ACID AND ALKALINE PHOSPHATASE IN WHITE CELLS

Data for the Lymphocyte and the Polymorphonuclear Leukocyte of Man and the Rabbit

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A GREAT many tissues contain phosphatases, or more correctly phosphomonoesterases, active at either pH 4.5–5.5 or at pH 9.5–10.0.[1–3] For convenience, these enzymes will be referred to as "acid" and "alkaline" phosphatase respectively. Recently, Cram and Rossiter[4] studied the phosphatases of the rabbit polymorphonuclear leukocyte. Unlike the red cell, where the maximum activity is at pH 4.5–5.5,[5–7] the maximum phosphatase activity of the rabbit polymorphonuclear leukocyte is in the pH range 9.5–10.0. Because of the difficulty of obtaining cell suspensions containing one cell type only, similar data are not available for the rabbit lymphocyte, or for either the polymorphonuclear leukocyte or the lymphocyte of man.

In this study the problem has been approached from a statistical point of view. Data have been assembled on the acid and alkaline phosphatase activity of mixed white cell suspensions obtained from the blood of both man and the rabbit by several different procedures.

Methods

Cell Preparations. Mixed white cell suspensions were obtained from freshly-drawn human blood either by centrifuging in a constricted centrifuge tube as used by Butler and Cushman[8] for the determination of ascorbic acid in white cells, or by flotation on a solution of gum acacia as described by Spear.[9] White cells were obtained from the rabbit by these two methods and also from a peritoneal exudate by the method of deHaan,[10] the details of which have already been described.[4] In each instance the cells were washed twice with isotonic saline before the enzyme activity was determined. Heparin was used as an anticoagulant.

Total and differential white cell counts were made by the usual methods. Five individual dilutions were made on each suspension and one total white cell count done on each dilution. The figure recorded was the mean of these five counts. For the differential counts, 500 cells were examined on each slide. The cells were divided into polymorphonuclear leukocytes (which included basophils and eosinophils) and lymphocytes (which included monocytes). In general, the percentage of polymorphonuclear leukocytes was greatest in suspensions obtained by the peritoneal exudate method of deHaan and least in those obtained by the flotation method of Spear. With this method the polymorphonuclear cells tended to clump together. These clumped cells were removed from the suspensions by filtration through fine gauze.

Phosphatase Determination. The phosphatase activity was determined by the method of King and Armstrong,[11] in which the phenol, liberated by the hydrolysis of disodium phenyl phosphate, is measured by the method of Folin and Ciocalteu.[12] The test was always run in duplicate. For the determination of acid phosphatase the final pH was 4.9 and for the determination of alkaline phosphatase it was 9.9. Details of the method have already been published.[4]

In much of the previous work on white cell phosphatases, cell suspensions were used.[13–16] The difficulty of extracting phosphatases from the cell is well known[1,2] and it is likely that the full enzyme activity of the cells has rarely been measured. This difficulty has now largely been overcome by extracting the cells with a solution of saponin. Rossiter[17] has recently shown that surface-active substances such as

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saponin, bile salts, or alkyl sulfate liberate phosphatase from white cells into the surrounding fluid The cells of 0.2 ml. suspension were extracted by 0.5 ml. 1 per cent saponin, a quantity of saponin which previously has been shown to be optimal.

Recording of Results. The results for both acid and alkaline phosphatase are expressed as the amount of phenol (in mg.) liberated by 100 ml. of cell suspension in one hour. This figure is equal to the number of King-Armstrong units, as defined by Watkinson, Delory, King and Haddow,[18] per 100 ml. cell suspension for the acid phosphatase, and four times the number of King-Armstrong units, as defined by King, Haslewood, Delory and Beall,[19] per 100 ml. cell suspension for the alkaline phosphatase.

