standpoint of good health-physics practice and for better understanding of the metabolism of radionuclides in the human body. To make such surveys is becoming increasingly easier, since human spectrometers and whole-body counters have been or are being built at many universities and atomic energy installations throughout the world (*8*).

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2 November 1959

Laminarase of Euglena gracilis

Abstract. An enzyme in extracts of the protistan *Euglena gracilis* splits the polysaccharide laminarin (β-1:3-glucosan). Its optimal *p*H is 5.0, and it is activated by Mn^{++} ions.

The storage polysaccharide (paramylon) of the protistan *Euglena gracilis* has been reported to be a β-1:3-glucose polymer (*1*) probably very similar in structure to laminarin (*2*), a polysaccharide produced in large quantities by several species of Phaeophycea (brown algae), notably *Laminaria* (*3*). Because of this similarity it seemed of interest to look for a laminarin-splitting enzyme in *Euglena.* Laminarase has been described in extracts of higher plant tissues (*4, 5*).

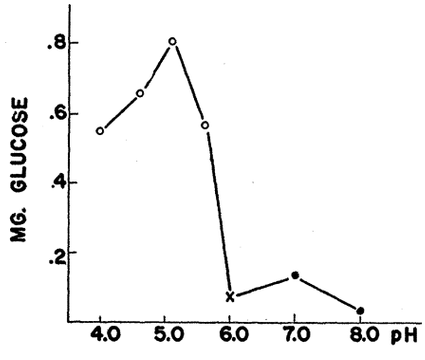

Fig. 1. Effect of *p*H on activity of *Euglena* laminarase. Assay was carried out as described in the text, but without Mn^{++}. Final buffer concentration, 0.1*M*. Open circle, acetate; cross, phosphate; solid circle, "Tris" (*7*).

Euglena gracilis (strain Z, Indiana collection strain No. 753) was grown as described elsewhere (*6*). The cells were harvested by centrifugation, suspended in approximately 5 vol of acetone at −15°C, homogenized for 30 seconds in a Waring blender, collected by filtration and washed with additional cold acetone, and given a final ether wash until most of the chlorophyll had been removed. The acetone powder was further dried overnight under reduced pressure. Extraction of the enzyme was carried out by stirring 5 gm of the *Euglena* acetone powder for 4 hours at 4°C in 50 ml of 0.04*M* acetate buffer, *p*H 6.0, containing NaCl at a concentration of 0.5*M* and Versene at 0.002*M*. The extract was dialyzed overnight against 0.01*M* "Tris" buffer (*7*), *p*H 7.3. The laminarase assay was based on the colorimetric determination of the reducing groups liberated by the action of the enzyme on the polysaccharide with 3,5-dinitrosalicylic acid, a reagent much used for the determination of amylase (*8*). The final conditions of the laminarase assay were as follows: 5 mg of laminarin (*9*) was incubated with occasional stirring for 2 hours at 37°C with 0.25 ml of the *Euglena* extract, in a final volume of 1.0 ml of 0.1*M* acetate buffer, *p*H 5.1, containing $10^{-3}M$ Mn^{++}. The reaction was stopped by the addition of 1.0 ml of the dinitrosalicylic acid reagent (*8*) and 3 ml of water. The excess laminarin was removed by centrifugation, and the supernatant was heated in a boiling-water bath for 5 minutes. After cooling, the volume was adjusted to 10 ml, and the color intensity was determined with a Klett-Summerson photoelectric colorimeter with a green filter (No. 54). The amount of reducing material liberated was expressed as milligrams of glucose.

The optimal *p*H of the *Euglena* laminarase was found to be 5.0, as shown in Fig. 1. It is of interest that the same value has been reported for the enzyme from higher plant tissues (*5*). Another similarity was the relative heat lability of the *Euglena* enzyme: at 55°C and *p*H 7.3, 61 percent of the activity was lost in 4 minutes, while complete inactivation occurred within 8 minutes. A time course of the laminarase reaction is given in Fig. 2. When the effects of various metal ions on the enzyme were tested Fe^{+++}, Cr^{+++}, Ni^{++}, Ba^{++}, and Mg^{++} were found to be slightly inhibitory or inactive, while Hg^{++} was strongly inhibitory at concentrations of 10^{-4} and $10^{-3}M$. It was of particular interest that 10^{-4} and $10^{-3}M$ Mn^{++} strongly stimulated the enzyme. Activations from 50 percent to over 100 percent, depending on the enzyme preparations, were observed. Smaller activations resulted from the addition of Co^{++} or Ca^{++} at the same concentrations.

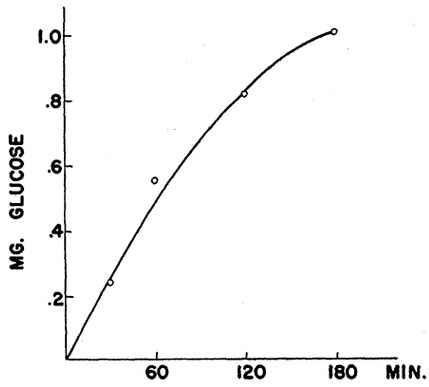

Fig. 2. Time course of laminarase reaction. Assay was carried out as described in the text, but without Mn^{++}.

Fractionation experiments now in progress tend to indicate that more than one enzyme may be involved in the breakdown of laminarin. Examination of the products of the enzymatic degradation may further clarify this point. In *Euglena,* it appears that laminarase probably serves to mobilize the reserve carbohydrates, especially since we were unable to find any amylase activity in the organisms (*10*).

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