

ABSTRACTS OF MEMOIRS

RECORDING WORK AT THE PLYMOUTH LABORATORY

BAKER, P. F. & MCNAUGHTON, P. A., 1976. Kinetics and energetics of calcium efflux from intact squid giant axons. *Journal of Physiology*, **259** 103-144.

The Ca efflux from intact squid axons consists of three major components: one that is activated by Ca_0 , one that is activated by Na_0 and a residual flux that persists in the nominal absence of both Ca_0 and Na_0 . The properties of these components have been investigated in unpoisoned axons and in axons poisoned with cyanide.

Under all conditions the shape of the curve relating Ca_0 to Ca_0 -activated Ca efflux approximates to a section of a rectangular hyperbola, consistent with simple Michaelis activation.

The external Ca concentration giving half-maximal activation of Ca_0 -activated Ca efflux is about $2 \mu M$ in unpoisoned axons immersed in Na-ASW, but on poisoning changes progressively to values in the range 1-10 mM. The residual efflux from unpoisoned axons may reflect activation by traces of Ca present immediately external to the axolemma.

The apparent affinity for Ca_0 of Ca_0 -activated Ca efflux is very similar in unpoisoned axons immersed in sea waters containing Na, Li, Tris or K as major cation, whereas in poisoned axons the affinity in Na and Li is about the same but higher than that in choline and Tris.

In unpoisoned axons Ca influx increases linearly as Ca_0 is increased from $2 \mu M$ to 110 mM. The absolute value of the Ca influx from 10 μM - Ca_0 is less than 1% of the Ca_0 -activated Ca efflux at this external Ca concentration. In poisoned axons the sizes of Ca_0 -activated Ca efflux and Ca influx were similar at all Ca concentrations examined.

The shape of the curve relating Na_0 to Na_0 -activated Ca efflux approximates to a section of a rectangular hyperbola in unpoisoned axons but is clearly sigmoidal in axons that have been fully poisoned with cyanide. The sigmoidal shape develops progressively during poisoning.

The external Na concentration giving half-maximal activation of Na_0 -activated Ca efflux is about 60 mM in unpoisoned axons and often exceeds 300 mM in fully poisoned axons.

In both unpoisoned and poisoned axons, at constant Na_0 , replacement of 200 mM-Tris by 200 mM-K reduced the Na_0 -activated Ca efflux reversibly.

The magnitude and kinetics of both Ca_0 -activated Ca efflux and Na_0 -activated Ca efflux were unaffected by injection into the axon of a solution containing 220 mM-EGTA and 100 mM- $CaCl_2$. Subsequent exposure to cyanide reduced the amplitude of the Ca efflux to about one third, and the kinetics of both components of the Ca efflux changed to that characteristic of the poisoned state. Injection of ATP produced a transient increase in efflux and restored a Na_0 -activation curve close to that seen in unpoisoned axons. AMP and cyclic AMP were ineffective.

Application of cyanide in the presence of oligomycin produced a large increase in Ca efflux. Subsequent injection of ATP had little effect on the size of the efflux but altered the kinetics of Na_0 -activation so that they resembled more closely those seen in unpoisoned axons.

These results are discussed and shown to be consistent with a model in which the Ca pump must bind ATP for efflux to occur.

BRYAN, G. W., 1976. Some aspects of heavy metal tolerance in aquatic organisms. In *Effects of Pollutants on Aquatic Organisms* (ed. A. P. M. Lockwood), pp. 7-34. Cambridge University Press.

In most waters the concentrations of heavy metals are very low (Riley & Chester, 1971), although higher natural concentrations occur in rivers and estuaries which are associated with outcropping metaliferous lodes. As a result, the concentrations of heavy metals in natural waters can easily be increased to levels which aquatic organisms have not previously encountered. The mechanisms possessed by organisms for handling natural fluctuations in the availability of heavy metals assume particular importance under contaminated conditions. The degree of protection afforded varies from species to species, so that under contaminated conditions the ecological balance may be tilted as the more tolerant organisms are favoured. From the public health point of view, these protective mechanisms determine the degree of contamination of edible fish and shellfish, even if the organisms themselves are unaffected.

This paper is not primarily concerned with the manifold toxic effects of heavy metals but deals with some of the processes which determine the tolerance of aquatic organisms to heavy metals.