TABLE 1.—*Acid and Alkaline Phosphatase of Suspensions of Human White Cells*

Method of obtaining cells	Cell Count (10^3 cells/cu. mm.)		Phosphatase Activity (mg. phenol/100 ml. cell suspension/hr.)	
	Polymorphonuclear leukocytes	Lymphocytes	Acid	Alkaline
Ac.	1630	340	10.50	0.51
Ac.	820	420	1.50	0.00
Ac.	1940	400	9.00	0.87
Ac.	990	1660	11.30	1.62
Ac.	1960	520	5.10	0.30
Ac.	740	1310	7.50	1.11
Ac.	1340	630	4.60	0.96
Ac.	1310	990	2.50	0.90
Sp.	230	670	1.70	0.00
Sp.	520	1830	3.00	4.50
Sp.	430	800	1.10	0.93
Sp.	650	1510	3.80	2.55
Sp.	440	1030	3.20	0.00
Sp.	620	970	3.40	1.44
Ac.	1020	1590	1.10	1.32
Ac.	1350	1650	6.20	1.02
Ac.	5560	7100	26.40	3.63
Sp.	2840	540	11.50	0.00
Sp.	2880	920	9.20	1.14
Ac.	1560	3030	6.40	0.33
Ac.	2040	750	17.30	1.38
Ac.	2710	1530	25.10	1.50
Ac.	720	2050	2.90	1.68
Ac.	1240	3030	15.50	1.77
Ac.	730	1880	2.50	2.13

Ac., Acacia method of Spear.[9] Sp., Spinning method of Butler and Cushman.[8]

RESULTS

Human White Cells

Table 1 shows that for the human cells the activity of the acid phosphatase was usually greater than that of the alkaline phosphatase. When the lymphocyte count was high, the acid phosphatase activity tended to be high and, to a lesser extent, when the polymorphonuclear leukocyte count was high, the alkaline phosphatase activity was high. The acid and the alkaline phosphatase activity were, therefore, each plotted against (a) the polymorphonuclear leukocyte cell count and (b) the

lymphocyte cell count (fig. 1), and the regression equations and the coefficients of correlation (table 2) were determined in each instance.

There was an obvious correlation between the acid phosphatase activity and the lymphocyte count ($r_{13} = 0.80 \pm 0.07$) and a less significant correlation between the acid phosphatase activity and the polymorphonuclear leukocyte cell count ($r_{12} = 0.52 \pm 0.15$). On the other hand, there was a statistically significant correlation between the alkaline phosphatase activity and the polymorphonuclear leukocyte count ($r_{12} = 0.59 \pm 0.13$) and an insignificant correlation between the alkaline phosphatase activity and the lymphocyte count ($r_{13} = 0.20 \pm 0.20$).

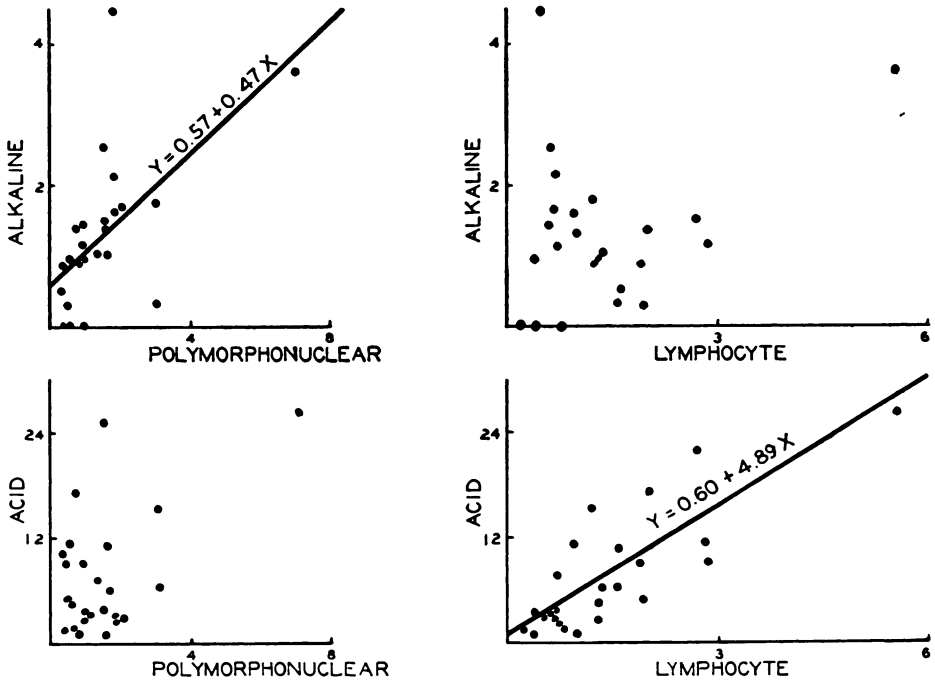

FIG. 1.—Relation between phosphatase activity and cell count of suspensions of human white cells. Abscissa: Polymorphonuclear leukocyte or lymphocyte count in 10^3 cells per cu. mm. Ordinate: Alkaline or acid phosphatase activity in mg. phenol per 100 ml. cell suspension per hr.

