Pompe's Disease: Detection of Heterozygotes by Lymphocyte Stimulation

Abstract. *A technique has been developed for the detection of inborn errors by multiple enzyme analysis of lymphocytes stimulated by phytohemagglutinin. Its practicality has been demonstrated in Pompe's disease in which there is a deficiency of acid α-1,4-glucosidase (E.C. 3.2.1.20).*

Human lymphocytes treated with phytohemagglutinin (PHA) undergo morphologic transformation and increased protein synthesis, followed by mitosis (*1*). There is also a concomitant increase in the total activity of several lysosomal enzymes, acid phosphatases (E.C. 3.1.3.2, acid β-glycerophosphatase and acid phenolphthalein phosphatase), and aryl sulfatase (E.C. 3.1.6.1) (*2*) and α-glucosidase (E.C. 3.2.1.20) (*3*). In some experiments, β-glucuronidase (E.C. 3.2.1.31) activity increased with stimulation (*2, 3*). Increased activities of nonlysosomal enzymes [glucose-6-phosphate dehydrogenase (E.C. 1.1.1.49) and lactate dehydrogenase (E.C. 1.1.1.27)] have also been demonstrated (*3*).

Pompe's disease is associated with a deficiency of acid α-1,4-glucosidase activity (*4*). Sometimes the heterozygote can be detected with unseparated white blood cells (*5, 6*), but this technique does not appear to be reliable for all heterozygotes. Measurement of enzyme activity during stimulation of lymphocytes with PHA might provide another way to detect inborn errors of metabolism and the detection of heterozygotes.

Blood in heparin was obtained from two patients with Pompe's disease (glycogen storage disease type II), five heterozygotes and eight controls. Two techniques were used for both cell separation and enzyme assay. In one, lymphocytes were separated on a column of glass beads (*7*) and suspended in Eagle's minimum essential medium modified for suspension culture (Grand Island Biological) containing 15 percent autologous serum. The PHA-P (Difco, 0.1 ml/10 ml of medium) was added to half the sample, and the culture was placed in an atmosphere containing 5 percent CO_2 at 37°C. Cells were harvested at 0 and 56 hours for determination of acid α-glucosidase (*6*), acid *p*-nitrophenyl phosphatase (*8*), and protein (*9*) (Table 1). The second technique consisted of partial purification (90 to 95 percent) of lymphocytes by gravity sedimentation. Lymphocytes were cultured as above except that fetal calf serum was used, and they were harvested at 48 hours. Assays were performed for acid α-1,4-glucosidase (*10*), β-glyceryl acid phosphatase (*2*), and protein (*9*) (Table 1). In some experiments assays were also performed for neutral α-1,4-glucosidase (E.C. 3.2.1.20) (*10*).

Neither affected individual had detectable lymphocyte acid glucosidase activity before or after stimulation with PHA. Normal activities of the two types of acid phosphatase and of neutral glucosidase were found at zero time, and an increase after stimulation similar to that found in the controls was seen in patients and heterozygotes.

The heterozygotes could not be distinguished reliably from controls at zero time. After stimulation with PHA, acid glucosidase activities were suggestive of, but not uniformly diagnostic for, heterozygosity. However, comparison of PHA-induced changes in the relation of acid glucosidase activity to acid phosphatase activity permitted reliable identification of the heterozygotes (column 7, Table 1). These numbers are obtained by dividing the ratio of α-glucosidase activity to acid phosphatase activity in cells stimulated by PHA by this ratio in unstimulated cells. This result is due to the fact that the response of acid glucosidase activity in stimulated lymphocytes of heterozygotes is less than that of the other lysosomal enzymes, while in controls the responses are similar. In fact, the relative increase of acid glucosidase in controls was greater than that of acid phosphatase as shown by values of greater than 1 in column 7 of Table 1. It is not known whether the difference observed in the heterozygotes represents altered rates of synthesis, activation, or degradation of the enzyme. Identical relations in the three types of individuals were found when comparing α-glucosidase activity with that of another lysosomal enzyme, *N*-acetyl-β-glucosaminidase. The nonlysosomal neutral α-glucosidase responded to stimulation with increased activity both in the patient and the control tested. Although identification of heterozygotes is frequently possible by a lack of increase of the pertinent enzyme, the calculation of the ratios is more generally useful, especially in those experiments in which removal of polymorphonuclear leukocytes was incomplete. Such incomplete removal of cells high in enzyme activity may mask a true increase.

Comparison of relative changes in enzyme activity in PHA-stimulated lymphocytes from patients and, more

Table 1. Activity of enzymes before and after stimulation with phytohemagglutinin. Units are as follows: columns 1 and 4, micromoles of maltose hydrolyzed per minute per gram of protein [for method used in control 8, patient 2, and heterozygotes 4 and 5, see (*10*), for others, see (*6*)]; columns and 2 and 5, micromoles of inorganic phosphate released per hour per microgram of protein [for method, see (*8*)] except in control 8, patient 2, and heterozygotes 4 and 5, in which the units are micromoles of paranitrophenol released per hour per microgram of protein [for method, see (*2*)].

