A Comparison of Human Leukocyte Phosphatase Activity toward Sodium β-Glycerophosphate, Adenosine 5′-Phosphate and Glucose 1-Phosphate

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For a number of years this laboratory has been interested in the phosphatase activity of isolated human leukocytes and the marked lability of leukocyte alkaline phosphatase in disease. Leukocyte alkaline phosphatase has been demonstrated to be essentially limited to the granulocytic cells and to be characteristically low in chronic myelocytic leukemia. Elevated values are noted in polycythemia vera when leukemoid features are present and in many, though not all, cases of idiopathic metaplastic disease resembling chronic myelocytic leukemia. Moreover, leukocyte alkaline phosphatase may be increased many fold on a per cell basis in a variety of "stressful" conditions such as acute pyogenic infection, trauma, hemorrhage, myocardial infarction, etc. Similarly, a well-marked elevation in unit cell alkaline phosphatase may be induced over a 72-hour period by the administration of large doses of ACTH or 17-OH corticosteroids to normal subjects. This pattern of response is absent or diminished greatly in chronic myelocytic leukemia.

The above experiments have been largely conducted employing sodium β-glycerophosphate as substrate. The experiments documented below attempt to define leukocyte phosphatase activity in terms of ability to hydrolyze two important physiologic metabolites, the monoesters adenosine 5′-phosphate and glucose 1-phosphate.

Adenosine 5′-phosphatase has been demonstrated in various tissues by Reis and in blood serum by Dixon and Purdom. In 1952, Swenseid, Wright and Bethell studied the ability of human leukocytes from leukemic subjects to hydrolyze adenosine 5′-phosphate. Utilizing this substrate, they found phosphatase activity in cell populations from subjects with chronic lymphocytic and chronic myelocytic leukemias. Maximal activity was present in the acid pH range, and the conclusion was drawn that adenosine 5′-phosphatase was predominately an acid phosphatase in human leukocytes. It was not realized at the time how labile leukocyte alkaline phosphatase may be, and that values obtained in leukemic leukocytes cannot, because of inherently different metabolic patterns, be extrapolated to nonleukemic leukocytes from either normal subjects or subjects with other diseases.

The data reported below have been obtained with the following objectives in mind: (1) to reassess the ability of human leukocytes to hydrolyze phos-
phosphorus from the naturally occurring and metabolically important esters adenosine 5'-phosphate and glucose 1-phosphate; (2) to investigate the question of whether the phosphorus-hydrolyzing activity toward these substrates and toward sodium β-glycerophosphate at pH 9.9 is mediated by the same phosphomonomesterase or same group of phosphomonomesterases capable of hydrolyzing phosphorus from all three esters, or whether, in addition, phosphatases more specific for one or another of the esters under study can be demonstrated. It is recognized that the evidence bearing on the last point will be indirect and inferential in character rather than absolute, but the remarkable lability of leukocyte alkaline phosphatase in various diseases and in response to ACTH and 17-OH corticosteroids affords an unusual opportunity to obtain significant data in this regard. More specifically, data will be reported on (a) the parallelism of phosphorus-hydrolyzing activity toward all three substrates in different disease states and in normal subjects; (b) the responsiveness of phosphatase activity towards glucose 1-phosphate and sodium β-glycerophosphate when 17-OH corticosteroids are administered; (c) the comparison of phosphatase activity in substrate mixtures with that in individual substrates. It should be noted in the latter respect that if different enzymes are concerned with the hydrolysis of different substrates, some additive effect would usually be noted in substrate mixture experiments, while if all three substrates are hydrolyzed by the same enzyme or group of enzymes active against a number of phosphomonoesters, this effect would not be expected. Further, though phosphomonomesterase activity may not be operative at exactly the same rate with different substrate esters, nonetheless, if a single enzyme or group of enzymes is involved for all these substrates, variations in activity—whether due to leukemia, other disease, or to steroid medication—should exhibit parallelism. The very marked variations in activity patterns in different cell populations afford a rather unique opportunity to employ this parameter.