Acid Phosphatase. Since there was a significant correlation between the acid phosphatase activity and either the lymphocyte count or the polymorphonuclear leukocyte count, it was necessary to determine whether there was a significant partial correlation between the acid phosphatase activity and the cell count for each of the cell types, or whether the lesser correlation between the acid phosphatase activity and the polymorphonuclear leukocyte count was merely the result of there being a significant correlation between polymorphonuclear leukocyte count and lymphocyte count.

It was also desirable to determine how much of the acid phosphatase activity was in each of the two cell types, since correlation coefficient data give little hint

as to the distribution of the enzyme. From the figures of table 1 the following multiple regression equation was derived:

$$Y_{acid} = 0.39 + 0.52\ X_{poly.} + 4.51\ X_{lymph.} \quad (1)$$

where Y_{acid} is the acid phosphatase activity in mg. phenol/100 ml. suspension/hr., $X_{poly.}$ is the polymorphonuclear leukocyte cell count in 10^3 cells/cu. mm. and $X_{lymph.}$ is the lymphocyte count in 10^3 cells/cu. mm. In figure 2 the observed acid phosphatase activity is plotted against the value calculated from this equation.

TABLE 2.—*Correlation between Acid and Alkaline Phosphatase Activity and Cell Count of Human White Cells*

	Type of Correlation	Alkaline Phosphatase		Acid Phosphatase	
		Coefficient of Correlation	S. E. of r	Coefficient of Correlation	S. E. of r
r_{12}	Coefficient of correlation between phosphatase activity and polymorphonuclear leukocytes	0.59	±0.13	0.52	±0.15
r_{13}	Coefficient of correlation between phosphatase activity and lymphocytes	0.20	±0.20	0.80	±0.07
r_{23}	Coefficient of correlation between polymorphonuclear leukocytes and lymphocytes	0.57	±0.14	0.57	±0.14
$r_{12.3}$	Coefficient of partial correlation between phosphatase activity and polymorphonuclear leukocytes (lymphocytes excluded)	0.59	±0.13	0.42	±0.20
$r_{13.2}$	Coefficient of partial correlation between phosphatase activity and lymphocytes (polymorphonuclear leukocytes excluded)	−0.20	±0.20	0.72	±0.10
$R_{1.23}$	Coefficient of multiple correlation between phosphatase activity and polymorphonuclear leukocytes and lymphocytes	0.61	±0.13	0.80	±0.07

It can be seen from the multiple regression equation that, on the average, the lymphocyte had approximately 9 times more acid phosphastase activity than had the polymorphonuclear leukocyte. Even although there was much less acid phosphatase in the polymorphonuclear leukocyte than in the lymphocyte, it was desirable to determine whether this lesser acid phosphatase activity was correlated with the polymorphonuclear leukocyte cell count. Table 2 shows that the coefficient of partial correlation between the acid phosphatase activity and the lymphocyte count with the polymorphonuclear leukocyte excluded was statistically significant ($r_{13.2} = 0.72 \pm 0.10$), while that between the acid phosphatase activity and the polymorphonuclear leukocyte count with the lymphocyte count excluded was much less so ($r_{12.3} = 0.42 \pm 0.20$). The latter figure barely exceeds twice its standard error and so is of dubious significance. This means that the significant correlation between the acid phosphatase activity and the polymorphonuclear leukocyte cell count ($r_{12} = 0.52 \pm 0.15$) was the result of a significant correlation between the polymorphonuclear leukocyte count and the lymphocyte count ($r_{23} =$

0.57 ± 0.14), rather than a correlation between acid phosphatase activity and polymorphonuclear leukocyte cell count per se. The finding that the coefficient of multiple correlation between the acid phosphatase activity and the two cell types ($R_{1.23}$ = 0.80 ± 0.07) was not greatly different from the coefficient of partial correlation between the acid phosphatase activity, and the lymphocyte count with the polymorphonuclear leukocyte count excluded ($r_{13.2}$ = 0.72 ± 0.10) would indicate the insignificant role played by the polymorphonuclear leukocyte in the acid phosphatase activity of mixed white cell suspensions.

