Acid Phosphatase in Gaucher's Disease

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Increased acid phosphatase activity in the serum and tissues of patients with Gaucher's disease has now been recognized for two decades, but as yet no relation has been established between the enzyme and the etiology and progress of the disease. Here, we review results obtained by various investigators, ranging from a consideration of the methods used for the evaluation of serum acid phosphatase in Gaucher's disease to the most recent findings regarding the purification and characterization of two acid phosphatase isoenzymes from the spleen from patients with Gaucher's disease. We also discuss the intracellular location of tissue acid phosphatase in patients with Gaucher's disease and its contribution to the increased activity in serum.

Additional Keyphrases: investigative history of clinical, biochemical aspects · intermethod comparison · isoenzymes in serum, plasma, tissues, cells · chromatography on agarose, polyacrylamide, etc. · sphingolipid storage disease · needed · research · leukemic reticuloendotheliosis · Hodgkin's disease · "hairy cell" leukemia · cancer · histochernistry of Gaucher's disease · SP and SP₂ acid phosphatase · tissue of origin · screening · sensitivity to tartrate · physical properties and microheterogeneity of purified isoenzymes · relation to intracellular iron

Introduction and History

Acid phosphatase (orthophosphoric-monoester phosphohydrolase, acid optimum; EC 3.1.3.2) is a ubiquitous enzyme that catalyzes the hydrolysis of various orthophosphate esters.

Increased serum acid phosphatase activity in Gaucher's disease was first reported in 1956, by Tuchman et al. (1), when by chance they noted an above-normal value for acid phosphatase activity in the serum of a 52-year-old woman with a lytic bone lesion in whom the diagnosis of Gaucher's disease was confirmed by positive pathological findings in a bone marrow aspirate drawn 18 years after her splenectomy.

With regard to the question of tissue acid phosphatase in the disease, Crocker and Landing (2) asserted that Farber was the first investigator to appreciate the presence of increased amounts of acid phosphatase activity in the Gaucher cell.

Farber's observations, first recorded in 1941, were later described in a review of Gaucher's disease that was published in 1952 (3). Using homogenates of spleen, Crocker and Landing (2) confirmed that tissue acid phosphatase activity is also above normal in Gaucher's disease.

Since Tuchman and coworkers reported their observations, supranormal values for serum acid phosphatase have been documented in several hundred cases of Gaucher's disease. In fact, the association of increased serum acid phosphatase activity with all three clinical forms of Gaucher's disease is such a common finding that some clinical laboratories now use some type of serum acid phosphatase determination as a noninvasive screening procedure for assisting in the diagnosis of Gaucher's disease in infants, children, or adults. Despite the diagnostic significance of information on acid phosphatase activity in serum and storage organs in patients with Gaucher's disease, we know of no published review of this specific subject in the past two decades.

Our purpose here is to review what has been learned during the last 20 years regarding acid phosphatase activity in Gaucher's disease. We have focused our attention primarily on the clinical aspects and practical considerations that pertain to the increased acid phosphatase activity in the serum of persons with this particular lipid storage disease. We also summarize the results of studies that have been directed towards characterizing the various forms of acid phosphatase that are increased in Gaucher's disease. For more general reviews of the clinical significance of acid phosphatase in other diseases the reader should consult the excellent and informative publications by other investigators (4–7) that have appeared in recent years.

A Brief Description of the Clinical and Biochemical Aspects of Gaucher's Disease

Gaucher's disease is the most common of the sphingolipid storage diseases. It represents an autosomal recessive inborn error of metabolism in which there is an accumulation of the glucosylsphingosine glucocerebroside in intracellular storage deposits of lysosomal origin (8–12). The disease is characterized by the presence of lipid-laden histiocytes throughout the reticuloendothelial system, particularly in the red pulp of the spleen, lymph nodes, liver sinoids, and bone marrow. The cytoplasm of "Gaucher cells" is filled with rod-shaped, membranous inclusion bodies composed mainly of glucocerebroside (13–15). Glucocerebroside, or "ceramide-glucose" as it is sometimes called, is an intermediate in the biosynthesis and catabolism of complex membrane glycosphingolipids and ganglosides. The accumulation of this lipid is due to the profound deficiency of the lysosomal hydrolase, β-glucocerebrosidase (EC 3.2.1.45) (16, 17).

The clinical presentation of Gaucher's disease is grouped into three classifications, all of which exhibit severely decreased tissue glucocerebrosidase activities and increased serum and tissue acid phosphatase activity (2).

The form most commonly encountered is referred to as adult, type I, non-neuronopathic Gaucher's disease. Hepatosplenomegaly is the major visceral manifestation of the adult form, although anemia, thrombocytopenia, and erosion of the cortex in the long bones are also common complications (8, 12). The age at onset and the clinical severity of adult Gaucher's disease vary considerably. Some patients exhibiting hepatosplenomegaly are diagnosed during their first decade, others remain relatively symptom-free for most of their lives.

The second type of Gaucher's disease is designated as in-
fantile or type 2, neuronopathic. Patients with this form of the disease are characterized not only by visceral complications such as hepatosplenomegaly but also by severe central nervous system involvement resulting in strabismus, muscle hypertonicity, and retroflexion of the head. Infantile Gaucher’s disease manifests itself several months after birth and usually results in death from anoxia or respiratory infection before the age of two.

Juvenile, or type 3, subacute neuronopathic Gaucher’s disease is relatively rare. Although the symptomatology varies, patients with this form of the disease have both neurological and visceral involvement, but they generally survive longer than those with the type 2 form of the disease.

The biochemical basis for the variable clinical presentation of Gaucher’s disease has not yet been elucidated. Several studies suggest that there is a correlation between residual tissue glucocerebrosidase activity and the disease severity (16, 18, 19). However, two other groups of investigators failed to demonstrate such a correlation (20, 21).

Methods for Determining Acid Phosphatase Activity in Serum

The Bodansky method. One of the earliest techniques used to assay serum acid phosphatase activity was the procedure of Bodansky (22), in which sodium β-glycerophosphate is the substrate. The assay is performed in diethylbarbiturate buffer at pH 5.0, requires relatively large volumes of serum (0.5–1.0 mL), and involves a rather lengthy (1–3 h) incubation. After the reaction is quenched with trichloracetic acid, the amount of inorganic phosphate liberated during the incubation period is estimated colorimetrically. One “Bodansky unit” of enzyme activity is defined as that amount of enzyme liberating 1 mg of phosphorus per hour, and enzyme activity is expressed on the basis of 100 mL of serum. The reference interval is regarded as 0–1.1 Bodansky units (23, 24).

Although β-glycerophosphate is a relatively specific and particularly useful substrate for detecting the increased acid phosphatase activity in the serum from patients with metastatic carcinoma of the prostate (5), the Bodansky “acid glucocerebrosidase” method frequently fails in identifying serum from cases of Gaucher’s disease where increased acid phosphatase activity can be readily demonstrated by assays in which other phosphatase substrates are used. This problem was first appreciated by Tuchman et al. (25) when they observed that of 12 serum samples from cases of adult Gaucher’s disease that demonstrated increased acid phosphatase values in assays with phenyl phosphate as substrate, only four displayed increased activity when assayed by the Bodansky method. We have had a similar experience in our laboratory: we were able to demonstrate a five- to ten-fold increase in acid phosphatase in serum from patients with Gaucher’s disease by a fluorometric phosphatase assay (26); the same sera had been previously scored as having a normal acid phosphatase value when analyzed by one of the routine colorimetric methods used in the clinical laboratory. In summary, when considering a diagnosis of Gaucher’s disease, one should avoid using a serum acid phosphatase screening procedure in which β-glycerophosphate is used as the phosphatase substrate. Sobotka et al. (27) recognized this fact in 1959 when they wrote: “Since many laboratories determine [acid] phosphatase with glycerophosphate as substrate, they were bound to miss the high phosphatase values in Gaucher’s disease.”

