



Tungsten Microelectrode for Recording from Single Units

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Table 1. Inhibition of growth of sarcoma 180 by 6-azauridine in tissue culture.

Concn. of 6-azauridine (μ mole/ml)	Growth as compared with control (%)
0	100
0.01	88
0.03	83
0.1	50
0.3	16

Table 2. Reversal by uridine of the inhibitory action of 6-azauridine (0.2 μ mole/ml) on sarcoma 180 in tissue culture.

Concn. of uridine (μ mole/ml)	Growth as compared with control (without inhibitor or uridine) %
0	37
0.002	59
0.006	84
0.02	94
0.06	94
0.2	96

on sarcoma-180 *in vivo* might be attributable to a metabolite of the analog formed by the liver or other normal tissues. An attractive possibility for consideration, as a metabolite of 6-azauracil, was its riboside (6-azauridine), since this derivative is formed by certain microorganisms and inhibits the growth of 6-azauracil-resistant strains that emerge when *Streptococcus faecalis* is grown in the presence of 6-azauracil (5). The 6-azauridine used was prepared both biosynthetically (using *S. faecalis* 8043) and by chemical synthesis (6, 7).

Of the sarcoma-180 cells (8), 200,000 were introduced into each culture flask (Earle's T-15) (9) in which 2 ml of Eagle's medium containing 10 percent horse serum was present (4). After 24 hours, this medium was replaced by Eagle's medium containing 5 percent dialyzed horse serum and various concentrations of 6-azauridine; the medium was renewed daily. After 7 days, the protein content of the cell layer was determined, using the method of Oyama and Eagle (10). In the controls, without inhibitor, a three- to four-fold increase in cell protein, as compared with that observed 24 hours after inoculation, was obtained. Both the biosynthetically and the chemically prepared 6-azauridine showed the same high activity in inhibiting the growth of sarcoma-180; the results obtained with the biosynthetic material are shown in Table 1.

The activity of 6-azauridine was an-

tagonized by uridine: as is shown in Table 2, the molar ratio of metabolite to antagonist necessary to abolish almost completely the action of 6-azauridine (at the level tested, 0.2 μ mole/ml) was 1/10. Even with a tenfold higher concentration of uridine, under these conditions, no evidence of toxic effects on the cells was observed. Deoxyuridine was also active in reducing the inhibitory activity of 6-azauridine; however, quantitative data concerning its activity and its possible toxicity for the cells have not yet been obtained. Further work will be concerned with the action of related analogs and their nucleosides, the effects of other possible reversing agents and their comparative activity, and the effects of the agents on other cell lines in tissue culture (11).

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4. ———, *Science* 122, 501 (1955).
5. R. E. Handschumacher, *Biochim. et Biophys. Acta*, in press.
6. ———, in preparation.
7. The small amounts of 6-azauridine were generously supplied by R. E. Handschumacher of our laboratories.
8. The sarcoma 180 cells used in the tissue-culture studies were derived from a strain isolated and generously supplied by George Foley, Children's Cancer Research Foundation, Boston, Mass.
9. W. R. Earle and F. Highhouse, *J. Natl. Cancer Inst.* 14, 841 (1954).
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11. We wish to express our gratitude especially to the Jane Coffin Childs Memorial Fund for Cancer Research for a grant which permitted the equipment of a laboratory for tissue-culture studies and contributed to the costs of the research. This program also is supported by grants from the American Cancer Society and the U.S. Public Health Service. One of us (R. S.) is grateful for post-doctoral fellowship support by the Stiftung für Stipendien auf dem Gebiete der Chemie, Switzerland.

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Tungsten Microelectrode for Recording from Single Units

An electrode has been developed to fill the need for an easily made, sturdy device capable of resolving single-neuron action potentials at least as well as the commonly used micropipette. It was designed to be used not only in acute animal experiments in the central nervous system, but also in cases where pipettes may be especially prone to break, such as in chronic unrestrained preparations, with muscle, and in the human being during neurosurgical pro-

cedures. Early experience made it clear that, while tip diameters of the order of 20 μ may at times be adequate for resolution of unitary spikes recorded extracellularly, tips 5 μ or less are much more satisfactory, and that intracellular recording usually demands tips of less than 1 μ in diameter. Since steel wire becomes too fragile near the tip when thus sharpened and also requires too thick a shaft, tungsten was selected as by far the stiffest, easily available metal.

