HCl, finally against distilled water, and the product was lyophilized.

The hexose content of the lysozyme-galactosylamine reaction product determined by the orcinol method was 2.0% (1.6 galactose/mol protein) in urea and 2.8% (2.2 galactose/mol protein) in guanidine HCl solution. The lactosylamine derivative obtained in guanidine HCl solution contained 8.8% of hexose (3,6 lactose/mol protein). The coupling of D-glucosamine under the same conditions yielded a product containing 1.5% (1.2 ml glucosamine/mol protein) glucosamine, as determined by the Morgan-Elson method.

The presence of galactose, galactose plus glucose, or glucosamine respectively was demonstrated in the acid hydrolysates of the products by thin layer chromatography[5].

The electrophoretic mobility of the galactosylamine and of the glucosamine derivatives in acrylamid gels in the presence of sodium dodecyl sulfate[6] was essentially the same as of the untreated lysozyme. The lactosylamine derivative migrated somewhat slower, as could be expected from the gain in molecular weight due to the carbohydrate residues.

Peptide maps of the carboxymethylated[7] products were performed on silica thin layer[8] (Figure 1) after trypsin digestion[9]. Sugar-containing peptides were demonstrated on thin layer electrophoregramms detected successively by ninhydrine and by orcinol-sulfuric acid reagents[8] (Figure 2).

The fingerprints of both the lactosyl- and the galactosylamine derivatives differ significantly from that of the lysozyme. The absence of the C-terminal leucine on the peptide map of the lactosylated products indicates that the C-terminal carboxyl was quantitatively modified by the lactosylamine. The presence of 3.6 mol of lactose in one mol of protein, as well as the presence of several sugar-containing peptides in the trypsin digest, suggest that the major part of the lactosylamine must be bound to the free carboxyl groups of glutamic and/or aspartic acid residues (2 glutamic and 7 aspartic acid residues were found in lysozyme by CANFIELD and ANFINSEN[9].

The orcinol reaction of the trypric peptides of the galactosylated product on the electrophoregrams of silica thin layers was much weaker than those of the lactosylamine derivatives. The leucine spot on the peptide map of the galactosylderivative was almost as accentuated as on the corresponding peptide maps of the unchanged lysozyme. This suggests that none of the available carboxyl groups of this product is glycosylated quantitatively under the conditions used.

The presence of the sugars in a non-dialysable, electrophoretically homogenous protein derivative, as well as the presence of sugar-containing peptides in its tryptic digest, indicate that this simple method permits the introduction of well defined oligo- and monosaccharides into protein molecules under mild conditions by means of a naturally occurring glycosylamine bond.

Résumé. Mono- et oligosaccharides ont été attachés au lysozyme par une liaison glycosylaminique, en faisant réagir les protéines activées sur les groupements carboxyliques par carbodiimides, avec des glycosylamines.

E. MOCZAR

Laboratoire de Biochimie du Tissu Conjonctif, Faculté de Médecine, Université Paris-Val de Marne, 6 rue du Général Sarrail, F–94000 Créteil (France), 25 May 1973.

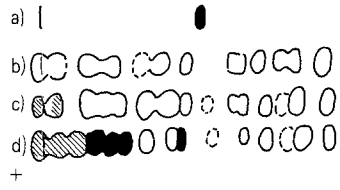

Fig. 2. Electrophoregramm of the tryptic peptides on silica thin layer. Buffer: pyridine-acetic acid-water 1:10:89, 2 h, 300 V. ○, ninhydrine positive sports; ●, ninhydrine and orcinol positive spots, a) glycosylgalactosylhydroxylysine; b) lysozyme; c) galactosylamine-; d) lactosylamine derivative.

[5] E. MOCZAR and M. MOCZAR, in *Progress in Thin Layer Chromatography* (Ed. A. NIEDERWIESER and G. PATAKI; Ann Arbor-Humphrey, London 1970), p. 169.
[6] G. FAIRBANKS, T. L. STECK and D. F. M. WALLACH, Biochemistry *10*, 2606 (1971).
[7] P. BUTLER, J. I. HARRIS, B. S. HARTLEY and R. LEBERMANN, Biochem. J. *112*, 679 (1969).
[8] E. MOCZAR, J. Chromat. *76*, 417 (1973).
[9] R. E. CANFIELD and C. B. ANFINSEN, J. biol. Chem. *238*, 2689 (1963).

