Table 1. Ready production of the "D"-like antibody.

Injection	Number	
	"D"-like antibody produced	No antigenic response
Heat extracts of Rh-negative red blood cells (rbc)	13	7
Heat extracts of Rh-positive (R_2r) rbc	4	4
Heat extracts of Rh-positive (D-- /D--) rbc	6	6
Rh-positive (R_2R_2) rbc	7	2
Washed sediments from heat extracts of Rh-positive rbc	6	4
Washed sediments from heat extracts of Rh-negative rbc	5	5

antigen, and 6 out of 12 produced anti-D-like specificity, but only one gave strong reactions. Of the 20 animals injected with heat extracts of Rh-negative blood, 13 produced good to moderate effects. In all cases the sera were absorbed with Rh-negative blood in dilutions of 1:5 or 1:10 and tested with red cells suspended in saline.

Ponder and Ponder (*3*) had shown that the heat extracts when ultracentrifuged yielded a sediment consisting of tiny fragments and "myelin" forms released from the red cells by heating. These were readily visible by phase microscopy. Subsequently, Murray and Clark (*2*) demonstrated that guinea pigs, when injected with the washed sediment derived from heat extracts of Rh-negative blood and centrifuged at 10,000 rev/min, produced an antibody which they believed showed anti-D specificity. This observation was confirmed in our experiments with the heat extracts of Rh-positive blood, sedimented at 30,000 *g* and washed three times at the same or higher centrifugal fields. Of ten animals, each injected twice with the sediment derived from the heat extracts of a total of 20 ml of whole blood, six produced good antibody response. In similar experiments with the sedimented particles from heat extracts of Rh-negative blood, five out of ten guinea pigs produced the antibody.

The production in guinea pigs of an antibody with "D"-like specificity upon injection of Rh-positive, Rh-negative, or rhesus red cells was demonstrated.

Table 1 records the results of injecting (*4*) guinea pigs with heat extracts (50°C) of Rh-negative red cells, heat extracts of two series of Rh-positive red cells, one series with Rh-positive red blood cells (R_2R_2), the washed sediment from heat extracts of Rh-positive blood, and also from Rh-negative blood. In each of these series there is a high incidence of production of the "D"-like antibody.

The relation of the "D"-like antigen in Rh-positive and Rh-negative red cells or their extracts and in rhesus red cells to the usual D antigen of Rh-positive blood requires extensive study. Thus far, the "D"-like antibodies produced in guinea pigs seem to differ from human anti-D in several respects, among which the following are cited: (i) Rh-positive red cells blocked with a powerful blocking anti-D [or selected cells sensitized with anti-c (hr′)] are still agglutinated by the "D"-like antibody produced in guinea pigs injected with rhesus red cells or heat extracts of Rh-positive or Rh-negative blood. (ii) The "D"-like antibodies induced by any material containing the "D"-like antigen when exposed to Rh-positive, Rh-negative, or rhesus red cells yield eluates showing "D"-like specificity, while Rh-negative red cells, possessing as they do the "D"-like antigen, when exposed to human anti-D do not yield active eluates.

No antibody to the "D"-like antigen is obtained if the heat extracts are prepared from either trypsinized Rh-positive or Rh-negative red cells (*5*).

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4. The technical assistance of Thomas J. Pluhar is acknowledged.
5. A fuller discussion of these findings is in preparation.

14 November 1960

Effect of Strychnine upon the Electrical Activity of an Isolated Nerve Cell

Abstract. The effect of strychnine upon the electrical activity recorded from the axon and the soma of an isolated nerve cell (the nonadapting stretch receptor cell of the crayfish) was studied. Protracted exposure of the soma of the cell to strychnine prolongs the duration of the intracellulary recorded action potential, as has been described in other excitable tissues treated with quaternary ammonium ions. During the plateau in the falling phase of the soma spike, the axon is usually firing repetitively. The stimulation of the inhibitory fiber produces a premature termination of the prolonged spike.

