was about two times higher than that by the β anomer. In the medium with the mixture of both anomers, the value (20.4 ± 2.4) of secreted insulin lay between those in the media with the α or β anomer alone. Considering the rapid mutarotation of one anomer to the other during the incubation, the real difference between the effectiveness of the α and β anomers on insulin secretion is considered to be more than that shown in Fig. 2.

It was recently reported that there was a significantly greater protection of the pancreatic beta cells by the α anomer of D-glucose against the diabetogenic effect of alloxan as compared with the β anomer (*8*). The protective effect of D-glucose has been assumed to occur at the beta cell membrane (*9, 10*), where the receptor and transport systems for D-glucose are considered to be involved. However, insulin secretion does not seem to be governed by the D-glucose transport itself, since phlorizin inhibited D-glucose transport, whereas the D-glucose stimulated insulin release remained unaffected by phlorizin (*11*).

These and our results suggest the possibility that the pancreatic beta cells distinguish the α and β anomers of D-glucose for triggering insulin secretion at the receptor site of the cell membrane.

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Distinct Alkaline Phosphatase in Serum of Patients with Lymphatic Leukemia and Infectious Mononucleosis

Abstract. *A distinct alkaline phosphatase (phosphatase N) was demonstrated in the serum of patients with acute lymphatic leukemia, chronic lymphatic leukemia, and infectious mononucleosis. This enzyme closely resembles that extracted from the thymus of mice with lymphoma or lymphatic leukemia, both in its electrophoretic mobility and its substrate specificity. The phosphatase N activity was related to the clinical state of patients with lymphatic leukemia and disappeared with recovery from infectious mononucleosis.*

A distinct alkaline phosphatase, which we designated phosphatase N (*1*), appears in the thymus of AKR, C57BL/6, and SJL/J mice with lymphoma or lymphatic leukemia (*2–4*). Several characteristics of phosphatase N distinguish it from normal alkaline phosphatases. A unique alkaline phosphatase band is present after polyacrylamide gel electrophoresis of thymic extracts of leukemic AKR mice. This band is absent in thymic extracts of normal AKR mice (*2*). In animal studies it was found that phosphatase N catalyzes the hydrolysis of monoesters of orthophosphoric acid, but, in contrast with normal alkaline phosphatase, does not affect the *S*-substituted monoester of thiophosphoric acid (*2, 3*). The unique capacity to differentiate between oxygen and sulfur links between the alcohol moiety and the phosphoryl group is a strong indication that the enzymatic mechanism of phosphatase N is different from that of other alkaline phosphatases (*5*). This substrate specificity was used for routine documentation of the presence of phosphatase N and for quantitative measurements of it.

Phosphatase N was present in thymic extracts of leukemic mice in which the disease was induced by Gross strain or Rich strain of murine leukemia virus or by whole-body irradiation. The enzyme appeared before dissemination of the disease. Phosphatase N in the spleen, mesenteric lymph nodes, peripheral lymph nodes, kidney, and liver appeared concurrently with dissemination (*6*). To date, this enzyme has been found only in murine lymphoma and leukemia (*2–4*).

We explored the possibility that phosphatase N might appear also in the serums of patients with acute lymphatic leukemia (ALL), chronic lymphatic leukemia (CLL), and infectious mononucleosis (IM). These disorders represent a range of lymphoproliferative disorders from a highly malignant disease (ALL) to a self-limited one (IM). If phosphatase N appears in the serums of these patients exclusively, it may serve as a diagnostic tool. Furthermore, if the diagnostic value of this enzyme is established, a change in the amount present following treatment might be used in the evaluation of the effectiveness of therapy.

Serums from 6 patients with ALL (newly diagnosed or in relapse); 5 patients with untreated CLL, 22 patients with clinically and serologically active IM, and 10 normal controls were examined. In determining whether phosphatase N appears in patients with lymphoproliferative diseases we first demonstrated that an additional alkaline phosphatase appears in their serums and that it has the same electrophoretic mobility in polyacrylamide gel electrophoresis as the band obtained with thymic extracts of mice with lymphatic leukemia. This band was absent in normal controls.

