Isolated Neuron Preparation for Studies of Metabolic Events at Rest and during Impulse Activity

Abstracts. *Application of the Cartesian diver technique to the slowly adapting stretch receptor organ of Crustacea permits quantitative measurements to be made of some metabolic processes in a single intact neuron during different functional states.*

In the course of recent electrophysiological studies on the properties of the nerve cell of the slowly adapting stretch receptor organ of Crustacea, it became apparent that this preparation may permit investigation of some metabolic events in a single neuron during different functional states (at rest and during impulse activity).

This neuron can be easily isolated, it can survive for several hours (even days), and it is capable of repetitive impulse activity. This activity can be induced not only by stretching the muscle bundle in which the dendrites are embedded (see *1*) but also by altering the concentration of the K^+ and Ca^{++} ions in the external medium (*2*). The Cartesian diver technique of Linderstrom-Lang (*3*) with its modifications (*4*) can therefore be used. By this technique O_2 uptake as well as the activity of several enzymes (see *5*) can be measured in a single intact cell.

The preparation consists of the cell body of the neuron of the slowly adapting stretch receptor organ of a crayfish, a segment of axon approximately 800 μ long and a portion (approximately 560 μ) of the muscle bundle in which the dendrites of the neuron are imbedded. These are the minimal dimensions compatible with survival for most of the preparations obtained from young specimens (*Procambarus alleni* or *Orconectes virilis*, 3 to 5 cm in total length). The axon belonging to the fast-adapting receptor can be split away from the preparation, but the risk of injury is high. The contribution made to the measurements by the metabolism of this portion of axon as well as by the muscle bundle can be estimated in separate experiments.

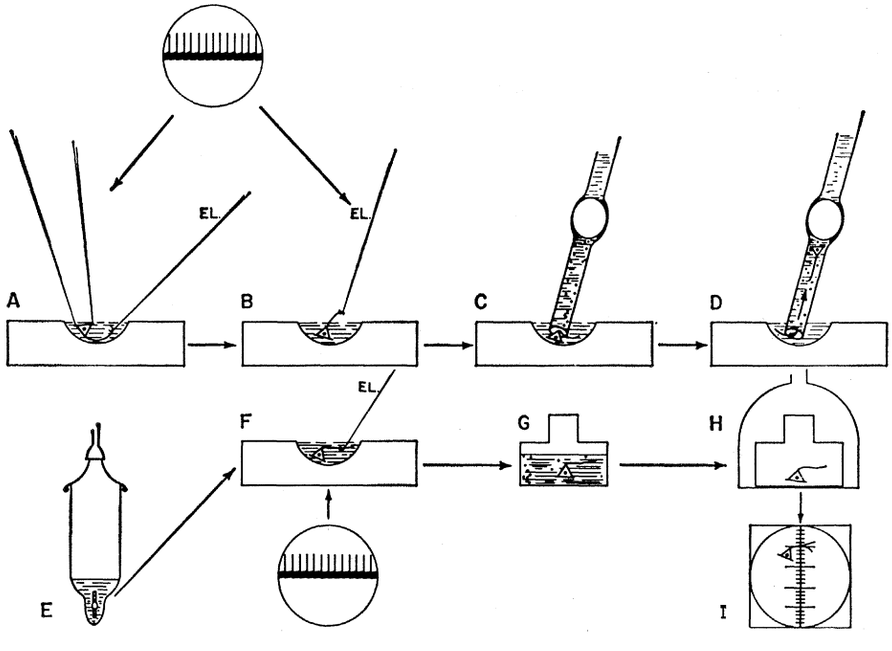

Fig. 1. Experimental procedures. The cell was isolated (*A*). Its ability to respond to stretch with repetitive impulse activity was tested after the muscle bundle had been cut by recording from the axon by means of the wire electrode *EL* (*B*). Thereafter the preparation was introduced by suction into the microdiver (*C*) and placed at the desired position (*D*). The microdiver was subsequently sealed and, after a period of equilibration, measurements were made (*E*). The diver was then opened, and the functional integrity of the cell was tested as in *B* (*F*). Steps *C* to *F* can be repeated several times without apparent damage to the cell. At the end of the experiment the preparation was placed in alcohol (95 percent) containing a minute amount of eosin to permit the cell to be seen in subsequent steps (*G*). After dehydration the alcohol was boiled off at room temperature under high vacuum (*H*) and the preparation (or part of this) was weighed with quartz microbalances (*I*).

