belle, or even the fine abundance of a generous matriarch, repeated on a cosmic scale: *das Ewig-Weibliche* . . .).

In justice to Michael Bullock, who appears to be the translator in question, I should like to point out that according to the *New English Dictionary, girdle* is a respectable variant form of *griddle* "by metathesis of *r*." It is defined as a "circular plate of iron which is suspended over the fire and upon which cakes are baked or toasted." The first example of this usage is dated about 1400. There is an entry for *girdle* in this sense as a combining form, "as *girdle-cake*. . . ."

According to Nancy Mitford, a recognized authority on *U* diction, "girdle-cake" might be mentioned casually in a conversation in London. Fanny Wincham (whose husband, pastoral theologian at Oxford, has been named ambassador to Paris) and Uncle Matthew are having tea:

"Delicious girdlecake."

"Comes from the Shelter—they've got a Scotch cook there now" [*Don't Tell Alfred* (Harper, New York, 1961)].

An American edition of *Man's Conquest of the Stars* might well explain that the galactic system has the form of a griddle cake, a hot cake, a pancake; but it seems that the English edition is within its rights.

MARY ANN HARRELL
4607 Connecticut Avenue, NW, Washington, D.C.

Electroplax and Nerve Activity

In his article "Chemical factors controlling nerve activity" (*1*), D. Nachmansohn refers to a protein which I have isolated from the electric organ of the electric eel as the "physiological acetylcholine receptor." Although initial results suggested such a role for the protein (*2*), recent studies on its properties led to a change in my interpretation of the nature of the material. This new interpretation was presented at the 1st International Pharmacology Meeting, held in Stockholm in August 1961; since the paper has not yet appeared in print (*3*), I am writing to restate my views.

The following points must be considered in assessing the possible function of the protein.

1) Binding of acetylcholine (ACh) and some depolarizing agents to the protein is very weak as compared to their high activity in physiological processes.

However, other depolarizing agents—for example, noracetylcholine-12, norcholine-12, and pyridinealdoxime dodeciodide—have a high affinity for the protein, yet these compounds in vivo are considerably less active than ACh (*4*). Moreover, the binding of noracetylcholine-12 and norcholine-12 is about equal, though as analogues of ACh and choline, respectively, large differences in affinity would be expected.

2) Schoffeniels and I recently found that binding of *d*-tubocurarine (0.02 mg/ml) to the purified protein is not affected by carbamylcholine (1 mg/ml). Competition between these drugs for the physiological receptor is well documented.

3) Immunohistochemical studies reveal that the protein is localized in or near the conducting and nonconducting membranes of electroplax (*5*). The ACh receptor would be expected to be localized only at the conducting membrane.

4) The amount of purified protein obtained from electric tissue is much greater than would be expected for the ACh receptor substance of the end-plate region.

From these and other considerations it is now concluded that the protein is a membrane component which is distinct from the physiological ACh receptor substance. Nevertheless, the protein may have a role in the electrical activity of conducting membranes. Thus, the effectiveness with which a series of drugs blocked axonal conduction paralleled quite closely their affinity for the protein (*6*). *d*-Tubocurarine, chlorisondamine, and protamine, which bind strongly to the protein, also block activity in squid axons, but only after treatment of the nerve with cobra venom (*6*). Compounds which combine weakly with the protein (for example, ACh, carbamylcholine, decamethonium, neostigmine, and dimethylaminoethyl acetate), in 0.1M concentration, did not affect squid-axon activity, even after treatment of the nerve with cobra venom. Acetylcholine in $10^{-6}M$ concentration acts at the neuromuscular junction. These compounds do block activity in detergent-treated sciatic nerve, but only in 0.1M concentration (*7*). *d*-Tubocurarine is far more active, while choline, which ordinarily has 1/1000 the physiological activity of ACh, equaled it in activity (*7*). Although *d*-tubocurarine blocks at the node of Ranvier (*8*), Dettbarn has found no effect of acetylcholine at this locus (*9*),

where the active membrane is covered by a thin and porous structure.

