d.h. der kleinste nach unseren Forderungen noch zulässige Umfang n einer Teilstichprobe ist gleich dem dreifachen Wert der in Prozent ausgedrückten relativen Standardabweichung dieser Stichprobe. Nach dieser Regel lässt sich der kleinste zulässige Umfang der Stichproben auf einfache Weise abschätzen. Ist beispielsweise der Mittelwert einer Stichprobe $M = 60$ und die Standardabweichung $\sigma = 10$, so beträgt der kleinste zulässige Umfang n der Stichprobe nach Formel (4) $n = 3\,(10 \cdot 100/60) = 50$, d.h. es müssen für jede Teilstichprobe (also für jede der untersuchten gleichstrukturierten Regionen eines Organs) mindestens 50 Bildfelder ausgemessen werden.

Sollte es aus technischen Gründen nötig sein, kann man den Umfang der Teilstichproben noch weiter verringern, indem man eine grössere Veränderung des Mittelwertes beim zufälligen Auftreten eines Extremwertes zulässt oder sich mit der geringeren Sicherheitsschwelle von $2\,\sigma$ für den eingesetzten Extremwert begnügt. Da nach Formel (3) f und p in reziprokem Verhältnis zueinander stehen, hat ein Erhöhen der tolerierten Veränderung des Mittelwertes auf 1,5% die gleiche Wirkung wie ein Senken des eingesetzten Extremwertes auf $2\,\sigma$. Diesen Wert sollte man in keinem Fall unterschreiten, da sonst, wie die t-Tabelle von Student zeigt, die Irrtumswahrscheinlichkeit zu stark anwächst. Für das oben erwähnte Beispiel würde sich dabei ein unterster für den Umfang der Stichprobe noch zulässiger Wert von $n = 33$ ergeben.

Anderseits wird das Überschreiten eines Wertes von 4 für den Faktor f/p im Interesse einer höheren Messgenauigkeit der Teilstichprobe wenig sinnvoll sein, da dann die Irrtumswahrscheinlichkeit bereits unter $1‰$ gesunken ist und eine weitere Vermehrung der Messungen nur noch eine sehr geringe Steigerung der Sicherheit mit sich bringt.

Wie sich der optimale Umfang der Gesamtstichprobe mit Hilfe des Mutungsintervalles schätzen lässt, so ermöglicht das angegebene Verfahren, für die Teilstichproben den kleinsten Umfang zu bestimmen, der den gestellten Forderungen noch genügt.

Summary. If, for technical reasons, the sample size of stereological measurings has to be kept as small as possible, a method is given to determine the smallest number of measurings tolerable. It should never be less than twice the relative standard deviation of the sample, expressed as percentage.

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A New Method for Determination of α-Amylase

There are various methods available for the determination of the enzyme, α-amylase, based on (a) decrease in viscosity of starch solution, (b) decrease in turbidity of starch suspension, (c) decrease in the starch-iodine colour intensity and (d) the increase of reducing groups.

In the past there were many attempts to simplify the native substrate for α-amylase, but so far this approach has met with only partial success. For example, α-*p*-nitrophenyl maltosides have been used as substrates for amylases[1–3]. However, it was found that α-amylases exhibit lower affinity (by several orders of magnitude) for the low molecular weight substrates. It is therefore assumed that α-amylase requires many points of attachment to long chain substrate molecules to obtain full catalytic activity.

A new method for the determination of α-amylase activity was reported by RINDERKNECHT et al.[4]. Starch grains were labelled covalently with a coloured substance, Remazolbrilliant Blue R. A suspension of such material was then incubated with an α-amylase source for a given length of time. The reaction was terminated by addition of diluted acetic acid. The mixture was then filtered and the filtrate was assayed colorimetrically.

For some years, we have been working on the development of an α-amylase method based on the use of new types of substrates. The synthesis of these substrates involves essentially 2 major steps. First, soluble starch was coloured under alkaline conditions using Cibacron blau F 3 G-A. This procedure resulted in the formation of covalent bonds between starch molecules and dye molecules. Second, the coloured starch was cross-linked by the addition of 1,4-butandiolglycidether. This gave a three-dimensional, insoluble network which swells in water. The degree of swelling could be controlled by the amount of cross-linking. The enzymatic hydrolysis of such insoluble starch derivatives yields soluble starch split products carrying the coloured marker.

The resulting blue starch polymer is homogenized in a Waring Blendor to small particle size and washed several times in distilled water and several times in 0.02 *M* Phosphate Buffer (pH 7.0) containing 0.01 *M* NaCl. This blue cross-linked starch polymer in the form of small gel grains is then resuspended in the above mentioned Phosphate buffer.

