reported the serum phosphohexose isomerase levels in five groups of patients. The normal control group had a mean serum isomerase of 17.7, S.D. ±8.6; a group of 41 patients with nonmalignant disease, a mean of 16.2, S.D. ±11.3; 11 cases of leukocytosis of infection, a mean of 18.2, S.D. ±13.4; 12 patients with chronic lymphocytic leukemia had a mean value of 15.2, S.D. ±9.5; and 16 cases of chronic myelocytic leukemia, a mean value of 67.9, S.D. ±19.9 units.

When the patients with chronic myelocytic leukemia received treatment either in the form of x-radiation or Myleran, the isomerase decreased as the leukocyte count fell, and, thereafter, tended to parallel the rise and fall of the circulating granulocytes. An example of this is shown in figure 1. The isomerase reflected no aspect of the disease other than the total number of granulocytes; it did not reflect a shift to cell immaturity, clinical deterioration, splenomegaly, or other clinical or laboratory findings.

That leukocytes are rich in isomerase had been demonstrated previously by Bodansky. The results on the isolated leukocytes showed the granulocytes to contain somewhat more than three times the phosphohexose isomerase activity of the lymphocytes. We were unable to demonstrate any qualitative difference in the isomerase of serum and leukocytes because the enzyme from both sources was unaffected by the presence of magnesium or fluoride ions in a .001 M concentration and both were inhibited by zinc. This inhibition by zinc was partially reversed in each case by cyanide. This does not prove that the enzyme from both sources is the same, but the similar response to these agents suggests identity.

It seemed that the elevated isomerase in the chronic granulocytic leukemia
group was either being released during the rapid formation and proliferation of granulocytic cells or was released into the circulation as they were destroyed. Leukocytosis was produced in dogs by injection of Piromen and in some, an elevation of serum isomerase accompanied the leukocytosis, but this was not a constant finding (fig. 2). The rise in enzyme activity accompanying leukocytosis might be explained either on the basis of myeloproliferation or increased leukocyte destruction associated with the increase in circulating granulocytes. In two dogs, the initial leukocyte decrease often associated with Piromen injection was accompanied by a sharp rise in serum isomerase. This would be more in keeping with the second hypothesis.

Cameron, Courtice, and Jones\textsuperscript{5} using 2 and 3 mg./Kg. of HN\textsubscript{2} in dogs, found progressive necrosis of the germinal centers in lymph nodes and spleen to begin in 3 to 6 hours after injection, the necrotic debris having been entirely cleared away in 24 hours. The bone marrow showed little change in five hours, the maximum destruction of myeloid tissue taking place 24 to 72 hours after injection. In our experiments, the dogs receiving nitrogen mustard showed a consistent isomerase pattern of two peaks occurring at 6–10 hours and 30–70 hours after injection. The first corresponds closely in time to the decline or disappearance of the lymphocytes; the second and larger peak to the falling granulocyte count. Under these conditions myeloproliferation as a cause of increased enzyme activity can be excluded. It would seem that here we are getting enzyme released into the circulation from damaged or disintegrating cells. This might originate in many tissues damaged or destroyed by nitrogen mustard, but the biphasic curve associated with the decrease in lymphocytes and granulocytes, the first and smaller peak coinciding in time with the maximum destruction of lymphocytes both in blood and lymphoid tissue and the second larger peak with the destruction of granulocytes and myeloid tissue, strongly suggests that the increased enzyme activity originates at least in part in these two cell series and their precursors.

The life span of the lymphocyte in chronic lymphocytic leukemia has been estimated to be about 30 days, while that of the granulocyte in chronic myelocytic leukemia is thought to be from 3 to 13 days.\textsuperscript{6, 7} That is, the rate of destruction of granulocytes is at least three times that of the lymphocytes. Thus, in patients with comparable leukocyte counts, there are approximately three times as many granulocytes destroyed per unit time in cases of chronic myelocytic leukemia than there are lymphocytes destroyed in chronic lymphocytic leukemia. In our previously reported series the mean leukocyte count of the chronic myelocytic leukemia group was 93,000 per cu. mm. and the mean isomerase level was 67.9 units. The chronic lymphocytic leukemia group had a mean leukocyte count of 95,000 per cu. mm. with a mean isomerase level of 15.2 units. As the isomerase content of the granulocyte is approximately three times that of the lymphocyte, there would be 9 to 10 times as much isomerase being liberated from the disintegrating cells originating in the peripheral leukocyte pool in the myelocytic group than in the comparable lymphocytic group. One would not expect the isomerase levels to be 10 times higher in the chronic myelocytic leukemia group because of resting enzyme levels, contribution from other tissues, and enzyme inactivation and destruction.
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The mechanism of enzyme release on leukocyte destruction would explain the difference in serum enzyme level in chronic lymphocytic leukemia and chronic myelocytic leukemia. In the latter group the enzyme richer granulocytes are breaking down with a rapidity that allows the serum isomerase to rise. In chronic lymphocytic leukemia the longer life span of the cell and its lower concentration of enzyme do not contribute enough isomerase per unit time to cause an increase in the serum enzyme activity.

SUMMARY

1. The phosphohexose isomerase activity of the serum of patients with chronic myelocytic leukemia closely parallels the rise and fall in total granulocyte count.
2. In separated leukocytes the isomerase activity of the granulocytes was found to be approximately three times that of the lymphocytes.
3. The serum isomerase activity was estimated in dogs with induced leukocytosis and leukopenia. The enzyme activity bore a closer relationship to leukocyte destruction than to leukocyte production.
4. These findings suggest that the elevated serum phosphohexose isomerase activity found in patients with chronic myelocytic leukemia probably originates from disintegrating granulocytes.

SUMMARIO IN INTERLINGUA

1. Le activate de isomerase de phosphohexosa in le sero de patientes con chronic leukemia myelocytic monstra un strie parallelismo con le augmento e abassamento del total numerazione granulocytic.
2. In leucocytos separate, il essa trovate que le activitate del isomerase in le granulocytos eseva approximativamente tres vices plus forte que illo in le lymphocytos.
3. Le activitate de isomerase in le sero eseva estimate in canes con inducute leucocytosis e leucopenia. Le activitate del enzyma exhibiva un plus striete correlation con le destruction de leucocytes que con le production de leucocytes.
4. Iste constatationes suggere que le elevate activate de isomerase de phosphohexosa que es incontrate in le sero de patientes con chronic leukemia myelocytic resulta probablemente ab le disintegration de granulocytes.

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