Reports

Glass Insulated Platinum Microelectrode

Abstract. Microelectrodes for electrophysiological use have been prepared easily and quickly by electrolytically sharpening platinum iridium alloy wire and coating with molten glass. The desirable combination of the electrical characteristics and strength of the platinum iridium wire with the exceptional durability of glass insulation has long been known, but earlier methods of fabrication were difficult and tedious.

Electrophysiological changes in single neurons are often most easily detected with an insulated core metal microelectrode. This type of electrode usually has a much lower noise level and a better high frequency response than the fluid-filled micropipette. Many designs have been proposed for making metal microelectrodes, but in spite of the investigations of the mechanical, electrical, and geometrical properties (*1*), the designs are usually determined empirically. The considerations that enter into the design can be more easily understood through a short discussion of the advantages and defects of the currently popular electrodes.

A stainless-steel electrode can be made cheaply and readily in large quantity. It appears to record satisfactorily in certain situations (*2*). It is a strong electrode, but the varnish insulation is troublesome, principally because it lacks durability. In addition, the electrode is apt to be excessively unstable electrically. The tungsten electrode recently described by Hubel (*3*) can now be made easily and features great strength, but it also suffers from electrical instability and the inadequacies of the existing varnishes. The indium-filled glass electrode of Dowben and Rose (*4*), as modified by Gesteland *et al.* (*5*), is somewhat difficult to prepare but has excellent electrical characteristics and appears to record well in a variety of situations, but its tip is easily damaged and it cannot be reworked or reused.

Platinum has always been a favorite electrode metal of electrophysiologists and has been used successfully in a wide variety of situations. Wilska (*6*) has described a sharpened platinum iridium alloy wire microelectrode insulated with glass which seems to combine all the ideal features of a good microelectrode and to exclude a number of undesirable ones. It records well from electrophysiological units, is quite selective, possesses high mechanical strength, and the insulation is outstanding. Wilska's method of manufacture is to grind the wire to a point and then shrink a heated glass capillary over it. This requires a high degree of skill and elaborate equipment both in pointing of the wire and in coating it with glass. We have worked out a method for fabricating this type of electrode which requires little skill or equipment and is much less time consuming.

The technique is as follows: A length of 8 to 10 mil, 70 percent platinum, 30 percent iridium alloy wire (*7*) is straightened by passing it, while under tension, through a small flame. One end of a short length of wire is immersed in a solution of 50 percent sodium cyanide, with 30 percent sodium hydroxide added to prevent the formation of hydrogen cyanide. An electrolyzing current is applied from an a-c source between the wire and a carbon rod inserted into the bath. The initial shaping is accomplished at 6 to 10 volts a-c (root-mean-square) accompanied by vigorous agitation of the bath. A magnetic stirrer is very convenient for this purpose. Final polishing with a much smaller voltage (0.8 volt a-c) yields smooth, gradually tapering electrodes having tips less than 1 μ in diameter. Agitation is not necessary during this step. The taper is controlled by the length of the wire inserted into the bath. Repeated withdrawal of the tip from the bath is not necessary in these processes. After pointing, the electrode should be washed in distilled water, air dried, and stored until a day or so before using, at which time it should be glass coated.

This procedure is accomplished by pushing the tip through a small drop of molten glass adhering to a V-shaped electrically heated loop of 15 mil platinum wire. Corning No. 7570 solder glass is used for coating because of its extremely low working point, 560°C. Corning No. 0041 potash soda lead glass, which has a much higher working point (990°C), has also been used successfully. Although this glass is somewhat stronger, it is more difficult to obtain uniform coatings with it. The thermal expansion coefficient of the wire is about 8.5×10^{-7} cm/cm °C, and any glass with a similar coefficient would probably be suitable without cracking. The wire is pushed through the molten drop until an adequate length of it is coated. The tip may be pulled back through the drop or the shank may be pulled up through the top of the loop. The temperature of the loop must be increased as the thicker shank of the wire is inserted inside the glass drop, as the increased heat loss decreases the fluidity of the glass. The glass wets the platinum iridium wire and covers the entire electrode surface, including the tip, with a thin insulating coat. However, to record properly, the extreme tip must be bare of glass, and a procedure must be employed to remove the right amount of insulation and provide the best interface between the electrode

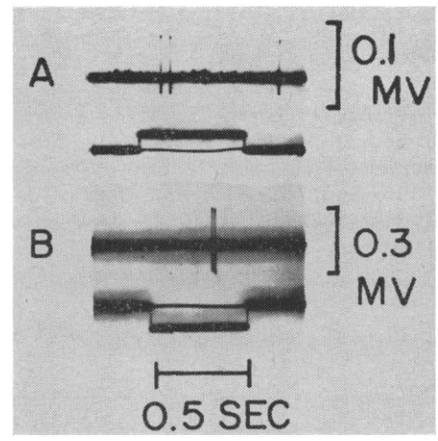

Fig. 1. Single unit action potentials recorded with the same platinum iridium electrode from ganglion cells in the retina of a goldfish (A), and the retina of a frog (B). In (A), upward step in lower trace indicates the duration of illumination used for stimulus. In (B), downward step indicates duration of interruption in illumination used for stimulus.

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Type manuscripts double-spaced and submit one ribbon copy and one carbon copy.

Limit the report proper to the equivalent of 1200 words. This space includes that occupied by illustrative material as well as by the references and notes.

Limit illustrative material to *one* 2-column figure (that is, a figure whose width equals two columns of text) or to *one* 2-column table or to *two* 1-column illustrations, which may consist of two figures or two tables or one of each.