The effects of strychnine upon the activity of a single nerve fiber are known (*1*). Strychnine has also been extensively used in electrophysiological investigations of central nervous system activities. In these experiments, however, the inferences which can be drawn concerning the actions of the compound are limited because of the complex structural and functional arrangements in the central nervous system. We have not seen in the literature a study of the effect of strychnine upon the electrical activity of a single, isolated nerve cell.

The stretch receptor of Crustacea (*2*), which has been studied physiologically by several investigators (*3, 4*), is a suitable preparation for such a study. The soma of the isolated nerve cell can be easily penetrated by microelectrodes, and both excitatory (the generator potential set up by stretch) and inhibitory stimulation may be applied.

Nonadapting abdominal stretch receptors of crayfish were dissected and mounted on a device similar to that described by Eyzaguirre and Kuffler (*4*). The fast-adapting receptor was cut off. The preparation was suspended in a plastic box containing Van Harreveld solution. The axon was raised into a layer of mineral oil where platinum electrodes recorded the discharge. Micropipets filled with potassium chloride were used to penetrate the cell; they were mounted in a bridge circuit permitting simultaneous recording and stimulation. After the addition of a strychnine sulfate solution (1 percent) to the perfusion fluid (final concentration about 0.03 percent), the exceedingly regular discharge of the receptor to a steady stretch was broken and bursts of spikes were recorded from the axon, which were followed by pauses.

The cell must be depolarized, either by stretch or by application of a pulse of cathodal current through the impaling microelectrode, for the strychnine type of activity to appear. The threshold to direct stimulation was not altered appreciably, but a short burst of spikes (two or three) was initiated at a rheobasic current. The membrane resistance and the resting potential were not altered. The frequency of occurrence of the bursts increased with increasing degree of stretch, while the number of spikes in the bursts decreased. A total desynchronization with a return to the regular discharge of the receptor could be obtained if a high degree of stretch was applied or if calcium ions were added to the solution.

The changes described are due to the action exerted by strychnine mostly upon the membrane of the cell (and possibly the initial portion of the axon) rather than upon the axonal membrane (at the concentration used). When the muscle bundle and the soma of the cell were kept in oil, with only several millimeters of the axon being lowered in

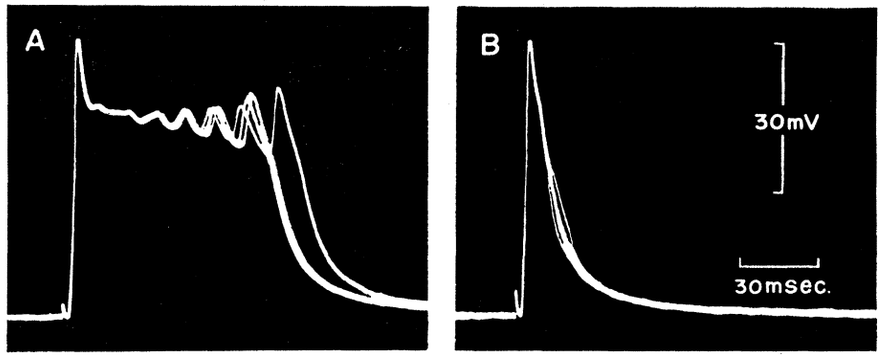

Fig. 1. (*A* and *B*) Superimposed action potentials evoked by antidromic stimulation. In *B*, inhibitory fiber stimulation caused a premature termination of the prolonged action potential.

the saline containing strychnine before being raised again into the oil layer, no alteration of activity took place. When the cell was lowered into the saline, the changes in activity described soon appeared.

After 5 to 10 minutes of exposure to strychnine, only long bursts of spikes (from five to 20 or more) were recorded from the axon except at extreme degrees of stretch. Intracellular recording from the soma in this condition showed: (i) no effect of strychnine on the membrane potential—the membrane's resistance was not changed; (ii) a prolongation of spike potential by a plateau which developed during the falling phase of the spike and which could be as long as several seconds; (iii) a series of oscillations during the plateau. The amplitude of these oscillations increased toward the end of the plateau.