The Gutman method. The Gutman procedure for determination of serum acid phosphatase with phenyl phosphate (28) is essentially a modification of the King and Armstrong assay for serum alkaline phosphatase (29); thus the activity is expressed in King–Armstrong units. One such unit equals 1 mg of phenol liberated per hour at 37 °C per 100 mL of serum. The 10-mL assay mixture, which contains 0.5 mL of serum in citrate buffer (0.1 mol/L, pH 4.9), with 5 mmol/L disodium phenylphosphatase as substrate, is incubated for 3 h, and the liberated phenol is quantitated with Folin–Ciocalteau reagent (28). A modification of this procedure was used by Tuchman et al. (1, 25) when they first documented increased values for serum acid phosphatase in Gaucher’s disease. Since then the “acid phenylphosphatase” assay of Gutman has been used more commonly than any other to suggest or support a diagnosis of Gaucher’s disease. The reference interval for serum acid phosphatase in men is reported by various investigators to be 0.6 to 4.7 King–Armstrong (K.A.) units (5). Using phenyl phosphate as the substrate, Sobotka et al. (27) confirmed the finding of Tuchman’s group and demonstrated increased serum acid phosphatase activity in each of 24 cases of Gaucher’s disease: the acid phosphatase values for the Gaucher patients ranged from 4.8 to 14.3 K.A. units, with a mean of 8.4.

Although phenyl phosphate has proven to be an effective substrate for detecting above-normal acid phosphatase activity in serum of Gaucher’s disease patients, there have been documented cases of Gaucher’s disease in which the Gutman procedure failed to demonstrate an increased value for serum acid phosphatase (2). It is therefore fair to conclude that the Gutman assay is an acceptable procedure to use when considering the diagnosis of Gaucher’s disease; however, it should be realized that 10–15% of the patients with Gaucher’s disease will exhibit serum acid phosphatase values below the upper limit of the control range. The phenylphosphatase assay usually reveals a two- to sixfold increase (relative to the mean for the control group) in serum acid phosphatase activity in Gaucher’s disease (1, 2, 25). It should be emphasized that—because serum acid phosphatase values vary considerably among infants, adolescents, and adults (2)—it is important that one use appropriate age-matched control specimens to determine a reference interval for serum acid phosphatase.

p-Nitrophenyl phosphate. In a direct colorimetric assay introduced originally by Hudson et al. (30) p-nitrophenyl phosphate was incorporated as the acid phosphatase substrate in 0.05–0.10 mol/L sodium acetate buffer at pH 4.5–5.5. The most common colorimetric substrate currently in use in clinical laboratories is p-nitrophenyl phosphate. The primary advantage of this procedure is that once the reaction has been terminated by addition of concentrated base, the extent of p-nitrophenol release can be estimated directly from the absorbance of the solution at 410 nm and the molar absorbivity (molar absorption coefficient) for p-nitrophenolate anion (ε = 13.9 × 10³ L mol⁻¹ cm⁻¹). The principal disadvantages of this method, as well as of the Gutman and Bodansky procedures, are that relatively large volumes of serum (0.1–1.0 mL) are required as the source of enzyme and relatively long incubations (0.5–3.0 h) are needed.

Our own experience with p-nitrophenyl phosphate as a serum acid phosphatase substrate leads us to consider this colorimetric substrate about as effective as phenyl phosphate in the phosphatase assay in demonstrating increased serum acid phosphatase activity in patients with Gaucher’s disease. In one particular study (26) in which we used p-nitrophenyl phosphate as the substrate in incubations conducted in 0.1 mol/L sodium acetate buffer at pH 4.5, serum from six of eight cases of adult Gaucher’s disease exhibited increased acid phosphatase values, the greatest increase relative to the control mean value being about 4.5-fold. However, two of the eight cases showed no significant increase. We have encountered other cases of adult, non-neuronopathic Gaucher’s disease, particularly in persons with barely detectable hepatosplenomegaly, who show unremarkable total serum acid phosphatase values by the p-nitrophenyl phosphate assay (R. H. Glew, unpublished observation).
Other colorimetric phosphatase substrates. α-Naphthyl phosphate and naphthol AS-BI phosphate are two additional chromogenic phosphatase substrates that we find useful in identifying patients with Gaucher’s disease. However, these compounds are not very widely used as phosphatase substrates in clinical laboratories. Using these substrates, Li et al. (7) reported four- to eightfold increases in plasma acid phosphatase activity in two individuals with adult, type 1 Gaucher’s disease.

The fluorometric 4-methylumbelliferyl phosphatase assay. In 1970, Cornish et al. (31) demonstrated that 4-methylumbelliferyl phosphate could be used effectively in the clinical laboratory as a substrate for determination of serum alkaline phosphatase (EC 3.1.3.1). Figure 1 shows the phosphatase reaction for 4-methylumbelliferyl phosphate: the substrate, 4-methylumbelliferyl phosphate, is nonfluorescent in an acid medium (pH <7), but the product of the reaction, 4-methylumbellifere, is fluorescent when the solution is made alkaline by the addition of base, usually glycine–ammonium hydroxide buffer (0.3 mol/L glycine, pH 10.5).

The advantages of speed and sensitivity offered by the fluorogenic phosphatase substrate encouraged us to investigate its usefulness as a reagent for estimation of serum acid phosphatase activity in Gaucher’s disease (26). Furthermore, the successful application of 4-methylumbelliferyl phosphate as an acid phosphatase substrate in studies (32) on the subcellular fractionation and isolation of rat liver lysosomes suggested to us that this fluorogenic phosphate derivative should be a useful substrate for the estimation of serum acid phosphatases of lysosomal origin that are increased in Gaucher’s disease. In our initial study (26) we reported optimum assay conditions for determination of serum acid phosphatase activity in Gaucher’s disease serum. Maximum activity was observed at pH 5.5 to 6.0 in 0.1 mol/L sodium citrate buffer. In exploring the effect of various compounds added to the assay medium, we noted that very high concentrations (0.5 to 3.0 mol/L) of 2-mercaptoethanol enhanced the acid phosphatase activity in serum from patients with Gaucher’s disease. Fortunately, and to our surprise, the acid phosphatase activity present in control serum was markedly (50–90%) inhibited by the same concentration of 2-mercaptoethanol. Taking advantage of this differential effect of 2-mercaptoethanol on the acid phosphatase activities in Gaucher and control serum, we established the following assay for serum acid phosphatase, which has proven to be an effective screening procedure for Gaucher’s disease. The fluorometric assay contains the following constituents in a final volume of 100 μL: 5 μL of serum enzyme, 50 μL of aqueous 4-methylumbelliferyl phosphate (5.0 mmol/L), 10 μL of sodium citrate buffer (1.0 mol/L, pH 6.0), and 35 μL of 8.6 mol/L 2-mercaptoethanol, prepared by diluting neat 2-mercaptoethanol 0.6/1 with distilled water. Reaction mixtures are incubated at 37 °C for 15 min and the reaction is terminated by adding 2.9 mL of the glycine–ammonium hydroxide buffer. A reference blank is prepared in the same manner for each serum specimen, except that the glycine–ammonium hydroxide buffer is added immediately after serum has been introduced into the incubation medium. Samples are mixed thoroughly and their fluorescence is determined (we use a Turner Model 111 fluorometer). Empirical fluorescence values are converted to micromoles of substrate hydrolyzed by using readings for a standard solution of 4-methylumbelliferyl phosphate. It is convenient to prepare a 0.10 mmol/L stock solution of 4-methylumbelliferyl phosphate in chloroform in a Teflon-lined screw-cap tube and to construct a standard curve (i.e., fluorescence values vs mass of 4-methylumbelliferyl phosphate) by adding 5, 10, and 15 μL of the standard solution to 3.0 mL of the glycine–ammonium hydroxide buffer. The acid phosphatase assay as described is linear with up to 0.5 millimolars of enzyme for 30 min. One unit of enzyme activity is defined as 1 μmol of substrate hydrolyzed per minute. Control values for the fluorometric serum acid phosphatase assay, determined by use of 12 serum specimens from normal adults, were as follows: range, 0.5–1.8 milliunits/mL of serum; mean, 0.8 milliunits/mL (26). Using this fluorometric acid phosphatase procedure, we have found a significantly increased acid phosphatase activity in serum from each of 22 cases of Gaucher’s disease, including patients representing both the neuronologic (types 2 and 3) and the non-neuronologic, visceral (type 1) form of the disease. Heterozygous carriers of Gaucher’s disease exhibit acid phosphatase values in the control range.