The electrode consists of an electrolytically sharpened tungsten wire insulated to the tip with a suitable lacquer. A wire 125 μ (5 mils) in diameter and about 1 inch long is bent slightly near one end which is then mounted in a 27-gage hypodermic needle. Because crimping of these needles results in perfectly satisfactory electric contact, no attempt has been made to solder the tungsten. Electropolishing is then carried out by a method analogous to that described by Grundfest *et al.* (1) for steel: the terminal few millimeters are immersed in a saturated aqueous potassium nitrite (KNO₂) solution, and an alternating current is passed between the wire and a nearby carbon rod, using 2 to 6 v, which may be conveniently obtained from a 6.3-v filament transformer fed by a Variac (2). The optimum voltage is not critical, but currents that are too low or too high tend to cause pitting.

If the wire is kept stationary and if the polishing is allowed to continue until all bubbling ceases, a rather abrupt pencil-like point is obtained which has a tip of ultramicroscopic dimensions (from 0.5 to 0.05 μ in diameter). Such a result is explained by the fact that the meniscus height depends on the diameter of the wire, which decreases as the polishing proceeds. The suddenness of the taper may give rise to excessive dimpling of the tissue to be penetrated. This may be avoided by lowering and raising the wire during all but the final stages of polishing, thus producing almost any degree of taper. A hydraulic drive with two oil-filled syringes and plastic tubing may be used for this, as well as for the coating.

Fig. 1A shows an electromicrograph of a wire sharpened as described; the tip measures about 0.4 μ . Tips of this size or less are consistently obtained without particular skill or practice.

Sharpened electrodes are washed in detergent and coated with a clear lacquer (3) that has been allowed to thicken to an almost honeylike consistency by exposing to air at room temperature for some hours. Under a dissecting microscope (6 to 40 magnifications), the wire is lowered into a beaker brimful of freshly stirred lacquer, and then slowly raised. When the tip

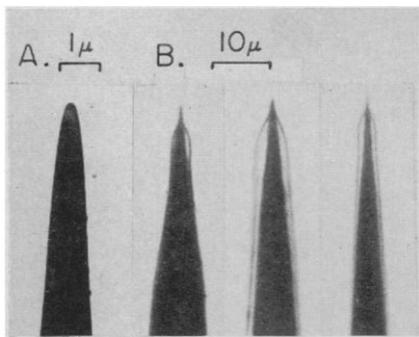


Fig. 1. (A) Electromicrograph of an uncoated, sharpened tungsten wire; (B) optical photomicrographs of coated electrodes immersed in water to show the coating.

emerges and lacquer runs up from the tip, the tip is quickly redipped up to the bead so formed, and this is repeated until the lacquer no longer runs up. The receptacle must be full and preferably small, and it must be used in a region where air is circulating to prevent thinner vapor from condensing on the tip and washing off the coating. The electrode is allowed to dry for 24 hours; it need not be baked.

Coarse testing is carried out by watching bubbling under the microscope when direct current is passed through the electrode in 0.9-percent NaCl solution using 6 to 12 v (electrode negative). This may be done for the shaft (at 10 diameters) and for the very tip (at 100 to 850 diameters). For electrodes to be used for intracellular work, the resistance may be measured as the electrode is immersed slowly in saline. Any abrupt changes after contact indicate flaws in the coating which may have failed detection in previous tests.

Fig. 1B shows several coated electrodes that were photographed under water with an optical microscope to show the coating, which is not otherwise visible and which usually extends well beyond where it can be seen by this method, as proved by the bubble tests.

Resistance measurements have been made using a Wheatstone bridge with rectangular pulses or short bursts of sine waves for a signal and a cathode-follower input stage as a detector (4). For small currents (of the order of 10^{-7} amp or less), resistance is fairly independent of direction and magnitude of current, and it varies widely from electrode to electrode, averaging perhaps 75 Mohm but ranging from 25 to 200 Mohm at low frequencies (100 cy/sec). At high frequencies (5 to 10 kcy/sec), the impedance drops to about 0.5 to 5 Mohm even when only the very tip is immersed. Measurements made while recording single-unit action potentials by shunting the electrode to ground with

a variable resistance and calculating resistance from the drop in spike voltage have varied from 2.5 to 10 Mohm.