A Microlathe for Constructing Miniature Multibarrel Micropipettes for Iontophoretic Drug Application

Various techniques for the construction of multibarrel micropipettes for the micro-iontophoretic application of drugs to neurones have been published. CURTIS[1] has described a large multibarrel electrode (Figure 1) which must be handmade by an experienced glassworker, a limitation which together with the large drug volume required, reduces its utility. HERZ et al. (in KRNJEVIC[2]) have described a technique using cemented glass tubes which are twisted together just prior to pulling. Unfortunately, while this technique produces excellent small-sized electrodes, considerable time must be devoted to acquiring the technique; the electrodes tend to be very variable in their properties and dimensions and the tip length can seldom be longer than 5 mm.

The microlathe described here permits the construction of electrodes having 3 or more barrels with reproducible characteristics and with small (1.5–2 mm) diameter tip extensions of up to 2 cm or more which permits their use in investigating deep brain nuclei. This microlathe, the design of which is based on the conventional glassworkers lathe, allows simultaneous rotation, heating, fusing, and drawing apart of the softened glass electrode blank. The essentials of the lathe are evident from Figure 1.

An aluminium optical bench (B) forms the bed of the lathe upon which a platform (P) can be moved by means of a rack and pinion (R)[3]. It is imperative that the pair of gears on the head and tailstock should have the same

[1] D. R. CURTIS, *Physical Techniques in Biological Research* (Ed. W. L. NASTUK, Academic Press, New York 1964) Vol. 5, p. 144.
[2] K. KRNJEVIC, *Methods of Neurochemistry* (Ed. R. FRIED, Marcel Dekker, New York 1971), p. 129.
[3] Edmund Scientific Co., Barrington N. J., USA; Catalogue Numbers: Bench 60,573, Platform 40,891.

ratios since both chucks must rotate in synchrony. Any suitable gears can be used, those in the lathe described came from a bombsight computer.

Drawing out the tubing is accomplished by moving the rotating tailstock (T) away from the heater (H) while the glass is still soft, by means of a rack and pinion mechanism (R). A keyway in the lower driving shaft (D) engages with a pin in the lower tailstock gear (L) and couples the rotary motion to the tailstock, allowing it to continue to rotate while the hot tubing is drawn out.

A hollow shaft (S) through which the lower drive shaft slides should extend roughly 25 mm on either side of the lower tailstock gear in order to minimise binding of the drive shaft as the tail stock of rocked forward and back. At the outer end of this hollow shaft is fitted the pin which engages with the keyway.

A geared DC motor (M) powered by a simple variable voltage supply so as to give a chuck rotational speed of 60–120 rpm, is used to drive the lathe. Care excerised in the construction and alignment of the lathe so that it runs freely, will mean that only a small drive motor will be required since the softened glass imposes little load on the mechanism. Three-jaw electric drill chucks are used to hold the glass tubing and the inner surfaces of the jaws should be as smooth as possible to prevent shattering the tubing as the chucks are tightened.

A simple 8 to 9 mm diameter single layer heating coil of 20 g Nichrome wire forms the heating coil (H). Power for the heater is obtained through a step-down transformer (10 V 10A) which has a full wave thyristor domestic lamp dimmer in series with the primary winding. Alternatively, a Variac can be used instead of the dimmer.

Electrode blanks are constructed using 1.3 mm (or smaller) diameter thin walled Pyrex capillary tubing. It is preferable to use tubing which is slightly larger in diameter for the centre tube (roughly + 0.01 mm). If the tubing is purchased in bulk, then grading prior to cutting is easily performed using a pair of vernier calipers.

The electrode glass should be pre-cut and the ends fire polished; the side tubes being half the length of the centre tube. Following this the tubes are washed in chromic acid, followed by distilled water, dry acetone and then air dried.

An assembly jig which can be fabricated from a plastic 1 ml 'Tuberculin' syringe (Figure 2a) is used to aid assembly and minimize hand contact with the glass. A pair of spring wire clips can be constructed to hold the bundle of glass together (Figure 1, C) or alternatively the bundle can be bound with fine wire (Figure 2a).

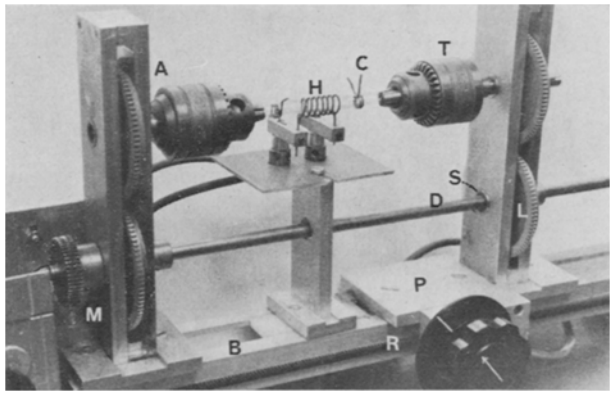

Fig. 1. Overall view of the lathe. The spring clips (C) used to hold the glass bundles together can be clearly seen. The large knob (with arrow) in the lower RHS controls the tail stock movement. To the bottom left of the headstock (A) can be seen the drive motor worm gear and pinion (M). The other parts identified by letters are described in the text.