CORNER, E. D. S. & HARRIS, R. P., 1976. Hydrocarbons in marine zooplankton and fish. Part I. Zooplankton. In *Effects of Pollutants on Aquatic Organisms* (ed. A. P. M. Lockwood), pp. 71–85. Cambridge University Press.

In recent years detailed analyses of sea water, as well as marine species ranging from micro-organisms to vertebrates, have demonstrated the presence of hydrocarbons, both aliphatic and aromatic, which are similar in nature to components of petroleum and petroleum products. Also present, however, are other hydrocarbons that are characteristic of living organisms.

In the open sea concentrations of hydrocarbons are generally low and the origin of these compounds is not always easily determined. By contrast, in areas affected by massive contamination the hydrocarbons may be present in high concentrations and can be directly related to the particular source of pollution. These two different situations have prompted studies of both short-term and long-term effects of petroleum hydrocarbons, the former being principally concerned with toxicity, tainting and behavioural responses, and the latter with uptake, metabolism and release or possible accumulation.

This two-part paper reviews the background to these studies and then discusses pertinent experiments that are still in progress, the first section being mainly concerned with zooplankton and the second with fish.

GIBBS, P. E., CLARK, A. M. & CLARK, C. M., 1976. Echinoderms from the northern region of the Great Barrier Reef, Australia. *Bulletin of the British Museum (Natural History)*, (Zoology), **30**, 101–144.

This paper gives records of about 140 species of echinoderms from the northern region of the Great Barrier Reef collected during the Royal Society–Universities of Queensland Expedition in 1973. One ophiuroid species is new to science, namely *Amphiura phrixocantha* sp.nov. Ten other ophiuroids, two echinoids and one holothurian are new to the Australian fauna and the ranges of eight more ophiuroids and one holothurian are extended to the Great Barrier Reef Province from other parts of Australia. In addition, a few unpublished records from relevant material in the British Museum collections are included and notes are given on several species of especial interest. In contrast to most shallow-water tropical collections, the one from this Expedition includes a high proportion of burrowing species from soft substrates. A list of some animal associates of the specimens collected is appended.

KEYNES, R. D. & ROJAS, E., 1976. The temporal and steady-state relationships between activation of the sodium conductance and movement of the gating particles in the squid giant axon. *Journal of Physiology*, **255**, 157–189.

Comparisons were made between the kinetics and steady-state properties of the sodium conductance changes and of the sodium gating currents, in the squid giant axon perfused with caesium fluoride and maintained at a high membrane holding potential. After an initial delay of 10–150 μ sec whose size was a function of the holding potential and pulse amplitude, the permeability rise on depolarization followed cube law kinetics. Values of the conductance time constant τ_m and of the gating current time constant $\tau(V)$ were determined for membrane potentials ranging between -140 and $+70$ mV. Both lay on nearly symmetrical bell-shaped curves with maxima somewhat under 500 μ sec (at 6.3 °C), falling close to -35 mV membrane potential. The midpoints of the m_∞ and steady-state charge distribution curves lay between -25 and -35 mV, and both had a slope of about 0.014 mV⁻¹ at the midpoint. The ratio of the limiting slopes of the curves for sodium conductance and for gating charge distribution plotted against potential was close to 3:1, confirming the previous conclusion that each sodium channel behaves as if it incorporated three gating sub-units. At a high holding potential, the time constant for shutting off the sodium conductance at the end of a large pulse was shown to be close to one third of the gating current time constant, as predicted by cube law kinetics. The failure of this relationship to hold good for small depolarizing pulses can readily be explained in terms of a multi-state gating

system. The agreement thus observed between the behaviour of the sodium conductance system and the properties of the mobile charges whose movements generate the asymmetrical displacement current provides useful support for the identification of these charges as the sodium gating particles.

PINGREE, R. D. & MARDELL, G. T., 1976. Bucket S.T.D. measurements. *Deep-Sea Research*, **23**, 551-555.

It is shown that both bubble effects and small temperature differences between the conductivity cell and the temperature sensing elements are important considerations when using conventional S.T.D. systems for monitoring sea surface salinity whilst steaming. By carefully attending to these details, merely placing an S.T.D. in a bucket flushed with a hose will provide a system capable of absolute accuracy of 0.01‰ and resolution 0.001‰.