In our laboratory, for the purpose of detecting the elevated serum acid phosphatase in Gaucher’s disease, we have found the pH 6.0–fluorometric assay to be superior to the more traditional acid phosphatase procedures involving phenyl phosphate or p-nitrophenyl phosphate as the phosphatase substrate. In about 20% of the cases where the diagnosis of Gaucher’s disease was later confirmed by biochemical (i.e., demonstration of glucocerebroside deficiency) and histological (i.e., identification of Gaucher cells in a bone-marrow aspirate) methods, initial screening for the presence of increased serum acid phosphatase activity by these colorimetric methods failed to detect increases that were later documented by using the fluorometric procedure.

The fluorometric assay is particularly useful when there is a suspicion of Gaucher’s disease in an infant or small child, because relatively small volumes of serum are required (5–10 μL). Although of diagnostic value, serum acid phosphatase determination should be regarded only as a screening procedure: a positive result for serum acid phosphatase must be followed by either examination of a bone-marrow aspirate for Gaucher cells or by biochemical analysis of leukocytes or fibroblasts for glucocerebroside (9). Since there have been reported cases of biochemically proven Gaucher’s disease where Gaucher cells were absent from the bone-marrow aspirate (33), the finding of an above-normal acid phosphatase value compels one to analyze peripheral leukocytes or fibroblasts for glucocerebroside (34) or acid β-glucosidase (34). The “pH 5.5–α-taurocholate” β-glucosidase assay procedure of Peters et al. (35, 36) provides a convenient method for confirming the diagnosis of Gaucher’s disease; in this micro-scale assay 4-methylumbelliferyl-β-D-glucopyranoside is used as the substrate, and <0.5 mL of whole blood is required. Because the bone-marrow aspirate represents a relatively painful and invasive procedure, we recommend that suspected cases of Gaucher’s disease first be evaluated for serum acid phosphatase and leukocyte glucocerebroside. However, it is important to realize that other pathologic conditions such as carcinoma of the prostate and Niemann–Pick disease are associated with increased serum acid phosphatase activity when acid phosphatase is determined fluorometrically (727).

Quantitation of serum acid phosphatase isoenzymes in Gaucher’s disease by ion-exchange mini-column chromatography and polyacrylamide gel electrophoresis. Although various colorimetric or fluorometric assays are useful for de-

![Fig. 1. Principle of the fluorometric determination of acid phosphatase activity by use of the artificial substrate 4-methylumbelliferyl phosphate](image-url)
decting increased serum phosphatase activity in patients with Gaucher’s disease, the problem remains of distinguishing Gaucher’s disease from other clinical conditions—most frequently prostatic carcinoma, which also gives rise to increased acid phosphatase activity in serum.

It is now apparent that human serum contains several forms or isoenzymes of acid phosphatase and that Gaucher’s disease is characterized by an increase in a specific serum isoenzyme. The major serum acid phosphatase in Gaucher’s disease can be quantitated and differentiated from the major isoenzyme in the serum of patients with prostatic carcinoma by polyacrylamide gel electrophoresis (38, 39) or ion-exchange chromatography (38, 39). Li et al. (7) used polyacrylamide gel electrophoresis at pH 4.3, followed by an appropriate stain, to separate and make visible the serum isoenzymes and allow cases of Gaucher’s disease, prostatic carcinoma, and other disorders to be distinguished. The specific serum isoenzymes can be quantitated by scanning a stained gel at the absorbance maximum of the phosphatase reaction product (37). Electrophoretic analysis of serum and tissue acid phosphatase isoenzymes is discussed in greater detail below.

Merce (38, 39) has recently reported the use of ion-exchange chromatography on DEAE-Sephadex to distinguish between serum from patients with Gaucher’s disease and serum from patients with prostatic carcinoma. In this method, 1 mL of serum is applied to a mini-column containing DEAE-Sephadex equilibrated in 50 mmol/L Tris·HCl, pH 8.0. Fractions collected from successive elutions with 0.1, 0.2, and 0.3 mol/L sodium chloride are assayed for acid phosphatase activity. Most of the acid phosphatase in serum in Gaucher’s disease is contained in the 0.1 mol/L eluate; in contrast, the principal acid phosphatase in the serum of patients with prostatic carcinoma is eluted with 0.2 mol/L sodium chloride. Mini-column chromatography of serum acid phosphatase isoenzymes can be performed economically, rapidly, and efficiently in the clinical chemistry laboratory, as evidenced by the successful and widespread use of this kind of procedure for quantitation of creatine kinase (EC 2.7.3.2) isoenzymes in the serum of patients with suspected myocardial infarction (40).

Although prostatic acid phosphatase is sensitive to L-(+) tartrate and the predominant serum acid phosphatase in Gaucher’s disease is tartrate-resistant (7), we have not found L-(+) tartrate to be a particularly useful reagent for distinguishing sera from patients with prostatic carcinoma and Gaucher’s disease.

Isoenzymes of Acid Phosphatase That Are Increased in Serum and Tissues in Gaucher’s Disease

The possibility of a relative increase in more than one species of acid phosphatase in the Gaucher cell was first suggested by the results of the histochimical studies performed by Hibbs et al. (41). However, their finding of the phosphatase product generated at acid pH in two subcellular locations does not necessarily mean that there are two distinct acid phosphatases in the same cell (see the next section). The first direct evidence for multiple forms of acid phosphatase in serum and tissues of patients withGaucher’s disease was provided by Li and coworkers (7, 42). Using electrophoresis in polyacrylamide gel (7.5%) at pH 4.3 followed by exposure of the gels to an acid phosphatase activity stain at pH 4.0, it was possible to distinguish acid phosphatase isoenzymes in plasma, leukocytes, and extracts of various tissues. With this procedure, they identified seven isoenzymes in extracts of human leukocytes.

The isoenzymes were designed 0, 1, 2, 3, 3b, 4, and 5, based on their cathodal mobility: isoenzyme 0 does not penetrate the gel and isoenzyme 5 has the greatest mobility. The resulting electrophoretic patterns are referred to as “zymograms.” Isoenzymes 1 through 4 from leukocytes exhibit a degree of cell specificity: isoenzymes 1, 2, and 4 are present in neutrophils and isoenzymes 1 and 4 in monocytes; isoenzyme 3 is the only acid phosphatase in platelets. Isoenzyme 3b was found only in the primary blast cells that are present in the circulation in various types of leukemia. A strong isoenzyme 5 band was demonstrated in the characteristic “hair cell” leukocytes from patients with leukemic reticuloendotheliosis and in the granulomatus spleen tissue of a patient with Hodgkin’s disease and was the only isoenzyme detected by their activity stains that was absolutely resistant to inhibition by 50 mmol/L L-(+) tartrate.

In 1970, Li et al. (42) presented evidence that acid phosphatase isoenzymes 0 and 1 were prominent in extracts of spleen from a patient with Gaucher’s disease. Isoenzyme 0 remained in the stacking gel when electrophoresed (pH 4.3), exhibited a pH optimum of 4.5, and had a relative molecular mass of 143,500, as approximated by sucrose density-gradient centrifugation. Removal of Gaucher cells from the spleen extract by filtration through cotton before electrophoresis led to the conclusion that isoenzyme 0 is confined to Gaucher cells, whereas isoenzyme 1 is associated with splenic leukocytes. However, in 1973 this same group of investigators reported that acid phosphatase isoenzymes 1, 3, and 5 were all present in extracts of spleen from a different Gaucher patient (7). In our laboratory, isoenzyme 0 has not been observed in zymograms of five different Gaucher spleen homogenates, but isoenzymes 1 and 5 have consistently appeared as the major acid phosphatase species.

Isoenzymes 1 and 3 are the major acid phosphatases contained in extracts of various human tissues—including testis, kidney, lung, bone, intestine, and muscle (43). Isoenzyme 2 is the predominant acid phosphatase species in prostate but was not detected in other tissues except as a minor fraction in pancreas and spleen.