For further details see "Suggestions to Contributors" [*Science* **125**, 16 (1957)].

and the tissue. Since the glass coating is thinnest at the tip, where an applied voltage has its strongest gradient, preferential breakdown of the insulation at this location is easily done electrically. The best electrical stability and lowest impedance may be obtained by coating the exposed area with platinum black. Fortunately, the two procedures may be combined into one by utilizing the platinizing voltage as the insulation rupturing voltage. The platinizing bath is a 1 percent solution of platinous chloride (stronger solutions appear to be detrimental to the coating). Current from a 15-volt d-c source in series with a 1-megohm resistor is passed for 15 to 30 seconds between the electrode and a platinum wire in the solution, the electrode being negative. A stream of tiny bubbles from the tip indicates a good electrode. Bubbles elsewhere indicate that the insulation is leaky and that the electrode needs to be recoated with glass. After electrodes have been used in biological material, they should be cleaned by bathing them overnight in distilled water.

Electrodes of this type have recorded successfully from the ganglion cells of the vertebrate retina, the optic nerve of the squid, mechanoreceptors on the antennae of mosquitoes, and many others. Some of the neurons recorded from were sensory nerve fibers and ganglion cells less than 10 μ in diameter for which most other types of electrodes are too noisy. Neurons giving impulses of 40 μv or more could easily be isolated from the surrounding neural activity. Figure 1 shows records made by using the same electrode first in the retina of a goldfish and then in the retina of a frog. With our recording system the electrode usually has a noise level of 20 μv and will discriminate impulses larger than 40 μv.

The electrode is durable enough to record well after penetrating the cartilaginous "skull" of a squid (*8*), and it should also be able to record successfully after penetrating other tough structures such as the dura of the mammalian brain (*9*).

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References and Notes

1. See the following papers for measurements of the various parameters involved, extensive discussions, and bibliography: A. L. Byzov, *Biophysics (U.S.S.R.) (English translation)* **2**, 248 (1957); J. A. B. Gray and G. Svaetichin, *Acta. Physiol. Scand.* **24**, 278 (1951); R. E. Pattle, "The construction and electrical properties of microelectrodes for physiological work," *Porton Tech.* Paper No. 383, 9 Nov. 1953; G. Svaetichin, *Acta. Physiol. Scand.* **24**, 863, 5 (1951).
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9. The opinions and assertions contained herein are ours and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large. This research was supported in part by National Science Foundation grant G-7086.

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Isolation of Uridine Diphosphate–Glycosyl Compounds from the Slug

Abstract. An examination of the acid-soluble nucleotides of *Limax maximus* Linné revealed, among others, several uridine diphosphate–glycosyl compounds. Nucleotides isolated and identified were the uridine diphosphates of glucose, acetylglucosamine, and acetylgalactosamine.

Numerous reports have indicated the occurrence in various snails and other mollusks of sulfated polymers (mucins) which contain one or more of the sugars glucose, galactose, glucosamine, and galactosamine (*1*). Since current research trends support the hypothesis that polymer syntheses are intermediated by nucleotide-activated precursors (*2*), it seemed worth while to investigate the nucleotide content of the slug, *Limax maximus* Linné (*3*).

Nucleotides were extracted from 24 slugs (156 g) by homogenization with 150 ml of ice-cold 10-percent trichloroacetic acid in a Waring blender. An equal volume of ethanol was added, the precipitate was centrifuged off and discarded, and the supernatant solution was treated essentially by procedures previously described (*4*). Nucleotides in the extract were freed of salts by charcoal treatment (*5*) and then adsorbed onto a Dowex-1-formate column. The column was washed with 3*M* formic acid and the uridine diphosphate–glycosyl compounds were then eluted with 4*M* ammonium formate (*p*H 2.8).

Salts were again removed by charcoal treatment and the nucleotides were chromatographed on Dowex-1-formate with a linear gradient (*6*) which changed from 3*M* formic acid to 4*M* ammonium formate, *p*H 3. A single peak of the five obtained from the column contained over 80 percent (42 μmole as uridine) of the 260-mμ absorbing material. Only the material from the single large peak was further investigated. After processing by the charcoal procedure (about 25 percent recovery), the material gave 250 mμ to 260 mμ and 280 mμ to 260 mμ ratios of 0.76 and 0.42, respectively, thus suggesting the presence of uridine compounds. This material was then separated into three major ultraviolet absorbing bands by paper chromatography on Whatman No. 1 paper, with ethanol-M ammonium acetate (7.5:3, vol/vol) as solvent, first at *p*H 7.5 and then at *p*H 3.7 (*7*).

The slowest moving band contained at least 30 percent uridine diphosphate–glucose, as determined by comparing 260 mμ measurements with assay by uridine diphosphate–glucose dehydrogenase (*8*) and acid hydrolyzable glucose content as determined with glucose oxidase (*9*). The remainder of the material in the first band has not been identified.

The second band exhibited R_F values on paper chromatograms similar to known uridine diphosphate–acetylglucosamine and was found to contain acid-hydrolyzable acetylhexosamine (0.1*N* HCl/10 min at 100°C) (*10*). To determine whether or not more than one amino sugar was present, the material was hydrolyzed for 2 hours in 2*N* HCl and then was chromatographed on a 1- × 50-cm Dowex 50-H column by the procedure of Gardell (*11*). Two hexosamine-positive peaks (*12*) emerged from the column with R_F values suggestive of glucosamine and galactosamine, and were further identified as such by (i) comparison of R_F values with knowns on paper chromatograms with use of *n*-butanol-pyridine water (6:4:3 vol/vol) as solvent and silver nitrate to visualize sugar spots (*13*), and (ii) by degradation with ninhydrin by the procedure of Stoffyn and Jeanloz (*14*) to yield arabinose and lyxose, respectively.

The third, fastest-moving ultraviolet absorbing band contained a reducing compound (*15*) which has not been identified.

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