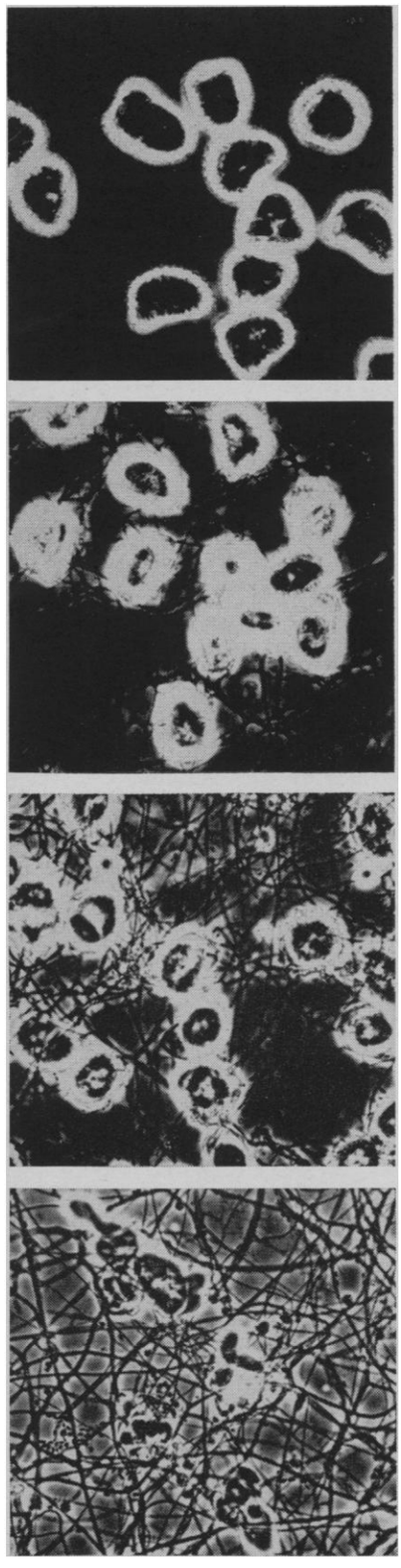

Fig. 1. *Hemileia vastatrix* spores: (top) before inoculation with *Verticillium* sp.; (upper middle) 2 days after inoculation, showing *Verticillium* beginning to attack rust spores; (lower middle) 10 days after inoculation with *Verticillium* sp.; (bottom) 2 weeks after inoculation, showing complete digestion of the *Hemileia* spores and full sporulation of *Verticillium*.

of *Verticillium* sp. The mold developed luxuriantly on the rust spores during the first 2 days. After about 2 weeks very few rust spores capable of taking up lactophenol blue were found, though sometimes it appeared that the walls of these spores had not been attacked (see Fig. 1).

The existence of a fungilytic secretion was demonstrated on solid media. One petri dish was inoculated with *Helminthosporium gramineum* and incubated. After 7 days, standardized colonies of *Verticillium* sp. were prepared on each plate and the incubation was continued. The plates were observed for 4 weeks and during this time a marked attack on the *Helminthosporium* mycelium was seen. The same kind of experiment was repeated with various other molds and in most experiments the *Verticillium* grew directly on the fungus mycelium.

Plates prepared with heat-inactivated rust spores were also inoculated with *Verticillium* and after incubation for 1 week each colony was surrounded by a clear zone, which suggested lysis of the test organisms. Presumably the same agent was responsible both for the partial lysis on agar media and for the direct attack on the rust spores in aqueous suspension.

The possibility that the lytic agent was an enzyme was investigated. One-milliliter portions of a suspension of spores or minute fungus colonies (previously thoroughly washed), were added to 4-ml portions of: (i) sterile water; (ii) filtrates of liquid *Verticillium* sp. cultures (glucose-asparagine yeast extract solution); (iii) heated culture filtrates. Triplicate tubes were incubated and samples were removed at intervals (up to 2 weeks) for microscopic examination. In the water tubes the contents of the rust spores were almost entirely stainable with lactophenol blue. The condition of the spores in the heated culture filtrates was essentially the same as in water. The spores in the unheated culture filtrates had almost completely lost their ability to take up the strain.

It seems from these results, and from those of the experiment in which strong direct attack has taken place on the test organisms in growing cultures or in water (that is, under conditions of nutrition unlikely to favor antimetabolite production), that some thermolabile lytic substance was produced by the isolated strain of *Verticillium*. This heat lability and the pattern of activity, suggest that the lytic substance is an enzyme.

Attempts to obtain a cell-free enzyme preparation were made with the culture filtrates. The filtrates were frozen and freeze-dried. The freeze-dried material could be stored at 5°C for several weeks, without loss of activity. Attempts to purify the lytic enzyme by procedures of protein fractionation are in progress.

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19 December 1961

## Effect of Acetylcholine on the Electrical Activity of Somatic Nerves of the Lobster

*Abstract.* The effects of acetylcholine (ACh) on axons of the walking leg of lobster have been investigated. In $10^{-2}M$ concentration the ester lowers the resting potential by approximately 7 mv within 10 minutes. Simultaneously, the spike height is decreased. Complete but reversible block ensues in 10 to 15 minutes. At lower concentrations essentially similar but smaller effects are observed. Choline chloride and sodium acetate, at concentrations of $10^{-2}M$, are without any effect. In conjunction with the evidence accumulated for the essential role of acteylcholine in the control of ion movements, the demonstration of the ability of acetylcholine to reversibly depolarize axons of somatic nerve fibers appears pertinent.

