Summary. The effect of the glycolytic inhibitor 2-deoxy-D-glucose on the oxidation of ^{14}C-labelled substrates of the tricarboxylic acid cycle was investigated in ascites carcinoma cells of the mouse. In the presence of high concentrations of glucose, deoxyglucose stimulates the oxidation of pyruvate C-2 and C-3 to CO_2, but not the oxidation of succinate C-1,4 to CO_2. While deoxyglucose causes, in the absence of glucose, an inhibition of the oxidation of exogenous palmitate C-1 to CO_2, it causes, in the presence of glucose, a stimulation of the palmitate oxidation. There is a possible correlation between the effect of deoxyglucose on the intracellular ATP metabolism and the effect of deoxyglucose on the palmitate oxidation.

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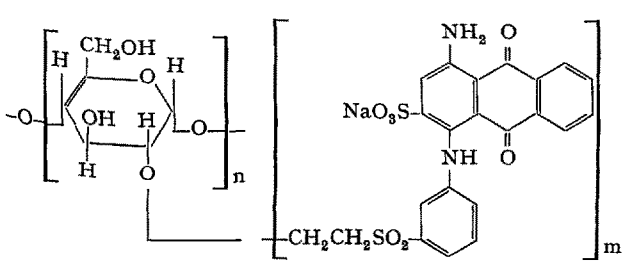
A New Method for the Determination of α-Amylase

The significance of serum amylase as a diagnostic indicator of pancreatic and other diseases has prompted the development of numerous techniques for the determination of this enzyme during the past 40 years. The methodology which includes viscosimetric, turbidimetric and saccharogenic procedures and assays based on the starch-iodine reaction has been reviewed recently by SEARCY, WILDING and BERK[1].

In this communication we report a new method for the determination of α-amylase which we believe to be superior in simplicity and specificity to those published to date. It is based on the use of a starch substrate labeled covalently with Remazolbrilliant Blue R (RBB)[2] as represented in Figure 1.

Both soluble and insoluble starches can be labeled by the following procedure[3]. Potato or corn starch (50 g) is suspended in 500 ml of water and stirred vigorously at 50 °C. A solution of 5.0 g of Remazolbrilliant Blue R in 500 ml of water is added to the suspension. During the following 45 min, 100 g of sodium sulfate is added in several portions. The reaction mixture is then treated with a solution of 5.0 g of trisodium phosphate in 50 ml of water and stirring at 50 °C is continued for a further 75 min. The mixture is centrifuged and the supernatant discarded. The dark blue RBB-starch is resuspended in water and again centrifuged. Washing in this manner is continued until the supernatant is completely colorless. The product is now rinsed twice with methanol and dried in a vacuum desiccator over phosphorus pentoxide. If a soluble starch is used in the labeling process the final reaction mixture is subjected to exhaustive dialysis against tap water in order to remove excess dye, salts, and other low molecular products.

The new amylase assay is illustrated by the following procedure. A suspension of RBB-starch (2%) in 4.5 ml of 0.02 *M* sodium phosphate buffer (pH 7.0) containing 0.05 *M* sodium chloride is placed in a 15 ml wide-mouth vial with a plastic closure. A solution (0.5 ml) of porcine pancreatic α-amylase[4] containing 0-1200 Somogyi units/100 ml is added to the suspension and the vial is



Starch Remazolbrilliant blue ($m \ll n$)

Fig. 1.

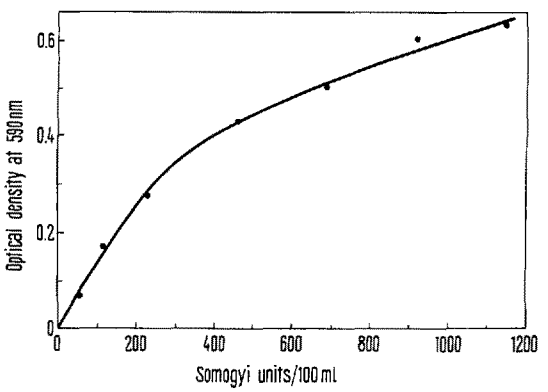


Fig. 2. Colorimetric determination of α-amylase with RBB-starch.

stoppered and placed in a Dubnoff shaker at 37 °C. After shaking for 15 min, enzyme action is terminated by addition of 2 ml of dilute acetic acid, reducing the pH to 4. The mixture is filtered and the filtrate assayed colorimetrically at 595 nm against a blank prepared similarly but with omission of α-amylase. Figure 2 summarizes the results obtained with serial dilutions of α-amylase ranging from 0–1200 Somogyi units/100 ml. A near-linear relationship between enzyme concentration and optical density readings exists within a concentration range of 0–250 Somogyi units/100 ml, covering the values encountered in normal sera.

Development of a micromethod and clinical-chemical evaluation of the new technique are in progress. Automated procedures using RBB-starch in conjunction with dialysis are also under investigation[5].

Zusammenfassung. Es wird eine einfache und empfindliche Methode zur Bestimmung der α-Amylase mit Hilfe eines durch Remazolbrillantblau R kovalent markierten Stärkesubstrates beschrieben. Die Bestimmung der Enzymwirkung erfolgt durch colorimetrische Messung der Spaltprodukte nach Entfernung des überschüssigen Substrates.

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[5] This work was supported by USPH grants No. AM 04683-07 and AM 08293-04.