chick heart and adult mouse muscle fibroblasts, embryonic chick retinal epithelial cells and SZ_3B16 mouse melanoma[4] cells. The film could be cut into patterns with a scalpel, or stripped away with a silicone rubber wedge of desired width, to provide clean edges demarcating junctions between the prepared substrate and the underlying glass coverslip.

Incubation of all tissue explants so far tested (embryonic chick heart, foetal human connective tissue and adult human skin) appeared to be at least as successful on the fused substrate as on dried collagen. The mean outgrowth (based on 2 measurements at right angles) from 10 chick heart explants incubated for 24 h on fused collagen was probably significantly greater than that from 10 explants incubated on standard dried collagen film ($t = 2.9126$, d.f. 14; $0.02 > p > 0.01$).

Cells of permanent in vitro lines of mouse melanoma[4], human melanoma[5] and Ehrlich Lettré mouse ascites carcinoma[6] all grew successfully on fused collagen. However, no significant difference in mitotic index (based on 10 counts of 1000 cells) could be found among SZ_3B16 mouse melanoma cells seeded at a uniform density on fused collagen, dried collagen and glass.

Routine fixation of cells on fused collagen with formol saline and subsequent staining with Mallory's aqueous haematoxylin were entirely successful. So far E. M. sectioning has been limited to the substrate alone but control of the depth of film and the facility with which the latter can be removed from the coverslip for subsequent processing suggest that it will be perhaps even more convenient to handle than standard dried collagen[3]. The effects of heat treatment on microcrystalline collagen[7] have not yet been studied. If this also produces a satisfactory substrate, ethylene oxide sterilization and the subsequent period of delay before use[7] could be eliminated.

Summary. Coverslips coated with rat-tail collagen dried at 37 °C were placed in a hot-air sterilizing oven at 160 °C for 2 h. The resulting transparent sterile film was found to be a useful multipurpose substrate for cell culture and for subsequent histological sectioning.

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A Simple Stimulus Isolation Unit Using a Complementary Metal Oxide Semiconductor Switch

Most neurophysiological experiments involve both the electrical stimulation of an excitable tissue (by passing an electrical current between 2 points) and the recording of the response (by measuring the voltage difference between 2 other points). If the current flows between a source and ground, the electrical recording is distorted, usually for several msec after the end of the stimulus[1]. This 'stimulus artifact' is greatly reduced or supressed by circulating the current through a path that is independent of the ground used for recording. There are at least 4 types of device stimulus isolation unit (SIU) that perform this function: The output of the secondary windings of a transformer ('inductorium'), the high-speed relay[2], the radiofrequency SIU[3,4] and the opto-isolator SIU[4–6]. The transformer output is necessarily AC. With high-speed relays it is difficult to obtain pulses of short duration (below 1 msec) without 'chatter'. The radiofrequency SIU is essentially a high frequency transformer whose output is rectified and filtered to produce DC outputs[3,4] and the opto-isolator SIU use a light beam to control the flow of current driven by a battery[4–6]. The last two SIU are multi-component instruments that could be replaced by just one battery, to drive the current, and one switch, to turn on and off the current. Here I am presenting a new type of SIU, with most of the features of the older instruments, whose construction is very simple and whose cost (excluding batteries) is ~ US $7.00. It consists of a very fast dual complementary metal oxide semiconductor (CMOS) switch that turns on or off the current flow between

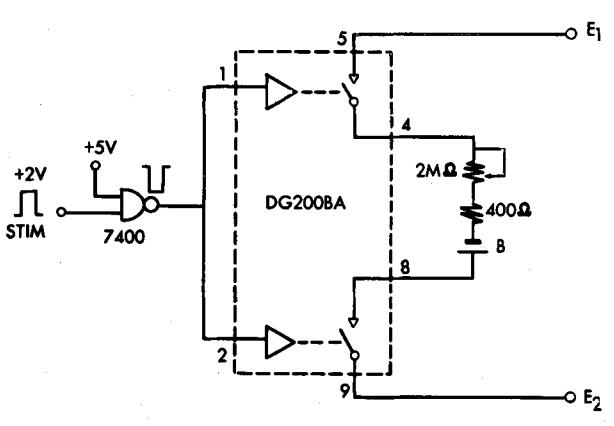

Fig. 1. Diagram of a CMOS SIU. The stimulating current flows between electrodes E_1 and E_2 when both switches are closed. The duration of the current pulse is determined by the duration of the positive square pulse (STIM), and the magnitude, by a 2 $M\Omega$ variable resistor. The numbers correspond to the pin numbers of Siliconix DG 200BA. B represents two 9.8 V mercury batteries, Mallory TR137.

