front page of the New York *Times* carried a picture of scientist *X*, together with an article describing the experiment he was undertaking. When *X* discussed his experiment at a scientific meeting 6 weeks later he reported reluctantly that, despite hard work at great speed, he had not yet been able to reach any conclusions. At the same meeting *Y* announced that he had successfully carried out the experiment and obtained results in agreement with the theory; shortly thereafter *Y* published his findings. It was not until some 2 months later that *X*, in a "Letter," was able to report his own experiment, which also confirmed the theoretical expectation. He pointed out, however, the necessity of controlling the temperature of the experiment quite carefully to avoid introducing large extraneous effects; indeed, since *Y* had not taken such precautions, his findings lacked significance. In this instance an important experiment was performed in a short time and ultimately in a reliable way. But the example shows vividly the actual circumstances under which the experiment was carried out—the announcement of an experiment before it was undertaken, the newspaper publicity, the hurried activity of two scientists working under pressure to be the first to publish—and the lack of sufficiently careful work which may result from these conditions.

While much more could be said about the differing patterns of competition in various sciences and about the rapid changes taking place in many of these disciplines, my aim has not been to treat the topic exhaustively. It is sufficient if the perspectives of the outside observer have been broadened, to make him aware that the scientist is not just somebody concerned with new ideas and techniques, but that he carries out his work in a human, and sometimes all too human, context.

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Chemical Factors Controlling Nerve Activity

Analysis reveals the underlying chemical system that generates the currents responsible for nerve impulses.

David Nachmansohn

One of the characteristic features of living cells is their high potassium ion (K^+) concentration in contrast to the low K^+ concentration in the outer environment. The reverse is true for the sodium ion (Na^+) concentration. However, only conducting cells, nerve fibers, and muscle fibers make use of these concentration gradients for generating the electric currents which propagate impulses. These currents are carried by ions. During activity, Na^+ moves into the interior, and this movement is followed by an outflow of an equivalent amount of K^+ (*1*). There is a strong and rapid rise of sodium conductance and an equally rapid return to the initial stage. Subsequently, potassium conductance, already high in the resting state, increases but slightly, and the changes are relatively slow (*2*). These facts raise immediately the fundamental question: What is the special mechanism which enables conducting cells to use ionic concentration gradients, the source of electromotive force, for the generation of electricity?

It is difficult to see how electricity in a fluid system such as the living cell can be generated without chemical reactions. Conducting cells must be endowed with a special chemical system controlling the movements of ions in a specific way. Any doubt as to the chemical nature of this process has been removed by the recent heat-production measurements of A. V. Hill and his associates (*3*). They found that the initial heat can be separated into two phases: a strong positive heat, coinciding with electrical activity, followed by a negative heat during recovery. The conducting membrane is only 50 to 100 angstroms thick. Per gram of active material, the positive heat amounts to about 3 millicalories. This is about the same amount of heat as that produced per gram of muscle during a twitch.

What is the chemical reaction? About 30 years ago, acetylcholine was linked to a special phase of nerve activity. It was assumed to be released from nerve endings and to act as a neurohumoral transmitter on the effector cell, nerve or muscle. The observations were based on classical methods of pharmacology. However, the idea of a special mechanism at nerve endings which is basically different from that in axons was opposed by many electrophysiologists. The facts were not questioned, but the interpretation was. A new approach appeared imperative.

The rapid development of biochemistry, especially the spectacular rise of protein and enzyme chemistry during the last few decades, has provided powerful tools for analyzing cellular function in terms of physics and chemistry. An approach with biochemical methods was initiated 25 years ago. The enzymes effecting hydrolysis and the formation of acetylcholine were analyzed, the sequence of energy transformations was established, and a number of chemical reactions were correlated with physical events. Central to these studies have always been the proteins and enzymes, especially those linked specifically to the action of acetylcholine. They have been isolated and purified from the

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electric organs of electric fish. This material is particularly suitable. The organs are the most powerful bioelectric generators created by nature and are, moreover, highly specialized in their function, but the electricity is nevertheless basically similar to that of nerve and muscle fibers. The organs contain only 2 percent protein and 93 percent water, and isolation of the proteins associated with acetylcholine action is thereby greatly facilitated. These organs have been used, since 1937, for the analysis of the chemical basis of bioelectricity. The information obtained from the analysis of the proteins in vitro has been applied wherever possible to the study of the events in the living cell.

It soon became apparent that the original theory must be modified. A new concept was proposed which explains the original observations and reconciles the facts with the views of electrophysiologists. The action of acetylcholine is an intracellular or, rather, intramembranous process. It is responsible for the sudden changes in conductance that take place in conducting membranes during activity. The acetylcholine system is the specific chemical system with which all conducting cells of the animal kingdom are endowed and which enables them to generate the electric currents which propagate nerve impulses (*4*, *5*).