Normal human serum catalyzes the hydrolysis of both *p*-nitrophenyl phosphate, which is a monoester of orthophosphoric acid, and cysteamine-*S*-phosphate, which is a monoester of thiophosphoric acid. In our ten normal subjects, the ratio of hydrolysis of these substrates ranged from 1.46 to 1.76, with a mean of 1.60. The activity of phosphatase N can be expressed as a percentage of total serum alkaline phosphatase activity by the expression

$$100\ (V_{\text{p-NPP}} - 1.6\ V_{\text{CASP}})/V_{\text{p-NPP}}$$

where $V_{\text{p-NPP}}$ is the velocity of hydrolysis of *p*-nitrophenyl phosphate and V_{CASP} is the velocity of cysteamine-*S*-phosphate. According to this equation phosphatase N ranges from -9 to $+9$ percent in 10 normals and in 55 control patients.

The rate of hydrolysis of both substrates was measured spectrophotometrically, the change of absorbancy of the product being plotted versus time. For *p*-nitrophenyl phosphate hydrolysis, the formation of *p*-nitrophenol was determined. For cystea-

mine-*S*-phosphate hydrolysis, the formation of cysteamine was established with the aid of 5,5′-dithiobis(2′-nitrobenzoic acid) according to the method of Ellman (*7*). In this method, the 5-thio-2-nitrobenzoic acid formed as a result of disulfide interchange with cysteamine was measured spectrophotometrically.

In all of the patients with lymphoproliferative disease, phosphatase N was present. For ALL, the range of phosphatase N was 26 to 100 percent of the total alkaline phosphatase activity; for untreated CLL, the range was 35 to 39 percent; and for active IM, the range was 23 to 100 percent except for one patient who had 13 percent (Fig. 1). In 6 of the 22 cases of IM, more than 80 percent of the measurable alkaline phosphatase activity was due to phosphatase N. This suggested that an inhibitor of the normal alkaline phosphatase might be present in the serums of these patients. To evaluate this hypothesis, we mixed serums of IM patients with those of normal individuals; the measured alkaline phosphatase activity in the mixtures appeared to be 30 to 50 percent lower than the sum of alkaline phosphatase activities measured separately with *p*-nitrophenyl phosphate as substrate. The inhibition of alkaline phosphatase activity in normal serum was proportional to the amount of IM serum added.

The correlation of phosphatase N with treatment response in the leukemia patients and with disease activity in IM was investigated. Of two patients with ALL in whom a hematologic remission was achieved with chemotherapy, one patient showed a drop in the level of phosphatase N from 43 to 14 percent. In the other, phosphatase N activity remained constant despite clinical improvement.

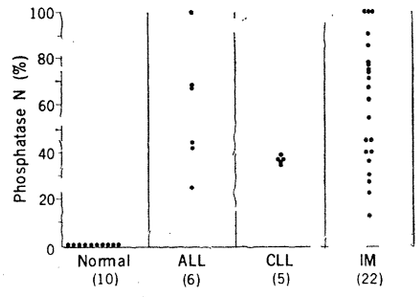

Fig. 1. Phosphatase N (percentage of total alkaline phosphatase activity) in serum of normal controls and subjects with ALL, CLL, and IM. Each dot represents one case; the total number of cases for each category is given at the bottom.

Follow-up data are available for two patients with CLL. Neither patient had received treatment during the period between the first and second determinations, and marked clinical deterioration had taken place in both. Rises in phosphatase N activity from 39 to 98 percent in one patient and from 37 to 93 percent in the other were found to parallel sharp elevations in peripheral white blood cell counts. Chemotherapy was begun for the former patient, and, along with a partial hematologic response, the phosphatase N activity decreased from 98 to 70 percent.