In most normal tissues only little isoenzyme 5 activity is usually present, but in leukemic reticuloendotheliosis the bone marrow and spleen become infiltrated with reticulum cells that are rich in this particular species of acid phosphatase (44). In fact, isoenzyme 5, tartrate-resistant acid phosphatase has proven to be a reliable marker enzyme for diagnosis of this type of leukemia (45).

Lam and Yarn (46) purified isoenzyme 5 from human leukemic spleen by more than 270-fold, to apparent homogeneity. This purified preparation exhibited a specific activity of 133 units per milligram (one unit equals 1 μmol of p-nitrophenyl phosphate cleaved per minute) with greatest activity between pH 5 and 6. With regard to substrate specificity, they showed that the enzyme from hairy cells catalyzes hydrolysis of both organic and inorganic pyrophosphates: p-nitrophenyl phosphate, α-naphthyl phosphate, nuclease triphosphates, and pyrophosphate were all hydrolyzed. No significant hydrolysis of the phosphate group of AMP, glucose 6-phosphate, α- and β-glycerophosphate, pyridoxal phosphate, or phosphoserine was detected. Ferrous ion or magnesium ion, at 1 mmol/L final concentration, inhibited enzyme hydrolysis of ATP by about 90%. α-Phenanthroline, a reagent that forms a complex with ferrous ion, inhibited enzyme activity by 40% at a concentration of 0.15 mmol/L. The relative molecular mass of the isoenzyme 5 from hairy cell spleen was estimated to be 84,000 by sucrose gradient centrifugation.

As we shall see in a later section, the characteristic phosphatase of reticulum cells is hairy cell leukemia, which has the same electrophoretic mobility in acid (pH 4.3) polyacrylamide gels as the type 5 isoenzyme present in the Gaucher cell, is

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2 Nonstandard abbreviations used: DEAE, diethylaminoethyl; SP, sulfopropyl; CM, carboxymethyl; QAE, quaternary aminoethyl; Tris, tri(hydroxymethyl)methylamine.
apparently distinct in terms of physical-chemical structure and kinetic properties from the type 5 isoenzyme found in patients with Gaucher’s disease.

In addition to their studies of tissue extracts, Lam et al. (7) also investigated the acid phosphatase isoenzymes in human serum and plasma by zymogram analysis. They demonstrated that normal plasma contains primarily isoenzyme 5 activity, while serum also contains isoenzyme 3 that is released from platelets during blood clotting. Increased plasma acid phosphatase activity was found in cases of prostatic carcinoma (isoenzyme 2), Paget’s disease (isoenzyme 5), and multiple myeloma (isoenzyme 5). Patients with leukemic reticuloendotheliosis or Hodgkin’s disease had normal serum acid phosphatase values when assayed with use of p-nitrophenyl phosphate or α-naphthyl phosphate. Most of the acid phosphatase in Gaucher plasma was resistant to inhibition of L-(+) tartrate and migrated to the position of isoenzyme 5 on acid polyacrylamide gels. Patients with eosinophilia also had increased isoenzyme 5 in their plasma, derived presumably from circulating eosinophils. Although plasma acid phosphatase values in diseases involving proliferation of circulating cells—such as thrombocytopenia and various types of leukemia—were generally not markedly increased, zymogram patterns revealed specific increases in the predominant isoenzymes indigenous to the abnormal cells. The nature of the isoenzymes of acid phosphatase present in serum and tissues of patients with Gaucher’s disease has also been investigated by use of cation-exchange column chromatography. Using mini-columns of SP-Sephadex, one can readily demonstrate the presence of two species of acid phosphatase that are increased in detergent (e.g., Triton X-100) extracts of Gaucher spleen. Crude, unfraccionated extracts of Gaucher spleen with detergent usually contain two to three times more acid phosphatase activity than extracts of control spleen. Chambers et al. (47) demonstrated that when an aliquot of crude Gaucher spleen extract is chromatographed on a column containing SP-Sephadex ion-exchange resin equilibrated in sodium acetate buffer (10 mmol/L, pH 5.8) containing 0.1 mol/L sodium chloride, two peaks of acid phosphatase activity can be resolved (Figure 2) and that each is elevated relative to the control. In nine preparations of Gaucher spleen, 65 to 95% of the total acid phosphatase activity recovered from the column did not bind to the resin, but eluted in the 0.1 mol/L sodium chloride wash fractions. This isoenzyme is designated the “SP1” acid phosphatase. Analytical recovery of total acid phosphatase activity from the ion-exchange column was 55 to 75%. More recently we have used preparative-scale chromatography on SP-Sephadex to separate the acid phosphatase isoenzymes from two different Gaucher spleens. Analytical recoveries of acid phosphatase activity were 100%, and we found the SP1 isoenzyme to represent 30 and 50% of the total acid phosphatase activity.

The SP1 acid phosphatase exhibits the electrophoretic mobility of a type 1 isoenzyme and is truly an “acid” phosphatase, because its pH optimum is 4.5. As shown in Table 1, the SP1 enzyme is completely inhibited by 25 mmol/L L-(+) tartrate, 25 mmol/L sodium fluoride, or 5 mmol/L molybdate, but is resistant to inhibition by 25 mmol/L dithionite and 5 mmol/L cupric ion.

The other prominent acid phosphatase species present in detergent extracts of Gaucher spleen is recovered in the 0.5 mol/L sodium chloride eluate from the SP-Sephadex column and exhibits the electrophoretic mobility of a type 5 isoenzyme. This second acid phosphatase isoenzyme, designated “SP11,” exhibits optimal activity at pH 5.5, and is strongly inhibited by 25 mmol/L sodium fluoride, 25 mmol/L sodium dithionite, 5 mmol/L molybdate, or 5 mmol/L cupric ion (Table 1). The SP11 component is readily distinguished from the SP1 acid phosphatase component on the basis of the resistance of the former to inhibition by 25 mmol/L L-(+)-tartrate. Thus, in terms of resistance to tartrate, the SP11 component is similar to the acid phosphatase that predominates in the serum of persons with Gaucher’s disease.

About 75% of the acid phosphatase activity in control spleen preparations does not bind to SP-Sephadex. However, the principal acid phosphatase in control tissues does not appear to be the same as the Gaucher spleen SP1 acid phosphatase by several criteria, including K_m and pH optimum (R. H. Glew, unpublished observations). Purification and characterization of the acid phosphatases from control tissues, along with careful immunochemical studies, will be needed to answer the fundamental question of whether the increased acid phosphatase activity in the Gaucher cell results from increased de novo synthesis of a unique phosphatase protein or repre

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**Table 1. Comparison of Various Compounds as Inhibitors of the SP1 and SP11 Acid Phosphatases from the Spleen of a Patient with Gaucher’s Disease**

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Final concentration, mmol/L</th>
<th>% Inhibition</th>
<th>SP1</th>
<th>SP11</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-(+)-Tartrate</td>
<td>25</td>
<td>93</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Sodium dithionite</td>
<td>25</td>
<td>5</td>
<td>93</td>
<td></td>
</tr>
<tr>
<td>Cupric sulfate</td>
<td>5</td>
<td>7</td>
<td>94</td>
<td></td>
</tr>
<tr>
<td>2-Mercaptoethanol</td>
<td>2500</td>
<td>92</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>Ferrous sulfate</td>
<td>5</td>
<td>30</td>
<td>69</td>
<td></td>
</tr>
<tr>
<td>Sodium fluoride</td>
<td>25</td>
<td>100</td>
<td>93</td>
<td></td>
</tr>
<tr>
<td>Ammonium molybdate</td>
<td>5</td>
<td>100</td>
<td>97</td>
<td></td>
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</tbody>
</table>

*The following compounds had no effect on the activity of either the SP1 or SP11 acid phosphatase when tested at the indicated final concentrations: formyl-aldehyde (25 mmol/L), magnesium chloride (25 mmol/L), EDTA (5 mmol/L), sodium oxalate (5 mmol/L), α-phenanthroline (1 mmol/L), p-chloromercuribenzoate (0.1 mmol/L), ethanol (1 mmol/L), and ascorbate (30 mmol/L).*
sents the modification or overproduction of an enzyme structure that is already present in normal tissues.

