this "late" message is copied from the original infecting genome or from replicas remains unanswered.

[1] Cohen, S. S., *Federation Proc.*, **29**, 641 (1961).
[2] Kornberg, A., S. Zimmerman, S. R. Kornberg, and J. Josse, these PROCEEDINGS, **45**, 772 (1959).
[3] DeMars, R. I., *Virology*, **1**, 83 (1955).
[4] Wiberg, J. S., M. L. Dirksen, R. H. Epstein, S. E. Luria, and J. M. Buchanan, these PROCEEDINGS, **48**, 293 (1962).
[5] Hall, B. D., A. P. Nygaard, and M. H. Green, *J. Mol. Biol.*, **9**, 143 (1964).
[6] Sekiguchi, M., and S. S. Cohen, *J. Mol. Biol.*, **8**, 638 (1964).
[7] Edlin, G., *J. Mol. Biol.*, **12**, 363 (1965).
[8] Leive, L., *Biochem. Biophys. Res. Commun.*, **18**, 13 (1965).
[9] Korn, D., J. J. Protass, and L. Leive, *Biochem. Biophys. Res. Commun.*, **19**, 473 (1965).
[10] Lowry, O. H., N. J. Rosebrough, A. J. Farr, and R. J. Randall, *J. Biol. Chem.*, **193**, 265 (1951).
[11] Levinthal, C., D. P. Fan, A. Higa, and R. A. Zimmerman, in *Cold Spring Harbor Symposia on Quantitative Biology*, vol. 28 (1963), p. 183.
[12] Hurwitz, J., J. J. Furth, M. Malamy, and M. Alexander, these PROCEEDINGS, **48**, 1222 (1962).

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*SIMILARITY OF EFFECTS OF OXYGEN, SULFUR, AND SELENIUM ISOLOGS ON THE ACETYLCHOLINE RECEPTOR IN EXCITABLE MEMBRANES OF JUNCTIONS AND AXONS**

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Excitable membranes have, during electrical activity, the ability of changing their permeability to ions in a specific, rapid, and reversible way. The assumption of a purely physical nature of these changes, as proposed by Hodgkin,[1] has become untenable in the light of several recent developments. The strong initial heat production coinciding with electrical activity[2, 3] provides evidence for the assumption that chemical reactions control the permeability cycle. Failure to affect electrical parameters significantly by drastic modifications of the ion composition in the internal and external fluid of the axon[4] is hard to reconcile with the views referred to as the "ionic theory" of conduction. On the other hand, a large amount of evidence has accumulated over the last few decades in favor of the assumption that the action of acetylcholine (ACh) is essential for controlling the permeability cycle in all excitable membranes during electrical activity.[5–8] The original hypothesis that ACh is a chemical mediator between two cells was based on results obtained with classical methods of pharmacology. The assumption proved unacceptable in the light of biochemical investigations on cellular, subcellular, and molecular levels, an approach more adequate for the analysis of an event taking place in a membrane of less than 100 A thickness in a few millionths of a second.

Experimental data indicate that ACh apparently induces a conformational change in the ACh-receptor protein with a shift of charge, thereby initiating a chain

of reactions resulting in increased permeability. ACh esterase rapidly inactivates the ester by hydrolysis, reversing the conformational change and restoring the permeability barrier prevailing in resting condition. As required by theory, potent competitive and specific inhibitors of either of the two proteins postulated to be active in the primary event—esterase and receptor—block electrical activity rapidly and reversibly. According to biochemical data and a combination of electron microscopy with histochemical staining techniques, the ACh system is organized within or adjacent to the excitable membrane and tightly bound to it. No alternative explanation has been proposed for the abundant biochemical data on which the role of ACh in the permeability cycle is based, although there have been occasional objections raised on the basis of isolated observations taken out of context and usually arising from misconceptions of basic biochemical notions.

Recently, Mautner and his associates synthesized a number of sulfur and selenium isologs of ACh, choline, and related compounds.[9, 10] The molecular size and shape of these isologs is very similar, so that the ability to fit the active sites of the ACh receptor or ACh esterase should not be affected appreciably. In contrast, electron distribution in the stereoisomers may differ markedly, as indicated by kinetic, spectroscopic, and dipole measurements of isologous esters.[11–13] Thus, it appeared possible that these isologs, although isosteric, may differ in their abilities to bind to receptor sites and in their abilities to induce conformational changes of ACh esterase or of ACh receptor. This assumption is compatible with the observation that the pharmacological effects were modified greatly when the ether oxygen was replaced by sulfur or selenium.[14] It is interesting to note that the above compounds are hydrolyzed by ACh esterase at very similar rates.