ROBERTS, B. L. & RYAN, K. P., 1976. Myelinated synapse-bearing cell bodies in the central nervous system of *Scyliorhinus canicula* (L.). *Cell and Tissue Research*, **171**, 407-410.

Some neurons of the anterior lateral-line lobe of dogfish (*Scyliorhinus*) have synapse-bearing perikarya enclosed by layers of compact myelin. The identity of these cells, which have myelinated axons and unmyelinated, synapse-bearing dendrites, is unknown.

WHITFIELD, M., 1975. Applications of ion-selective electrodes in the development of a chemical model for sea water. *Proceedings of the Society for Analytical Chemistry*, **12**, 56-60.

For the major electrolyte components in sea water we now have a wealth of theoretical models but a dearth of good thermodynamic data. Consequently we are unable to decide which model gives the more accurate picture of the chemical properties of sea water. Ion-selective electrodes can provide much valuable information on the variation of mean-ion activity coefficients in sea water - particularly over the oceanographic temperature and pressure range and their past application and potential usefulness are reviewed. Few detailed studies have been made of the minor components in sea water and the emphasis is still placed on the accumulation of accurate *in situ* observations. Techniques for the *in situ* use of ion-selective electrodes are briefly summarized and indications are given of possible future applications.

WHITFIELD, M., 1976. Stratified waters hold the key to the past. *Nature, London*, **263**, 8-9.

Recent work on the sediments underlying contemporary stratified waters in the Black Sea and the East African rift lakes is set in the context of current thinking about the evolution of the oceans. It is emphasized that the latest information suggests that the oxygenation of the oceans has not progressed smoothly but has suffered many partial reversals over the last few billion years. This suggests that concepts in fields as diverse as sedimentary geochemistry and paleoecology might have to be reviewed.

ZAMMIT, V. A. & NEWSHOLME, E. A., 1976. Effects of calcium ions and adenosine diphosphate on the activities of NAD⁺-linked isocitrate dehydrogenase from the radular muscles of the whelk and flight muscles of insects. *Biochemical Journal*, **154**, 677-687.

The activity of NAD⁺-linked isocitrate dehydrogenase from the radular muscle of the whelk (*Buccinum undatum*) is stable for several hours after homogenization of the radular muscle. Consequently, the enzyme from the whelk muscle is suitable for a systematic investigation of the effects of Ca²⁺ and ADP.

The sigmoid response of the enzyme activity to isocitrate concentration is markedly increased by raising the Ca²⁺ concentration from 0.001 to 10 μM, but it is decreased by ADP. Similar effects are observed for the enzyme from the flight muscle of the locust (*Schistocerca gregaria*) and the water bug (*Lethocerus cordofanus*). The percentage activation by ADP of the enzyme from either the whelk or the insects is greater at 10 μM-Ca²⁺, and 50% of the maximum activation is obtained at 0.10 and 0.16 mM-ADP for the enzyme from whelk and locust respectively at this Ca²⁺ concentration. At 10 μM-Ca²⁺ in the absence of added ADP, the apparent K_m for isocitrate is markedly higher than in other conditions. Ca²⁺ concentrations of 0.01, 0.1 and 0.2 μM cause 50% inhibition

of maximum activity of the enzyme from the muscles of the whelk, locust and water bug respectively.

The opposite effects of Ca^{2+} on the activities of isocitrate dehydrogenase and mitochondrial glycerol phosphate dehydrogenase from muscle tissue are consistent with the hypothesis that changes in the intracellular distribution of Ca^{2+} control the activities of these two enzymes in order to stimulate energy production for the contraction process in the muscle. Although both enzymes are mitochondrial, glycerol phosphate dehydrogenase resides on the outer surface of the inner membrane and responds to sarcoplasmic changes in Ca^{2+} concentration (i.e. an increase during contraction), whereas the isocitrate dehydrogenase resides in the matrix of the mitochondria and responds to intramitochondrial concentrations of Ca^{2+} (i.e. a decrease during contraction). It is suggested that changes in intramitochondrial Ca^{2+} concentrations are primarily responsible for regulation of the activity of NAD^+ -isocitrate dehydrogenase in order to control energy formation for the contractile process. However, when the muscle is at rest, changes in intramitochondrial concentrations of ADP may regulate energy formation for non-contractile processes.