For testing, 1 ml of blue starch polymer suspension (containing 0.143% of dry plymer) was used. To 1 ml samples, 20 µl portions of crude porcine pancreas extract were added. The α-amylase extract was prepared from 10 mg of porcine pancreatin powder from ORGANON, BATCH 32 A/59, which was suspended in 10 ml of the above phosphate buffer. The mixture was stirred for 5 min at room temperature and centrifuged at 5000 rpm for 10 min. The clear supernatant was the source of crude α-amylase extract.

The enzymatic reaction was performed at 37 °C for 0, 10, 20 and 30 min. The samples were shaken during the incubation time. The enzymatic hydrolysis was terminated by placing the tubes in a boiling water bath or

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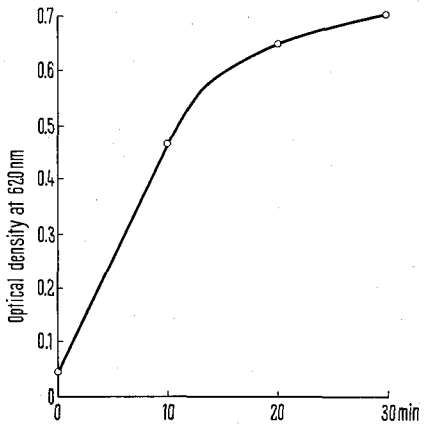

Fig. 1. Enzymatic degradation of blue starch polymer by crude α-amylase from porcine pancreas at various incubation periods. The crosses on the curve are averages of 2 determinations.

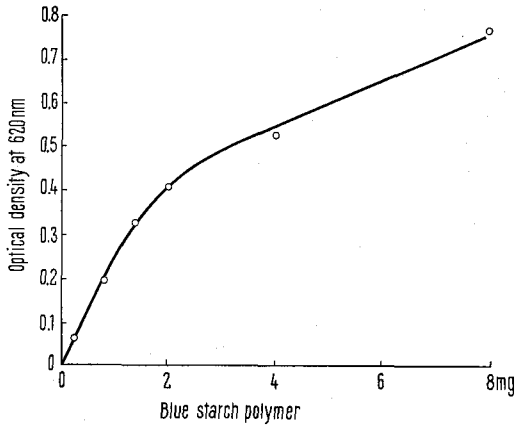

Fig. 2. Substrate concentration curve of blue starch polymer No. 290 hydrolysed by a constant amount of crude α-amylase from porcine pancreas. The crosses on the curve are averages of 2 determinations.

changing the pH. The soluble blue starch products were separated from the insoluble blue substrate by a brief centrifugation and the optical density of the blue supernatant was measured at 620 nm. The release of soluble blue starch split products, in respect to time of hydrolysis, is shown in Figure 1. The release of soluble blue starch products was found to be a linear function of enzyme concentration. It was also observed that the appearance of soluble blue starch products gives a Michaelis-Menten curve type (Figure 2).

This is quite a simple and sensitive method for α-amylase determination which, when further standardized, could find a wide application[5].

Zusammenfassung. Für die Bestimmung der enzymatischen Aktivität der α-Amylase wurde ein neues Substrat synthetisiert. Die Synthese kam in der Hauptsache durch die Farbstoffbindung an wasserlösliche Stärke und die anschliessende Vernetzung sowie durch den Zusatz von Diepoxid zustande.

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Control of Glass Microelectrodes for Intracellular Recordings

Since the work of Ling and Gerard[1] many techniques to prepare, fill and stock microelectrodes have been developed. The characteristics of recording electrodes may alter significantly electrophysiological data[2] and so a careful control of methods and results is necessarily the first step in this kind of research. In the present study a method of emptying and examining glass microelectrodes under the electron microscope is described. The correlation between external tip diameter and electric resistance is analyzed and 2 electrode filling procedures are compared.

Micropipettes were prepared from Kimax 46485, 0.7 to 1.0 mm capillary tubing (wall thickness 0.10–0.21 mm) using an Alexander-Nastuk puller[3], the parameters of heating and pulling force kept constant within the limitations of the machine. The microelectrodes were mounted on microscope glass slides and filled either by boiling or by a modification of Tasaki's method[4]. The boiling procedure consisted of two 15 min periods of gentle boiling in 3 *M* KCl, separated by cooling interval of 20 min. Tasaki's method was simplified to include the following steps: (a) boiling at 28–40 °C in absolute ethanol under reduced pressure for 10 min; (b) cooling at room temperature for 10–20 min until bubbles disappear; (c) immersion in distilled water for 5 min; (d) immersion in 3 *M* KCl for 20 min under reduced pressure

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