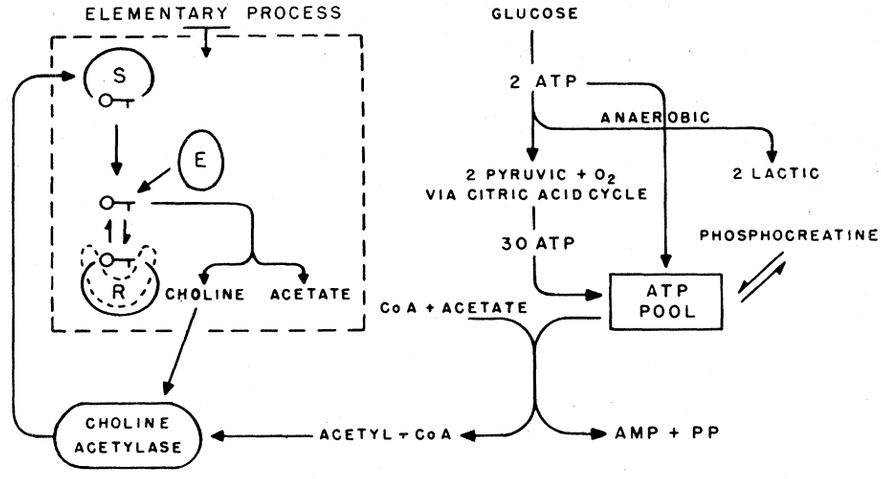


Fig. 1. Sequence of energy transformations associated with conduction and integration of the acetylcholine system into the metabolic pathways—a schematic presentation of the elementary process (see text). *S*, storage protein; *E*, cholinesterase; *R*, acetylcholine receptor protein, O— acetylcholine. [Nachmansohn (*4*, *5*)]

The Acetylcholine System

The picture of the elementary process which has emerged from these studies may be described as follows (Fig. 1). Acetylcholine is bound, in resting condition, to some protein (or conjugated protein). Any stimulus reaching the membrane releases the ester, acetylcholine, which then reacts with a receptor protein. In this reaction some change of the protein is produced, probably a change of configuration. It is this reaction which is responsible for the increase in sodium conductance. It is the trigger by which the ionic concentration gradients, the potential source of electromotive force, become effective and by which the action currents are generated. The ester-receptor complex is in dynamic equilibrium with free ester and receptor protein; the free ester is attacked by cholinesterase (acetylcholine esterase) and rapidly inactivated by hydrolysis, the receptor—and the sodium conductance—being thus permitted to return to the original condition. The barrier is reestablished. The extraordinary speed of the hydrolysis by cholinesterase, which has a turnover time of 30 to 50 microseconds (*6*), permits the rapid restoration of sodium conductance. It explains the ability of nerve fibers to conduct several hundred or thousand impulses per second.

The development was reviewed, in 1959, in a monograph (*5*). In the present article some recent advances and new evidence for the concept proposed are discussed.

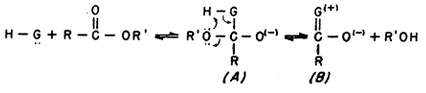
One of the major lines of evidence for the crucial role of the acetylcholine system in the generation of bioelectricity has been the demonstration that it is impossible under any condition to separate cholinesterase activity from electrical activity. If the picture of the elementary process proposed is correct, block of enzyme activity should stop electrical activity. A variety of extremely potent and specific inhibitors of cholinesterase have been applied to a great variety of fibers. In all types of fibers of the animal kingdom tested—central and peripheral, cholinergic and adrenergic, motor and sensory, vertebrate and invertebrate—electrical activity invariably failed when enzyme activity was blocked.

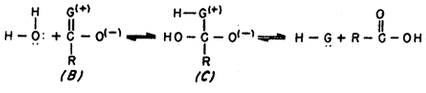
Some physiologists objected to this evidence on the basis that the concentrations of inhibitor used were unconventionally high, many times as high as at the nerve endings. It is not difficult to explain this apparent discrepancy. The conducting membrane of the axon, in contrast to that of the junction, is surrounded by structural barriers. The outside concentration is, therefore, irrelevant. Only the concentration at the site of action is pertinent. When the nerve axons are exposed, for instance, to 1 milligram of a powerful inhibitor of cholinesterase (such as diisopropyl phosphofluoridate) per milliliter, less than 1 microgram per gram is found inside the axon at the time when electrical activity fails. Moreover, a functional interdependence of electrical and chemical activity has been demonstrated in intact fibers. If electrical and chemical activity are measured in intact crab fibers exposed to inhibitors of cholinesterase, electrical activity fails when the enzyme activity falls to 20 percent of the initial level, regardless of the type of the inhibitor used and its concentration (*7*). Only the rate at which this level is reached varies.