The monocellular electroplax preparation of *Electrophorus electricus* developed by Schoffeniels[15, 16] and greatly refined by Higman, Bartels, and Podleski[17–19] is, because of many extraordinary features, particularly suitable for evaluating the interaction between ACh and related compounds with the ACh receptor. The reaction of micromolecules with the receptor is readily measured in this preparation by recording the effects on various electrical parameters. The preparation is highly sensitive to changes of molecular structure, and the results obtained are reproducible to a high degree of accuracy over a wide pH range. Receptor activators depolarize the membrane, apparently by inducing conformational changes, whereas receptor inhibitors react with the active site, but are unable to induce conformational changes; they block the electrical response by competition with ACh, but do not depolarize. Using this preparation, striking differences were found between the potency of oxygen, sulfur, and selenium isologs.[20] As an illustration, the effects of choline and of its sulfur isolog may be mentioned: choline induces only partial depolarization even at a concentration as high as 1×10^{-1} M, while cholinethiol depolarizes fully at a concentration of 5×10^{-5} M, i.e., it is only about ten times weaker than ACh.

The isologs tested on the electroplax act on the excitable membrane only at the level of the junction. More than 90 per cent of the surface of the excitable membrane of this cell is surrounded by structural barriers impervious for most quaternary nitrogen derivatives, such as ACh and curare. Nerve and muscle fibers also have strong permeability barriers. The powerful effects of ACh, curare, and related compounds on the junctions and their failure to act on conducting membranes were

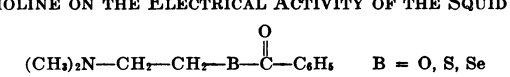
chiefly responsible for the concept of a basic difference between the mechanism of the permeability changes in the excitable membranes of axons and junctions. The evidence that potent, specific, and competitive inhibitors of either the ACh receptor or the esterase block electrical activity in all excitable membranes was not convincing to the supporters of the neurohumoral transmitter theory unless a direct effect of ACh and curare on electrical activity of axons could be demonstrated. Direct effects were recently demonstrated either on special preparations where the structural barrier, although present, was not strong enough to protect the receptor of the axon against the action of externally applied ACh and curare[21, 22] or where the barriers were reduced by chemical pretreatment. For instance, ACh and curare had the postulated effects on electrical activity of the squid giant axon after a brief exposure of the axon to low concentrations of cottonmouth moccasin venom.[23, 24] Examination with electron microscopy by Dr. D. Robertson revealed that this pretreatment had produced a marked disintegration of the Schwann cell covering the excitable membrane. Radioactively labeled ACh and curare were found inside the axon, while they did not penetrate before the venom treatment.

If, as theory postulates, the action of ACh is due to its effect on a specific receptor protein, it must be expected that the active site of this protein should be similar in all excitable membranes, whether in the axon or at the junction. If this were the case, O, S, and Se isologs should exhibit differences of potency in the squid axon similar to those observed at the junctions of the electroplax. This would offer a novel supportive evidence for the similarity of the macromolecule reacting with these isologs and its essentially similar function.

2-Dimethylaminoethylbenzoate, the tertiary analogue of benzoylcholine, and its sulfur and selenium isologs were selected for testing the above hypothesis. Benzoylcholine is an intermediary form between ACh and local anesthetics, such as procaine and tetracaine:[25, 26] it may be, according to experimental conditions, either a depolarizing or blocking agent, and therefore it is relatively difficult to evaluate its mode of action. The tertiary analogue, on the other hand, is purely a blocking agent, i.e., a receptor inhibitor. Tested on the synaptic junctions of the electroplax, the O isolog is the weakest, the S isolog is a stronger, the Se isolog is the strongest inhibitor (unpublished data of G. Webb and H. G. Mautner). When applied to the squid axon, the S isolog, as may be seen from Table 1, is about ten times more potent than the O isolog in blocking electrical activity of untreated squid axons. The

TABLE 1

EFFECTS OF OXYGEN, SULFUR, AND SELENIUM ISOLOGS OF THE TERTIARY ANALOGUE OF BENZOYLCHOLINE ON THE ELECTRICAL ACTIVITY OF THE SQUID GIANT AXON*



B	−M conc.	Decrease of Action Potential (%) Control	Venom-treated
O	2.5×10^{-3}	—	60, 80
	1×10^{-2}	15, 20	100, 100
S	5×10^{-4}	—	40
	1×10^{-3}	20, 30	100, 100
	3×10^{-3}	100, 100	—
Se	3×10^{-4}	10, 20, 30	100, 100
	1×10^{-3}	70, 100, 100	—

