Leukocytic Alkaline Phosphatase

Behavior During Prolonged Incubation and Infection in Normal and Leukemic Leukocytes

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THE PRESENCE of alkaline phosphatase in segmented neutrophils has been well established. Although the origin, nature and function of this enzyme are poorly understood, variations from the normal leukocytic alkaline phosphatase activity have been demonstrated by biochemical and histochemical methods. In chronic myelogenous leukemia the enzyme is greatly deficient whereas in neutrophilic leukemoid reactions and in certain myeloproliferative disorders, there is a greatly increased activity.[1-4] Observations presented in this report on the behavior of alkaline phosphatase after prolonged incubation and during pyogenic infection have provided more striking evidence of the enzymatic differences in morphologically similar cells in some of the above disorders.

METHODS AND MATERIALS

The details of procedures carried out in these studies were described in a previous publication.[4] Peripheral blood smears were obtained on cover slips and fixed with 95 per cent alcohol or a methyl alcohol formalin mixture. The histochemical methods used were a slight modification of the Gomori calcium-cobalt method[5] and a modified azo dye technic.[6] The degree of alkaline phosphatase activity was graded by + − system.

The level of alkaline phosphatase activity was determined biochemically on separated leukocytes at the same time that histochemical studies were done. The method used was that of Valentine and Beck[2] but leukocytes were separated by EDTA (sequestrene) and dextran as previously described.

In these studies leukocytes were obtained from normal individuals, patients with chronic myelogenous leukemia and myeloproliferative disorders.

EXPERIMENTAL RESULTS

Biochemical studies demonstrate relatively little alkaline phosphatase activity in segmented neutrophils of normal individuals. Histochemically with either the cobalt-calcium method, incubating the preparations for 1 hour at 37 C or with the azo dye technic for 10 minutes at room temperature, approximately 25 per cent of segmented neutrophils give a + − to 2+ reaction. However on prolonged incubation in nine normal individuals the alkaline phosphatase activity of polymorphonuclear leukocytes became greatly increased; in 24 hours over 80 per cent of the cells were positive, a great majority giving 3+ and 4+ reactions (see chart 1).

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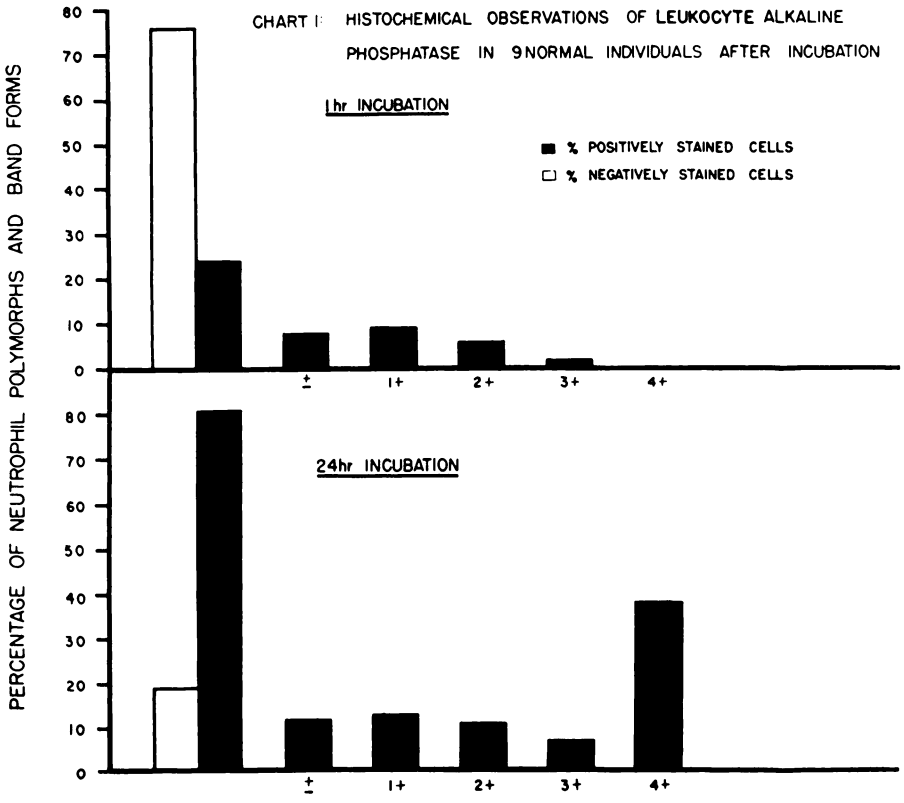