**Methods**

The method of leukocyte isolation has been previously described. The technic used for all alkaline phosphatase determinations was similar to that previously reported, except that the total volume of all materials used was reduced to ½ in order to conserve the quantity of cells required for each experiment. In all instances, 0.1 ml. of a leukocyte suspension containing 30,000 to 50,000 leukocytes per cu. mm. was added to each flask.

In all experiments the final concentration of sodium β-glycerophosphate was 0.04 molar, adenosine 5'-phosphate 0.002 molar and glucose 1-phosphate 0.002 or 0.004 molar. Magnesium chloride in a final concentration of 0.001 molar and saponin in a final concentration of 0.1 per cent were also added in all incubation mixtures. Barbiturate buffer at pH 9.9 was used as previously described for all phosphatase determinations at an alkaline pH, and acetate buffer at pH 5.0 was used for all determinations at an acid pH. The different substrate molarities were made necessary because of solubility and technical reasons and, while the limitations affect total phosphorus hydrolyzed, they do not limit comparisons as defined above in the objectives of this investigation. It is recognized that utilization of all three substrates at the same molarity would have some inherently desirable features. Adenosine 5'-phosphate is poorly soluble, necessitating its use at low concentrations. Sodium β-glycerophosphate could have been used at this same low concentration. However, this would introduce difficulties in the substrate mixture...
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experiments where the addition of a final concentration of 0.002 molar adenosine 5'-phosphate to 0.002 molar sodium β-glycerophosphate results in doubling of the phosphate bond molarity of the mixture in comparison to that present in the individual substrate experiments. In contrast, using sodium β-glycerophosphate at 0.04 molar concentration and adenosine 5'-phosphate at 0.002 molar results in only minimal change in phosphate bond molarity in the combined substrate studies. Since it was found that substantial hydrolysis of phosphorus occurs at both molarities in single substrate experiments, it was felt that additive effects, if present, could be readily demonstrated in the mixtures. Further, the molarities used also permitted the detection of wide ranges in activity in different cell populations under analysis.

Incubation time was one hour at 37 C. Enzyme activity was then terminated by the addition of trichloroacetic acid in all experiments employing sodium β-glycerophosphate and/or adenosine 5'-phosphate as substrates. Incubation mixtures employing glucose 1-phosphate were inactivated by transferring the tubes to a boiling water bath for five minutes. These were then immersed in ice water for five minutes. Deproteinization was then carried out by the addition of sulphuric acid in an amount sufficient to bring the final concentration to 0.01 molar and to lower pH to 5.2. All tubes were then centrifuged at 2000 rpm for 20 minutes. This modification was made since it was found that lowering the pH below 5.2 with trichloroacetic acid resulted in hydrolysis of the substrate.

Color development was carried out as previously described.1 Slight residual cloudiness was observed occasionally in experiments with glucose 1-phosphate after color development. This was removed readily by centrifugation. All results are hereafter expressed as milligrams of phosphorus liberated per hour by 10⁶ leukocytes from the substrate at 37 C.

RESULTS

A. Comparison of Results Employing Adenosine 5'-Phosphate and Sodium β-glycerophosphate as Substrates at pH's 9.9 and 5.0

Results are summarized in figure 1. Comparison of mean values is made in 14 normal cell populations, cell populations from 5 subjects with neutrophilic leukocytosis and cell populations from 4 subjects with chronic myelocytic leukemia. The subjects grouped for convenience as having neutrophilic leukocytosis had various underlying pathologic states, such as infection, which experience has shown result in elevation of unit cell alkaline phosphatase values. Experiments in which both sodium β-glycerophosphate and adenosine 5'-phosphate were combined in the incubation mixture in the same molarity as when employed separately were done only in determinations at pH 9.9.