Heater temperatures and heating time prior to drawing the tubing must be determined empirically. Sufficient heat must be given to fuse the tubes laterally without causing the lumen of the tube to shrink and the rotational rate should be such as to just prevent noticeable sagging of the softened glass bundle, but not enough to cause the softened tubes to fly apart – about 60 rpm appears to be adequate. Some very slight sagging and the resultant rolling action of the softened glass helps the side tubes to fuse to the centre one.

After from 40–60 sec of heating (coil bright yellow) the rotational rate is increased to about 100 rpm while drawing the blank out. It is normally sufficient to move the tail stock back 1 to 1.5 cm to obtain a reasonably long tip length – although if longer electrodes are required the tail stock can be moved as far as desired. The heater should be turned off when the pulling process starts and the electrode blank should be allowed to continue rotating until cool before removing.

The finished blanks (Figure 2a) are then drawn using a conventional vertical or horizontal electrode puller and with as small a heating coil diameter as possible. Both electrodes should be usable.

Centrifugation has proven to be the best method of filling the electrodes since the difficulties of inserting glass fibres into drawn blanks and the vagaries of pulling caused by the fibres makes this method a completely unsatisfactory one for these electrodes. To this end a collar of 22 g copper wire (Figure 2a, C) is slipped over the shank of the electrode (from the tip end) and a small amount of fast-setting epoxy cement is spread carefully around the collar and warmed gently over a small flame. This warming serves to liquefy the epoxy which then flows between the tubes and the collar and then sets rapidly reinforcing this unfused section of the electrode.

A conventional solid centrifuge rotor[4] is used, since swing-out bucket heads produce too much air drag, causing both slow running and overheating of the centrifuge. The rotor is adapted for electrode centrifugation by plugging opposite holes with two identical bored hard rubber stoppers, into which a hard plastic sleeve (against which the wire collar bears) are inserted (Figure 2c). Such sleeves can be constructed from plastic disposable hypodermic syringe needle covers. In order to reduce air drag still further the vacant rotor holes should be taped over on both the top and bottom.

Electrodes are filled using thin (PE 10) catheter tubing, the solutions being filtered through a 0.4 μm 'Millipore' filter before use, and the filling tube rinsed in filtered distilled water before and after inserting into the electrodes. Care must be taken to exclude air bubbles and not to overfill the barrels. Model maker's paints are used to identify the barrels.

Just prior to centrifugation the electrode tips are broken back to give tip diameters of from 3–7 μm, by pushing them, under microscope control, into a polished metal block. This seems to be the most reliable and controllable method of doing this; the electrode is clipped onto the transverse carrier of the mechanical stage, and the block mounted over the condenser lens.

An additional advantage of electrodes constructed in this manner is that a connector can be constructed to connect the drug barrels to the current source (SPENCER[5]), which greatly facilitates the changing of electrodes during an experiment. Such a connector is illustrated in Figure

[4] I. E. C. 800 Series Centrifuge.

[5] H. J. SPENCER, Med. biol. Engng. *9*, 683 (1971).

