Myoid Conduction in the Siphonophore *Nanomia bijuga*

MYOID conduction has been suspected in the Cnidaria for some sixty years[1], but has only recently been demonstrated[2,3]. There are no recordings available of activity at the cellular level; until now potentials from cnidarian muscles have been recorded with large extracellular electrodes covering a number of active units.

The stem of the siphonophore *Nanomia bijuga* is covered with a layer of epithelio-muscular cells some 45 μm thick (Fig. 1); this is far thicker than similar cells in many other cnidarians. The muscle fibrils run longitudinally and are non-striated.

Portions of the stem, about 4 cm long, were pinned onto the wax base of a cooled chamber and covered with seawater maintained at 13° C. Cells were successfully impaled by glass microelectrodes filled with 2.8 M potassium chloride having resistances of between 60 and 80 MΩ. Resting potentials of between −50 mV and −80 mV were obtained with 63 mV being the mean of twenty penetrations. This value is in agreement with the values given for anthomedusan epithelia[3]. Many preparations showed spontaneous activity with cell interiors depolarizing 17–20 mV (Fig. 2). The rise time of these potentials is approximately 10 ms, but repolarization takes up to 3 s. These events were conducted without decrement along 2 cm of the stem with a conduction velocity of 20 cm/s as measured by a pair of extracellular suction electrodes.

If the preparation was shocked by square wave pulses of 1 ms duration delivered by a suction electrode to the surface of the stem two distinct types of potential changes were seen in the conducting cells. The type of potential change was dependent on stimulus strength.

The electrical constants of this preparation were not known, and so the exact current delivered below the stimulating electrode could not be calculated. With the distance between the microelectrode and the stimulating electrode at 1 cm, shocks delivered at a potential of 3 V gave no change in the potential of the cell interior (Fig. 2). At 4 V there is a distinct depolarization of the cell membrane and at 6 V an even greater change so that the interior of the cell became 4 mV more positive. In such cases the rate of increase of positive charge inside the cells is very slow. Events such as these which I have called sub-threshold pulses are not propagated but are probably conducted with decrement. At a specific stimulus strength (about 9.5 V) for any one preparation the penetrated cell will fire giving a rapid decrease in the potential across the outer membrane of the cell. This event I have called a supra-threshold pulse and it seems to be identical to the spontaneous pulses already described.

Each supra-threshold pulse causes a slight contraction of the longitudinal muscle fibres, often sufficient to dislodge the microelectrode. Contractions during spontaneous firing never caused the microelectrode to come out of the cell. If a micro-

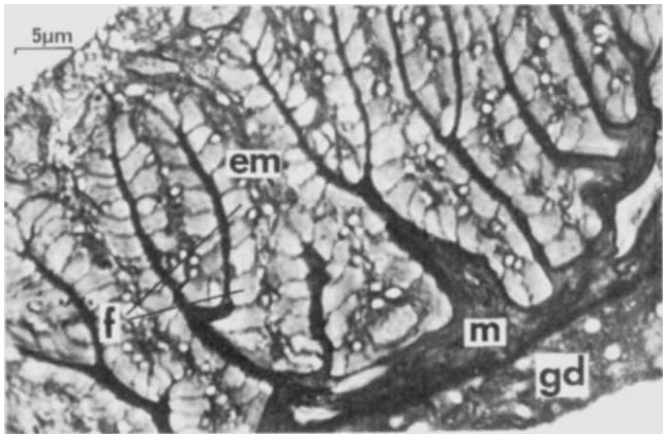

Fig. 1 Transverse section of stem of *Nanomia*, 0.5 μm 'Epon' section (Richardson's stain); em, single layer of epithelio-muscular cells; f, fibrils of epithelio-muscular cells; gd, gastro-dermis; m, mesogloea.