Despite the difference in molarity, absolute values are only moderately lower when adenosine 5'-phosphate is employed as substrate than when sodium β-glycerophosphate is used with normal cell populations. However, the discrepancy is more marked when high activity cell populations are employed. The limitations of molarity differences would be expected to be more marked in the latter instance. However, the results clearly show that at pH 9.9, the ability to hydrolyze phosphorus from sodium β-glycerophosphate or adenosine 5'-phosphate appears to follow the same pattern. It is elevated above normal in the nonleukemic leukocytes in both instances and is comparably low in both instances in chronic myelocytic leukemia. Moreover, in combined substrate experiments no additive effect is observed as would usually be expected were two independent enzymes, one a nonspecific phosphomonesterase and the other specific for adenosine 5'-phosphate, present. The results suggest that
at pH 9.9, both substrates are probably being acted upon by the same enzymic or group of enzymes. This is most clearly delineated in subjects with marked phosphatase activity.

The results indicate that at pH 5.0 the activity employing both substrates is variable, but less so. If adenosine 5'-phosphate alone is considered, it is apparent that "adenosine 5'-phosphatase" can be demonstrated either to be more or less active at pH 5.0 than 9.9, depending entirely on which cell populations are compared.

Similar experiments were conducted employing cell populations other than those charted in figure 1. These included populations from subjects with chronic lymphocytic leukemia, infectious mononucleosis, myeloid metaplasia, polycythemia vera and carcinoma with metastases. In all cell populations the activity using the two substrates paralleled each other—being high, low or normal in both instances, depending on the cell population under analysis. No additive effect was noted in combined substrate experiments in any of these additional cases.

B. Comparison of Results Employing Glucose 1-Phosphate and Sodium β-Glycerophosphate as Substrates at pH 9.9

Results are summarized in figure 2. No results are presented at pH 5.0 for glucose 1-phosphate, since nonenzymatic hydrolysis occurs at this pH with
FIG. 2.—A comparison of human leukocyte phosphatase activity at pH 9.9, employing sodium β-glycerophosphate and glucose 1-phosphate as substrates. Cell populations from normal subjects with disease states known to produce elevated alkaline phosphatase values and leukocytosis, and subjects with chronic myelocytic leukemia (C.M.L.) are compared. Values are expressed as milligrams of phosphorus hydrolyzed per hour by 10⁸ leukocytes at 37 C.

Again, it is apparent, however, that at pH 9.9, the same pattern of activity pertains for both sodium β-glycerophosphate and glucose 1-phosphate. Quantitative differences are due to necessary differences in molarity and possibly to some difference in the rate of hydrolysis of different esters. Again, it is clear from figure 2 that there is no additive effect when the combined substrate is employed. In fact, values are obtained intermediate between those for the two substrates individually, suggesting that here, too, an enzyme or enzymes concerned with hydrolyzing the monoester phosphate linkage, rather than enzymes with particular specificity for either of the two substrates, are concerned. The pattern is not so clear in the data where chronic myelocytic leukemic cell populations are concerned, possibly because the low values in this group and the range of experimental error do not permit clear definition. Further, additional experiments in a variety of disease states other than those recorded in figure 2 confirm the data analyzed above.

Figure 3 shows a single instance in which large doses of hydrocortisone were given at four-hour intervals for three days. The characteristic pattern of increasing activity in leukocyte alkaline phosphatase reaching a peak in 72 hours applies equally to glucose 1-phosphate and sodium β-glycerophosphate as substrates. The hydrolyzing activity for both falls markedly three days after the medication is discontinued. It appears, accordingly, that a specific glucose 1-phosphatase cannot be distinguished on this basis at pH 9.9.
Fig. 3.—Effect of administration of hydrocortisone on phosphatase activity of human leukocytes, employing sodium β-glycerophosphate and glucose 1-phosphate as substrates at pH 9.9. Hydrocortisone was administered in the amount of 80 mg. every four hours for three days and then discontinued. Values are expressed as milligrams of phosphorus hydrolyzed per hour by 10^9 leukocytes at 37 C. Results are given before administration of hydrocortisone, at 24 and 72 hours after initiating the drug, and 3 days after stopping the medication.