Recently, a new and refined technique was used by Dettbarn (*8*). Electron microscope studies of Robertson (*9*) have shown that at the Ranvier nodes of myelinated frog nerve fibers the conducting membrane is covered by a thin and porous structure only. Using a single fiber of a frog sciatic nerve and applying Staempfli's technique to measure the electrical activity of a single Ranvier node, Dettbarn obtained, with 300 micrograms of eserine per milliliter, reversible block of electrical activity in 30 seconds. With 30 to 40 micrograms per milliliter, electrical activity is blocked in a few minutes. This effect is comparable to the conventional concentrations used on junctions. With the refined technique, a thousandfold increase in the potency of the inhibitor has been obtained.

Organophosphorus Compounds

An instructive illustration of the way in which the analysis of chemical and molecular forces in the proteins contributed to an understanding of nerve function resulted from the use of organophosphorus compounds. These compounds are widely used as insecticides, and some of them are potential chemical warfare agents. Their fatal action is due to the irreversible inactivation of acetylcholinesterase. The mechanism of their reaction with the enzyme became obvious once the mechanism of the hydrolytic process was established. Analysis of the molecular forces acting between the substrate and the enzyme has revealed that the active surface has two functionally and spatially separated sites: an "anionic" site attracting the quaternary nitrogen group of the ester by Coulomb and van der Waals forces, and an "esteratic" site which has an acidic and a nucleophilic group; the latter group forms a covalent bond with the electrophilic carbon of the carbonyl group (Fig. 2). The hydrolytic process takes part in two steps, as follows:





The enzyme is symbolized by the acidic group (H) and the nucleophilic group (G). In the first step the alcohol (choline) is eliminated by an electronic shift. As a result, an acetylated enzyme is formed. This acetylated enzyme reacts rapidly, in microseconds, with water to form acetate and restored enzyme (*10, 11*).

In the case of organophosphorus compounds a phosphorylated enzyme is formed which does not react with water, or reacts only very slowly, in days or weeks (*12*). For all practical purposes the enzyme is inhibited, and death of the animal ensues.

Once the mechanism of action was known, it seemed possible to reverse the reaction. A nucleophilic group should be able to displace the phosphoryl group from the nucleophilic group in the active site of the enzyme. Hydroxylamine was known, from Hestrin's experiments, to form hydroxamic acid from acetate in the presence of cholinesterase (*13*). It does this by attacking the carbon of the carbonyl group. Hydroxylamine indeed reactivates the phosphorylated enzyme, but it is a slow process, taking hours and requiring high concentrations (*12*). Wilson suggested the attachment of the active nucleophilic group to a quaternary nitrogen at proper atomic distance. This quaternary group should promote the interaction between reactivator and phosphorylated enzyme, just as acetylcholine is greatly superior to ethylacetate as a substrate, due to the attraction of the quaternary nitrogen group to the anionic site by Coulomb and van der Waals forces. Among a number of compounds suggested by Wilson and synthesized by Sara Ginsburg, pyridine-2-aldoxime methiodide (2-PAM) proved to be an extremely powerful reactivator (*14*). Kewitz *et al.* (*15*) applied the compound to mice and showed that it is an extremely potent antidote against insecticides and nerve gas poisoning, especially in combination with atropine. It is effective in animal experiments against 50 to 100 times the lethal doses (LD_{100}) of organophosphates. It has been successfully applied on a large scale, by Namba and Hiraki (*16*) in Japan, to human beings, victims of severe insecticide poisoning, and many lives have been saved. The extraordinary potency and reactivating power were surprising. Wilson (*17*) and his associates started, therefore, to explore the basis for this potency of PAM as a reactivator. They were able to demonstrate that PAM and phosphorylated esterase have molecular complementariness—that is, perfect fit; if the cationic nitrogen is attracted to the anionic site, the active oxygen atom is just one bond length away from the phosphorus atom.

Kewitz (*18*) has shown in animals treated with PAM that the compound acts by repairing the specific biochemical lesion produced by the organophosphorus compound—that is, by reactivating the phosphorylated enzyme in vivo. These observations were recently extended by Rosenberg (*19*) to the brains of intoxicated animals. The possibility of reactivating phosphorylated cholinesterase raised the question of whether electrical activity of a conducting membrane, after being irreversibly blocked by organophosphate, could be restored by the reactivation of the enzyme. Pyridine-2-aldoxime methiodide is unsuitable for such experiments, since it is lipid-insoluble and would not penetrate through the structural barrier. A lipid-soluble derivative of PAM, benzoyl pyridine oxime methiodide, which is a good reactivator of