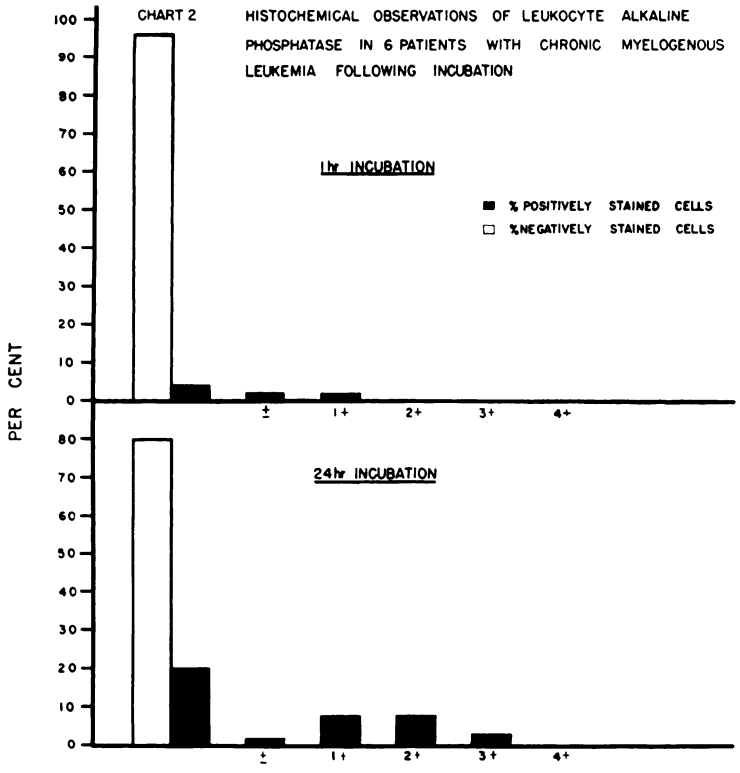

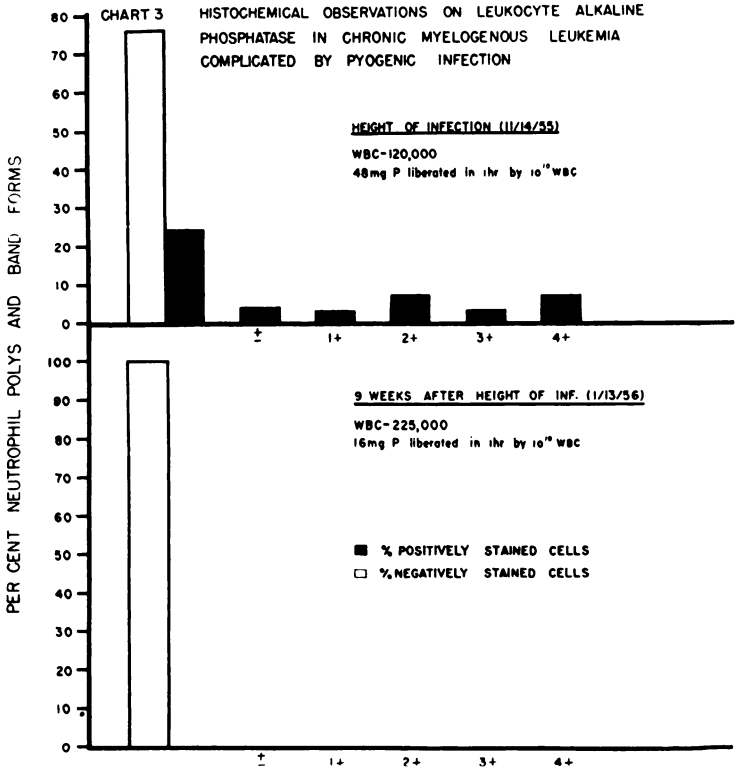

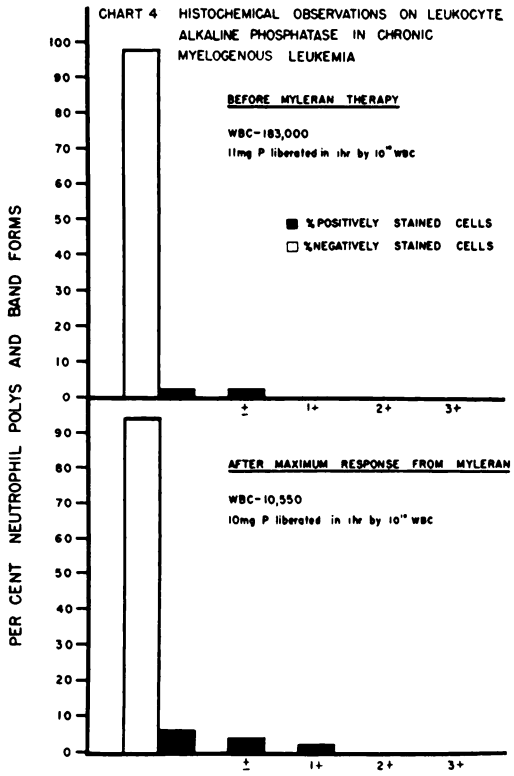

In marked contrast, studies on the polymorphonuclear leukocytes of six patients with chronic myelogenous leukemia revealed that about 4 per cent of segmented neutrophils showed a + − to 1+ reaction following 1 hour incubation. After 24 hours incubation, about 20 per cent of segmented neutrophils became positive while 80 per cent showed no evidence of alkaline phosphatase activity (see chart 2).

Technical objections have been raised to the use of the calcium-cobalt method because of diffusion, especially when prolonged periods of incubation have been employed. However, with the use of a high concentration of calcium ions and a high pH (9.9), diffusion artefacts were avoided in these studies.

TABLE 1.—*Effect of Therapy on the Biochemical Measurements of Alkaline Phosphatase on Separated Leukocytes in Chronic Myelogenous Leukemia*

Patient	Date	Therapy	Wbc	Alkaline* Phosphatase
F.T.	9/16/54	Myleran started 4 mg. a day	132,500	5.6
	10/13/54	Myleran total 108 mg.	20,000	15 mg.
	11/12/54	No Rx. since 10/13/54	58,000	8 mg.
	11/19/54	Myleran started 6 mg. a day	62,000	11 mg.
	12/27/54	Myleran stopped total 144 mg.	12,700	9 mg.
	2/24/55	No Rx. since 12/24/54	22,000	9 mg.