Two forms of acid phosphatase can also be resolved from the serum of patients with Gaucher’s disease [47] by chromatography on SP-Sephadex in sodium acetate buffer (10 mmol/L, pH 5.8). The SP1 serum acid phosphatase is eluted from the column with a dilute (0.1 mol/L) solution of sodium chloride, and the more basic SPII serum enzyme is eluted with 0.5 mol/L sodium chloride. The SP1 serum acid phosphatase has properties similar to those of the SPII isoenzyme of Gaucher spleen, including its electrophoretic mobility in acid polyacrylamide disc gels (7), the $K_m$ for 4-methylumbelliferyl phosphate, its resistance to inhibition by L-(-)-tartrate, pH optimum, and its high affinity for cation-exchange resins such as SP-Sephadex [47].

Figure 3 shows typical profiles obtained when sera from a control individual and a patient with type 1 non-neuropathic Gaucher’s disease are chromatographed on mini-columns of SP-Sephadex. In contrast to Triton X-100 extracts of Gaucher spleen, which often contain equal amounts of the SP1 and SPII type acid phosphatases, Gaucher serum contains mostly acid phosphatase of the SPII type, 20- to 50-fold more than in control sera. Control serum, so fractionated, yields about equal amounts of the homologous isoenzymes. Both the SP1 and the SPII species of acid phosphatase are increased in serum from both nonneuropathic and non-neuropathic cases of Gaucher’s disease, but nevertheless the differences between control and patient acid phosphatase activities are greatest in the SPII fraction, and increased serum SPII acid phosphatase is highly characteristic of Gaucher’s disease. Moreover, acid phosphatase isoenzyme 2 from the serum of patients with prostatic carcinoma appears in the 0.1 mol/L sodium chloride eluate and is therefore easily distinguished from the SPII acid phosphatase (R. H. Glew, unpublished observation).

### Intracellular Location of Tissue Acid Phosphatase in Gaucher’s Disease

Acid phosphatase has a dual localization in the Gaucher cell. Hibbs et al. [41] used histochemical techniques and electron microscopy to identify subcellular locations of acid phosphatase activity in splenic Gaucher cells at various stages of lysosomal involvement. Both sodium $\beta$-glycerophosphate and cytidine monophosphate were used as substrates in incubations that were carried out at pH 5.0. Acid phosphatase activity in Gaucher cells was localized to the intertubular spaces of the cytoplasmic storage deposits that are believed to be of lysosomal origin. The amount of acid phosphatase activity varied with the structure and apparent stage of development of the lysosomal inclusion bodies. The most intense acid phosphatase reaction was localized to the intertubular spaces of the more compact, smaller cytoplasmic bodies indigenous to smaller Gaucher cells. In larger Gaucher cells that contained dilated (often fused) storage deposits, acid phosphatase activity was restricted to those areas of the cell that contained the more tightly packed tubules, and it was absent from the larger, distended bodies. In cells of the splenic sinuosids, acid phosphatase was not confined to the lysosomal storage bodies but was also shown to be present in portions of the endoplasmic reticulum that were contiguous with dense cytoplasmic deposits. These particular deposits contained some Gaucher cell-like tubular structures, but they did not yet harbor significant acid phosphatase activity. These investigators postulated that the dense bodies inherit their acid phosphatase from the endoplasmic reticulum as they develop and concurrently take on the characteristic tubular appearance. The apparent tubules are, in fact, not hollow rods but actually stacks of sheets of membrane composed largely of glucocerebrosides [14, 15]. As these bodies increase in size the tubules become detached and their content of acid phosphatase activity appears to diminish. Consequently, very little acid phosphatase activity is apparent on the distended tubular bodies of the largest Gaucher cells. In the most mature of the Gaucher cells these bodies appear to fuse occasionally with the plasma membrane, whereupon they excrete or discharge their contents into the extracellular space.

In their histochemical studies, Hibbs et al. did not address the question of whether the acid phosphatase activities in the endoplasmic reticulum and the lysosomes of the Gaucher cell were due to a single species of phosphatase or if a distinct isoenzyme was associated with each organelle. Our studies on the substrate specificity of purified acid phosphatases from Gaucher spleen (see the section on Progress . . . ) indicate that CMP and $\beta$-glycerophosphate, the substrates used by Hibbs et al. [41], are cleaved only by the SP1 acid phosphatase isoenzyme. Consequently, their technique for localizing acid phosphatase in Gaucher spleen would not have detected the SP1 acid phosphatase. The extralysosomal acid phosphatase they observed in the endoplasmic reticulum was probably also of the SP1 variety and progenitor of the lysosomal enzyme. The same investigators demonstrated the hydrolysis of thiamine pyrophosphate at pH 7.2 in the Golgi apparatus and of ATP in the plasma membrane gap junctions of the Gaucher cell. Both of these compounds are excellent substrates for the SP1 acid phosphatase, but not for the SP1 isoenzyme. Therefore, some of the hydrolysis of these compounds could have been the result of residual activity of the SPII acid phosphatase at neutral pH and may indicate an extralysosomal site for the isoenzyme. In this regard, it would be useful to study subcellular fractions of fresh Gaucher spleen and to analyze and compare the properties of the lysosomal acid phosphatase associated with the glucocerebroside-rich storage deposits with those of microsomal and Golgi acid phosphatase from the same tissue.

The question of the subcellular location of the two principal acid phosphatases of the Gaucher cell has been answered in part by the isolation of the acid phosphatase-rich intralysosomal storage deposits by equilibrium ultracentrifugation in a sucrose density-gradient. Extraction of the purified glucocerebroside-rich deposits with the detergent Triton X-100, followed by chromatography of the solubilized acid phosphatase, presented an excellent opportunity to study the enzyme's properties.
phatase activity on a SP-Sephadex column, revealed that essentially all of the acid phosphatase was of the SP₁, tar-
trate-sensitive type (47). The subcellular localization of the
SP₁₁ acid phosphatase remains to be determined.

The demonstration by Hibbs et al. (41) of acid phosphatase
activity in two different organelles of the Gaucher cell is
reminiscent of the findings of Lin and Fishman (48), who
demonstrated the presence of two different acid phosphatases
in mouse kidney cells. One isoenzyme was localized to the
microsomal fraction, was not inhibited by L-(+)-tartrate, and
had a pH optimum of 6.3, while the other was of lysosomal
origin, had a lower pH optimum (5.6), and was inhibited by
L-(+)-tartrate. Extralysosomal locations of acid phosphatase
have been demonstrated in various tissues (49-52).

Also noteworthy in this regard is the report (53) concerning
the tartrate-resistant acid phosphatase that is prominent in
the reticulum cells of patients suffering from hairy cell leu-
kemia, also called "leukemic reticuloendotheliosis." Lam et
al. (53) showed that the type 5 acid phosphatase isoenzyme
has a dual localization in the hairy cell, being present in both
the lysosomal and microsomal fractions separated by sucrose
gradient centrifugation. It is tempting to conclude from their
study that a single molecular species of acid phosphatase can
exist in two different organelles of the same cell. However, one
must be cautious in drawing conclusions based on the co-
electrophoresis of enzymes on polyacrylamide gels, because
two distinct protein structures can exhibit the same electrophoretic
mobility under a given set of experimental conditions.
In fact, Lam et al. (53) demonstrated that the microsomal and
lysosomal type 5 isoenzymes from hairy cell spleen eluted at
unique positions when chromatographed on CM-cellulose
columns.

Once antibodies directed against the several distinct acid
phosphatases of the Gaucher cell are available, it will be of
interest to inquire into the subcellular location of the various
acid phosphatase isoenzymes by using immunological-ultra-
structural methods of analysis. The availability of appro-
priate antibodies will also make it possible to investigate the
cross reactivity and structural relatedness of the increased
serum and tissue acid phosphatases in Gaucher's disease and
other disorders.

The Origin of Serum Acid Phosphatase in
Gaucher's Disease
Crocken and Landing (2) suggested that the increased
serum acid phosphatase activity in patients with Gaucher's
disease arises by spillage of the enzyme from the glucocere-
brosidase-rich storage cells of the spleen and other tissues. They
observed that serum acid phosphatase values decreased pre-
cipitously (by 40 to 76%) in five children after splenectomy.
In the months after splenectomy the serum acid phosphatase
values in four of these same five cases gradually returned to
what they were pre-operatively. In the fifth case, the serum
acid phosphatase value remained low for six months. The
concept of tissue leakage of acid phosphatase was substan-
tiated by the observation that cultured Gaucher spleen cells
excrete the enzyme into the growth medium (54).