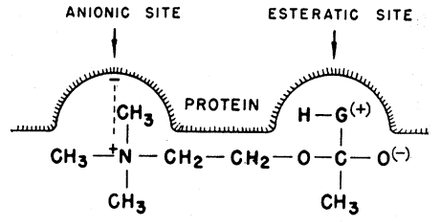


Fig. 2. Schematic presentation of the interaction of the active groups in the surface of cholinesterase and the substrate: the Michaelis-Menten complex. H represents the acidic group and G, the nucleophilic group, in the enzyme surface. [Nachmansohn and Wilson (*11*)]

phosphorylated enzyme and was synthesized by Sara Ginsburg, was used by Hinterbuchner (*20*). He exposed two preparations to an organophosphorus compound (paraoxon) until electrical activity was irreversibly blocked; this took from about 8 to 10 minutes. After that the control was washed with Ringer's solution. Activity was not restored. On washing the other preparation with benzoyl pyridine oxime, electrical activity reappeared. Thus, electrical activity, irreversibly blocked, has been restored by a specific chemical reaction—that is, by the removal of a phosphoryl group from the enzyme.

The Receptor Protein

I should like now to turn to another protein of the acetylcholine system, the receptor protein. Although a receptor for acetylcholine has long been postulated on a theoretical basis, experimental evidence for its existence was offered only several years ago in experiments with intact electroplax from the electric organs of electric fish. It was shown that acetylcholine and related compounds (such as, for instance, carbamylcholine, decamethonium, and procaine) in very low concentrations block electrical activity without affecting cholinesterase activity. Even concentrations 200 times as high as those required for block did not markedly affect the enzyme activity (*21*). Obviously, these compounds act on a similar but different cell constituent, the long-postulated receptor.

In tests of the effects of these compounds on the electrical characteristics, a striking difference became apparent: one type of compound blocked electrical activity with simultaneous depolarization; the second type blocked but did not depolarize the membrane. Depolarization implies, of course, increased con-

ductance, postulated to be the physiological function of acetylcholine. In general, acetylcholine and other methylated quaternary nitrogen derivatives blocked with depolarization, whereas tertiary analogues blocked but did not depolarize. Apparently, acetylcholine and other related compounds produce a change in the receptor resulting in a state of membrane conductance similar to the physiological one during electrical activity. The tertiary analogues combine with the active site but do not produce the change. In analogy with enzyme chemistry, we refer to the one type as receptor activators, to the other as receptor inhibitors.

The striking difference between quaternary compounds and their tertiary analogues appears particularly pertinent in the light of binding studies made with the enzymes cholinesterase and choline acetylase. If the binding of quaternary inhibitors (for example, tetramethylammonium and trimethyl ethanolamine) is compared to the binding of their tertiary analogues (trimethylammonium and dimethyl ethanolamine), the extra methyl group does not increase the binding (*22*). But the enzyme activity with the corresponding esters is greatly increased by the extra methyl group. The rate of acetyl enzyme formation increases tenfold. An analogous situation prevails with choline acetylase (*23*). How can we explain this remarkably powerful action of one methyl group on the enzymic process? A quaternary nitrogen group has a tetrahedral structure and is more or less spheric in shape. The extra methyl group is not in touch with the protein surface unless the protein changes its shape and envelops the quaternary group. Some support for the assumption of a change of configuration comes from observations on the entropy of activation of hydrolysis: that of the acetylcholine is very favorable as compared to that of the tertiary analogue. The difference due to the presence of the extra methyl group is about 25 to 30 entropy units (*24*). The problem is being investigated at present by Allen Gold. If the assumption is borne out, it may explain the trigger mechanism of the action of acetylcholine on the receptor protein. A small local folding of a helical or nonhelical section of a protein chain may remove a positively charged amino group from a strategically located point, thereby permitting the flow of ions through the membrane. A 2- to 4-angstrom shift of the charged group may be sufficient to account for the proposed trigger action. We know indeed that for one molecule of acetylcholine metabolized, about 1000 sodium ions may flow into the interior.