These findings are consistent with a newer concept (*3*)—that the purified electroplax protein may be identical with, or may closely resemble, the component of conducting membranes with which drugs combine when they produce their effects. It differs from the physiological ACh receptor, particularly with respect to its affinity for ACh, carbamylcholine, decamethonium, neostigmine, and dimethylaminoethyl acetate. On this basis one might explain the weak action of these compounds on nerve conduction, in contradistinction to their high potency at the end-plate.

SEYMOUR EHRENPREIS
Department of Pharmacology, Georgetown University Medical Center, Washington, D.C.

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Ehrenpreis raises the question whether the protein which he isolated from electric tissue is indeed the acetylcholine (ACh) receptor protein. He explains why he now disagrees with the interpretation accepted by him when he worked in my laboratory.

Isolation of proteins has become a commonly used procedure, and in the case of enzymes no problem exists as to identification. In the case of the receptor protein the main difficulty confronting us has indeed been the question whether the protein isolated is identical with the physiological receptor postulated to react with ACh in the elementary process of conduction. This identification is impossible solely on the basis of test-tube studies. It has been achieved essentially in the studies on intact cells—in particular, in the studies on the monocellular electroplax preparation carried out by Henry Higman, Philip Rosenberg, and Eva Bartels and developed during the last 2 years to a high degree of sensitivity for evaluating structure-activity relationships (*1*, *2*). Ehrenpreis was not directly associated with these investigations; his

opinion is, therefore, based on second-hand and, unfortunately, incomplete information.

When my associates and I first offered evidence, in experiments on the electroplax, for the existence of a cell constituent distinctly different from ACh-esterase, we were struck by the observation that the compounds, reacting with the receptor only and not affecting the esterase, blocked electrical activity in two different ways: one type blocked with, the other without, depolarization. The former type had the biological action on the receptor postulated for ACh, the latter had only an inhibitory effect. In analogy with enzyme chemistry we referred to the first type as receptor activators, to the second as receptor inhibitors (*3*). Acetylcholine, carbamylcholine, dacamethonium, and others belong to the first type, procaine, tetracaine, curare, and others to the second type.

1) When the protein in question was isolated by Ehrenpreis, he tested by equilibrium dialysis the binding strength of a series of tertiary, monoquaternary, and diquaternary compounds, known from the electroplax studies to interact with the receptor. The protein studies were still quite preliminary and are being carried out only now on a quantitative basis, by S. Beychok and H. B. Higman.

Nevertheless, it became indeed apparent, as I mentioned in my Herter lecture (*4*), that a marked difference exists between the binding strength in vitro and the potency of action on the living cell when receptor activators and inhibitors are compared. When several series of receptor inhibitors were tested, there appeared a parallelism between their binding strength and their efficiency in blocking electrical activity. A striking example of this parallelism is offered by the local anesthetics procaine, tetracaine, and dibucaine—compounds closely related in structure to ACh, but tertiary nitrogen derivatives and receptor inhibitors (*1*, *5*). It has been, moreover, shown in experiments on the electroplax that tetracaine and ACh compete for the same cell constituent (*2*).

According to the quite preliminary studies in vitro it seems that the binding strength of receptor activators, such as ACh, carbamylcholine, prostigmine, and so forth, to the protein may be weak when compared with their high potency on the cell. However, it must be stressed that the available information is tentative and far from being quantitative. No binding constants have been determined thus far. Binding between micro- and macromolecules is necessary for interaction. For the proposed physiological role of the receptor protein it is necessary to postulate a large rate constant for the combination of receptor and ACh. This, of course, yields no information about the equilibrium (binding) constant. Clearly, if the rate of dissociation is correspondingly great, the equilibrium constant for binding, by definition, will be low. Moreover, we do not know how many of the receptors and how many active sites on each receptor must be activated for a maximum response. In view of all these uncertainties, no statement is at present justified as to the quantitative relationships until extensive further studies will have clarified the situation.