	4/12/55	Myleran started 6 mg. a day	41,000	7 mg.
	8/15/55	Myleran stopped total 352 mg.	4,500	12 mg.
	12/8/55	No Rx. since 8/15/55	5,800	12 mg.
I.C.	9/13/54	None	65,000	7 mg.
	10/6/54	Myleran started 2 mg. a day	35,000	7 mg.
	11/26/54	Myleran stopped total 142 mg.	11,400	14 mg.
	11/18/55	No Rx. since 11/26/54	13,500	10 mg.
	3/8/55	No Rx. since 11/26/54	19,500	14 mg.
M.B.	8/16/54	Myleran started 4 mg. a day	183,000	11 mg.
	10/18/54	Myleran stopped 10/4/55 total 164 mg.	28,000	16 mg.
	1/4/55	No Rx. since 10/4/55	89,000	6 mg.
	2/8/55	Myleran started 1/17/55 138 mg. to date	48,000	7 mg.
	3/15/55	Myleran to date 238 mg.	13,600	10 mg.
	4/26/55	Myleran stopped 3/29/55 total 266 mg.	10,550	8 mg.
	8/30/55	No Rx. since 3/29/55	9,900	7 mg.
W.P.	11/22/54	None	114,000	7 mg.
	12/6/54	Myleran started 11/27/55 40 mg. to date	125,000	10 mg.
	1/31/55	Myleran stopped 1/13/55 total 188 mg.	29,500	12 mg.
	2/21/55	No Rx. since 1/13/55	25,500	12 mg.
	3/28/55	Myleran started 3/15/55 26 mg. to date	50,000	8 mg.
	6/6/55	Myleran 182 mg. to date	25,250	7 mg.
	7/28/55	Myleran stopped total 340 mg.	11,100	7 mg.
	10/7/55	No Rx. since 7/28/55	57,500	12 mg.
	11/15/55	P^{32}-5 mc. by mouth 11/10/55	87,000	10 mg.
	12/19/55	No Rx. since 11/10/55 No further drop in WBC	64,000	7 mg.

* Expressed in Mg. Phosphorus liberated by 10^{10} WBC in 1 hour at 37° C.