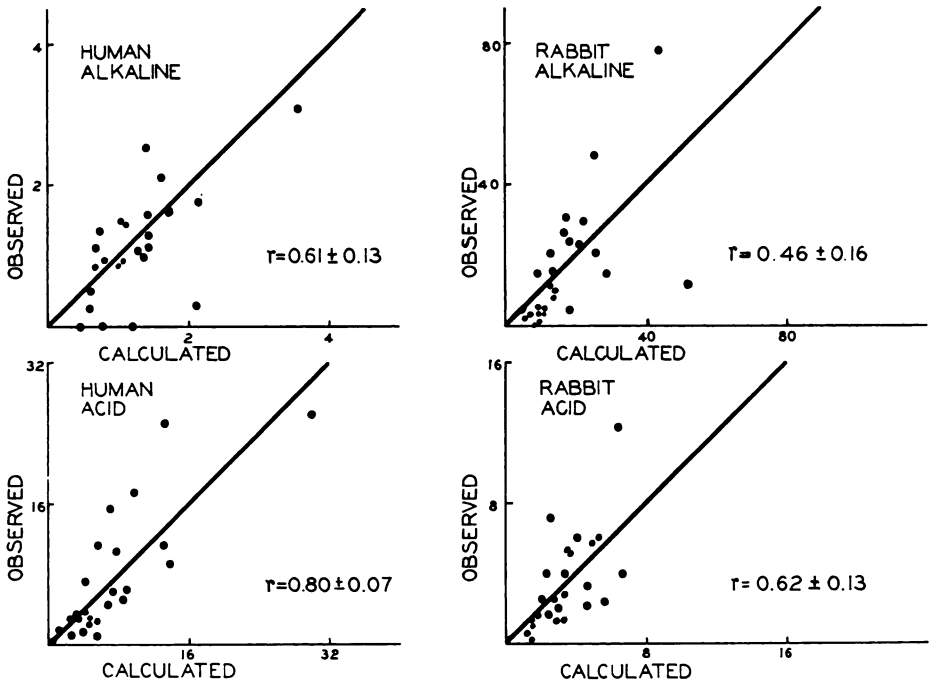

FIG. 2.—Relation between observed phosphatase activity and phosphatase activity calculated from the polymorphonuclear leukocyte and lymphocyte cell count using the multiple regression equations presented in the text. The phosphatase activity is expressed in mg. phenol per 100 ml. cell suspension per hr.

Alkaline Phosphatase. For alkaline phosphatase activity the position was the reverse. The multiple regression equation was found to be:

$$Y_{alkaline} = 0.71 + 0.55\ X_{poly.} - 0.19\ X_{lymph.} \quad (2)$$

where $Y_{alkaline}$ is the alkaline phosphatase activity in mg. phenol/100 ml. suspension/hour, and $X_{poly.}$ and $X_{lymph.}$ have the same significance as in Equation 1. The observed alkaline phosphatase activity is plotted against the value calculated from this equation in figure 2.

The regression equation shows that most of the alkaline phosphatase activity was in the polymorphonuclear leukocyte. Also the coefficient of partial correlation between the alkaline phosphatase activity and the polymorphonuclear leukocyte

count with the lymphocyte count excluded was statistically significant ($r_{12.3}$ = 0.59 ± 0.13), whereas that between the alkaline phosphatase and the lymphocyte count with the polymorphonuclear leukocyte count excluded was not significant ($r_{13.2}$ = −0.20 ± 0.20). Again the multiple correlation between the alkaline phosphatase activity and the two cell types ($R_{1.23}$ = 0.61 ± 0.13) was not greatly different from the coefficient of partial correlation between the alkaline phospha-