But binding forms only part of biological activity. It is a prerequisite, permitting something additional to happen. We know from enzyme chemistry that binding of competitive inhibitors may be very strong but that the complex is nevertheless inactive. The dis-

sociation constant of the prostigmine–ACh-esterase complex is 10^{-7}, that of acetylcholine–ACh-esterase is 10^{-3}, but the activity in the latter case is extraordinary. The enzyme has a very high turnover number with ACh; it is one of the fastest acting enyzmes known. Moreover, if one compares ethanolamine with the mono- and dimethyl aminoethanol, one finds that each methyl group increases the binding by a factor of 7. Addition of a third methyl group does not contribute to binding at all. But if we compare the enzyme activity toward aminoethyl acetate with that toward the ester containing one, two, and three methyl groups, we find that the trimethyl ester (ACh) has a tenfold higher rate of acetyl enzyme formation than the dimethyl derivative. In the hydrolytic process there is a large increase of ΔS^*, the entropy of activation, in going from the dimethyl to the trimethyl ester, indicating that in the active phase some molecular rearrangement of the enzyme must take place in the activated complex (*6*). Butyrylcholine is more strongly bound to the enzyme than is ACh, but its V_{max} is 1/150 as large.

A difference similar to that found with ACh-esterase has been observed in the reaction of mono-, di-, and trimethylaminoethyl acetate with the receptor of the electroplax (*7*). The dimethyl compound has a potency 10 to 20 times that of the monomethyl analogue. This is a factor not too different from that found in the interaction with the enzyme and may conceivably be attributed to increased binding by the methyl group. But the trimethyl analogue, ACh, has a potency 200 times that of the dimethyl analogue. It would be extremely difficult to attribute such an enormous increase in potency to the contribution to binding of one additional methyl group, especially since there is good reason to believe, on the basis of the esterase studies, that this third methyl group does not contribute at all to binding to the protein. Clearly, factors other than binding help to determine the potency of action.

The long-chain analogues of ACh offer a special problem. Owing to strong van der Waals forces they react quite strongly with a great number of proteins in addition to those of the ACh system, and also with other macromolecules; their mode of action is quite complex and still under investigation. They cannot be readily classified in either of the two categories.

2) It is true that carbamylcholine in a concentration of 1 mg/ml did not show competitive action with curare in a concentration of 0.02 mg/ml; but Ehrenpreis failed to mention that at a higher ratio curare binding was almost completely suppressed by carbamylcholine. The former finding just means, again, that the binding of the activator (carbamylcholine) is very much poorer than that of the inhibitor (curare). The binding of an activator in equilibrium may easily be 1000 times poorer than that of an inhibitor.

3) The immunochemical studies referred to by Ehrenpreis consisted of a few exploratory and quite tentative experiments carried out by a student. Unfortunately, the protein used as the antigen for preparing antibodies was not pure, and the data are, therefore, questionable. The results were full of contradiction, and the experiments were discontinued. In any case, the presence of ACh receptor in the membrane surrounding the whole cell would be consistent with the presence of ACh-esterase. As in several other nonconducting membranes, the system may be

associated with ion flux regulation, even though the membrane has not the prerequisites for impulse conduction.

4) The amount of receptor protein seems to represent approximately 0.1 to 0.2 percent of the total protein; this percentage is far from surprising for an organ so highly specialized in its function. Acetylcholine esterase forms about the same fraction of the total protein. The evaluation of the amount of the receptor in the end-plate region is completely speculative and can easily be off by 1 to 2 orders of magnitude. If it were true that there was not more receptor protein in the electroplax than was expected to be present at the synapse, then this fact would form a serious difficulty for the assumption that the ACh system plays an essential role in the generation of bioelectric potentials.