To study a protein by studying reactions on the intact cell is unsatisfactory. It appeared necessary to isolate the protein. The first notable attempt to isolate the receptor was made by Chagas (*25*) and his associates in Rio de Janeiro, and they deserve great credit for their initiative. They injected a radioactive curare-like compound, the triethiodide of gallamine, into electric tissue and prepared an extract which they dialized against distilled water. The compound appeared bound to components of the extract. But this binding could be due to Coulomb and van der Waals forces. Indeed, curare readily combines with all kinds of macromolecules rich in negative charges, such as acidic polysaccharides and nucleic acids (*26*). But the complex is readily dissociated by increasing ionic strength. It was therefore decided to try a different approach. Ehrenpreis (*26*) prepared extracts from electric tissue, fractionated them with ammonium sulfate, and tested the binding of curare to the proteins in equilibrium dialysis, according to the method of Klotz (*27*), against phosphate buffer at *p*H 7.5 and ionic strength 0.1. No binding of curare was observed when freshly prepared extract was used, but with a fraction obtained with ammonium sulfate at 60 percent of saturation, and a fourfold concentration, binding was obtained. Moreover, some of the protein was precipitated by curare. Most of the protein which could be precipitated by curare was in the fraction obtained with ammonium sulfate at 30 percent of saturation. When this protein was separated and dialyzed against phosphate buffer at *p*H 7.5 and ionic strength 0.1, part of the protein went into solution, but one part remained precipitated. This part could only be solubilized at a *p*H of 9. Curare has, in addition to the two quaternary nitrogen groups, two phenolic hydroxy groups. These groups dissociate at *p*H 9. Possibly they form hydrogen bonds with this particular protein. Urea dissociates the complex. This difference between the curare complex formed with this particular protein and that formed with other proteins and macromolecules is most fortunate because it permits separation of the protein in a relatively simple way and essentially as one component. According to ultracentrifuge and electrophoretic studies, 90 percent or more of the purified preparation is formed by this one protein. Its molecular weight is around 100,000 (*28*).

Now came the crucial question: How can we identify this protein and decide whether it is the acetylcholine receptor protein? Such a difficulty obviously does not arise in enzyme purification. At this point the availability of a monocellular preparation which had been developed with great resourcefulness and originality by Schoffeniels (*29*) became decisive for the identification of the protein as the physiological acetylcholine receptor.

Monocellular Electroplax

A single electroplax is dissected from the bundle of Sachs, the electric organ situated at the tail end of *Electrophorus*; there, the extracellular space separating the individual cells is large (Fig. 3). The electroplax is more or less rectangular in shape, varying between 5 and 15 millimeters in length, 1 and 1.5 millimeters in height, and 0.5 and 1.0 millimeter in thickness. The two opposite faces of the cell differ in function and structure: the caudal face is innervated and conducting, the opposite face is nonconducting and has many digitations, which greatly increase the surface. Each cell is located in a compartment formed by connective tissue. The technique requires complete removal of the connective tissue of the adjacent compartment situated close to the conducting membrane.

As shown in Fig. 4, the cell is kept between two nylon sheets, one with a window adjusted to the dimensions of the cell, the other with a grid consisting of nylon threads by which the cell is pressed against the window. The sheets are mounted between two blocks of Lucite (Fig. 5), each containing a pool. When the two blocks are fixed, the two pools of fluid are completely separated by the cell. Ions or other chemicals dissolved in the solution cannot pass from one side to the other except through the cell. The fluid of one pool bathes the conducting membrane, that of the other bathes the nonconducting membrane.

This unique preparation permits separate analysis of the properties of the two types of membrane. It is extraordinarily sensitive and versatile. Compounds that react with the acetylcholine system affect electrical activity in low concentrations; acetylcholine, for in-

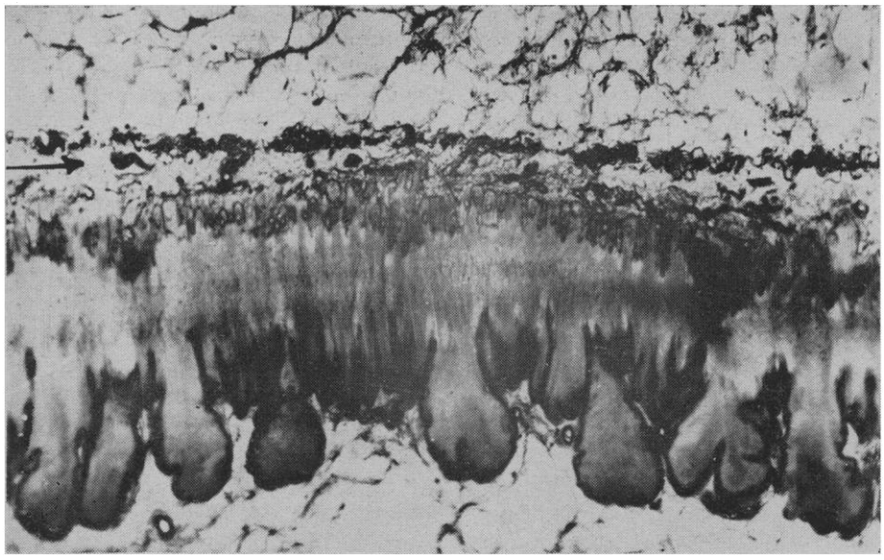


Fig. 3. Photomicrograph of a cross section of an electroplax from the bundle of Sachs of *Electrophorus*. Innervated membrane on the top, nonconducting membrane on the bottom. The arrow marks the space between the connective tissue of the adjacent department and the innervated membrane. The connective tissue must be removed by dissection. (About × 146) [Nachmansohn *et al.* (*41*)]

stance, blocks conduction reversibly at a concentration of $10^{-7}M$. The various electrical characteristics can be studied with intracellular electrodes. Figure 6 illustrates the difference between a receptor activator (carbamylcholine) and a receptor inhibitor (tetracaine) tested with this preparation. The former blocks the electrical activity, and simultaneously a depolarization takes place; with the latter, the resting potential remains unchanged when electrical activity ceases. Both of the effects are readily reversible. The preparation permits the correlation of electrical characteristics with chemical factors and with ion flux.