Three characteristic cases of IM are presented in Table 1. In patient M.P., the percentage of phosphatase N dropped from 100 to 0 percent with clinical recovery and correlated well with the heterophile and ox cell lysin titers. In patient R.W., in whom there was no clinical improvement during a 1-month follow-up period and no change in heterophile and ox cell lysin titers, the percentage of phosphatase N did not change. Patient N.B. had a low total alkaline phosphatase activity when first studied, with 100 percent phosphatase N. The percentage of phosphatase N decreased more slowly than did the heterophile and ox cell lysin titers. The total alkaline phosphatase activity rose considerably during the recovery of this patient.

In order to determine the degree of specificity of phosphatase N for lymphoproliferative disorders, we examined the serums from patients with other diseases: 5 patients with systemic lupus erythematosus, 15 with rheumatoid arthritis, 20 with ulcerative colitis, 5 with breast cancer, and 10 with cancer of the colon. No phosphatase N could be detected in any of these patients.

These results suggest that measurement of phosphatase N in the serums of patients with lymphoproliferative diseases may be useful as an indicator of the presence and activity of the disease. However, more extensive study is needed before phosphatase N can be used as a diagnostic tool.

An important question raised by this study is the origin of the phosphatase N. At least three possibilities should be considered: (i) phosphatase N is present in minute amounts in the normal lymphocytes and appears in the serum as a result of the increase in circulating lymphoid cells, whether these are benign or malignant; (ii) phosphatase N appears concurrently with other cellular changes associated with neoplastic transformation; (iii) the synthesis of phosphatase N is related to the presence of a virus.

Evidence against the first hypothesis comes from studies in mice which showed that phosphatase N was absent in lymph nodes, thymus, and spleen of three strains of normal animals (*2*, *3*). Furthermore, phosphatase N could not be detected in cell cultures of resting or phytohemagglutinin-stimulated normal peripheral blood lymphocytes (*8*).

The second possibility seems particularly appropriate in regard to CLL, for which evidence for a viral etiology is lacking. Since phosphatase N appears also in IM, however, it seems unlikely that malignant transformation of lymphocytes is the sole prerequisite for the appearance of this enzyme in the serum.

The third possibility, that the synthesis of phosphatase N is virus-induced, seems to us a likely hypothesis. In mice, the viral etiology of lymphatic leukemia is well documented (*9*). The presence of Epstein-Barr virus has been implicated in IM (*10*). Phosphatase N has been found in the presence of different types of murine tumor viruses,

Table 1. Correlation of phosphatase N activity with serological titers in three characteristic cases of infectious mononucleosis.

Date	Heterophile agglutinin titer	Ox cell lysin titer	Alkaline phosphatase activity		Phosphatase N (%)
			$V_{p\text{-NPP}}$	V_{CASP}	
Patient M.P., age 17, female					
9 January 1973	1:3072	1:5120	20.0	0.0	100
7 May 1973	1:24	1:160	10.0	6.6	0
Patient R.W., age 17, female					
20 January 1973	1:768	1:2560	9.5	3.5	41
21 February 1973	1:768	1:2560	17.8	4.0	64
Patient N.B., age 4, male					
17 November 1972	1:384	1:2560	4.1	0.0	100
14 January 1973	1:48	1:160	26.4	10.0	39
7 May 1973	1:24	1:40	33.3	14.7	29

for example, in mice infected with the Gross or Rich strain, and in tissue cultures infected with the Moloney strain (*11*). These are RNA viruses, whereas the Epstein-Barr agent is a DNA virus. It has been suggested that different virus strains may be responsible for different clinical states (*11, 12*). The appearance of the same protein, phosphatase N, in different mammalian species and in diseases characterized by lymphocytic proliferation and viral infection suggests that this protein reflects changes in lymphocytes or lymphocyte precursors that are induced by viral infection. Efforts to distinguish between viral transformation associated with malignancy (acute murine leukemia and possibly human lymphatic leukemia) and that associated with benign lymphoproliferative disorders (IM) may lead to a better understanding of the etiology of human leukemias (*13*).