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electrodes E_1 and E_2 (Figure 1). The current is set by means of a variable resistor. Both switches are driven by built in drivers that, in the case of the CMOS Siliconix DG-200, are closed by a logic low. A simple way of driving these switch drivers with a conventional electrophysiological stimulator is to apply +2V to one input of a 7400 NAND, while the other input is tied to +5V. The resulting logic low output is then used to drive the switches. There are several other CMOS switches (like Analog Devices AD 7513) that can perform the same function with equally good results.

The ground isolation (as well as the resistance between the terminals during the off state) is $>10^9$ Ω. The capacitance to ground during the on state is ~25 pF and the frequency response goes from DC to ~100 kHz. The maximal voltage output is ± 16 V and the maximal current is 40 mA. The *t* off (delay between the 50% point of the positive edge of the control signal and the 10% output) increases by increasing the electrical resistance between the electrodes and ground: For this reason the CMOS SIU should be used with low resistance stimulating electrodes (<100 k) and the preparation should be grounded (Figure 2). However, the ground electrode should not be in direct contact with any of the stimulating electrodes, to avoid destroying the isolation. If these simple precautions are taken, *t* off will consistently be < 20 μsec.

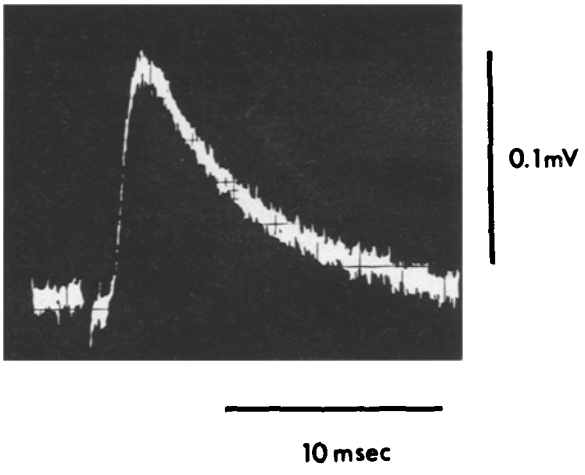

Fig. 2. End plate potential of a frog sartorius neuromuscular junction recorded using the fluid electrode technique[7]. The nerve was stimulated by the CMOS-switch SIU and the response was recorded with a DC amplifier with a band of 40 kHz. Notice the small size of the stimulus artifact, although the size of the response was 0.1 mV.

Summary. A simple stimulus isolation unit for extracellular stimulation is described. The current is provided by a battery and is controlled by a dual CMOS switch and a variable resistor.

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A Rapid Method for the Verification of Drug Injection into the Cerebral Ventricular System of the Rat

The study of catecholamines metabolism in the brain is at present carried out mainly by the introduction of these substances into the cerebral ventricular system. Likewise, in order to investigate the effects of different drugs acting on the central nervous system, the injection of these substances into different regions of the cranial cerebro-spinal fluid system is often used. While some investigators have used stereotaxic techniques[1–4], others have injected the drugs according to several anatomical reference points[5–8]. In both cases, however, it is necessary to be sure that the drug was injected in the previously selected site, especially when difficult and prolonged experiments are carried out (i.e. electrophysiological studies on the action of some substances on the central nervous system). When these sorts of experiments are done in large animals like dogs, cats and rabbits, the proof that the drug was injected in the selected target is easily checked by the extraction of a small volume of cerebro-spinal fluid. However, when experiments are done in small animals like guinea-pigs, rats and mice, this approach is not possible and the usual way to check that the drug has been injected into the selected target is

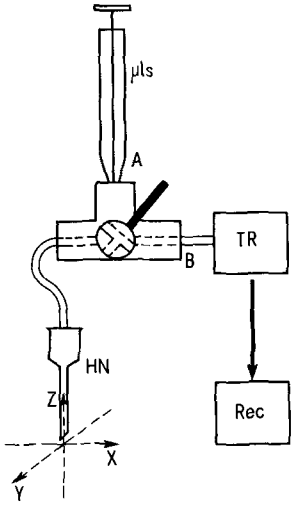

Fig. 1. HN, Hypodermic needle placed in stereotaxic coordinates (x, y, z); μlS, microliter syringe; TR, pressure to electrical signal transducer; Rec, single-channel recorder; A, position of the 3-way stopcock to connect HN to TR. B, position of the 3-way stopcock to connect HN to μlS.

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