Table 1. Comparison of mineral types between the substrate and diverticulum sand. The percentage (by weight) of each mineral type was determined on 500 mg of substrate sand and on 120 mg of diverticulum sand extracted from 34 sand dollars.

Sand mineral type	Color	Density (g/cm^3)	Percentage in sand in	
			Substrate	Diverticulum
Oxidized iron oxide	Red	5.0	0.2	0
Shell fragments		2.7	0.4	0
Oxidized silicates	Orange	2.9	1.4	0
Iron oxide	Black	5.2	9.8	78
Hydrolyzed feldspar and quartz	Translucent	2.7	10.4	0
Highly altered feldspar	Opaque	3.0	18.5	0
Highly altered silicates	Gray	2.8	28.2	0
Feldspar and quartz	Clear	2.7	31.1	22

as to why the diverticulum sand contains 22 percent of clear feldspar and quartz (Table 1), but not other minerals such as silicates, which are also abundant in the substrate.

The particle size of the sand was analyzed by sieving it through a set of Nitex nylon monofilament screen cloths; it was found that the young sand dollar stores only particles smaller than 500 μm in diameter, which constitute about 83 percent of the substrate. However, 70 percent of the sand extracted from the sand dollars is less than 300 μm in diameter, and this constitutes only about 20 percent of the substrate. This suggests that particle size is also a limiting factor. But considering the density and the size of sand particles together, it is still seen that the heavy sand grains are much preferred by the juveniles.

The distribution of *Dendraster* in the Puget Sound area is rather patchy and the population density varies greatly from place to place (*4*). If we assume that the storage of heavy sand is important in the survival of the juveniles, then it follows that the nature of the substrate is essential for the success of the newly recruited juveniles. Thus, differences in particle size and composition of the substrate may have a profound effect on the patterns of distribution and the differences in population density.

At the study site, 10-mm individuals are about 1 year old while 30-mm ones are 2 years old (*4*). This study indicates that at 2 years of age the animals are perhaps sufficiently large to withstand the shifting substrate and no longer need to store sand in the diverticulum. It is also noted that at this stage the gonads begin to grow; they will certainly compete with the diverticulum for coelomic space.

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5. I thank Dr. A. C. Broad for introducing me to this problem and Dr. R. Lambert for identifying the sand components. Technical assistance from G. Aronetz is acknowledged. Supported by a grant from the National Research Council of Canada.

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Capacitor Electrode Stimulates Nerve or Muscle without Oxidation-Reduction Reactions

Abstract. *Porous tantalum disks, available as "slugs" from the capacitor industry, have large available surface area and a thin insulating coating of tantalum pentoxide. When implanted, they fill with extracellular fluid and operate as capacitor-stimulating electrodes having high capacitance per unit volume. Capable of stimulating excitable tissue without generating electrochemical by-products, these electrodes should provide a safer interface between neural prosthetic devices and human tissue.*

During electrical stimulation of muscle or nervous tissue with conventional metal electrodes, charge transfer across the electrode-tissue interface occurs by a combination of oxidation-reduction reactions and double layer charging. Some of the products of the oxidation-reduction reactions are toxic to tissue and must not be allowed to accumulate. Also, these reactions may corrode the electrodes themselves. Clearly such reactions must be minimized or eliminated for long-term electrical stimulation.

Biphasic stimulating waveforms with zero net charge flow, or "balanced" waveforms, have been proposed by Lilly (*1*) and others to minimize electrochemical damage with metal electrodes. However, the particular anodic and cathodic reactions occurring during these waveforms are often asymmetrical and may yield net quantities of harmful products (*2*).

If a metal electrode is completely insulated with a very thin layer of perfect dielectric material, oxidation-reduction reactions are essentially eliminated. Even though electrons cannot pass through the insulating barrier, ions in the tissue can be attracted or repelled by charge on the electrode, and current pulses sufficient for stimulation may be delivered. Although a perfect dielectric layer on the electrode can never be achieved, residual electron leakage can be made so negligible that no significant oxidation-reduction reactions occur.

