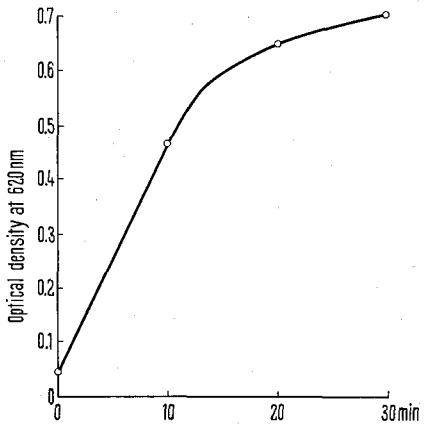

Fig. 1. Enzymatic degradation of blue starch polymer by crude α-amylase from porcine pancreas at various incubation periods. The crosses on the curve are averages of 2 determinations.

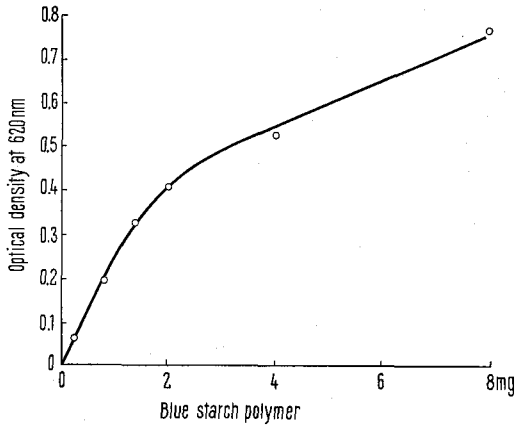

Fig. 2. Substrate concentration curve of blue starch polymer No. 290 hydrolysed by a constant amount of crude α-amylase from porcine pancreas. The crosses on the curve are averages of 2 determinations.

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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at a temperature below 45 °C. Electrodes were kept overnight in KCl and the procedures were repeated when they were not properly filled. The DC resistance of the microelectrodes was determined immediately after filling, using an Amatniek preamplifier by passing weak current pulse. Each electrode was then mounted on a labelled slide and prepared for electronmicroscopy as follows: (a) after a profuse rinsing in tap water, the slides were washed at least six times in distilled water and kept overnight in a large volume of clean water; (b) the next day they were rinsed in absolute alcohol and boiled under reduced pressure for 2 periods of 7 min, with a 10 min interval; (c) after this, the alcohol was removed by reduced pressure. This latter method for emptying KCl-filled electrodes gave the best results. Clean pipettes were obtained and could then be mounted for the electron microscope. Control pipettes not submitted to the filling procedure were separated from every batch and kept in distilled water for examination at the electron microscope.

Figure 1 shows how microelectrode tips were mounted for microscope study. A small square was cut from the center of an electron microscope grid. The grid was then placed on a narrow strip of cellulose adhesive tape fastened to a microscope slide so as to form a springy flexible mounting. A small drop of araldite was deposited over one side of the grid. The microelectrode, supported on a conveniently shaped wood stick, was carefully lowered into the araldite droplet. The tip was kept centered in the open square. After polymerization of the araldite the electrode was broken close to the grid edge and the grid was lifted cautiously with fine pointed tweezers. The microelectrode was easily found under the electron microscope (Phillips EM-100) and a certain length could be examined without interference of the grid wires. Heat dissipation is poor and consequently the beam intensity must be suitably controlled.

Measurements were taken for external tip diameter at a magnification of $\times$ 28,000. The electron microscope magnification was checked by comparison of measurements of the shank of the micropipette with those obtained at the same site with a light microscope.

A typical good tip and final tapering are shown in Figure 2.

Figure 3 shows that there is good correlation between external tip diameter and electrode DC resistance. Some discrepancy would be expected to occur because the internal tip diameter (which cannot be precisely measured under the electron microscope) is clearly the important one as far as conductivity is concerned. The observation of Figure 3 also shows that the DC resistance of electrodes filled by boiling is lower than that obtained with the modified TASAKI's method.