* With or without pretreatment for 30 min with 15 μg per ml cottonmouth moccasin venom. Each value represents one experiment. All the effects were reversible except that with Se after venom treatment. The fibers were kept in sea water at a pH of about 7.8 at room temperature.

effects of all three compounds become several times more potent after pretreatment with cottonmouth moccasin venom, but the same difference of potency persists between O and S and Se isologs. The Se isolog is the most potent of the three isologs; in treated and untreated axons, it is about three times as potent as the S isolog. Moreover, following venom pretreatment, the effect of the Se isolog is irreversible. Such irreversibility has been noted previously with succinoylselenocholine, but not with succinoylthiocholine, on junctions (P. Goodyer and H. G. Mautner, unpublished data). In view of the very high tendency of selenolesters compared to thiolesters to acylate certain nucleophiles, it may be hoped that selenolesters related to ACh may prove useful tools for labeling the receptor protein.

These preliminary studies show that the difference of potency of isologs acting on the ACh receptor of the axonal conducting membrane is similar to the differences in their relative ability to affect junctions. Replacement of the ether oxygen of 2-dimethylaminoethylbenzoate by sulfur and selenium progressively increases potency both in the action on the receptor of the axonal membrane and in the action on the junction. These findings are compatible with the postulate that the specific sites of reaction with ACh are similar in both locations.

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[1] Hodgkin, A. L., *Biol. Rev. Cambridge Phil. Soc.* **26**, 338 (1951).

[2] Abbott, B. C. A. V. Hill, and J. V. Howarth, *Proc. Roy. Soc. (London), Ser. B.*, **148**, 149 (1958).

[3] Abbott, B. C., J. V. Howarth, and J. M. Ritchie, *J. Physiol.*, **178**, 368 (1965).

[4] Tasaki, I., I. Singer, and T. Takenaka, *J. Gen. Physiol.*, **48**, 1095 (1965).

[5] Nachmansohn, D., *Chemical and Molecular Basis of Nerve Activity* (New York: Academic Press, 1959), p. 235.

[6] Nachmansohn, D., in *New Perspectives in Biology*, ed. M. Sela (Amsterdam: Elsevier, 1964), p. 176.

[7] Nachmansohn, D., *J. Mt. Sinai Hosp. N. Y.*, **31**, 549 (1964).

[8] Nachmansohn, D. *Ann. N. Y. Acad. Sci.*, in press.

[9] Guenther W. H. H., and H. G. Mautner, *J. Med. Chem.*, **7**, 229 (1964).

[10] *Ibid.*, **8**, 845 (1965).

[11] Guenther, W. H. H., and H. G. Mautner, *J. Am. Chem. Soc.*, **85**, 3458 (1963).

[12] Krackov, M. H., C. M. Lee, and H. G. Mautner, *J. Am. Chem. Soc.*, **87**, 892 (1965).

[13] Chu, S. H., and H. G. Mautner, *J. Org. Chem.*, **31**, 308 (1966).

[14] Scott, K. A., and H. G. Mautner, *Biochem. Pharmacol.*, **13**, 907 (1964).

[15] Schoffeniels, E., *Biochim. Biophys. Acta*, **26**, 585 (1957).

[16] Schoffeniels, E., and D. Nachmansohn, *Biochim. Biophys. Acta*, **26**, 1 (1957).

[17] Higman, H. B., and E. Bartels, *Biochim. Biophys. Acta*, **57**, 77 (1962).

[18] Higman, H. B., T. R. Podleski, and E. Bartels, *Biochim. Biophys. Acta*, **75**, 187 (1963).

[19] *Ibid.*, **79**, 138 (1964).

[20] Mautner, H. G., E. Bartels, and G. D. Webb, *Biochem. Pharmacol.*, in press.

[21] Dettbarn, W-D., *Nature*, **186**, 891 (1960).

[22] Dettbarn, W-D., *Life Sci.*, **12**, 910 (1963).

[23] Rosenberg, P., and T. R. Podleski, *Biochim. Biophys. Acta*, **75**, 104 (1963).

[24] Rosenberg, P., and F. C. G. Hoskin, *J. Gen. Physiol.*, **46**, 1065 (1963).

[25] Bartels, E., *Biochim. Biophys. Acta*, **109**, 194 (1965).

[26] Bartels, E., and D. Nachmansohn, *Biochem. Z.*, **342**, 359 (1965).