As much connective tissue as possible was removed under high-power magnification. The functional integrity of the preparation was tested by recording the impulse activity from the axon with a 30 μ wire electrode mounted in a microdrive. Although no forceps could be applied to the muscle bundle to produce stretch after it had been cut to the stated dimensions, stretch could still be applied when the axon was raised into the air because of the surface tension of the solution. Impulse activity was displayed on an oscilloscope and audio-monitored. Data are presented only from preparations which were capable of impulse activity with stretch before and after each microgasometric measurement (Fig 1).

To perform the microgasometric measurements the preparation was introduced into a capillary diver together with a small amount (approximately 0.5 μl) of the appropriate medium. The microdiver had a weight between 1 and 0.5 mg and a gas volume of 0.3 to 0.4 μl. For measurements of O_2 uptake the diver was charged with air, and the floating solution consisted of 0.1*N* NaOH. The sensitivity of the system is of the order of 10^{-4} μl of gas per hour with accuracy of ± 10 percent (see *4*).

Measurements were made for at least 1 hour. Since the same preparation could be introduced into a diver several times without apparent damage, as judged by its ability to produce impulse activity, it was possible to test the same neuron under different conditions. This was essential because of the scattering of values obtained from different preparations.

Oxygen uptake by the preparation in resting conditions (van Harreveld solution) and in the absence of any added substrate was 4.18×10^{-4} μl/hr ($\sigma = 2.1$; confidence interval for $p = .05$, 3.51 to 4.85). The O_2 uptake by the muscle tissue was measured in separate experiments (mean: 0.23 μl/mg hr; $\sigma = 0.05$; confidence interval for $p = .05$, 0.20 to 0.26) and the contribution by the muscle bundle to the total weight of the preparation (lipid free dry weight) was determined with a quartz microbalance (*6*). The respiration of the second large axon contained in the nerve was determined independently. When the above correction factors are taken into account, the endogenous respiration of this single crustacean neuron (60 to 85 μ long) with its dendrites and

800 μ of axon can be estimated to be 3.38×10^{-4} μl/hr ($\sigma = 1.7$; confidence interval for $p = .05$, 2.86 to 3.90). In the presence of glucose (10 to 100 mg/100 ml) a very substantial increase in respiration (up to 1.5-fold) was observed (*7*).

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References and Notes

1. C. A. Terzuolo and Y. Washizu, *J. Neurophysiol.* **25**, 56 (1962).
2. C. A. G. Wiersma, E. Furshpan, E. Florey, *J. Exptl. Biol.* **30**, 136 (1953); C. Edwards, C. A. Terzuolo, Y. Washizu, in preparation.
3. K. Linderstrom-Lang, *Nature* **140**, 108 (1937).
4. E. Zeuthen, *J. Embryol. Exptl. Morphol.* **1**, 239 (1953); E. Giacobini and J. Zajicek, *Nature* **177**, 185 (1956).
5. E. Zeuthen, in *General Cytochemical Methods*, J. F. Danielli, Ed. (Academic Press, London, 1961), vol. 2, p. 144; E. Giacobini, *J. Neurochem.* **2**, 169 (1962).
6. O. Lowry, *J. Biol. Chem.* **140**, 183 (1941).
7. Preparations which do not include the axon belonging to the fast-adapting receptor have been obtained only recently by G. Bonewell. Dr. R. E. McCaman from Indiana University constructed and calibrated the quartz microbalances. Work supported by U.S. Public Health Service grants B-2527 and 2G-572.