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References and Notes

1. In our designation of this heretofore unknown enzyme we used the symbol N for the Hebrew word *ne-elam* which means unknown. No reference to enzymatic activity toward a phosphoroamidate bond is intended.
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Cochlear Neurons: Frequency Selectivity Altered by Perilymph Removal

Abstract. *Removal of perilymph from the scala tympani of the guinea pig cochlea reversibly broadened the tuning curves of single spiral ganglion cells emanating from the basilar membrane. Thus, fluid continuity along the membrane is essential for normal cochlear function, in particular for sharp neural tuning curves. These data reveal a possible source of error in some estimates of basilar membrane motion and suggest a reappraisal of current concepts of the mechanism of sharp tuning in primary auditory nerve fibers.*

It has long been held that there is a serious discrepancy between the filtering properties of the basilar membrane and the frequency selectivity of single primary auditory neurons (*1–3*). Mechanical measurements, usually obtained at high sound pressures, indicate a broad tuning of the basilar membrane, whereas the tuning curves of primary neurons (obtained at threshold sound pressures), are much sharper. Rhode (*4*) and Rhode and Robles (*5*) have reported a nonlinearity in basilar membrane vibration which consists of a flattening of the input-output curves in the region of the peak of the membrane tuning curve. This nonlinearity results in a sharpening of the mechanical tuning curve at low sound pressures. Such a nonlinear behavior could be used to bring the neural and mechanical data into close agreement and thus eliminate discrepancies between basilar membrane and primary neural tuning (*6, 7*). There is, however, some conflict between these authors' findings and those of other workers. In particular, the failure to find a nonlinearity in the guinea pig cochlea (*3, 8, 9*) has lead some authors to state that the neural tuning curves cannot be explained solely on the basis of basilar membrane mechanics. Additional filtering processes have therefore been postulated (*2, 3, 10*). How much of this disagreement in mechanical measurements could be caused by species differences or by variations in technique is not clear. The results that are reported here show that removal of perilymph from the scala tympani could constitute a source of error in several of the mechanical measurements.

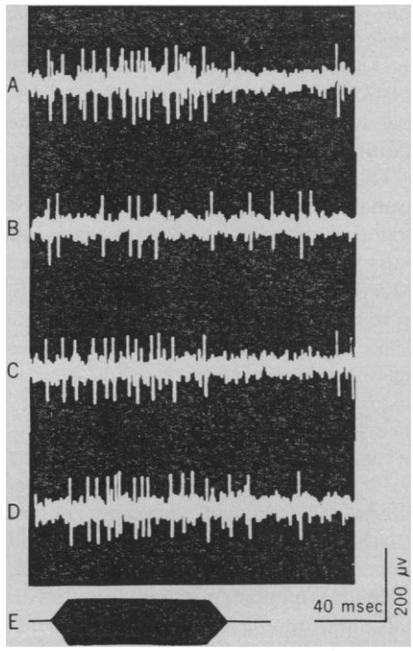

Fig. 1. Oscilloscope tracings of the response of a single ganglion cell in the guinea pig cochlea to a tone burst at the best frequency. The intensity of the tone burst was 10 db above threshold for the cell with the scala tympani full of perilymph. Responses were recorded (A) before draining perilymph, (B) with perilymph removed, (C) with perilymph only partially replaced, but covering basilar membrane, and (D) with scala tympani refilled. (E) Approximate position of tone burst.

The technique of recording from the bipolar cell bodies of the spiral ganglion has been described elsewhere (*7*). This procedure permits stable recording from single cells in a chosen and restricted frequency range in the basal cochlear spiral, while continuously monitoring the cochlea during experimental manipulations. The frequency selectivity of the ganglion cells was estimated by measuring the tuning curves (threshold versus frequency response curves) (*7, 11*).

As previously reported, sharp and sensitive tuning curves could be obtained from the ganglion cells after limited opening of the scala tympani (*7*). These curves are similar to those obtained from eighth nerve axons (*1, 2, 11*). From this, it is inferred that opening of the scala tympani without fluid removal does not substantially alter the neural tuning curves. On this point the results are in agreement with the findings of Evans (*12*), who recorded from the eighth nerve axons (the central processes of the spiral ganglion cells). However, in contrast