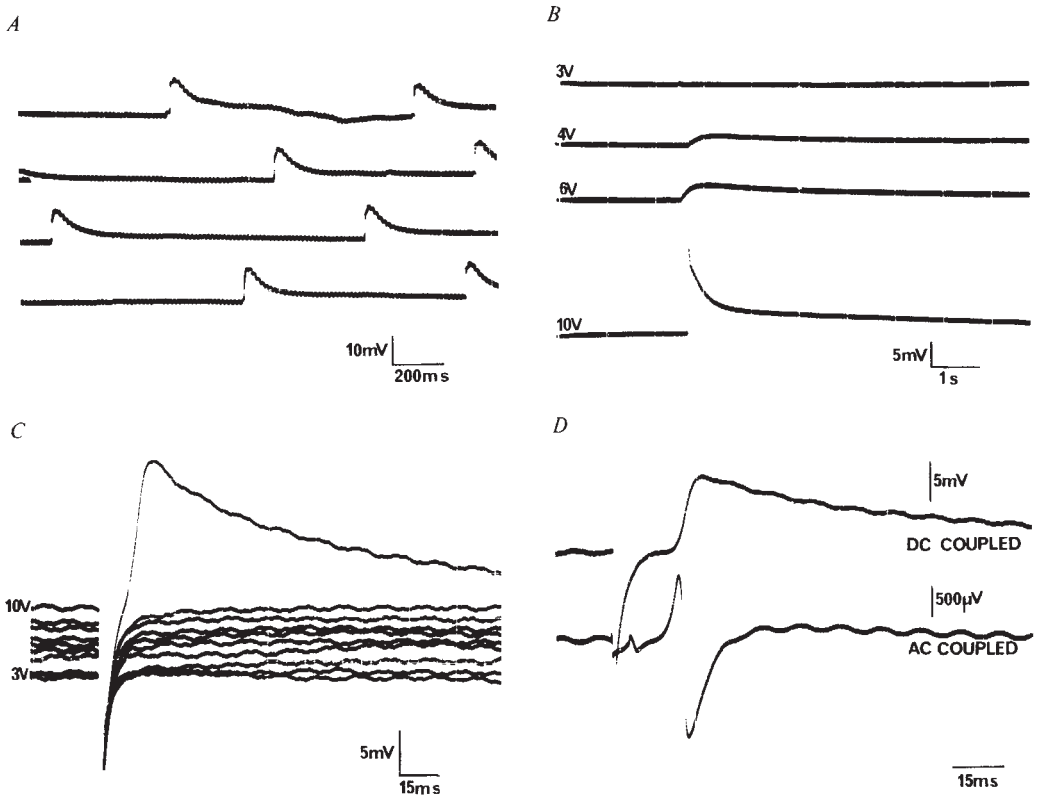

Fig. 2 *A*, Spontaneous supra-threshold pulses; *B*, electrically stimulated pulses showing change from sub-threshold pulses to a supra-threshold pulse as stimulus strength is increased; *C*, same as *B* but tracings super-imposed; *D*, a stimulated supra-threshold pulse as recorded by an intracellular electrode (upper trace), and an extracellular suction electrode above the intracellular electrode (lower trace).

electrode is placed directly beneath a suction electrode one can compare the passage of a supra-threshold pulse as recorded inside and outside the cell. The suction electrode in this case is covering more than one cell. The wave-shape and duration of this pulse, as recorded by the extracellular electrode, are very similar to those recorded from other hydrozoan epithelia[3-5].

Procion yellow and Niagara sky blue 6B injected iontophoretically diffused through a number of cells and so failed to show the exact location of the electrode tip. Use of a screw-micrometer to advance the electrode indicated that the tip in most cases would have been in or just above the muscle fibrils.

The preparation continued to propagate supra-threshold pulses when immersed in a solution containing one part seawater and one part isotonic magnesium chloride. Excess Mg^{2+} blocks nervous activity in other hydrozoans, so it seems that propagation of supra-threshold pulses does not depend on the nerve-net[3].

It is not yet possible to prove that these recordings are intracellular—they could conceivably be transepithelial pulses. Such pulses have been recorded between the enteron and surrounding water in *Hydra* but they have the opposite polarity to the pulses recorded in *Nanomia*[6]. If they are intracellular recordings then one might suppose that the cells in this conducting epithelium are connected by low resistance pathways as demonstrated by the local spread of sub-threshold excitation. This is a reasonable hypothesis since similar types of junctions have already been proposed for conducting epithelia and smooth muscles[7-9].