Comparison of filled and control pipettes from the same batch gives a clear indication that the modified TASAKI's method employed provides a better preservation of the tips.

The Table shows the means and standard deviations of the external tips of control and filled electrodes. The differences between the 2 groups are significant in the boiling method and non-significant in modified TASAKI's method.

Microelectrodes in the range of 10–40 $M\Omega$, corresponding to a tip diameter between 0.5 and 0.1 μ, were found useful

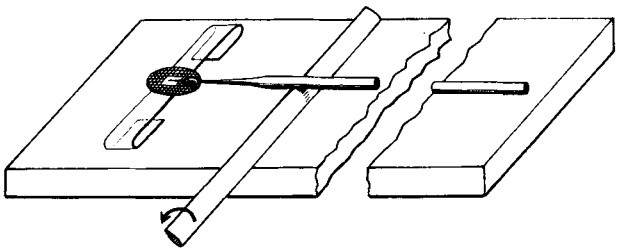

Fig. 1. Microelectrode mounting for electron microscopic observation.

Means and standard deviations of external tip diameters

	Modified TASAKI's procedure	Boiling procedure
Control electrodes	178 $\pm$ 7.4 nm (N = 14)	187 $\pm$ 7.9 nm (N = 18)
Filled electrodes	188 $\pm$ 7.7 nm (N = 14)	320 $\pm$ 170 nm (N = 17)

P values of comparisons between filled and control electrodes are statistically significant for those prepared by boiling procedure at the level of $P < 0.0025$ and nonsignificant for those prepared by modified TASAKI's procedure ($P < 0.15$). N, number of examined microelectrodes.

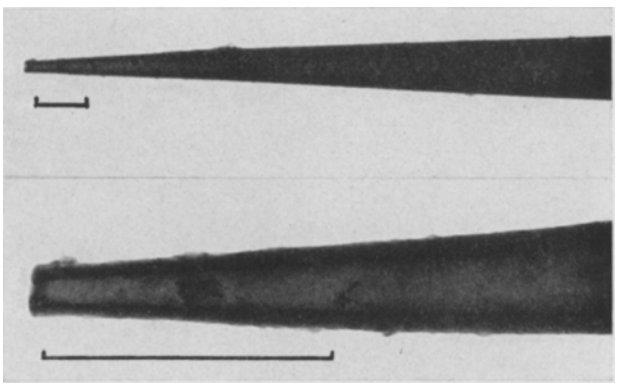

Fig. 2. Microelectrodes as seen under the electron microscope in order to measure external tip diameter. Electrode prepared by TASAKI's modified method. Calibration: 1 μ.

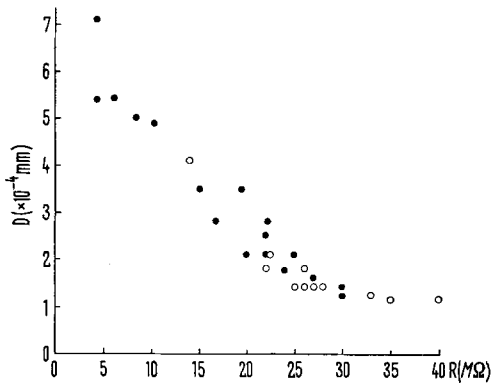

Fig. 3. Relation between electrode external tip diameter and electric resistance. ●, electrodes filled by boiling procedure; ○, electrodes filled by modified TASAKI's procedure.

for intracellular recording by a number of authors[5-7]. Electrodes with resistance under $5 M\Omega$ have very wide tips and are not normally used for that purpose, due to electrophysiological evidence of cell damage.

Electrophysiological properties of embryonic chick heart cells in vitro were studied, using electrodes prepared as described here, and it was verified that they caused no significant damage to the cells[8].

