Materials and methods. Mature specimens of *Blennius pholis* L., length 12.2–14.6 cm and weight 25–35 g, were collected from the rocky shores around Anglesey, tagged, and kept in the laboratory aquarium in tanks with circulating sea-water. Water temperature was continuously recorded and remained throughout the experiments, conducted June-August, within the range 15–17 °C. The fish were introduced to a diet of lug-worm, *Arenicola marina*, which after several days capture they accepted readily from the surface of the water immediately it was introduced. Social facilitation[5] appeared to play an important role in the early stages of artificial feeding. The fish were fed to excess on alternate days.

During experimental trials an 'Araldite' pellet, 2–3 mm diameter, was fed to each fish individually inside a piece of lug-worm. The time from feeding to appearance of the pellet in the faeces was recorded from each fish and used as a measure of the mechanical activity of the alimentary tract. Barium sulphate was mixed with the resin of the pellet to allow its progress to be monitored by X-ray screening. This enabled investigation of any differential effects of the drugs on the motility of arbitrarily defined fore-gut and hind-gut regions. This report however deals only with total clearance times i.e. the time elapsed between feeding and defaecation of the pellet.

Drugs were made up in teleost Ringer[6] and injected in 0.1 ml volumes into the coelomic cavity. 2 injections were administered during each experiment, approximately 1 h and 9 h after feeding. The following drugs were used: – acetylcholine chloride, acetyl-b-methylcholine chloride (methacholine), eserine sulphate, (Sigma), carbamylcholine chloride (carbachol), atropine sulphate, (B.D.H.).

Results and discussion. Of the 3 cholinesters tested only carbachol (0.05 mg/kg) stimulated motor activity of the alimentary tract. The mean clearance time, 17.7 h $\pm$ 0.99 ($\pm$ standard error), determined from 10 carbachol treated fish was significantly less ($P < 0.05$) than the mean clearance time, 20.1 h $\pm$ 0.61, determined from a group; of 35 untreated controls. Mean clearance times from fish treated with acetylcholine/eserine (1, 3 and 5 mg/kg) and methacholine (5 mg/kg) were not significantly different from those of their controls. These results may reflect the relative instability of acetylcholine or methacholine when released into the body fluids[7]. Both these compounds are more susceptible to cholinesterase activity than carbachol. Injection of an equimolecular concentration of the anticholinesterase, eserine, failed however to reveal any in vivo activity of acetylcholine which has been shown to contract isolated smooth muscle preparations of teleost gut [8–11], including preparations from *Blennius* (personal observation).

The action of carbachol in stimulating gut motilty may be via nicotinic or muscarinic receptors, or both, as this compound has been shown in mammals to be equally active at both sites[7]. Whilst it has been proposed that nicotinic receptors are the principal sites of cholinergic transmission in the teleost stomach[9] more recent evidence[4, 10, 11] has indicated that cholinergic transmission is predominantly muscarinic. Further evidence of a muscarinic site in *Blennius* is afforded by the observation that atropine, in otherwise untreated fish, decreased the rate of movement of food through the gut. The increase in clearance times was significant in 2 groups each of 10 fish treated with 3 mg/kg ($P < 0.05$) and 5 mg/kg ($P < 0.001$) atropine. Mean clearance times were respectively 21.3 h $\pm$ 0.27 and 22.7 h $\pm$ 0.47 compared with a mean control time of 19.9 h $\pm$ 0.44 determined from 36 untreated fish. If atropine acts as a selective muscarinic blocking agent, in the doses employed here, this would indicate the presence of cholinergic receptors on the smooth muscle cells. These results are consistent with other in vitro[11] and in vivo[4] studies reported from this laboratory[12].

Résumé. Les effets des cholinesters sur les mouvements spontanés du tractus alimentaire sont étudiés sur des poissons intacts. L'acétylcholine et la métacholine sont sans effets. Par ailleurs, une concentration de 0,05 mg/kg de carbachol produit un accroissement du mouvement spontané. Des concentrations de 3 et 5 mg/kg d'atropine inhibe le mouvement.

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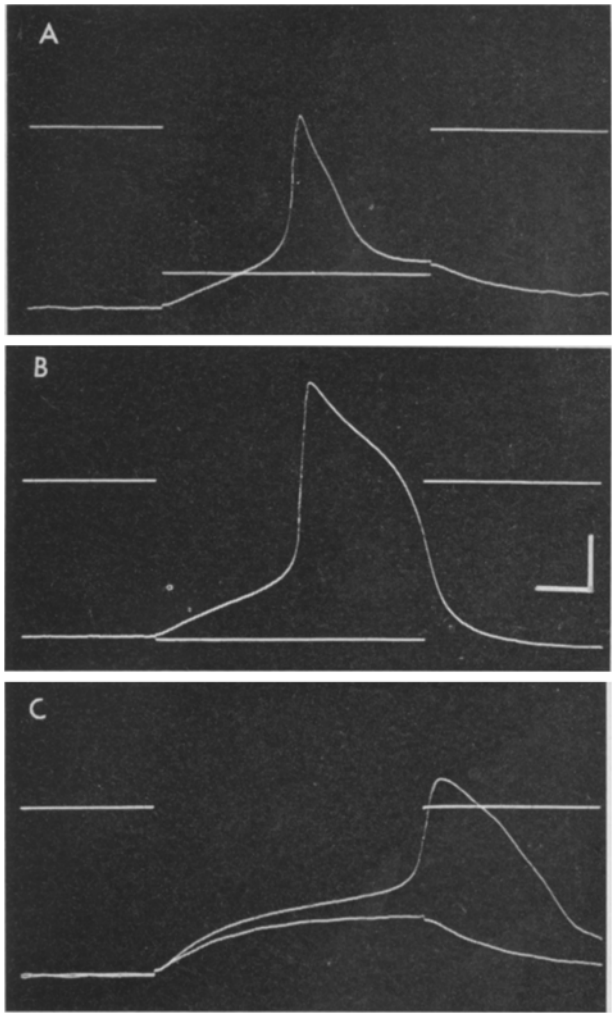
The Effects of the 'Calcium-Antagonist', Prenylamine, on the Action Potential of Crayfish Muscle (*Oronectes virilis*)