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Corrosion by the Sulphate-reducing Bacteria

THERE are several explanations for the aggravation of corrosion of iron and steel in oxygen-free conditions by sulphate-reducing bacteria. These are (*a*) stimulation of the cathodic part of the corrosion cell by the removal and utilization of the polarizing hydrogen by the bacteria[1]; (*b*) stimulation of the cathodic reaction by solid ferrous sulphides formed by the reaction of ferrous ions with sulphide ions produced by bacteria[2]; (*c*) stimulation of the anodic reaction, metal dissolution, by bacterially produced sulphide[3]; (*d*) local acid cell formation[4], and (*e*) formation of iron phosphide by reaction of the metal with bacterially reduced phosphates[5].

There is little evidence that (*d*) and (*e*) are significant except possibly in isolated cases, whereas (*c*) is probably important only at the start of the corrosion process due to the eventual formation of protective sulphide films in the presence of free sulphide ion[6].

Much support for (*a*), the cathodic depolarization mechanism first postulated by von Wolzogen Kühr, has come from work by Booth and Tiller[7]. Using mild steel electrodes at various controlled potentials, they observed a straight line relationship between the hydrogenase activity of the bacteria and the extra current required to maintain these potentials on the cathodes in the presence of resting-cell suspensions of sulphate-reducing bacteria, provided with benzyl viologen as electron acceptor. But the organisms used had been cultured on standard sulphate-containing medium and the resting-cell suspensions would therefore contain ferrous sulphide, both free and embedded in the mucopeptide material adhering to the cells. (The ferrous sulphide that forms during growth in sulphate media is impossible to separate completely from the bacteria except by chemical methods so severe that the bacteria themselves are destroyed.)

Using the same procedure as Booth and Tiller[7], we have observed a direct relation between the amount of chemically prepared ferrous sulphide added to a bacteria-free system, and the extra current required, as previously described. Moreover, organisms previously grown in a sulphate-free fumarate medium[8] showed little cathodic depolarization. This latter result could explain the low weight loss/time results obtained by Booth, Elford and Wakerley[2] with mild steel coupons in semicontinuous cultures of the bacteria in a sulphate-free fumarate medium. The similarity between the results of their potentiostatic experiments and those obtained by Booth and Tiller[7] suggests that there is some connexion between the rate of bacterial hydrogen uptake and the amount of ferrous sulphide present (this possibility was adumbrated by the observation of Goldner *et al.*[9] that particulate material stimulated hydrogen uptake). We have now confirmed this, using Warburg manometry to measure the rate of hydrogen uptake by bacteria grown on both sulphate-free and sulphate-containing media. When chemically prepared ferrous sulphide was added to the resting cell suspensions in the reaction flasks, substantially greater rates of hydrogen uptake were observed in nearly every case, using either sulphate or benzyl viologen as electron acceptor (Table 1).

This effect of ferrous sulphide may explain the wide variation encountered in manometric determinations of $-Q_{H_2}$ for the sulphate-reducing bacteria[10], since variable amounts of ferrous sulphide are carried over into the reaction flasks as is easily verifiable visually; the effect may also have a bearing on the observation[11] of a small hydrogen uptake by *Desulfotomaculum orientis*, a sulphate reducer usually considered to be hydrogenase-negative, and this question is being studied further.

Ferrous sulphide alone, though known to be cathodic to mild steel[2,12], is not a permanent cathode. Our weight loss/time experiments indicate that chemically or bacterially produced ferrous sulphides corrode by a first order reaction and that the eventual total corrosion is directly proportional to the

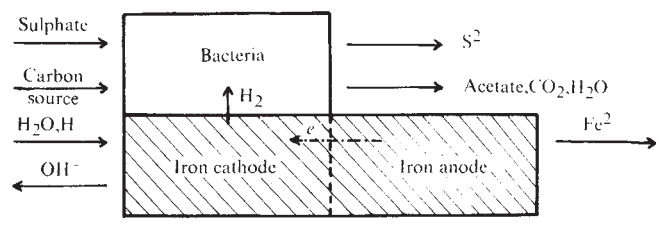

Fig. 1 Scheme illustrating the classic theory of cathodic depolarization by the sulphate-reducing bacteria.