TABLE 3.—*Acid and Alkaline Phosphatase of Suspensions of Rabbit White Cells*

Method of obtaining cells	Cell Count (10^3 cells/cu. mm.)		Phosphatase Activity (mg. phenol/100 ml. cell suspension/hr.)	
	Polymorphonuclear leukocytes	Lymphocytes	Acid	Alkaline
Pe.	2600	1010	2.00	29.00
Ac.	670	770	1.70	2.90
Ac.	220	260	1.40	2.60
Sp.	1210	540	2.50	3.50
Sp.	1410	420	1.70	4.30
Sp.	2330	700	2.50	15.60
Pe.	5710	2810	4.10	21.00
Pe.	1780	200	.20	11.60
Pe.	3450	1860	2.20	24.30
Ac.	1410	1160	2.80	4.70
Ac.	3120	1210	5.20	30.80
Pe.	3270	1920	3.40	27.40
Pe.	5050	1190	3.20	48.00
Pe.	2040	1150	3.90	21.50
Ac.	1100	210	1.20	3.50
Ac.	2040	760	7.10	8.60
Pe.	4070	890	1.30	29.40
Pe.	9120	1240	6.10	78.60
Ac.	2090	2880	12.20	15.60
Ac.	580	150	.70	00.00
Sp.	11,480	2190	6.00	12.50
Sp.	2750	2070	5.80	10.40
Pe.	3980	1060	1.30	22.90
Sp.	3230	610	4.10	5.20
Sp.	1860	2470	2.40	1.10

Ac., Acacia method of Spear.[9] Sp., Spinning method of Butler and Cushman.[8] Pe., Peritoneal exudate method of deHaan.[10]

tase activity and the polymorphonuclear leukocyte count with the lymphocyte count excluded ($r_{12.3}$ = 0.59 ± 0.13), indicating the minor importance of the lymphocyte in the alkaline phosphatase activity of the suspensions.

Rabbit White Cells

Table 3 shows that, unlike the human cells, the alkaline phosphatase activity of the rabbit cells tended to be greater than that of the acid phosphatase. Although the activity of either the acid or the alkaline phosphatase of the rabbit white cells

differed quantitatively from those of man, qualitatively both the distribution and the correlation of acid and alkaline phosphatase activity with cell count were similar in each species (fig. 3). As for the human white cells, so for the rabbit cells: the acid phosphatase activity was correlated with the lymphocyte count and less with the polymorphonuclear leukocyte count, and the alkaline phosphatase activity was correlated with the polymorphonuclear leukocyte count and less with the lymphocyte count.

Acid Phosphatase. Table 4 shows the significant correlation between the acid phosphatase activity and the lymphocyte count ($r_{13} = 0.61 \pm 0.13$), and the in-