Certain drugs that have been identified as 'Ca^{++}-antagonists' block excitation-contraction coupling in smooth muscle[1] and eliminate the inward flux of Ca^{++} during the action potential in the frog myocardium[2, 3]. The same drugs block the second, longer-lasting, delayed phase of Ca^{++} entry in the squid axon[4]. The present experiments were designed to test the effects of a Ca^{++}-antagonist on the action potential of crustacean muscle, which was the first experimental system in which Ca^{++} inflow was implicated in the genesis of spikes[5, 6].

Method. The observations were made on the contractor epimeralis and the superior extensor abdominalis medialis muscles of the crayfish, *Orconectes virilis*. The muscles were dissected in a van Harreveld's solution (v/H) containing 210.0 m*M* NaCl, 14.0 m*M* $CaCl_2$, 2.8 m*M* $MgCl_2$, 5.25 m*M* KCl, and 8.0 m*M* N-*Tris* (hydroxylmethyl) methyl-2-aminoethane sulfonic acid (TES) buffer pH 7.4. The Ca^{++}-antagonist tested was prenylamine lactate (N-(3,3-Diphenylpropyl)-α-methyl phenethylamine lactate; Segontin ®), which a was gift from the Hoechst Phar-

maceutical Co. These crayfish muscles frequently do not generate action potentials in normal v/H, so long, overshooting action potentials were established by adding procaine (10^{-3} w/v) to the v/H[7] or by transferring the preparation to 160 mM $SrCl_2$.

Results and discussion. Figure 1A shows an action potential generated by a crayfish muscle. The amplitude, duration, and the threshold for the action potential were unchanged when 0.01, 0.05 or 0.1 mM prenylamine was added to the bathing solution. However, within a few minutes after the addition of 0.25 mM prenylamine there was a marked lengthening of the duration, and a distinct increase in the threshold and in the amplitude (Figure 1 B). The prenylamine concentration was then raised to 1.0 mM. Within 10 min the amplitude of the action potential was reduced and the threshold was elevated. The preparation was then returned to procaine v/H, with repeated changes of the bathing solution. 1 h later the amplitude of the action potential remained at the depressed level and the threshold remained high (Figure 1 C). In some other experiments 1.0 mM prenylamine completely abolished active membrane responses, and repeated washing with v/H failed to restore excitability.



Responses of a crayfish contractor epimeralis muscle to depolarization. Calibrations: 1×10^{-7} amps, 20 mV, 20 msec. A) In v/H solution containing 10^{-3} (w/v) procaine. B) after 10 min in procaine v/H containing 0.25 mM prenylamine. C) 1 h after being returned from 1.0 mM prenylamine to v/H. For further description see the text.

The drug does not change resting potentials. In 3 experiments the membrane resistance was measured before and after treatment with 1 mM prenylamine, by passing hyperpolarizing current through 1 electrode and recording membrane potential with another. In one case there was about a 10% increase in input resistance following treatment with prenylamine, in the other 2 examples there was no detectable change. The mechanism by which prenylamine increases action potential duration and amplitude is uncertain. In 4 experiments in which prenylamine (0.01 to 0.5mM) was added to v/H that did not contain procaine, there was no development of overshooting action potentials. It is unlikely that prenylamine itself directly stimulates the Ca^{++} mechanism; probably it delays the increase in potassium permeability that brings about repolarization.

Prenylamine also blocks action potentials in which the inward current is carried by Sr^{++}. Once again, 0.25 mM prenylamine had no noticeable depressant effect, but the action potential was abolished when the concentration was increased to 1.0 mM or to 2.0 mM. In 7 experiments, there was no recovery when the preparation was returned to 160 mM $SrCl_2$.

The most unexpected observation was on the effects of pretreatment with prenylamine before the muscle fibers were exposed to procaine v/H or to 160 mM $SrCl_2$. In one series of experiments, a muscle from one side of the crayfish was placed in v/H, the muscle from the opposite side was placed in prenylamine. After 20 min both preparations were transferred to procaine v/H or to 160 mM $SrCl_2$. When the pretreatment was with 0.1 to 1.0 mM prenylamine, even 3 h later the muscles failed to generate action potentials, although the controls from the opposite side gave typical spikes. Pretreatment with the drug is effective at concentrations only 1/10 as high as those needed to block already developed action potentials. Procaine and Sr^{++} may make it more difficult for prenylamine to bind to its receptor.

The major conclusion is that prenylamine can block Ca^{++} spikes in crayfish muscle, but since there is also evidence for a decrease in stimulated potassium permeability, it is unlikely to act solely on the Ca^{++}-channel.

Zusammenfassung. Der organische Ca^{++}-Antagonist Prenylamin blockiert die leicht reversiblen Ca^{++}-Aktionspotentiale in der Muskelfaser des Flusskrebses *Orconectes virilis*. Etwas niedrigere Konzentrationen erhöhen die Schwelle und verlängern die Dauer, während schwache Konzentrationen von Prenylamin nur wirksam waren, wenn dieses verwendet wurde, bevor der Muskel dem Aktionspotentialerreger Procain oder Sr^{++} ausgesetzt war.

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