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Ion Exchange at Edge and Interlayer in Montmorillonites Differing in Size

Abstract. *Titrations of hydrogen-saturated montmorillonite with sodium hydroxide indicate that two kinds of hydrogen are exchanged on the surface of the clay. At low* p*H there is more titratable hydrogen in a fine-size fraction of the clay than there is in a coarse fraction. This indicates neutralization of hydrogen adsorbed at the crystallite edge. The hydrogen adsorbed on the interlayer flake surface is neutralized at a higher* p*H, and there are more titratable hydrogen ions for the coarse fraction than for the fine. This suggests that surface-charge density is a function of crystallite size, a proposal already made on the basis of other properties of clay.*

The technique described by Pommer and Carroll (*1*) for titrating separately the cation-exchange capacity (CEC) of the edge and surface of hydrogen-saturated montmorillonite offers exciting possibilities for further experimentation. Measuring the ion exchanged on the flake surface alone allows information to be gathered on the isomorphous substitutions on which this exchange is assumed to depend. Heretofore only measurement of the total exchange was possible; this included an unknown contribution from the exchange at the crystal edges. The method (*1*) is applied here to two fractions of montmorillonite, differing in particle size, in an effort to determine whether variations in the two cation exchange capacities are related to the size of the clay crystallite. The first equivalence point in the titration curve corresponds to a cation exchange capacity that increases as the size of the particles decreases. The exchange capacity corresponding to the second reaction tends to decrease with decreasing size of the particles.

As a result of careful and laborious supercentrifugation (*2*) a set of size fractions for several bentonitic montmorillonites was produced. For the Wyoming bentonite (commercial Aquagel) used here, the finest suitable fraction was 15N with ESD (equivalent spherical diameter) range of 0.05 to 0.02 μ. The coarse fraction was 15H with ESD ranges of 0.4 to 0.3 μ.

The fractionated clay samples were stored as dry powders. These were suspended by mixing with demineralized water in a blender. Dowex-50 resin in the hydrogen form (H-resin) was added to the suspension so that the exchange capacity was twice that of the clay. After the mixture had been stirred for 3 hours, it was separated by sieving and treated twice again with fresh H-resin.

A stock suspension of the hydrogen-saturated clay was prepared for each fraction. Samples of 10 ml were pipetted into covered polyethylene bottles. Varying quantities of 0.1*N* NaOH were added with enough water to bring the total volume to 15 ml. After 1 week the clay was separated by centrifugation, and the solutions were returned to the cleaned bottles. Measurements of *p*H were made on these solutions. The weight of clay used in the bottles for the titration was determined separately for each fraction by weighing samples of the stock suspension.

The data are plotted, according to the method of Pommer and Carroll (*1*), in Fig. 1. The first straight portion of the graph represents neutralization of one of the two acids, either hydrogen ions exchanged at the crystallite edge or hydrogen ions exchanged at the crystallite flat surface. The second straight portion of the graph represents neutralization of the other hydrogen-bearing exchange site. Carroll (*3*) has discussed the advantages and possible difficulties in this experimental technique for determining the relative amounts of exchange at each site.

Table 1. Cation exchange capacities for two montmorillonite fractions, differing in size. The sample weight for 15H was 0.0461 g and for 15N was 0.0469 g.

Acid titration	Meq/100 g	
	15H	15N
1st	30	41
1st and 2nd	152	139
2nd only	122	98

Because the concentrations of the two stock suspensions were slightly different, the weights of clay titrated for the two graphs were different, and the two curves are therefore not directly comparable. The significant points on the curves are the two points of intersection of the three lines, and these can be adjusted to represent exchange capacity in terms of 100 g of clay.

Table 1 shows the cation exchange capacity for the two fractions. The first reaction is completed after NaOH is added in the amount of 30 meq/100 g of clay for the coarse fraction (15H). The fine fraction shows the same reaction to be completed at 41 meq/100 g of clay. If the same reaction were titrated for both fractions, it would be necessary for sample 15H to contain almost one-third inert diluent in order to explain its difference from the measured cation exchange capacity of sample 15N. It is reasonable to conclude that the exchange capacity of the first reaction increases with de-

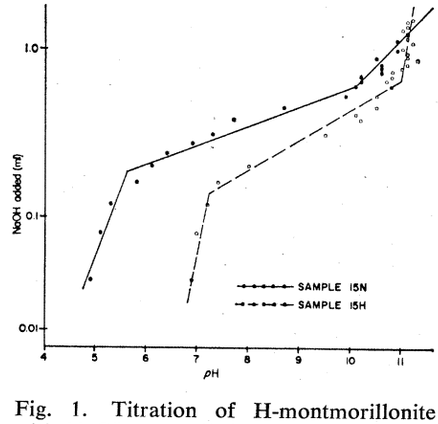

Fig. 1. Titration of H-montmorillonite with NaOH: two distinct reactions.