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* Present address: Department of Anthropology and Institute for Quaternary Science, University of Maine, Orono 04473.

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Insulin Secretion by Anomers of D-Glucose

Abstract. *Isolated rat islets were incubated for 5 minutes in the media containing either the α or β anomer of* D*-glucose (2 milligrams per milliliter). The amounts of secreted insulin and changes of anomers ratio were concomitantly determined. In spite of rapid mutarotation, significantly greater stimulation of insulin secretion was observed by α-*D*-glucose as compared with β-*D*-glucose.*

D-Glucose, but not L-glucose, is known to be the most important stimulus for insulin secretion (*1*). However whether the pancreatic beta cells distinguish the α and β anomers of D-glucose as insulin secretagogue has not been studied. We have observed that the α anomer of D-glucose was more effective than the β anomer in triggering insulin secretion from isolated rat islets of Langerhans.

Islets of Langerhans from the pancreases of fed male Wistar rats weighing 250 to 300 g were isolated by the collagenase digestion method (*2*). All incubations were performed at 37°C in gassed (95 percent O_2 and 5 percent CO_2) Krebs-Ringer bicarbonate solution containing 0.2 percent bovine plasma albumin as the basic medium. After a preliminary incubation period of 30 minutes, batches of 10 to 20 islets of comparable size were incubated for 5 minutes in 300 μl of the media supplemented with either the pure α or β anomer, or with the mixture of both anomers at the equilibrated ratio (α : β, 36 : 64) of D-glucose (*3*) in solution; α and β-D-glucose were prepared as described (*4*). These anomers were rapidly dissolved in the basic medium, which had been warmed to 37°C, by vigorous shaking just before use. The final concentration of D-glucose was 2 mg/ml. Ratios of D-glucose anomers in the incubation medium before and during the incubation were determined by our method with the use of β-D-glucose oxidase, mutarotase, and oxygen electriod of 5 minutes was measured by media at the end of the incubation period of 5 minutes was measured by double antibody radioimmunossay with reagents obtained from Dainabot Radioisotope Laboratory, Ltd. (Tokyo, Japan), against porcine insulin standard. Assay of mutarotase, which catalyzes the interconversion of D-glucose anomers, in isolated islets of Langerhans was performed by our method (*6*).

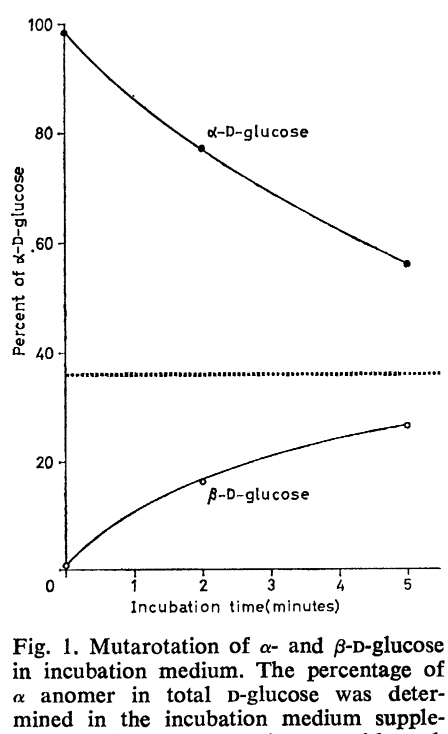

Fig. 1. Mutarotation of α- and β-D-glucose in incubation medium. The percentage of α anomer in total D-glucose was determined in the incubation medium supplemented with α- or β-D-glucose, with each 20 μl of the incubation medium taken out at the indicated times. Dotted line represents the percentage value of α anomer at equilibrium.

Changes of the ratio of anomers in the incubation media under our experimental conditions are shown in Fig. 1. The major portion of each anomer just before the incubation was more than 98 percent, and during the 5-minute incubation the α and β anomers were converted to the other anomers by 42.4 percent and 26.8 percent, respectively. Mutarotation is not considered enzymatic, since no mutarotase activity was found in the homogenate containing some 250 islets [0.375 mg as protein by the modified Lowry method (*7*)].

The amounts of insulin secreted during 5 minutes of incubation were measured under these experimental conditions (Fig. 2). Statistical analysis was performed by means of the paired *t*-test (one-tail). The amount of insulin (microunits per ten islets per 5 minutes) in the incubation medium without D-glucose was 10.5 ± 1.1, and those with α- and β-D-glucose were 25.5 ± 2.3 and 18.5 ± 1.9, respectively. Thus insulin secretion stimulated by the α anomer

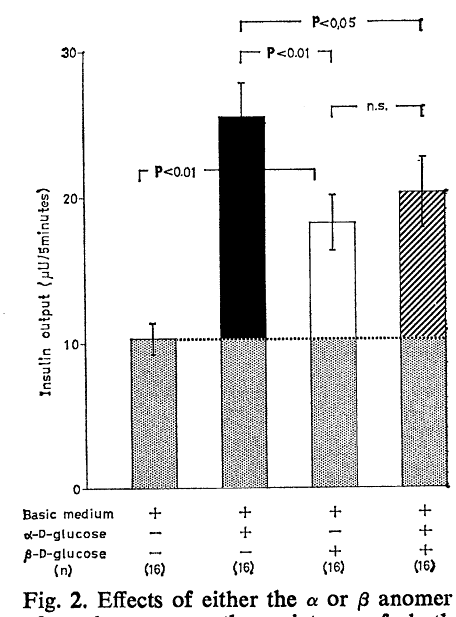

Fig. 2. Effects of either the α or β anomer of D-glucose, or the mixture of both anomers (α : β, 36 : 64) on insulin secretion from isolated islets of rat pancreas. Islets were incubated for 5 minutes with the anomers at the concentration of 2 mg/ml. Each column represents the mean of 16 observations with the standard error shown by the vertical line. The insulin output was measured in microunits per 5 minutes per ten islets.

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.

ATSUSHI NIKI
HATSUMI NIKI
Department of Internal Medicine, School of Dentistry, Aichigakuin University, Nagoya 464, Japan

ICHITOMO MIWA
JUN OKUDA
Department of Clinical Biochemistry, Faculty of Pharmaceutical Science, Meijo University, Nagoya 468

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2 July 1974

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-