A capacitor-stimulating electrode was first described by Mauro (*3*) in 1960 and recently improved by Schaldach (*4*). Neither of their electrodes is suitable for such applications as selective stimulation of cerebral cortex. Mauro's design is too bulky, and a small electrode of Schaldach's design will not hold the necessary charge. One method of increasing the amount of charge a miniature capacitor electrode will hold is by using a porous electrode material with a large accessible surface area. We have used a porous disk of tantalum for this purpose. Such a disk is termed a "slug" in the capacitor industry and is formed by fusing loosely packed tantalum powder into a conducting meshwork by a sintering process (see Fig. 1). Available surface area is increased to many times that of a smooth-surfaced electrode of comparable size. The insulating dielectric is tantalum pentoxide, a highly inert material. This dielectric layer is formed by anodization, and its thickness is directly proportional to the anodizing or forming voltage, about 20 Å per volt. When implanted, these electrodes fill with extracellular fluid and behave as electrolytic capacitors with extremely low leakage when operated at positive potentials. Our prototype electrodes were obtained as anodized slugs directly from the production lines of commercial capacitor manufacturers (*5*).

An important consideration in using a porous electrode is that organic ma-

terial may accumulate within the pores, increasing the electrical resistance to the ionic current which must flow to the innermost reaches of the electrode. This pore resistance leads to energy loss and places practical limits on the maximum thickness of electrode that can be used.

One electrode we use for selective surface stimulation of the cerebral cortex consists of a disk of tantalum meshwork 1.0 mm in diameter by 0.25 mm thick, with the oxide layer formed at 5 volts (see Fig. 2). To restrict ionic current flow to the front face of the disk, we shield the back and sides with a 10-μm-thick covering of vapor-deposited Parylene C (Union Carbide Corporation). This electrode has an available surface area of roughly 70 mm^2, a capacitance of 1.4 μf, a working voltage of 4 volts, and a d-c leakage resistance across the dielectric of greater than 500 megohms. It can deliver 0.5-msec positive rectangular current pulses up to 5 ma in amplitude. Negative or biphasic stimulus pulses may also be delivered if the electrode is operated about a positive bias potential. We have usually used positive constant-current stimulus pulses and have arranged for the electrode to slowly discharge, or "recover," between stimulus pulses. The discharge occurs through the tissue in the reverse direction to the stimulus pulse, and the time constant of this discharge may be regulated by the valve of an external resistor in the discharge path. A typical stimulating circuit with resulting waveforms is shown in Fig. 3. Care must be taken not to charge the capacitor electrode to a voltage higher than the original forming voltage. Should this occur, further anodization begins, which increases the thickness of the dielectric and decreases the electrode capacitance. Fortunately, this further anodization is a self-limiting process and serves as a safety factor in the event of an electrical fault in the stimulating circuit.

Electrodes similar to those shown in Fig. 2 have been tested in various ways. First a means was devised to demonstrate the advantage of the capacitor electrode over similar-shaped platinum electrodes in preventing oxidation-reduction reactions. Test electrodes were placed at either end of a strip of *p*H indicator paper saturated with unbuffered physiological saline. Current pulses similar to those used for stimulation were passed between the electrodes (1-ma to 4-ma pulses, 0.5-msec pulse width at 50 pulses per second). Monophasic current pulses passed between platinum electrodes gave marked color changes surrounding each electrode within a few seconds, indicating lowered *p*H at the anode and elevated *p*H at the cathode. After placing a low-leakage capacitor in series with the platinum electrodes to ensure zero net charge flow, *p*H changes were markedly reduced but were still detectable even with 1-ma stimulation pulses (the color change indicated lower *p*H at both electrodes under these conditions). The capacitor electrodes, connected directly to the stimulator with proper polarity, gave no detectable *p*H changes even with 4-ma stimulation pulses.

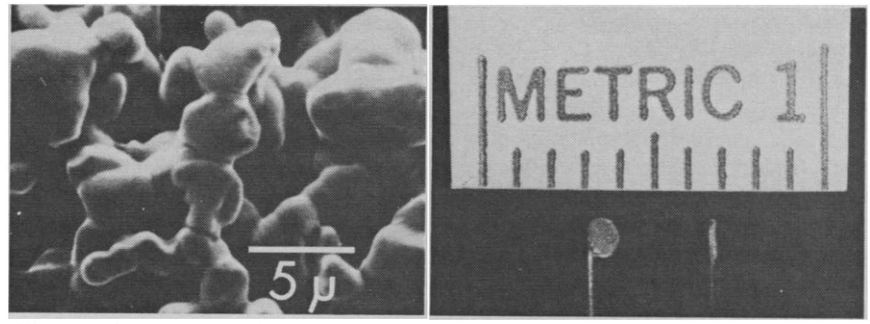

Fig. 1 (left). Scanning electron micrograph of fused tantalum meshwork showing large available surface area (*7*). Fig. 2 (right). Capacitor electrodes designed for surface stimulation of cerebral cortex.