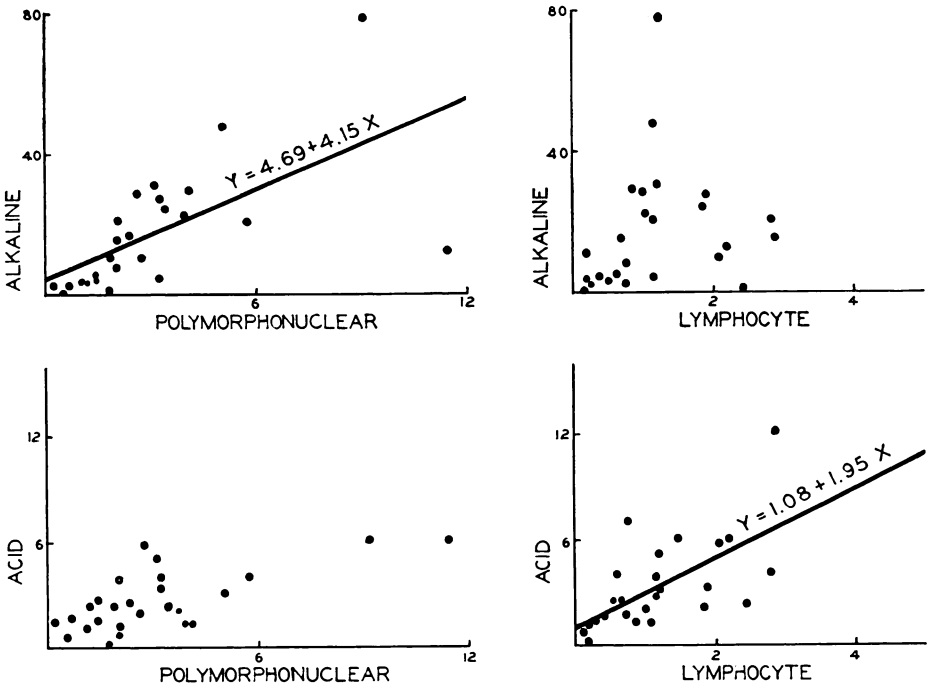

FIG. 3.—Relation between phosphatase activity and cell count of suspensions of rabbit white cells. Abscissa: Polymorphonuclear leukocyte or lymphocyte count in 10^3 cells per cu. mm. Ordinate: Alkaline or acid phosphatase activity in mg. phenol per 100 ml. cell suspension per hr.

significant correlation between the acid phosphatase activity and the polymorphonuclear leukocyte count ($r_{12} = 0.34 \pm 0.18$). The multiple regression equation was found to be:

$$Y_{acid} = 0.96 + 0.086\ X_{poly.} + 1.83\ X_{lymph.} \quad (3)$$

where the symbols have the same meaning as in Equation 1. The observed values of the acid phosphatase activity are plotted against the value calculated from the equation in figure 2.

The coefficient of partial correlation between the acid phosphatase activity and the lymphocyte count with the polymorphonuclear leukocyte count excluded was

significant ($r_{13.2} = 0.55 \pm 0.14$), whereas that between the acid phosphatase activity and the polymorphonuclear leukocyte count with the lymphocyte count excluded was not significant ($r_{13.2} = 0.10 \pm 0.20$). The insignificant nature of the polymorphonuclear leukocyte as a source of acid phosphatase is indicated by the finding that the coefficient of multiple correlation between the acid phosphatase activity and the two cell types ($R_{1.23} = 0.62 \pm 0.13$) was not greatly different from the coefficient of partial correlation between the acid phosphatase activity and the lymphocyte count with the polymorphonuclear leukocytes excluded ($r_{13.2} = 0.55 \pm 0.14$).

Alkaline Phosphatase. As for the human cells, the reverse was true for the alkaline phosphatase. The alkaline phosphatase activity was correlated significantly with

TABLE 4.—*Correlation between Acid and Alkaline Phosphatase Activity and Cell Count of Rabbit White Cells*

	Type of Correlation	Alkaline Phosphatase		Acid Phosphatase	
		Coefficient of Correlation	S. E. of r	Coefficient of Correlation	S. E. of r
r_{12}	Coefficient of correlation between phosphatase activity and polymorphonuclear leukocytes	0.61	±0.13	0.34	±0.18
r_{13}	Coefficient of correlation between phosphatase activity and lymphocytes	0.20	±0.20	0.61	±0.13
r_{23}	Coefficient of correlation between polymorphonuclear leukocytes and lymphocytes	0.44	±0.16	0.44	±0.16
$r_{12.3}$	Coefficient of partial correlation between phosphatase activity and polymorphonuclear leukocytes (lymphocytes excluded)	0.59	±0.13	0.10	±0.20
$r_{13.2}$	Coefficient of partial correlation between phosphatase activity and lymphocytes (polymorphonuclear leukocytes excluded)	−0.096	±0.20	0.55	±0.14
$R_{1\ 23}$	Coefficient of multiple correlation between phosphatase activity and polymorphonuclear leukocytes and lymphocytes	0.46	±0.16	0.62	±0.13

the polymorphonuclear leukocyte cell count ($r_{12} = 0.61 \pm 0.13$) but not with the lymphocyte cell count ($r_{13} = 0.20 \pm 0.20$). The multiple regression equation was found to be:

$$Y_{alkaline} = 6.12 + 4.44\ X_{poly.} - 1.95\ X_{lymph.} \qquad (4)$$

where the symbols have the same meaning as in Equation 2. The observed values of the alkaline phosphatase activity are plotted against the value calculated from this equation in figure 2.

It can be seen from the multiple regression equation that for the rabbit, as for man, most of the alkaline phosphatase activity was in the polymorphonuclear leukocyte.