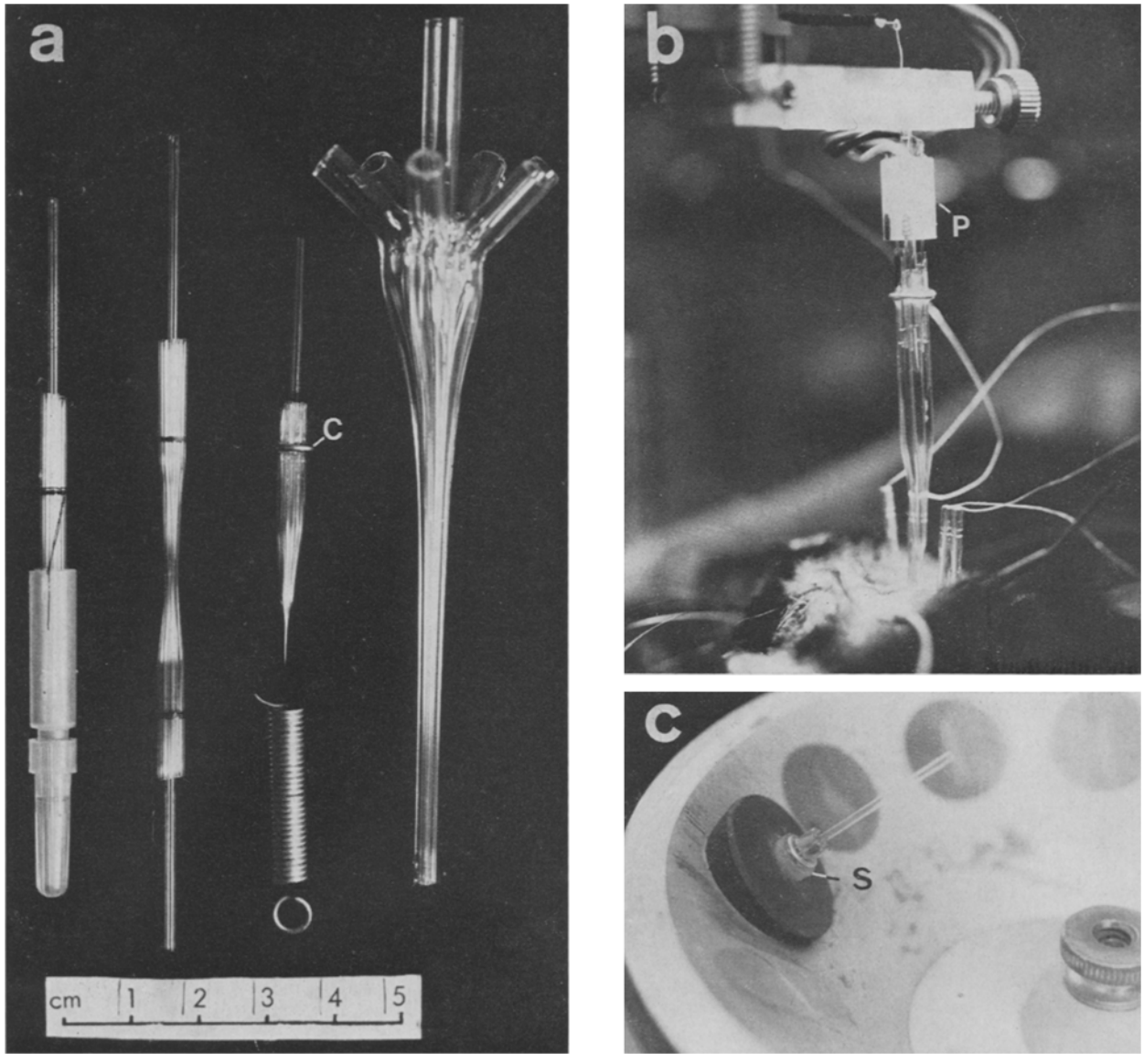

Fig. 2 (a) Stages in the construction of the electrode, together with an electrode of the type employed by CURTIS[1] for comparison. From the left; bundle of glass tubes wired together in the assembly jig; blank electrode assembly following fusion and drawing in the lathe and a finished electrode with the collar (C) fitted after having been pulled in a conventional microelectrode puller and ready for filling. Below the finished electrode is a coil of copper wire from which collar rings (below the coil) can be cut using a pair of diagonal wire cutters. (b) A microelectrode being used to record from the rat caudate nucleus. The connecting plug (P) is constructed from a length of 6 to 7 mm nylon rod (see text). The small vertical rods on the skull are stimulating electrodes. (c) Adapter for filling the electrodes by centrifugation. Since the rotor holes are oblique, the hole in the stopper must be sufficiently large to prevent its narrowing when the stopper distorts under centrifugal force. The plastic support (S) is a push fit into the stopper hold.

2b and can comprise part of the micro-electrode holder. Essentially the connector is constructed from a 6 to 7 mm diameter cylinder of nylon or teflon, perforated with a central hole for the central barrel, with one lateral hole per barrel, into which platinum wire contacts are fitted. The junction between the platinum wire and the cable should be insulated with 'formvar' lacquer or its equivalent and this junction should be within the plastic cylinder to reduce stray leakage paths. The connector should be rinsed after use with distilled water, followed by drying with acetone or absolute alcohol.

Résumé. On décrit un micro-tour pour confectionner de petites microélectrodes à plusieurs pipettes destinées à appliquer des drogues aux voisinage des neurones. Ces microélectrodes ont des tiges droites et leur géométrie et leurs particularités sont très uniformes. On peut faire facilement des électrodes à pointes longes (2 cm) et fines pour les introduire dans les structures profondes du cerveau. On expose aussi une méthode permettant de remplir les électrodes par centrifugation et d'établir un assemblage de connection.

H. J. SPENCER

Department of Physiology, Faculty of Medicine, University of Manitoba, 770 Bannatyne Avenue, Winnipeg (Manitoba R3E OW 3, Canada),
2 Juli 1973.