Response to Pyogenic Infection

In the presence of pyogenic infection, the polymorphonuclear leukocytes of normal individuals develop intense alkaline phosphatase activity. The biochemical method reveals a marked elevation of enzyme activity and histochemically nearly all segmented neutrophils are found to contain a strong reaction for alkaline phosphatase.

An untreated case of chronic myelogenous leukemia complicated by pneumonia and a breast abscess afforded the opportunity of observing the reaction to the infection in this disease. While a moderate rise in alkaline phosphatase was noted by the biochemical determination during the infection, this increased activity was confined to only 25 per cent of the polymorphonuclear leukocytes. As noted in Chart 3, only a small per cent of the cells were strongly positive and over 75 per cent were completely negative. Serial studies on this patient revealed a drop in the alkaline phosphatase values as determined biochemically over a period of weeks. Nine weeks after the height of the infection the patient's cells were completely devoid of alkaline phosphatase by histochemical methods and the biochemical studies confirmed the low level of phosphatase activity (see chart 3).

TABLE 2.—*Effect of Therapy on the Biochemical Measurements of Alkaline Phosphatase on Separated Leukocytes in Certain Myeloproliferative Disorders*

Patient	Date	Therapy	Wbc	Alkaline* Phosphatase
E.D.	11/16/54	None	43,000	116 mg.
Diagnosis:	11/30/54	Myleran 40 mg. to date	30,000	138 mg.
Agnogenic	12/13/54	Myleran stopped total 106 mg	16,200	165 mg.
Myeloid	1/19/55	No Rx. since 12/13/54	20,600	129 mg.
Metaplasia	4/18/55	No Rx. since 12/13/54	34,500	144 mg.
	6/13/55	X-ray Rx. to spleen 250R since 6/4/55	28,800	113 mg.
	7/26/55	No Rx. since 6/13/55	15,950	130 mg.
	8/30/55	No Rx. since 6/13/55	27,500	110 mg.
	12/6/55	No Rx. since 6/13/55	33,600	200 mg.
P.M.	7/26/55	P^{32}—5 mc. 7/1/55	44,000	129 mg.
Diagnosis:	8/23/55	No Rx. since 7/11/55	27,000	100 mg.
Polycythemia	9/13/55	No Rx. since 7/11/55 P^{32}—8 mc. 10/25/55	41,300	110 mg.
Vera	12/6/55	No Rx. since 10/25/55	30,100	80 mg.
	1/16/56	No Rx. since 10/25/55	20,550	110 mg.
		No further drop in WBC		
M.L.	9/28/55	None	28,400	100 mg.
Diagnosis:	10/31/55	X-ray Rx. to spleen 200R since 10/1/55	29,300	180 mg.
Polycythemia	11/9/55	No Rx. since 10/31/55	16,500	200 mg.
Vera	11/30/55	No Rx. since 10/31/55	8,950	200 mg.
		No further drop in WBC		

* Expressed in Mg. Phosphorus liberated by 10^{10} WBC in 1 hr. at 37 C.

Effects of Therapy

Biochemical and histochemical studies were carried out on 4 cases of chronic myelogenous leukemia and 3 cases of myeloproliferative disorders undergoing various forms of therapy. In 4 cases of chronic myelogenous leukemia, during prolonged remissions induced by Myleran*, no significant change was noted in alkaline phosphatase activity of leukocytes (see table 1 and chart 4).

In the myeloproliferative cases, despite marked drops in the leukocyte counts induced by Myleran, P^{32}, and x-ray, alkaline phosphatase activity measured by biochemical methods was not altered and histochemical studies showed that nearly all segmented neutrophils remained strongly positive (see table 2).

Discussion

It has been pointed out that after prolonged incubation strong alkaline phosphatase activity developed in a great majority of normal polymorphonuclear leukocytes but about 75 per cent of segmented neutrophils in chronic myelogenous leukemia failed to develop this activity. This absence of alkaline phosphatase activity may indicate that the polymorphonuclear leukocytes in chronic myelogenous leukemia are fundamentally deficient in the enzyme, although the presence of a potent inhibitor cannot be definitely ruled out.

The behavior of leukocyte alkaline phosphatase in the presence of pyogenic infection is of special interest. While practically all polymorphonuclear leukocytes in normal individuals became strongly positive during infection, in chronic myelogenous leukemia only a small portion of the segmented neutrophils demonstrated alkaline phosphatase activity. These observations on the effect of prolonged incubation and infection suggest that in chronic myelogenous leukemia two populations of leukocytes may exist, a small group of cells capable of alkaline phosphatase activity and a much larger population completely lacking in this enzyme activity.