The availability of a suitable monocellular preparation eliminated many difficulties encountered with previous preparations and, in general, became decisive for further progress. In particular it made possible identification of the protein isolated and assumed to be the physiological acetylcholine receptor. When Ehrenpreis tested the binding strength of the isolated protein with respect to a great variety of tertiary and mono- and diquaternary compounds related in structure to acetylcholine, he found great differences. When Rosenberg and Higman tested the effects of the same series of compounds on the electrical activity of the monocellular electroplax preparation, a striking parallelism became apparent. With other proteins and macromolecules the binding, if it exists at all, is weak, and moreover, no parallelism exists between binding strength and effect on electrical activity. It seems, however, that a difference exists between receptor activators and receptor inhibitors insofar as the binding is concerned. Acetylcholine and other activators are bound to the receptor protein, but the binding seems to be in general poorer than that of receptor inhibitors, and poor as compared to the effectiveness on the activity of the electroplax. A similar phenomenon is encountered in enzyme chemistry. Inhibitors are frequently much more strongly bound than substrates. The dissociation constant of the neostigmine- or physostigmine-cholinesterase complex is 10^{-7}; that of acetylcholine, 10^{-4}. Even in the case of substrates, the strength of binding by no means parallels the rate of reaction with the enzyme. Acetylcholine, for instance, is much more poorly bound to cholinesterase than butyrylcholine is, but it is a 150-times better substrate.

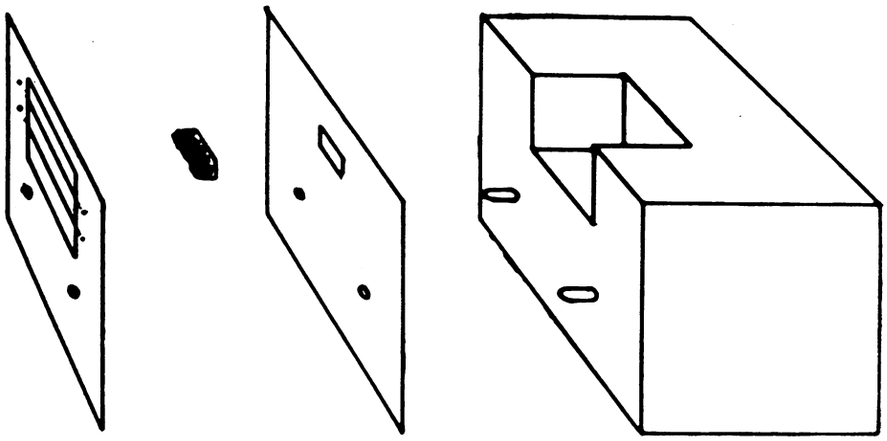


Fig. 4. Schematic diagram of the arrangement by which a single isolated electroplax separates two pools of fluid, showing the respective positions of one chamber for the pool of fluid, the sheet of nylon containing a window, the single electroplax, and the grid used for pressing the cell against the window. The other chamber is not shown (see Fig. 5). [Schoffeniels and Nachmansohn (*29*)]

Local Anesthetics and Curare

The properties of the protein in solution offer many interesting aspects. However, the reaction of this protein with local anesthetics, as observed both in the intact cell and in vitro, appears particularly pertinent. Local anesthetics such as procaine are strikingly related in structure to acetylcholine. In tetracaine one hydrogen atom on the N of the aniline is replaced by a butyl group. For many years it has been maintained that certain local anesthetics act by competing with acetylcholine for the active site of the receptor protein. Since they are receptor inhibitors, they act as antimetabolites, preventing acetylcholine from reacting with the active site. Some evidence for this assumption was offered with procaine on the monocellular electroplax (*29*). Recently, Rosenberg and Higman (*30*) compared the effectiveness of procaine, tetracaine, and dibucaine with respect to their effectiveness on the isolated single electroplax. They found tetracaine to be about 12 to 15 times as potent as procaine, and dibucaine twice as potent as tetracaine. When the binding strength of these three compounds with respect to the receptor protein was tested, a striking parallelism between binding strength and potency became

again apparent (*31*). The binding has a high degree of specificity.