Discussion

The data suggest that the ability to split phosphorus from adenosine 5'-phosphate, glucose 1-phosphate and sodium β-glycerophosphate is probably due to a single enzyme or identical enzymes at pH 9.9, since similar behavior is manifest in various disease states and, in the case of glucose 1-phosphate, when hydrocortisone is administered. The lack of additive effect when substrates are combined also tends to support this concept, since two enzymes working independently on different substrates at the same time would be expected to yield a higher value for phosphorus hydrolyzed than that obtained from only one substrate. The data do not preclude specific adenosine 5'-phosphatases or glucose 1-phosphatases at other pH values.

Our studies partially confirm those of Swenseid, Wright and Bethell, since hydrolytic activity toward adenosine 5'-phosphate was demonstrated by the leukocytes of all subjects studied. However, the present data indicate that the phosphorus hydrolyzing activity toward either adenosine 5'-phosphate or sodium β-glycerophosphate cannot be categorized as being more or less at pH 5.0 or 9.9 unless a given cell population under analysis is specified. This...
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is largely due to the fact that relative activity at the two pH’s reflects the extremely wide variations possible at pH 9.9. The relative activity toward both substrates is therefore greater at pH 5.0 in cells from subjects with chronic myelocytic leukemia with their characteristic low activity at pH 9.9 but may be just the reverse in cell populations from subjects with infection.

Summary

The ability of human leukocyte enzymes to hydrolyze phosphorus is compared in terms of the conventional substrate sodium β-glycerophosphate and the metabolically important phosphate esters, adenosine 5’-phosphate and glucose 1-phosphate. At pH 9.9, there is marked and comparable variation in phosphatase activity toward all three substrates, this being low in chronic myelocytic leukemia and high in the presence of infection and certain “stressful” states. Moreover, substrate mixture experiments show no increased hydrolysis of phosphorus when two substrates are present in the incubation mixture. Increased phosphatase activity toward both glucose 1-phosphate and sodium β-glycerophosphate resulted when corticosteroids were administered in large doses for 72 hours. The data, while not providing absolute proof, are compatible with the hydrolysis of phosphorus at pH 9.9, being due in the case of all three substrates to the activity of the same phosphomonoesterase or group of phosphomonoesterases. At pH 5.5, phosphatase activity toward both sodium β-glycerophosphate and adenosine 5’-phosphate was likewise demonstrated, but, in leukocytes, the pH of maximal activity varies from subject to subject and is dependent to a large extent on the amount of the highly variable “alkaline phosphatase” activity present in any given cell population at the time of analysis.

Summary in Interlingua

Le capacitate de enyzmas de leucocytos human de hydrolysar phosphoro esseva investigate comparativamente con (1) le substrato conventional, beta-glycerophosphato de natrium, e (2) e (3) substratos del metabolicamente importante esteres phosphatic, 5’-phosphato de adenosina e 1-phosphato de glucosa. A un pH de 9,9, il occurre marcate e comparabile variationes del activitate de phosphatase verso omne le tres substratos. Iste activitate es basse in chronic leukemia myelocytic e intense in le presentia de infecciones e de certe status characterizate per “stress.” In plus, nulle augmentate hydrolyse de phosphoro es manifeste quando duo substratos es presente in le mixtura de incubation. Un augmento del activitate de phosphatase verso 1-phosphato de glucosa e beta-glycerophosphato de natrium occurreva quando corticosteroides eseva administrate in grande doses durante 72 horas. Le datos non prova absolutemente sed permite le theses que le hydrolyse de phosphoro a un pH de 9,9 resulta, in caso de omne le tres substratos, ab le activitate del mesme phosphomonoesterase o del mesme gruppo de phosphomonoesterases. A un pH de 5,5, activitate de phosphatase eseva equalmente demonstrate, tanto verso beta-glycerophosphato de natrium como etiam verso 5’-phosphato
de adenosina, sed in leucocytes le pH del activitate maximal varia ab un
subjecto al altere e depende in grande mesura del variabilissime activitate de
"phosphatase alcalin" que es presente in un particular population de cellulas
al tempore del analyse.

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