Long-term stability testing included continuous pulsing of the capacitor electrodes in saline and in citrated human plasma at 25°C for 500 hours, with 3-ma, 0.5-msec pulses at 50 pulses per second. No changes in capacitance, d-c leakage resistance, or pore resistance occurred.

To determine the effects of chronic stimulation, capacitor and platinum electrodes were implanted on the motor cortex of a rhesus monkey. Twenty stimulation sessions averaging 5 hours each were performed over a 6-month period. The platinum electrodes were always capacitively coupled during stimulation. Stimulation waveforms for both types of electrodes were similar to those in Fig. 3, and all stimulation was monopolar with respect to a coiled platinum wire beneath the scalp, the platinum wire serving as an indifferent electrode. During each session, 2-second trains of 1.5-ma, 0.5-msec pulses at 50 pulses per second were applied sequentially to eight electrodes, four capacitor and four platinum, the sequence repeating every 45 seconds. Pulse thresholds for observable movement ranged from 1.2 to 3.0 ma and remained approximately constant for each electrode for the 6 months. If tissue damage occurred with either type

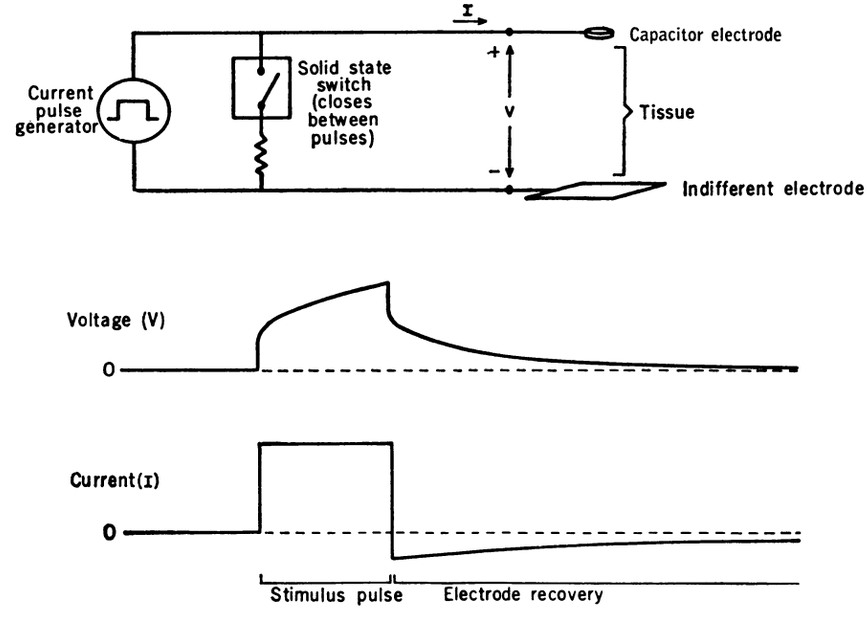

Fig. 3. Typical stimulating circuit with resulting voltage and current waveforms.

of electrode, it was not detected by the threshold criterion. Changes were observed, however, by appropriate analysis of voltage waveforms, in the polarization impedance of the platinum electrodes and in the pore resistance of the capacitor electrodes. Both of these quantities rose slowly between stimulation sessions and returned toward their original values during stimulation. The changes of the polarization impedance of the platinum electrodes were interpreted as accumulation and then oxidative removal of organic surface contaminants (*6*). Increase in the pore resistance of the capacitor electrodes was probably due to tissue ingrowth or accumulation of other organic material. The reason for decrease of this resistance during stimulation is yet to be explained. The long-term pore resistance stabilized at a level two to three times higher than that measured immediately after implantation.