Recently, Higman and Bartels (*32*) studied the competitive nature of the action of various receptor activators and inhibitors by testing the effects on the electrical characteristics with intracellular electrodes. They have accumulated a considerable amount of evidence for the competitive nature between the action of acetylcholine on the one hand and tetracaine and other receptor inhibitors on the other hand. For instance, a cell depolarized by acetylcholine is, even without removal of acetylcholine, completely repolarized by tetracaine or eserine as postulated by theory.

These results provide a chemical basis for the understanding of the action of certain local anesthetics. Moreover, since it is known that local anesthetics block electrical activity in all conducting fibers, the observations provide evidence that the acetylcholine receptor protein is present and essential for the generation of bioelectric currents in axons; this finding supplements the evidence previously obtained for the essentiality of acetylcholinesterase.

The strong binding of curare to the receptor protein has been discussed. The famous observation of Claude Bernard that curare acts only on the neuromuscular junction and does not affect conduction in nerve and muscle fibers formed for a long time the basis for the idea that there is a specific chemical mechanism at the junction. Some 15 years ago my associates and I offered experimental evidence that lipid-insoluble quaternary compounds cannot penetrate into the axon, apparently because of structural barriers (*33*). We proposed this as an explanation of why curare, acetylcholine, and other lipid-insoluble quaternary nitrogens do not affect conduction. Recently, Dettbarn (*34*) applied curare to the Ranvier nodes of a single isolated frog sciatic fiber, where, as we have seen, the axonal membrane is covered only by a thin and porous structure. He obtained rapid and reversible block of electrical activity. Walsh and Deal (*35*) treated desheathed frog sciatic fibers with a detergent, cetyltrimethyl ammonium bromide. After this treatment, curare, acetylcholine, neostigmine, and other quaternary compounds, inactive before, reversibly blocked electrical activity. A depolarizing action of acetylcholine was observed by Armett and Ritchie (*36*) on the desheathed cat vagus. Quite recently, Dettbarn and Davis (*37*) obtained a depolarization and a rapid and reversible block of electrical activity by acetylcholine applied to axons of somatic fibers of crustaceans. Finally, Rosenberg and Ehrenpreis (*38*) tried to reduce the structural barrier with enzymes. Among the great number of enzymes tested, cobra venom proved to be successful. After a 30-minute exposure of the giant axon of the squid to cobra venom (10 μg/ml), the venom was removed. The electrical activity was unimpaired. When curare was then applied, it rapidly and reversibly blocked electrical activity. Another venom, that of the cottonmouth moccasin, was found by Rosenberg (*39*) to be even more effective; after treatment with this venom, curare (in low concentrations) and acetylcholine blocked reversibly the electrical activity of the giant axon of the squid. In contrast, a series of tertiary nitrogen derivatives, related in structure to acetylcholine but lipid-soluble and show-

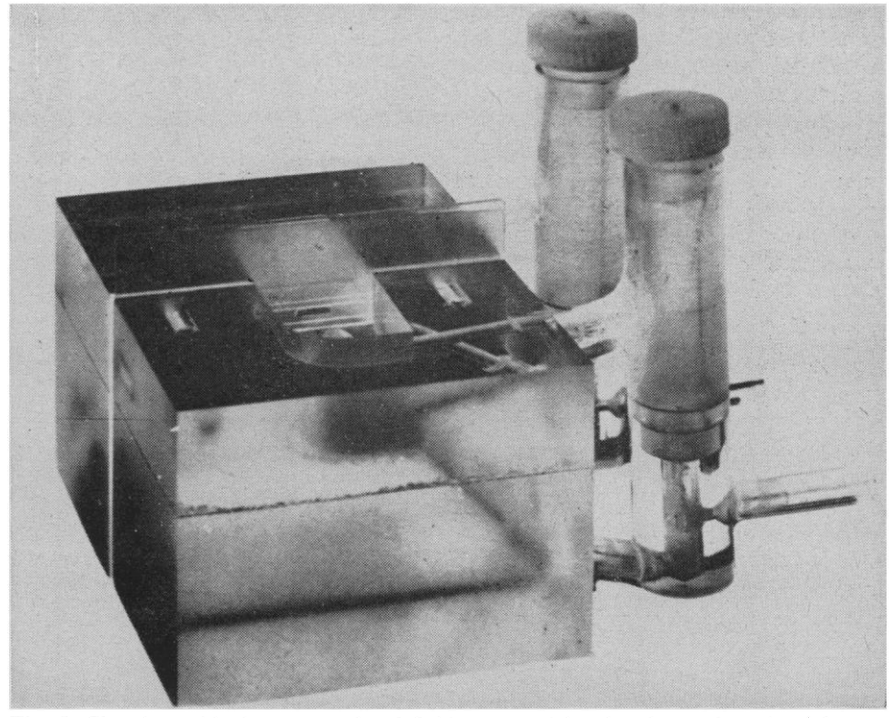


Fig. 5. Chamber with the two pools of fluid separated by the electroplax. At right are the air lifts for the two pools. [Schoffeniels (29)]