Administration of Myleran and P^{32} to patients with chronic myelogenous leukemia resulted in a marked drop in the leukocyte count. However, no alteration in unit leukocyte alkaline phosphatase activity occurred. In the myeloproliferative disorders treated with P^{32}, x-ray and Myleran the white cells decreased to near normal levels but the high leukocyte alkaline phosphatase activity persisted. Following therapy in the above disorders a return to a population of leukocytes displaying normal alkaline phosphatase activity might be expected. This did not occur in these studies and it is possible that all leukocyte precursors were equally influenced by the therapeutic agents employed.

In general, the experience in our laboratory has been similar to that reported in the literature. Low alkaline phosphatase activity has been consistently found in 14 typical cases of chronic myelogenous leukemia, whereas in 8 cases of leukemoid reactions associated with polycythemia vera and 4 cases of "agnogenic myeloid metaplasia," the phosphatase values were greatly elevated. However, as Valentine and his co-workers[3] have previously pointed out, there are occasional myeloproliferative disorders which present paradoxical findings. Recently in several cases involving the differential diagnosis between chronic myelogenous

* 1:4 Dimethanesulphonyloxybutane

leukemia and nonleukemic myelosis, difficulty was encountered in correlating the cytochemical and histologic evidence. In 2 cases autopsy findings were considered to be indicative of myelogenous leukemia, whereas histochemical and biochemical studies were typical of myeloid metaplasia. In the third case, the leukocytes were over 200,000 per cu. mm. and were practically devoid of alkaline phosphatase activity. Nevertheless, the pathological diagnosis was more suggestive of myeloid metaplasia than chronic myelogenous leukemia. It should be emphasized that in none of these 3 cases was the pathologic picture unequivocal and certain features of both disorders coexisted. It is possible that such cases represent myeloproliferative states in transition from benign metaplasia to leukemia.

In the present state of knowledge it is obvious that these atypical myeloproliferative problems cannot be resolved on a purely morphologic basis. Histochemical and biochemical methods have provided a means of differentiation in many of these myeloid disorders. However, further developments in cytochemical technics are required to furnish additional insight into metabolic and enzymic activities of leukocytes.

Summary and Conclusions

1. Evidence of a marked difference in alkaline phosphatase activity in the leukocytes of normal subjects and individuals with chronic myelogenous leukemia has been provided by observations on the behavior of the enzyme following prolonged incubation and during pyogenic infection.

2. Therapy with radiation and radiomimetic drugs in chronic myelogenous leukemia and myeloid metaplasia resulted in a marked fall in leukocyte count but no change was observed in the relative population of alkaline phosphatase positive and negative cells.

3. In our laboratory histochemical and biochemical values for leukocyte alkaline phosphatase have been similar to those reported in the literature for typical cases of chronic myelogenous leukemia and most leukemoid reactions. However, in certain cases parodoxical findings have been noted in which histochemical and biochemical studies were inconsistent with the pathologic diagnosis.

4. The inadequacies of purely morphologic criteria in these atypical cases were noted and the desirability of the further development of cytochemical methods has been pointed out.

Summario in Interlingua

1. Le demonstration de marcate differentias in le activitate de phosphatase alcalin in le leucocytos de subjectos normal e de individuos con chronic leucemia myelogene ha resultate ab observationes del comportamento del enzyma post prolongate periodos de incubation e in le curso de infectiones pyogene.

2. Le therapia per radiation e per drogas radiomimetic in chronic leucemia myelogene e in metaplasia myeloide resultava in un marcate reduction del numeration leucocytic, sed nulle alteration esseva notate in le fortia relative del populationes de cellulas con e sin activitate de phosphatase alcalin.

3. In nostre laboratorio le valores histo- e biochimic obtenite pro le phosphatase alcalin de leucocytos esseva simile al valores reportate in le litteratura pro casos typic de chronic leucemia myelogene e pro le majoritate del reactiones leucemoide. Tamen, in certe casos le resultatos esseva paradoxe in tanto que le studios histo- e biochimic non esseva de accordo con le diagnose pathologic.

4. Es notate le insufficientia de criterios de character purmente morphologic in tal casos atypic. Le desirabilitate de progressos additional in le disveloppamento de methodos cytochimic es signalate.

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