In conclusion, conventional metal electrodes generate oxidation-reduction products during stimulation of excitable tissue. Use of stimulation waveforms with zero net charge transfer may be sufficient in some cases to reduce these products to acceptable levels. However, large arrays of densely packed electrodes, such as those proposed for neural prosthetic devices, may require the extra margin of safety provided by capacitor electrodes.

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6. We thank B. Brummer of Tyco Laboratories, Inc., for advice on electrochemical matters.
7. Micrograph courtesy of P. Johnson and L. Hench of the University of Florida.

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Regulation of Muscle Acetylcholine Sensitivity by Muscle Activity in Cell Culture

Abstract. *Muscle in tissue culture provides a good system for studying long-term changes in surface membrane acetylcholine sensitivity. Muscle fibers stimulated intermittently over prolonged periods are less sensitive to iontophoretically applied acetylcholine and bind less ^{125}I-labeled α-bungarotoxin than inactive fibers.*

There is some evidence that the degree of muscle activity can influence the distribution of acetylcholine (ACh) sensitivity of the cell surface membrane. Direct electrical stimulation of denervated muscle can prevent the appearance of extrajunctional sensitivity that normally ensues (*1*). Muscle fibers that develop in culture from dissociated myoblasts are extremely sensitive to iontophoretically applied ACh. If it can be shown that ACh sensitivity is dependent on the degree of activity in vitro, then a tissue culture system may help elucidate the complex cellular mechanisms responsible for the change.

We previously noted that the chemosensitivity of several fibers (both innervated and uninnervated) that exhibited spontaneous twitches and action potentials was much lower than the mean of other inactive fibers (*2*). We now report additional experiments in which we have attempted to regulate activity over relatively long periods of time.

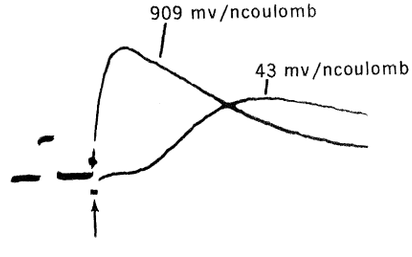

Fig. 1. Superimposed ACh potentials evoked at the same spot before and after a small displacement of the ACh electrode following a large current pulse. The slow response that began after a long delay (evoked with 0.219 ncoulomb) was converted to a rapidly rising response that began without a detectable latency (evoked with only 0.019 ncoulomb). Calibration of the square wave pulse, 5 mv and 10 msec, precedes the ACh response. Similar calibration pulses appear on oscilloscope traces in Fig. 2. The bar at the arrow indicates the duration of the ACh current pulse.

Muscle fibers were grown from myoblasts obtained from embryonic chick pectoral tissue by methods already described except that the drug cytosine arabinoside (ara-C) was not employed to eliminate fibroblasts (*2*). In older cultures, the background connective tissue anchors the muscle fibers and is more effective than a simple collagen layer in preventing them from pulling off the dish during periods of vigorous contraction. The techniques for intracellular microelectrode recording and ACh iontophoresis and for autoradiography have also been described (*2*).

In dense cultures, after an iontophoretic pulse the ACh response often started after a significant latency, an indication that the ACh pipette tip was located some distance from the receptors (*3*). Although some delay may have been due to a patchy distribution of receptors, the most significant factor was the diffusion barrier presented by the connective tissue. A more rapidly rising, larger response (Fig. 1) that occurred without a detectable delay followed a small vertical displacement of the ACh electrode tip [see (*4*)]. Only responses with less than a 5-msec latency were accepted.

Muscle fibers in 7- to 10-day cultures generate action potentials and twitch in response to a depolarizing stimulus (Fig. $2A_1$). Many fibers twitch spontaneously, but the amount of activity varies considerably from culture to culture and within a single culture over long periods of time. To regulate the activity we stimulated electrically one group of cultures through relatively large electrodes fastened in each plate (*5*).

Stimulated muscle fibers were compared to a group of fibers made inactive by the addition of 2.0×10^{-7} g of tetrodotoxin (TTX) per milliliter to the medium. The TTX, which blocks active Na^+ currents in other tissues (*6*), abolished spontaneous and stimulus-evoked action potentials within 1 minute at a concentration as low as 5×10^{-8} g/ml (Fig. $2A_2$).

In our experiments, stimulation (and TTX treatment) was begun on days 7 to 9 and continued for 2 to 6 days.