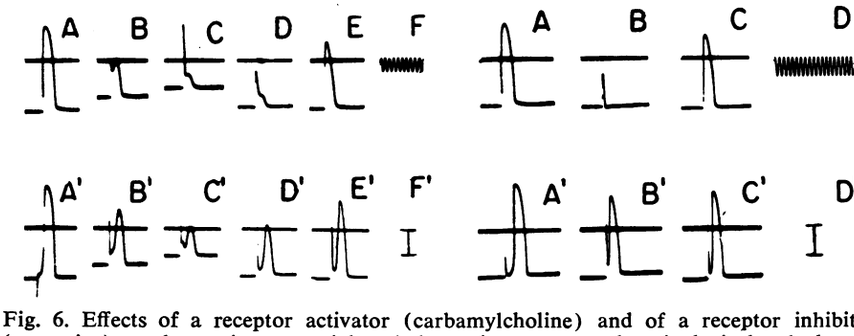


Fig. 6. Effects of a receptor activator (carbamylcholine) and of a receptor inhibitor (tetracaine) on the resting potential and the action current of a single isolated electroplax. The recordings were made with the cathode ray oscilloscope by means of microelectrodes, one of them intracellular. The value for the resting potential, measured by the distance between the two lines, is usually about 85 millivolts. There is overshoot during the discharge. (Left) *A–E*, direct stimulation; *A′–E′*, indirect stimulation; *A,A′*, control in Ringer's solution; *B,B′* and *C,C′*, at 1 and 4 minutes, respectively, after the addition of carbamylcholine ($5 \times 10^{-5} M$); (return to Ringer's solution at 5 minutes); *D,D′*, and *E,E′*, at 12 and 20 minutes, respectively. Calibration: *F*, 1000 cy/sec; *F′*, 50 millivolts. (Right) *A–C*, direct stimulation; *A′–C′*, indirect stimulation; *A,A′*, control in Ringer's solution; *B,B′*, at 5½ minutes after the addition of tetracaine ($5 \times 10^{-5} M$); (return to Ringer's solution at 6 minutes); *C,C′*, at 17 minutes (stimulus strength: *A′*, 30 volts; *B′*, 100 volts; *C′*, 60 volts). Calibration: *D*, 1000 cy/sec; *D′*, 50 millivolts. [Higman and Bartels (*32*)]

ing good and relatively specific binding to the receptor protein in solution, block conduction in the squid axon in concentrations surprisingly close to those previously observed on the synaptic junctions of electroplax.

Conclusion

The dramatic developments of biochemistry in the last few decades have greatly promoted our understanding of cellular function in terms of physics and chemistry, and we are reaching, in some fields, molecular levels. The few examples discussed in this article illustrate the approach to the analysis of the chemical factors that control nerve activity and the recent advances achieved (*40*).

References and Notes

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Science and the News

The Kefauver Hearings: The Drug Industry Finally Has Its Day and Does Quite Well

Last week the Kefauver committee entered its third, and presumably final, year of investigation of the drug industry. The hearings began in December 1959. Kefauver hopes to wind them up in the first few weeks of the new congressional session. What has taken so long is that Kefauver used most of the first two years for an intermittent but what must have been for the industry an excruciatingly prolonged exposé of what he saw as the industry's failure to properly serve the public interest.

Early last summer Kefauver produced a bill intended to reform the industry, and since then the hearings have been, technically at least, devoted to soliciting the views of interested parties on his "Drug industry antitrust bill." This "legislative" (as opposed to investigative) phase of the hearings began with the testimony of the American Medical Association in July, and reached a critical point last week with the testimony of the Pharmaceutical Manufacturers Association. Sandwiched in between was the testimony in September of Welfare Secretary Abraham Ribicoff, presenting the Administration's views. One of the curious aspects of all this testimony was that the drug industry's position turned out to be closer to the Administration's view than to that of its ally, the AMA.

Kefauver is asking for two quite different types of reform. The first half of his bill is concerned with amendments to the laws governing the Food and Drug Administration. In general approach, although not in detail, these amendments followed the recommendations of HEW, which are about the same now, under Ribicoff, as they were before the change in Administration. They would give FDA a stronger hand against makers of substandard drugs, require proof of efficacy as well as the presently required proof of safety before a new drug is allowed on the market, require the drug companies to provide wider distribution of information about new drugs, particularly regarding unfavorable side affects; and give the government authority over the choice of generic names for drugs.

Conflict

The AMA opposed all of these proposals, except the first, on which it took no position. Ribicoff supported, and the industry accepted, all of them, although not in the precise form Kefauver has suggested.

On generic names, for example, the AMA flatly opposed giving authority to the government, on the grounds that