The failure of acetylcholine (ACh) to affect axonal conduction in contrast to its action on synaptic junctions is frequently cited as a major objection to Nachmansohn's theory of the essentiality of ACh in axonal conduction (*1*). Mono- and diquaternary nitrogen derivatives, such as ACh and curare, are lipid insoluble. Nachmansohn has explained their failure to affect axons by the existence of structural barriers which prevent lipid insoluble compounds from reaching the conducting membrane; this is in contrast to the relatively greater permeability of the axon to the lipid soluble tertiary nitrogen compounds, such as eserine and the local anesthetics closely related in

structure to ACh. Evidence for this view was obtained in a great variety of experiments (*2*). However, curare acts on Ranvier nodes of single frog sciatic fibers (*3*) and Armett and Ritchie (*4*) have reported a direct action of ACh on the electrical activity of the desheathed rabbit vagus nerve but not on the sheathed nerve. We have now obtained the first direct effect of ACh on the electrical activity of sheathed somatic nerve fibers (*5*).

A small bundle of fibers was dissected out of the main nerve trunk of the walking leg of the lobster. The length obtained was about 5 cm and the diameter about 0.3 to 0.5 mm. The membrane potential was recorded with the "sucrose gap" method devised by Stämpfli (*6*) and described in detail by Straub (*7*). The action currents were recorded separately in a shallow bathing vessel equipped with external electrodes. The bathing solutions were constantly bubbled with 100 percent oxygen.

Filtered sea water containing 0.5 ml of 1.0*M* tris buffer (tris hydroxymethyl aminomethane) per liter was used. The *p*H was adjusted, if necessary, to 7.9 to 8.1 with a few drops of 0.1*N* HCl or NaOH. All solutions were allowed to equilibrate to room temperature (22 ± 2°C). The Na-free artificial sea water used in some experiments was similar to the natural one available at Woods Hole except for replacement of NaCl by choline chloride. Its composition in micromoles per milliliter was as follows: choline chloride, 430; KCl, 9.2; $CaCl_2$, 23.4; $MgSO_4$, 26.0. This solution was buffered in the same way as ordinary sea water.

Figure 1 shows the time course of the decrease of the membrane potential produced by ACh in 15 preparations. The onset of action usually occurs within 25 seconds after the solutions are changed. At least a part of this delay can be attributed to the clearing of dead space in the apparatus. The minimal effective concentration of ACh is about $10^{-3}M$, but higher concentrations were usually used. With $5 \times 10^{-3}M$ a depolarization of 4 to 6 millivolts is achieved within 4 to 6 minutes. The effect is reversible, usually completely, and the rate of recovery is slower than that of depolarization. The sensitivity of the preparation to ACh is close to that of the desheathed rabbit vagus in Armett and Ritchie's experiments, as may be estimated from their data (*4*).

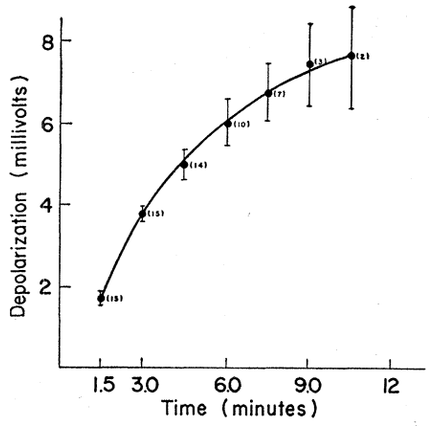


Fig. 1. Time course of the depolarization produced by acetylcholine ($10^{-2}M$). The vertical bars represent the standard error of the mean, and the numbers in parentheses represent the number of observations on individual preparations.

Successive reversible depolarizations may be readily achieved, but after three or four exposures some attenuation of rate and magnitude usually takes place. No effect was noted with sodium acetate or iodide or choline chloride in concentrations of $10^{-2}M$ and exposure times up to 15 minutes. Acetylcholine, in the same concentration, reversibly blocked conduction of the axons within 10 to 15 minutes. With lower concentrations the block occurred less rapidly.

Acetylcholine had no depolarizing effect in Na-free sea water maintained isotonic with choline chloride. This suggests that the effect in ordinary sea water may be due to an increased Na conductance. However, it must still be ascertained whether choline itself has an inhibitory effect at the high concentration used.

The high concentration of ACh required for the effects described may seem to be difficult to reconcile with the proposed physiological role, especially if compared with the low concentrations used at neuromuscular junctions. However, as mentioned above, much evidence has accumulated for the existence of structural barriers protecting the axonal membrane against the action of many chemicals. The high concentration of eserine required for blocking axonal electrical activity was also questioned until it was shown that when applied to single fibers, this specific and powerful inhibitor of cholinesterase rapidly and reversibly blocks electrical activity of Ranvier nodes at the concentrations effective at junctions, a 1000-fold increase in potency when compared to the action on the whole intact nerve fiber (*8*). Curare and ACh usually do not affect conducting membranes at all, even in extremely high concentrations, unless prior chemical treatment has reduced the barriers or special preparations are used (*3*, *4*, *9*). It is far more surprising to find a direct depolarizing action of ACh on the axon in the way predicted by theory than the necessity to use a high concentration. The effect is, of course, significant only in association with the many biochemical data accumulated (*1*). It is entirely possible that at the concentration used other chemicals may also block conduction. Clearly, this fact alone does not permit far-reaching conclusions, because to postulate that a compound has a physiological role to control ion movements and change conductance, a great number of prerequisites must be fulfilled. Only the ACh system has the properties required for the role proposed. The ACh system (acetylcholinesterase, choline acetylase, and the receptor) is present in all conducting fibers and its functional interdependence with electrical activity has been demonstrated. In the light of this and other information, the high concentration of ACh required to produce the postulated effect does not appear to be an argument against the physiological significance of the observations.

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