Discussion

Alkaline Phosphatase. The data presented show that for both man and the rabbit the alkaline phosphatase activity of a suspension of mixed white cells is confined to the polymorphonuclear leukocytes. The lymphocytes are inactive. This is a direct chemical confirmation of the histochemical findings of a number of workers[20–23] who have used the alkaline phosphatase technics of Gomori[24] and Takamatsu.[25] In the multiple regression equation for the activity of the alkaline phosphatase of the white cells of both man and the rabbit, the term involving the lymphocyte cell count was negative. This suggests that the lymphocyte may contain some inhibitor of alkaline phosphatase activity. However, the coefficient of partial correlation between alkaline phosphatase activity and lymphocyte count with polymorphonuclear leukocyte count excluded, although negative for both species, was not statistically significant. If such a hypothetical inhibitor exists, its concentration must be subject to a high animal-to-animal variation.

That there is a high concentration of alkaline phosphatase in polymorphonuclear leukocytes and also in other granulocytes can be deduced from observations on the alkaline phosphatase activity of the whole blood of patients with leukemia. Thus, an increased blood alkaline phosphatase has been reported in patients with myeloid leukemia[15, 26] and, when the cell count decreased after radiation therapy, the concentration of blood alkaline phosphatase also decreased.[26] In patients with eosinophilic leukemia and in animals with experimental eosinophilia the blood alkaline phosphatase also increased, suggesting that the eosinophil, like the neutrophil, is rich in alkaline phosphatase.[27] On the other hand, the blood alkaline phosphatase of a patient with lymphatic leukemia was unchanged, indicating that the lymphocyte is relatively poor in this enzyme.[28] The alkaline phosphatase activity of the serum of patients with either myeloid or lymphatic leukemia was also unchanged. However, when the white cell count decreased following radiation therapy, the serum alkaline phosphatase of patients with myeloid leukemia increased, there being no such change in the alkaline phosphatase of the serum of patients with lymphatic leukemia.[29] This again suggests that the lymphocyte is poorer in alkaline phosphatase than the neutrophil.

The activity of the alkaline phosphatase in the polymorphonuclear leukocytes of the rabbit is approximately eight times that of human cells. The activity of polymorphonuclear leukocyte alkaline phosphatase varies greatly from species to species. In experiments yet to be published it has been shown that the concentration of the enzyme is high in the polymorphonuclear leukocytes of the rabbit, rat, and guinea pig and almost absent in the polymorphonuclear leukocytes of the cat, dog and pigeon.

Acid Phosphatase. For both man and the rabbit the acid phosphatase is chiefly in the lymphocyte, although a lesser concentration is in the polymorphonuclear leukocyte. The findings for the rabbit are thus in agreement with the published observations of Cram and Rossiter.[4] The demonstration that there is little acid phosphatase in the polymorphonuclear leukocyte and much more in the lymphocyte is in keeping with the histochemical studies of Gomori [30] and others.[22, 31–33]

Human lymphocytes have more than twice the activity of rabbit lymphocytes. In other experiments it has been shown that the white cells of the rat and guinea pig have an acid phosphatase activity of the same order as that of the rabbit, whereas the activity of the white cells of the cat, dog and pigeon is much less. Whatever be the relative concentrations of acid and alkaline phosphatase in different species, the following generalization remains: the polymorphonuclear leukocyte is rich in alkaline phosphatase and poor in acid phosphatase, and the lymphocyte is rich in acid phosphatase and poor in alkaline phosphatase. Probably this pattern of phosphatase distribution has to do with the respective functions of these two cell types. At present so little is known of the function of tissue phosphatases generally that it does not seem profitable to speculate further upon the function of the phosphatases of the white cell.

Summary

1. The acid and alkaline phosphatase activity has been determined on a series of suspensions of white cells obtained from both man and the rabbit by several different methods.

2. A statistical analysis of the results shows that for both species the alkaline phosphatase of white cell suspensions is confined chiefly to the polymorphonuclear leukocyte and the acid phosphatase is chiefly in the lymphocyte, although the polymorphonuclear leukocyte contains lesser concentrations of this enzyme also.

3. Although this qualitative distribution was the same for both species studied, quantitatively the rabbit differed from man. The activity of the alkaline phosphatase of the rabbit polymorphonuclear leukocyte was eight times that of the corresponding human cell, while the activity of the acid phosphatase of the human lymphocyte was more than twice that of rabbit lymphocyte.

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