Subject	Before stimulation with PHA			After stimulation with PHA			
	Acid α-glucosidase 1	Acid phosphatase 2	Ratio of col. 1 to col. 2 3	Acid α-glucosidase 4	Acid phosphatase 5	Ratio of col. 4 to col. 5 6	Ratio of col. 3 to col. 6 7
				Controls			
1	3.6	5.9	0.61	6.2	8.6	0.72	1.18
2	4.0	5.2	0.77	6.7	7.6	0.88	1.14
3	4.2	5.7	0.74	6.8	8.1	0.84	1.14
4	3.6	6.1	0.59	5.8	8.7	0.67	1.14
5	3.2	6.1	0.52	5.9	7.9	0.75	1.44
6	3.2	5.1	0.63	6.1	7.9	0.77	1.22
7	4.0	5.8	0.69	6.3	8.8	0.72	1.04
8	0.92	0.28	3.29	3.1	0.57	5.44	1.65
				Patients			
1	0.0	5.7	0.0	0.0	7.9	0.0	
2	0.0	0.40	0.0	0.0	0.50	0.0	
				Heterozygotes			
1	3.1	3.1	6.0	2.6	7.7	0.34	0.65
2	3.4	3.4	5.9	2.6	8.7	0.30	0.52
3	3.1	3.1	5.1	2.0	8.0	0.25	0.41
4	1.9	1.9	5.94	1.8	0.50	3.60	0.61
5	5.7	5.7	6.63	2.7	0.52	5.19	0.78

important, from heterozygotes for familial metabolic disorders, should prove to be generally useful. For example, similar studies in another lysosomal enzyme disorder, acid phosphatase deficiency, have produced equally informative results (*11*). In the heterozygotes for this disorder, acid α-glucosidase from lymphocytes shows a normal response to stimulation with PHA, while comparison of increase of acid phosphatase to increase of acid glucosidase shows a relative lack of stimulation of acid phosphatase activity. This, as expected, is the opposite result of our findings in patients with Pompe's disease, where the relative lack of stimulation in the heterozygote is that of acid α-glucosidase activity.

This method of analysis may prove useful in the detection of heterozygotes, especially in those diseases in which the defect is not easily detected in uncultured blood specimens. Even in those diseases where the carrier state can be detected in cultured fibroblasts, this approach appears to be preferable in terms of time and expense of materials and labor. These considerations would also make it practical as a screening procedure.

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Ciliary Orientation: Controlled by Cell Membrane or by Intracellular Fibrils?

Abstract. *The cirri of the ciliate* Euplotes *all assume the "reversed" orientation whenever the cell is depolarized and the "forward" orientation whenever the cell is hyperpolarized. Potential changes arise spontaneously or are induced by electrical or mechanical stimuli. The orientation responses of the cirri are apparently independent of intracellular "neuromotor" fibrils previously assigned a coordinating function, as they persist after the fibrils are transected.*

Taylor (*1*) reported that coordination of orientation between the widely separated cirri and membranelles of the hypotrich *Euplotes* (*2*) was lost as a result of microsurgical transection of "neuromotor" fibrils which run through the cytoplasm from a "motorium" to the bases of the organelles. This report, widely quoted, was largely responsible for speculation, still prevalent, that intracellular systems of fibrils, performing a nervelike function, are involved in the coordination of ciliary activity. This speculation has received support from a report that "crawling movements and the characteristic avoiding reaction [in *Euplotes*] . . . are not performed normally after the large fibres, connecting anal cirri with membranelles, have been cut" (*3*). Taylor's experiments were also repeated with the aid of cinematography (*4*) with the opposite conclusion—namely, that sectioning of the fibrils does not destroy coordination between widely separated organelles.

We have reexamined this problem considering both conventional electrophysiological concepts and recent findings on the control of ciliary orientation in *Paramecium* (*5*, *6*). Our results verify the proposal (*4*) that orientation of the motile organelles of *Euplotes* is coordinated by changes in membrane potential which spread over the cell, and is independent of intracellular fibrils. Potential changes which control the orientation of cilia occur both spontaneously and in response to stimuli.

Experimental methods were similar

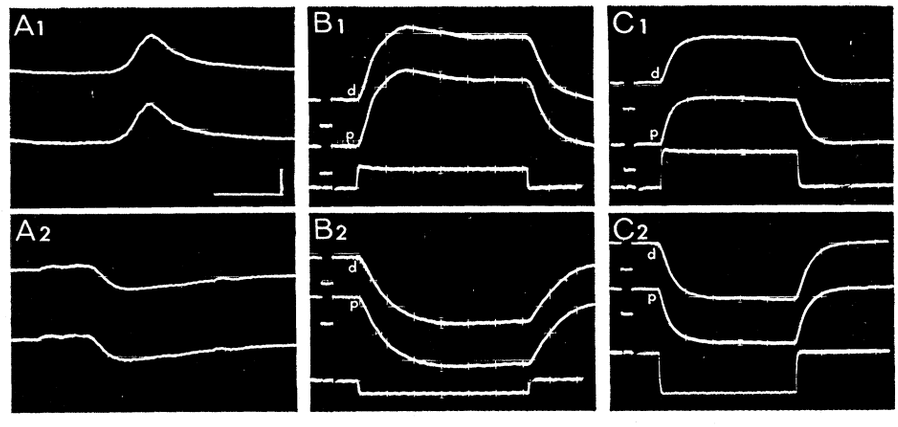

Fig. 1. Membrane potentials recorded with two intracellular electrodes inserted near opposite ends of a specimen. Separation of electrodes was 60 to 80 percent of cell length. (A_1) Spontaneous depolarizing wave; (A_2) spontaneous hyperpolarizing wave in same specimen; (B_1) outward current (bottom trace) injected with polarizing electrode inserted near proximal (*p*) recording electrode; (B_2) same specimen showing response to hyperpolarizing current; (C_1) similar to B_1 but with "neuromotor" fibrils visibly transected by a massive incision halfway between the proximal (*p*) and distal (*d*) recording electrodes; (C_2) same specimen as C_1, showing membrane potential recorded from both sides of incision in response to hyperpolarizing current injected near proximal electrode. Calibrations are 10 mvolt, 200 msec in A; 10 mvolt, 20 msec in B and C.