Lysosomal enzymes can leak from damaged cells under
conditions that leave the lysosomal membrane intact. It has
been observed that fixation of glial cells at too low an osmotic
pressure results in lysosomal acid phosphatase leaking through
apparently intact membranes (55). Perhaps lysosom-
al enzymes can escape as a result of other types of cell injury
such as that which occurs in the glucocerebrosidase-rich, glu-
cocerebrosidase-deficient storage cells in Gaucher's disease.
Furthermore, the damage that the lysosomal and plasma
membranes of the Gaucher cell incur conceivably is subtle,
and only certain proteins and enzymes with the proper size
or charge may be able to pass through the altered cellular
membranes.

As noted previously, Hibbs et al. (41) observed that the
lysosomal storage deposits of the Gaucher cell occasionally
fuse with the plasma membrane and appear to excrete their
contents into the extracellular medium. The acid phosphatase
isoenzyme present in the storage deposits of the Gaucher
cell has been shown to be of the tartrate-sensitive SP₁ type (47)
and could be the source of the increased tartrate-labile acid
phosphatase fraction observed in serum from Gaucher's dis-
ease. However, the serum SP₁ enzyme appears to be somewhat
different from the spleen SP₁ component separated on SP-
Sephadex columns. The enzyme in serum does not migrate
to the isoenzyme 1 position on acid polyacrylamide gels and
exhibits a different pH optimum (6.0 vs 4.5); these differences
may be the result of a modification of the spleen enzyme upon
its release into the serum.

The major acid phosphatase in Gaucher serum is of the
tartrate-resistant SP₁₁ type, and is probably not derived from
Gaucher cell lysosomal storage deposits, which contain tar-
trate-sensitive acid phosphatase of the SP₁ type (47). On the
other hand, the SP₁₁ acid phosphatase component of
Gaucher-disease serum does exhibit the same electrophoretic
mobility, affinity for cation-exchange resin, and sensitivity
to inhibitors (47) as the Gaucher spleen SP₁ isoenzyme, and
probably does arise by some form of direct tissue spillage.
Streifler (56) demonstrated a similarity in thermostability,
pH optimum, and electrophoretic properties of acid phos-
phatase isoenzymes from Gaucher serum and normal human
spleen lysosomes. However, the lysosomal acid phosphatase
from controls was strongly (98%) inhibited by 20 mmol/L
L-(+)

L-qtartrate, but the Gaucher serum acid phosphatase was not as strongly (48%) inhibited.

Although the reported (2) diminishing effect of splenectomy
on serum acid phosphatase values supports the concept of
spillage from the spleen, contradictory data in that same re-
port appear to negate such a straightforward conclusion. The
problem arises from the fact that Crocken and Landing (2)
demonstrated that Gaucher cell acid phosphatase was com-
pletely inhibited by L-(+)-tartrate, but the acid phosphatase
in serum from Gaucher patients, either before or after splen-
ectomy, was largely resistant to inhibition by L-(+)

L-tartrate. If simple tissue spillage provided the source of increased serum
acid phosphatase in Gaucher's disease, then the major acid
phosphatases demonstrable in spleen and serum should have
the same sensitivity to L-(+)
sodium tartrate. Unfortunately,
Crocken and Landing measured spleen acid phosphatase ac-
ivity with β-glycerophosphate, a substrate that is not cleaved
by the tartrate-resistant SP₁ acid phosphatase but which is
an excellent substrate for the SP₁ acid phosphatase (see the
next section). On the other hand, serum acid phosphatase
activity in the Gaucher patients they studied was determined
by using phenyl phosphate, which is a substrate for both the
SP₁ and SP₁₁ forms of serum acid phosphatase. Consequently,
the results of their study, taken at face value, would not sup-
port the concept of simple spillage from the spleen.

We have seen that while the SP₁ and SP₁₁ components of
acid phosphatase are present in about equal amounts in
Gaucher spleen, the SP₁₁ form of the enzyme predominates
in serum. There are at least two processes that could be in-
voked to modify the simple spillage hypothesis of Crocken and
Landing in such a way as to be consistent with the known
properties of the Gaucher spleen and serum acid phosphat-
ases: (a) selective secretion or release of the tartrate-resistant
SP₁ isoenzyme from the Gaucher cell and (b) selective inac-
tivation or excretion of the tartrate-sensitive SP₁ isoenzyme
in Gaucher serum.

If one considers the first suggestion, it is conceivable that
the SP₁₁ isoenzyme, which is considerably smaller than the SP₁

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Table 2. Serum Acid Phosphatase Activities in a Patient with Gaucher's Disease Before and After Splenectomy

<table>
<thead>
<tr>
<th>Days relative to surgery</th>
<th>Total serum acid phosphatase (units/L)</th>
<th>Isoenzyme ratio a</th>
</tr>
</thead>
<tbody>
<tr>
<td>-70 to -60</td>
<td>46.6 - 51.6 b</td>
<td>2.0</td>
</tr>
<tr>
<td>+3</td>
<td>23.0</td>
<td>1.6</td>
</tr>
<tr>
<td>+6</td>
<td>24.1</td>
<td>2.3</td>
</tr>
<tr>
<td>+70</td>
<td>54.0</td>
<td>2.2</td>
</tr>
<tr>
<td>+300</td>
<td>43.0</td>
<td></td>
</tr>
</tbody>
</table>

a Acid phosphatase isoenzymes were separated by column chromatography on SP-Sephadex.

b Values for control serum are: mean, 8.5 units/L and range, 4.4 - 12.7 units/L.

species (see the next section), escapes preferentially from the Gaucher cell and therefore much more of it than the SP1 isoenzyme is present in Gaucher serum. Alternatively, the SP1 and SP2 isoenzymes may both escape from the Gaucher cell with equal ease, in which case the SP1 isoenzyme would have to be more rapidly eliminated or inactivated in the Gaucher patient. Conversely, the SP2 acid phosphatase could be preferentially activated upon release into the plasma compartment. In this regard, recall the observation of Lam et al. (7) that isoenzyme 5 from extracts of Gaucher spleen actually increased in activity when incubated in plasma in vitro while the electrophoretically slower moving, tartrate-sensitive isoenzyme 1 that corresponds to the SP1 acid phosphatase remained unchanged.

We recently had the opportunity to follow serum acid phosphatase activity and isoenzyme profiles on SP-Sephadex before and after splenectomy in a 71-year-old man with Gaucher's disease. As shown in Table 2, total serum acid phosphatase activity, determined at pH 6.0 in sodium citrate buffer with 4-methylumbelliferyl phosphate as substrate, declined by about half after surgery. Within 70 days the serum acid phosphatase had returned to pre-operative values. We determined the relative amounts of the SP1 and SP11 isoenzymes in each of the serum samples by column chromatography on SP-Sephadex. The ratio of the SP11 to SP1 acid phosphatase remained constant at about 2:1 all during the study and was not influenced by the decrease in total serum acid phosphatase activity that occurred after splenectomy. The fact that the activity of acid phosphatase in serum never reached the control value, but was only decreased by half, indicates that the increased serum acid phosphatase probably originates not only from the spleen but also from other involved storage tissues and organs. The liver is probably the major source of the increased serum acid phosphatase in the splenectomized patient. The fact that the isoenzyme ratio did not change significantly after splenectomy indicates that there is probably no organ specificity with regard to the production or leakage of the two species of acid phosphatase that are increased. It would be worthwhile to see whether the proportion of acid phosphatase species is indeed the same in liver as in spleen.