Surprisingly, however, Ehrenpreis in his new interpretation admits that the protein may have some still-undefined function in the electrical activity of the conducting membrane. He argues that curare, but not ACh, affects axonal conduction either on special preparations [Ranvier node (*8*)] or after chemical pretreatment with detergent or cobra venom (*9*, *10*). Therefore, he concludes, the protein isolated is not the ACh receptor protein, which is present only at the synapse. He thus implicitly accepts the notion of a fundamental difference between synaptic transmission and axonal conduction.

The striking demonstration that curare blocks axonal conduction, and not only synaptic transmission as was believed for a century, should be reason enough for reevaluating the role of ACh in nerve activity, since it is generally accepted that curare acts as an antimetabolite of ACh. Tertiary, lipid-soluble analogues of ACh, such as local anesthetics and diphenylhydramine, act in similar concentrations and in a similar way on the synaptic junction of the electroplax and on the giant axon of the squid (*10*). These new developments support rather than invalidate the explanation offered many years ago—namely, that the failure of ACh to act on axonal conduction must be attributable to the presence of structural barriers surrounding axonal conducting membranes and preventing lipid-insoluble compounds, such as curare and ACh, from reacting with the ACh receptor protein. Cobra venom only reduces, but does not remove, the barriers, as is clear from the experiments reported (*10*). After ex-

posure to the venom of the cottonmouth moccasin, which is much more potent in reducing the barriers than is cobra venom, curare acts in much lower concentrations, and in this case ACh, too, is effective (*11*). At the Ranvier node the conducting membrane is covered not by heavy myelin but by a complex structure seen in the electron microscope; this structure apparently permits curare and tertiary analogues of ACh, the latter in extremely low concentrations, to act on the membrane, although ACh itself does not act. There are amazingly great variations in permeability of different axons with respect to very closely related nitrogen derivatives (*12*). In addition to the direct action of ACh after treatment with moccasin venom, direct effects have been observed on desheathed vagus (*13*) and even on sheathed somatic fibers (*14*). In these preparations the structural barriers seem to be insufficient to protect the membrane. Obviously, ACh must act in all these places on a macromolecule endowed with special properties and associated with axonal electrical activity; otherwise it could not produce its effects. Nevertheless, the physiological significance of these pharmacological actions, considered by themselves, may appear open to question. However, in conjunction with the huge amount of biochemical evidence accumulated in support of the interpretation that ACh has a role in the generation and propagation of electric currents—such evidence as the presence of ACh, ACh-esterase, and choline acetylase in the conducting fiber; the inseparable association of electrical and enzyme activity; and the effect of local anesthetics—the evidence of a direct action of ACh becomes pertinent and indicates the presence of an ACh receptor in the axon. Both the biological and the chemical arguments offered by Ehrenpreis fail to support his new interpretation.

DAVID NACHMANSOHN
College of Physicians and Surgeons, Columbia University, New York

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A Two-Way Affair

Edmund W. Sinnott [*Science* **135**, 278 (26 Jan. 1962)] assumes that such letters as he suggests [to individuals in the U.S.S.R.] would reach the person addressed. I have serious doubts about that. I know from experience that letters from the United States are not exactly welcomed behind the iron curtain. This includes letters to relatives.

The spread of good will must be a two-way effort to have any value. I wonder if Sinnott can show us a letter, similar in content to his, published in a leading journal of science of the U.S.S.R.

WILLIAM EISENMAN
160 West 77 Street, New York

As Eisenman points out, good will is a two-way affair, but this does not mean that we should wait for someone on the other side to make the first overture. There is a wide interchange of friendly correspondence between American and Soviet scientists, and my suggestion simply is that in connection with this, or as an extension of it, there be more formally expressed the desire for sincere good will between our peoples.

EDMUND W. SINNOTT
Yale University,
New Haven, Connecticut

Science Curriculum in Argentina

Garrett Hardin's review entitled "The 'two cultures' within biology" [*Science* **134**, 548 (1961)] has somewhat belatedly come to my attention.

With reference to his query and comment, "What is to be done? Possibly planning within universities can put a brake on the speciation process by requiring physical scientists to take at least one biology course . . . ," I