A premature termination of the prolonged spike potential was obtained by applying a short pulse of inward current during the plateau. The phenomenon was all-or-none in nature. Refractoriness was present after a prolonged action potential; this finding resulted when a second action potential was induced at different time intervals by direct or antidromic stimulation. The characteristics described are similar to those of the action potential recorded from cardiac muscle fibers (*5*), crayfish muscle fibers (*6*), frog spinal ganglion cells (*7*), toad spinal motoneurons (*8*) treated with quaternary ammonium ions, squid giant axon after injection of tetraethyl-ammonium (*9*), and nodes of a toad nerve fiber exposed to heavy metal ions (*10*).

It has been found that a premature termination of the prolonged spike potential can be obtained by stimulating the inhibitory fiber to the receptor with a single shock (Fig. 1). The effect is similar to that observed when an anodal pulse is applied to the cell. Strychnine, therefore, did not block the inhibitory synaptic processes, at least not under the conditions of our experiment. Finally, it was found that when calcium ions were added to the extracellular fluid the duration of spike potential became normal.

The spatial pattern of electrical activity during the plateau of the intracellularly recorded spike has been studied by recording with an external microelectrode along the surface of the axon and the cell body (see *11*). The site of origin of the spikes has been found, in agreement with the results of Edwards and Ottoson (*11*), to be in the axon, several hundred microns away from the soma. Only the first spike of the burst invades the soma, as indicated by record *B* of Fig. 2. It would seem that the sustained depolarization of the soma spreads to the axon, causing its repetitive firing. Whether the oscillations recorded in the soma eventually participate in the process of initiation of the spike in the axon is still to be ascertained.

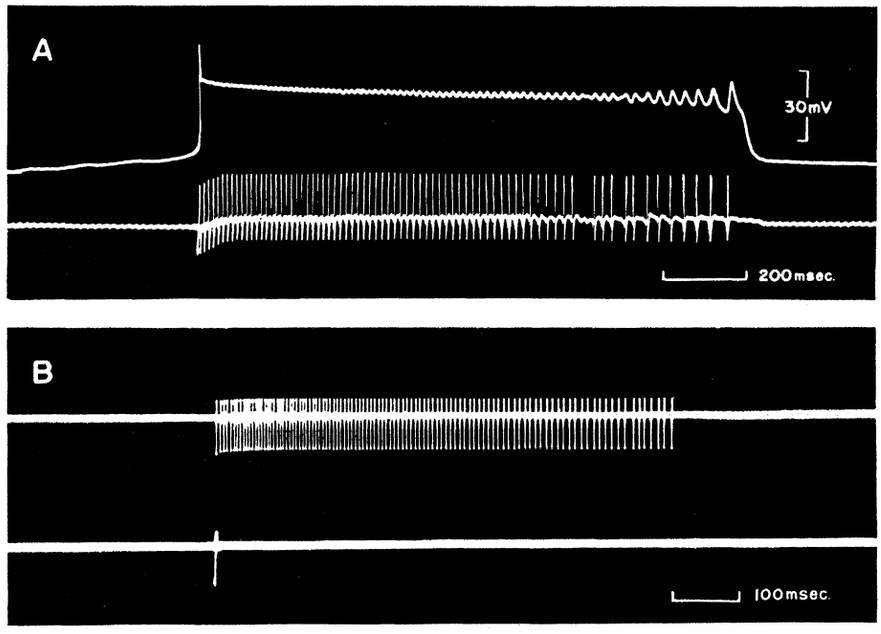

Fig. 2. (*A*) Intracellular record from the soma (upper trace) and extracellular record from the axon (lower trace) after application of strychnine. (*B*) The upper trace shows the discharge of impulses from the axon, while the lower trace is a microelectrode recording from the outside of the soma membrane. Note that inward current (down deflection) occurs in the soma only during the first spike of the burst.

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