Progress on the Purification and Characterization of the Properties of Splenic Acid Phosphatases in Gaucher's Disease

Until recently, in most investigations of the kinetic and physical-chemical properties of the acid phosphatase(s) in Gaucher's disease crude homogenates of Gaucher spleen have been used, little effort being directed toward purification and characterization of individual isoenzymes. Because it is now apparent that Gaucher spleen contains at least two principal forms of acid phosphatase (47), it is clear that earlier studies in which crude tissue homogenates were used as a source of enzyme actually yielded data that were attributable to the activities of a mixture of distinct enzymes. In view of the fact that the diagnostic value of acid phosphatase activity measurement in serum of patients with Gaucher's disease has been appreciated for nearly 25 years, together with the long-standing recognition that the Gaucher cell itself contains abundant acid phosphatase, it is surprising that the purification and characterization of tissue acid phosphatases in Gaucher's disease is still unreported. Indeed, much of what we know about acid phosphatase in Gaucher's disease comes from studies performed in 1960 by Crocker and Landing (2), using crude tissue extracts as a source of enzyme. They demonstrated that homogenates of Gaucher spleen have about a threefold increased acid phosphatase activity when assayed at pH 5 with β-glycerophosphate as the substrate. Despite this increased activity, total spleen phosphorous concentrations were normal. Based on results of a histochemical study, spleen acid phosphatase activity was shown to be neither activated by various divalent cations nor inhibited by formaldehyde or ethylenediaminetetraacetate. However, tissue acid phosphatase activity was decreased significantly by ferric and cupric ions, and was completely inhibited by sodium fluoride and L-(+)-tartrate. With sodium fluoride as substrate in assays performed at pH 5, the increased acid phosphatase activity in saline homogenates of Gaucher spleen was shown to be very sensitive to 10 mmol/L sodium fluoride (92% inhibition) and 10 mmol/L L-(+) tartrate (83% inhibition). Acid phosphatase activity in Gaucher-disease serum, with phenyl phosphate as substrate in incubations conducted at pH 5, was almost completely inhibited by 10 mmol/L sodium fluoride (90% inhibition), but formaldehyde (5 mL/L) or 10 mmol/L L-(+) tartrate had considerably less effect (30 and 18% inhibition, respectively).

Total spleen acid phosphatase exhibited a broad hydrogen ion concentration dependency, with optimum activity at pH 3.5 to 4.4. However, as we now appreciate, there are actually two species of acid phosphatase present in crude extracts of Gaucher spleen. Crocker and Landing used such a preparation to investigate substrate specificity and found that nucleoside triphosphates (particularly ATP), phosphoenolpyruvate, and thiamine pyrophosphate are all excellent substrates for Gaucher spleen acid phosphatase when assayed at pH 4.1.

Once we recognized the fact that Gaucher spleen contains a mixture of different acid phosphatases, we set about the task of separating and purifying the two principal isoenzymes, using 4-methylumbelliferyl phosphate as the phosphatase substrate. We have recently accomplished the extensive purification of both species of acid phosphatase from Gaucher spleen that correspond to isoenzymes 1 and 5 in the acid gel electrophoretic system of Li et al. (42). Table 3 summarizes the physical properties of these enzymes.

About 200 g of spleen from a patient with adult, type 1, non-neuropathic Gaucher's disease was homogenized in Tris·HCl buffer, pH 8.0, supplemented with Triton X-100. The 100 000 × g supernate was dialyzed and applied to a column of SP-Sephadex equilibrated with sodium acetate buffer (10 mmol/L, pH 5.5) containing 0.1 mol of sodium chloride per liter. The two major isoenzymes present in Gaucher spleen were readily separated by this procedure.

The SP11 acid phosphatase (a type 5 isoenzyme), which binds tenaciously to SP-Sephadex, was eluted from the resin by a linear gradient from 0.1 to 0.8 mol of sodium chloride per liter and constituted about half of the total acid phosphatase present in the homogenate of the Gaucher spleen. Fractions containing the SP11 acid phosphatase component were pooled and further purified (24 000-fold) to a final specific activity
of 1490 units (micromoles of 4-methylumbelliferyl phosphosphate hydrolyzed per minute) per milligram of protein.

Purification was accomplished by chromatography on CM-Sephadex, hydroxylapatite, Sephadex G-150, and concanavalin A-Sepharose. When stained for both protein and activity after disc-gel electrophoresis, the final enzyme preparation appeared as a tight doublet on electrophoresis in 150 g/L polyacrylamide gels at pH 4.3. We do not know whether the presence of two closely related acid phosphatase species in the SP11 preparation is ascribable to proteolytic digestion during the isolation process or is a demonstration of natural microheterogeneity. The latter would be unsurprising. Microheterogeneity may reside in the variable carbohydrate domain of the enzyme; we have found the enzyme to be a glycoprotein. The microheterogeneity in preparations of prostatic acid phosphatase is known to reflect the different numbers of neuraminic acid residues attached to a single enzyme protein (57). However, extensive digestion of the SP11 preparation from Gaucher spleen with neuraminidase (EC 3.2.1.18) did not generate a single electrophoretic species; instead it created several more anodally migrating species of acid phosphatase. The SP11 isoenzyme is a very basic protein, as evidenced by the high isoelectric point (pI, 8.5) determined for it by isoelectric focusing. This fact explains the tenacious binding of the SP11 form of acid phosphatase to SP-Sephadex columns at pH 5.5. Furthermore, the basic nature of the enzyme and its relatively small size (molecular mass about 34 000 daltons) account for its rapid cathodal mobility, which is characteristic of a type 5 isoenzyme under the acid disc-gel electrophoretic conditions of Li et al. (42). Results of electrophoreses in polyacrylamide gel in the presence of sodium dodecyl sulfate indicate that the enzyme may be composed of unequal subunits with relative molecular masses of 16 000 and 20 000.

Although the SP11 acid phosphatase of Gaucher spleen and the acid phosphatase of the hairy cell in leukemic reticuloendotheliosis have identical electrophoretic mobilities in acid disc gels, the two enzymes are distinct. Yam et al. (43) reported a relative molecular mass of 64 500 for the hairy cell acid phosphatase. Furthermore, the purified phosphatase from hairy cells has a specific activity that is less than 10% that of the purified SP11 component that we have isolated from Gaucher spleen. Finally, isoenzyme 5 from hairy cells is actually a pyrophosphatase (43), whereas the SP11 acid phosphatase isoenzyme from Gaucher spleen does not catalyze the hydrolysis of inorganic pyrophosphate.

With regard to substrate specificity (Table 4), the purified SP11 isoenzyme exhibits its highest activity on 4-methylumbelliferyl phosphate, p-nitrophenyl phosphate, and nucleoside triphosphates. Intense acid nucleoside triphosphatase (EC 3.6.1.15) activity was also demonstrated by Crocker and Landing, using crude homogenates of Gaucher spleen as a source of enzyme (2). The purified preparation of the SP11 acid phosphatase also exhibited a substantial amount of phosphoprotein phosphatase (EC 3.1.3.16) activity on the phosphoprotein phosphitin. The Gaucher enzyme has a substrate specificity and pH optimum that are very similar to the 23 000-dalton phosphoprotein phosphatase from bovine spleen (59).

The SP11 acid phosphatase component contained in the 0.1 mol/L sodium chloride eluate from the SP-Sephadex column was purified extensively on columns of QAE-Sephadex, DEAE-cellulose, hydroxylapatite, concanavalin A-Sepharose, Sephadex G-200, Cibacron Blue-Sepharose, and Sepharose 4-B. The purest preparation was greater than 90% pure as judged by polyacrylamide disc gel electrophoresis (pH 8.9) in the absence of sodium dodecyl sulfate. This particular species of acid phosphatase from Gaucher spleen has a relative molecular mass of about 180 000, as estimated from data obtained by gel filtration chromatography and velocity sucrose gradient sedimentation. The SP11 acid phosphatase yields two bands of protein when subjected to polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate; these bands have relative molecular masses of 67 000 and 111 000, bear a 1:1 molar relationship to each other, and both take up the periodic acid-Schiff stain, indicating that each subunit is a glycoprotein. This acid phosphatase isoenzyme of higher mass is a true acid phosphatase (pH optimum, 4.5) and is characterized by a relatively low isoelectric point (pI, 4.1). The large size and low isoelectric point account for the fact that the enzyme remains at the anode, as a type 1 isoenzyme, and barely penetrates acid polyacrylamide disc gels. The specific activity of our most nearly pure preparation of the SP11 isoenzyme on 4-methylumbelliferyl phosphate is about 40 units per milligram, much lower than that of the purified SP11 phosphatase (1500 units per mg). With regard to substrate specificities:

Table 4. Substrate Specificity of SP11 Acid Phosphatase

<table>
<thead>
<tr>
<th>Substrate Specificity (kilounits/g protein)</th>
<th>Specific activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Artificial substrates, 10 mmol/L</td>
<td></td>
</tr>
<tr>
<td>4-Methylumbelliferyl phosphate</td>
<td>1490</td>
</tr>
<tr>
<td>p-Nitrophenyl phosphate</td>
<td>1490</td>
</tr>
<tr>
<td>Phenolphthalein diphosphate</td>
<td>830</td>
</tr>
<tr>
<td>Phenyl phosphate</td>
<td>680</td>
</tr>
<tr>
<td>α-Naphthyl phosphate</td>
<td>430</td>
</tr>
<tr>
<td>Natural substrates, 10 mmol/L</td>
<td></td>
</tr>
<tr>
<td>Thiamine pyrophosphate</td>
<td>580</td>
</tr>
<tr>
<td>Phosphoenolpyruvate</td>
<td>410</td>
</tr>
<tr>
<td>GTP</td>
<td>960</td>
</tr>
<tr>
<td>CTP</td>
<td>840</td>
</tr>
<tr>
<td>ATP</td>
<td>1000</td>
</tr>
<tr>
<td>UTP</td>
<td>790</td>
</tr>
<tr>
<td>ADP</td>
<td>650</td>
</tr>
<tr>
<td>UDP</td>
<td>680</td>
</tr>
<tr>
<td>GDP</td>
<td>420</td>
</tr>
<tr>
<td>CDP</td>
<td>410</td>
</tr>
<tr>
<td>Phosvitin</td>
<td>60</td>
</tr>
</tbody>
</table>

* The following compounds were not substrates (specific activity <25 units per mg): α-glycerophosphate, β-glycerophosphate, glucose 1-phosphate, glucose 6-phosphate, mannose 6-phosphate, fructose 1,6-diphosphate, 3-phospho-D-glycerate, pyrurate, lactate, pyruvate, fumarate, malate, citrate, phosphate, pyrophosphate, diphosphate, triphosphate, tetrakis-hydroxymethylphosphionoate, ATP, GTP, UTP, CTP, AMP, GMP, UDP, CDP, GDP, and phosphatase.
specificity (Table 5), the SP1 acid phosphatase from Gaucher spleen hydrolyzes a large and diverse group of organic phosphates, including α-naphthyl phosphate, phenyl phosphate, 4-methylumbelliferyl phosphate, phosphoenolpyruvate, fructose 1,6-diphosphate, cytidine monophosphate, and phenolthalein diphosphate. Unlike the SPII isoenzyme, the SP1 acid phosphatase exhibits no phosphoprotein phosphatase activity, but will catalyze hydrolysis of O-phospho-L-serine.

The presence of low- and high-molecular mass forms of acid phosphatase in Gaucher spleen is consistent with the report of De Aravjo et al. (52), in which they described the separation and subcellular localization of low (≤30,000) and high (>100,000) relative molecular-mass species of acid phosphatase from rat brain, liver, and kidney. The acid phosphatases of low molecular mass were soluble cytosolic enzymes that were not affected by L-(+)-tartrate or p-mercuribenzoate and were highly substrate specific. The high-molecular-mass acid phosphatases were associated with lysosomes and were inhibited by L-(+)-tartrate and p-mercuribenzoate.

Currently, we are trying to isolate milligram quantities of the two major acid phosphatases of Gaucher spleen, so as to provide material for the preparation of antibodies and to support (a) comparative physical–chemical studies that include determination of amino acid and carbohydrate composition and (b) kinetic analyses and structure–function investigations.

### Directions for Future Research

The literature is replete with reports describing the isolation, purification, and characterization of the physical–chemical and kinetic properties of acid phosphatases from a wide variety of mammalian tissues. Despite our extensive knowledge concerning the substrate specificity, inhibitor sensitivity, subcellular location, subunit structure, and chemical composition of many of these enzymes, the physiological function of lysosomal and extralysosomal mammalian acid phosphatases remains obscure. The physiological function of the acid phosphatases in normal tissues is unclear, so it is not surprising that we are equally ignorant of the pathological consequences or role of the two principal acid phosphatase isoenzymes that are increased in Gaucher cells. Clearly, to gain answers to these questions, additional information is needed. Furthermore, it seems that we should focus new efforts and investigations initially on the more fundamental problems in the areas of structure–function and cell biology with the hope that progress in these basic areas will enlighten us as to the physiological role and possible pathological consequences of the high intracellular acid phosphatase activity in Gaucher’s disease.

In the search for physiological substrates, useful information would be obtained from studies on the ability of the acid phosphatases of the Gaucher cell to utilize various phosphorylated proteins as substrates. Since erythrocytes are phagocytized by reticuloendothelial cells that ultimately develop into Gaucher cells, it would be of interest to determine if the phosphorylated membrane proteins of the erythrocyte membrane (59) will serve as substrates for the SP1 or SPII acid phosphatases from Gaucher spleen. Appropriate phosphorylated protein substrates could be prepared by incubating erythrocytes with $[^\gamma^3P]ATP$. If orthophosphate is released from the labeled membrane by either acid phosphatase iso-enzyme, analysis of the membrane proteins by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate would reveal which phosphorylated proteins were serving as phosphatase substrates.

The results of recent studies by Kaplan et al. (60) on the determinants involved in the uptake of glycoprotein enzymes by cells suggest additional proteins that should be added to the list of potential phosphorylated protein substrates for acid phosphatases: the phosphorylated mannosyl residues of the glycoproteins that are recognized and taken up by cells may be cleaved by intracellular phosphatases. Perhaps through the removal of phosphate residues, acid phosphatases play a role in capturing enzymes that have been taken up by cells.

Finally, one should consider the proteins and enzymes that are phosphorylated by cAMP-dependent protein kinases as possible substrates for the acid phosphatases from the Gaucher cell. If any of these phosphoproteins are demonstrated to be substrates, then one may be able to construct hypotheses concerning the possible pathological effects that increases in a particular acid phosphatase might have on Gaucher cells located in the viscera or brains of patients with Gaucher’s disease.

With regard to possible pathological effects resulting from supranormal intracellular acid phosphatase activity, availability of pure preparations of the acid phosphatases from the Gaucher cell would make it possible to study the consequence of injecting these enzymes into single cells by micro-manipulation techniques. Perhaps biochemical and ultrastructural analysis of such cells would provide insight into the bases of the detrimental effects of these acid phosphatases.

Still further work is needed to establish the subcellular location of the SP1 and SPII acid phosphatases in the Gaucher cell by use of standard cell-fractionation techniques and immunological methods. The availability of antibodies directed
against pure preparations of the SP₁ and SP₁₁ enzymes from Gaucher spleen will permit the use of the immunoperoxidase technique or ferritin labeling to localize these acid phosphatases within the Gaucher cell.

The final area of needed research is a broad one involving physical–chemical and kinetic studies. Efforts should be directed at the preparation of sufficient quantities of pure SP₁ and SP₁₁ acid phosphatases from Gaucher spleen to support structural studies. It would be useful to characterize the primary structure of the subunits of these enzymes and to establish the composition and absolute structure of their oligosaccharide side chains. It would also be interesting to explore the role of hydrophobic domains in those acid phosphatases in an effort to improve our understanding of the manner in which they are inserted into, or associated with, cellular membranes; the use of synthetic lipid vesicles would facilitate these kinds of studies.

Finally, with regard to physiological function, it is noteworthy that during the digestion of erythrocytes by spleen macrophages the disappearance of hemoglobin is followed by an increase in the number of ferritin granules and in acid phosphatase activity in lysosomes (61). Is acid phosphatase activity involved in the processing of iron in the reticuloendothelial cell? It would be useful to explore the possibility of interactions between acid phosphatases and iron-containing proteins such as hemoglobin, ferritin, and transferrin. In that regard, it is noteworthy that a strong correlation has been noted between acid phosphatase activity and intracellular iron in Gaucher cells in different tissue (R. E. Lee, unpublished observations).

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References