Direct-current stability of the electrode seems adequate to coarse measurements, as shown by the absence of any obvious instability over periods of 5 minutes, using 100-mv pulses with an inkwriter and direct-coupled amplifier with over-all sensitivity of 40 mv/cm. As might be expected, the electrode must be connected to a high-impedance input if the low frequency response is not to be severely limited, which means that a grid-leak resistor must not be used in the input stage. No evidence for polarization is seen if input current is kept low. It should be noted that excessive grid current may give rise to considerable noise.

Single-unit records from the nervous system have been obtained to date in cats from posterior root fibers, spinal cord, brain stem (reticular substance, dorsal cochlear nucleus, and superior olive), cerebral cortex, and olfactory bulb (Fig. 2). Spikes presumably recorded from outside the cell, averaging 5mv (0.5 to 10 mv), resemble in form those described by Rose and Mountcastle for the indium micropipette (5). Such spikes have also been obtained from cat's cerebral cortex after inserting the electrode through the unincised dura mater. Other spikes, presumably intracellularly recorded, may reach 40 mv with conventional cathode-follower input, and 70 to 80 mv when negative capacitive feedback is adjusted short of ringing. Such spikes have been observed for up to $\frac{1}{2}$ hour with no loss of amplitude.

Finally, in fulfillment of the original

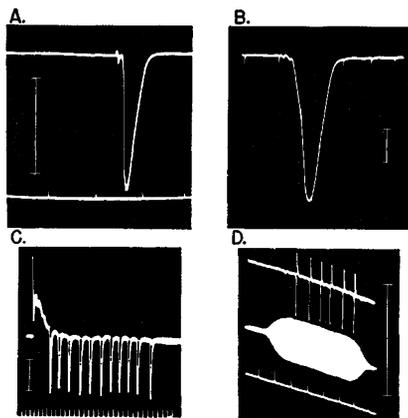


Fig. 2. Single-unit action potentials recorded from cat. (A) S_1 dorsal root, stimulation of same root. (B) Anterior horn cell, spinal cord, S_1 . Stimulation of ventral root. (C) "Renshaw cell," spinal cord, S_1 . Stimulation of ventral root. (D) Response in dorsal cochlear nucleus to a 4000-cy/sec tone. For all tracings, the amplitude is 5 mv, time is in milliseconds; positive deflections are downward.

objective, the electrode has been used for recording single units for periods of the order of 1 hour from cerebral cortex in chronic waking cats restrained only by a chest harness (6).

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References and Notes

1. H. Grundfest *et al.*, *Rev. Sci. Instr.* 21, 360 (1950).
2. I wish to thank C. Henson for his technical assistance and Irvin Levin, Instrumentation Division, Walter Reed Army Institute of Research, for suggesting the electrolytic process as applied to tungsten.
3. For example, Insl-x, E-33, clear, made by Insl-x Co., Inc., Ossining, N.Y.; or clear vinyl lacquer, S-986s, made by Stoner-Mudge, Inc., Pittsburgh, Pa.
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6. A description of this technique is in preparation.

28 November 1956

Selection of Body Sites for Fat Measurement

With increasing interest in the problem of obesity, workers are turning from such indirect measures as overweight, relative weight, and percentage-of-standard weight to direct measurement of body fat. However, when the superficial fat layers are measured by pinch calipers or by means of roentgenograms (1), the problem arises as to which of many possible sites to employ.

In order to investigate the standardized soft tissue, teleroentgenograms were taken on 100 American-born white males aged 21.0 to 22.9 years (2). With suitable shielding, gonadal radiation was limited to approximately 0.02 r. In all, six regions of the body were x-rayed (forearm, deltoid, thoracic, iliac, trochanteric, and lower leg). From the series of six regions contained on a single 7- by 17-in. film, fat measurements were made at 12 sites. These included the medial and lateral arm, the "pocket" formed by the triceps and deltoid muscles, the lower thoracic site, the iliac crest and spine, the upper and middle trochanteric sites, and the four quadrants of the lower leg (1, 3).

The group studied had a mean stature of 180 cm and a mean weight of 72 kg: it was reasonably representative of young adult American males. All fat thicknesses were positively intercorrelated, with values of r ranging from 0.32 to 0.96. In general, deltoid, thoracic, iliac, and trochanteric fat (areas of "central" fat) showed considerably higher group intercorrelations than "peripheral" or ex-