The present findings are in good agreement with the notion that avoiding heating under 40 °C preserves better the electrodes during the filling procedure[4,9]. However, when selection is made according to the resistance, the boiling method also yields adequate electrodes.

It is concluded that the usual selection of microelectrodes based on DC resistance measurements gives reliable information about adequacy of the recording device, regardless of the filling procedure. This statement is not likely to be exact if geometry and wall thickness of the tips are variable. These factors, however, tend to be similar when manufacturing procedure and materials are standardized.

Résumé. Une méthode pour vider et examiner des microélectrodes de verre au microscope électronique est décrite. On a trouvé une corrélation entre le diamètre du bout et la résistance électrique. Le diamètre de l'extrémité des électrodes dépend du procédé de remplissement et les meilleurs résultats sont obtenus par la méthode de TASAKI modifiée.

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[10] The authors wish to thank Dr. A. PAES DE CARVALHO and Dr. C. E. CHALLICE for their helpful criticism of the manuscript.
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Détermination quantitative de la radioactivité sur coupes autoradiographiques

Une méthode assez couramment employée pour obtenir des données quantitatives en partant des sections autoradiographiques faites sur l'animal entier consiste dans la mesure du noircissement du film par un microdensitomètre, suivie d'une comparaison avec un standard[1-3].

Dans le cas où une mesure quantitative plus précise est nécessaire, il est préférable de mesurer la radioactivité de la source même[4]. Dans des travaux précédents, d'ULLBERG par exemple, on cite l'emploi du compteur GM sans fenêtre ou à fenêtre mince pour la mesure directe des portions coupées en partant des sections autoradiographiques[5].

Dans cette communication, nous désirons décrire une méthode quantitative de mesure de la radioactivité en partant des sections autoradiographiques et faire quelques considérations sur les problèmes qui y sont liés.

Méthode. On coupe les portions des sections autoradiographiques, dont on veut faire la détermination quantitative, avec un bistouri et à l'aide d'un binoculaire stéréoscopique capable d'un agrandissement à 50 (Wild M5 48888).

On introduit les portions coupées dans des flacons de scintillation et on les solubilise avec de l'hydroxyde de hyamine ($1 M$ dans le méthanol) ou avec d'autres produits ayant la capacité de solubiliser les tissus, tels que, par exemple, le «Nuclear Chicago Solubiliser» (NCS) (0,6N dans le toluène). La quantité de hyamine ou de NCS capable de solubiliser dans un temps relativement court (quelques dizaines de minutes) 1 cm² de coupe de l'épaisseur de 30 μ est d'environ 0,3 ml.

Une fois les portions autoradiographiques solubilisées, on ajoute le liquide scintillant (20 ml de toluène + PPO + POPOP), on mesure la radioactivité (appareil Beckman LS-200 B) et on corrige enfin le «quenching» avec la méthode du standard externe. Dans la Figure 1 on peut observer la dépendance de l'efficacité du comptage de la quantité d'hyamine ajoutée, c'est-à-dire de la surface de coupe à solubiliser.

Le ruban adhésif transparent employé comme support des coupes, qui ne se solubilise pas, a une influence presque nulle sur l'efficacité du comptage en scintillation liquide. Si on ajoute dans un flacon de scintillation contenant un standard d'activité connue jusqu'à 20 cm² de ruban adhésif transparent, on a des variations de comptage qui

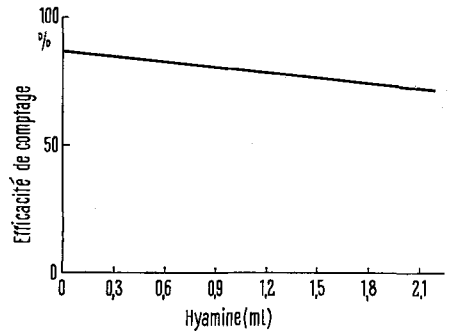

Fig. 1. Efficacité de comptage en fonction de la quantité d'hyamine ajoutée.

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