nuclear process come from the inhibiting effect of certain atoms and molecules on the reactivity of the recoil halogen products with methane (*17*). The additive substances possess ionization potentials (the energies required to remove electrons from them) nearly equal to or lower than those of the radioactive products formed in the neutron capture, and so exchanges of electrons between them can readily take place. Apparently, the formation of methyl halide may proceed through the reaction of positively charged recoil halogen ions with CH_4 molecules, and this mechanism is prevented by the neutralization of the halogens as a result of transfer of electrons.

A wide variety of phenomena are observed when the nucleus of a constituent atom of a molecule is transformed spontaneously or by nuclear reaction. The characteristics of the molecular disruption depend strongly on the type of nuclear transformation that occurs. In the illustrations described in this article, negative beta-decay generally causes only mild perturbation of the molecule, whereas violent molecular consequences follow the nuclear processes of isomeric transition, orbital electron capture, and radiative neutron capture.

References and Notes

1. A comprehensive review of this field of research is given by S. Wexler, in *Actions Chimiques et Biologiques des Radications*, M. Hassinsky, Ed. (Masson, Paris, 1965), vol. 8, pp. 103–241.
2. For illustrations, see the reviews of R. Wolfgang, *Annu. Rev. Phys. Chem.* **16**, 15 (1965) and I. G. Campbell, *Advance. Inorg. Chem. Radiochem.* **5**, 135 (1963); and the *Proc. Symp. Chem. Eff. Nucl. Transformations Prague* (1961); *ibid. Vienna* (1965).
3. S. Wexler and T. H. Davies, *J. Chem. Phys.* **18**, 376 (1950); *ibid.* **20**, 1688 (1952); S. Yosim and T. H. Davies, *J. Phys. Chem.* **56**, 599 (1952); J. L. Thompson and W. W. Miller, *J. Chem. Phys.* **38**, 2477 (1963).
4. S. Wexler, *Phys. Rev.* **93**, 182 (1954); M. L. Perlman and J. A. Miskel, *ibid.* **91**, 899 (1953).
5. A. H. Snell and F. Pleasonton, *J. Phys. Chem.* **62**, 1377 (1958).
6. T. A. Carlson, *J. Chem. Phys.* **32**, 1234 (1960).
7. A. H. Snell, F. Pleasonton, H. E. Leming, *J. Inorg. Nucl. Chem.* **5**, 112 (1957); S. Wexler, *ibid.* **10**, 8 (1959).
8. F. Cacace, G. Ciranni, R. Guarino, *J. Amer. Chem. Soc.* **88**, 2903 (1966); B. Aliprandi, F. Cacace, A. Guarino, *Proc. Symp. Chem. Eff. Nucl. Transformations Vienna*, 471 (1965).
9. S. Wexler, *J. Chem. Phys.* **36**, 1992 (1962).
10. R. H. Luebbe and J. E. Willard, *ibid.* **29**, 124 (1958); W. H. Hamill and J. A. Young, *ibid.* **20**, 888 (1952); A. A. Gordus and J. E. Willard, *J. Amer. Chem. Soc.* **79**, 4609 (1957); R. S. H. Chiang and J. E. Willard, *ibid.* **74**, 6213 (1952).
11. G. T. Seaborg, G. Friedlander, J. W. Kennedy, *J. Amer. Chem. Soc.* **62**, 1309 (1940); J. W. Kennedy, G. T. Seaborg, E. Segre, *Phys. Rev.* **56**, 1095 (1939).
12. T. A. Carlson and R. M. White, *Proc. Symp. Chem. Eff. Nucl. Transformations Vienna*, 23 (1965); *J. Chem. Phys.* **44**, 4510 (1966).
13. T. A. Carlson and R. M. White, *J. Chem. Phys.* **38**, 2930 (1963).
14. A. A. Gordus and C. Hsiung, *ibid.* **36**, 954 (1962).
15. L. Szilard and T. A. Chalmers, *Nature* **134**, 462 (1934).
16. S. Wexler and T. H. Davies, *J. Chem. Phys.* **20**, 1688 (1952).
17. G. Levey and J. E. Willard, *ibid.* **25**, 904 (1956); E. P. Rack and A. A. Gordus, *ibid.* **34**, 1855 (1961); *ibid.* **36**, 287 (1962); *J. Phys. Chem.* **65**, 944 (1961).

Ultrastructure and Cytochemistry of the Synaptic Region

The macromolecular components involved in nerve transmission are being studied.

Eduardo De Robertis

Some of the most outstanding findings of modern biology have been achieved through use of physical and chemical techniques in the study of macromolecular structure and function. Within this realm, now called molecular biology, form and function, which for centuries were considered separate entities, are inseparable. In the study of the nervous system this stage of analysis has been reached more slowly, and progress has been more difficult because of the extraordinary morphological and physiological complexity of this system. In recent years, with the use of fine microelectrodes for intracellular recording, it has been demonstrated that neurons have specialized loci of activity at which a variety of local and propagated potentials occur. Studies in which pharmacological agents and labeled ions are used have also revealed the existence of chemical receptors and of ionic mechanisms in specialized portions of a single nerve cell (*1*). Until recently, this wide variety of activities could only be correlated with the structures observable with light microscopy. Investigations of this sort showed the complexity of the fine neuronal processes, of the axons and dendrites, and of the intercellular connections, but they could not give any information on the subcellular components involved in such mechanisms. It is only since 1953 that, with the use of the electron microscope, some of the subcellular structures that underlie nerve activity could be demonstrated. This instrument, associated after 1959 with methods of cell fractionation, has led to the recognition of some important features in the submicroscopic and chemical organization of the synapse, defined here as the region at which the excitatory or inhibitory influences from one neuron are transmitted to another neuron or to an effector in an irreciprocal direction.

In 1877, Du Bois Raymond (*2*) first suggested that synaptic transmission could be either chemical or electrical, and both mechanisms have been found to occur in the peripheral and central nervous systems. However, chemical synapses are by far the most common and are the only ones that will be considered here from the point of view of ultrastructure and cytochemical organization. Chemical synapses are also endowed with other important but still little-known functions generally called trophic and plastic properties, which may be the neurological basis of conditioning and learning (*1*).

Hypotheses concerning chemical transmission are based on the assumption that a specific transmitter is synthesized and stored in nerve endings and that it is liberated when the nerve impulse arrives at the terminal. It is also postulated that the transmitter reacts with a chemical receptor situated in the postsynaptic element and that from this reaction a change in ionic permeability takes place, this change

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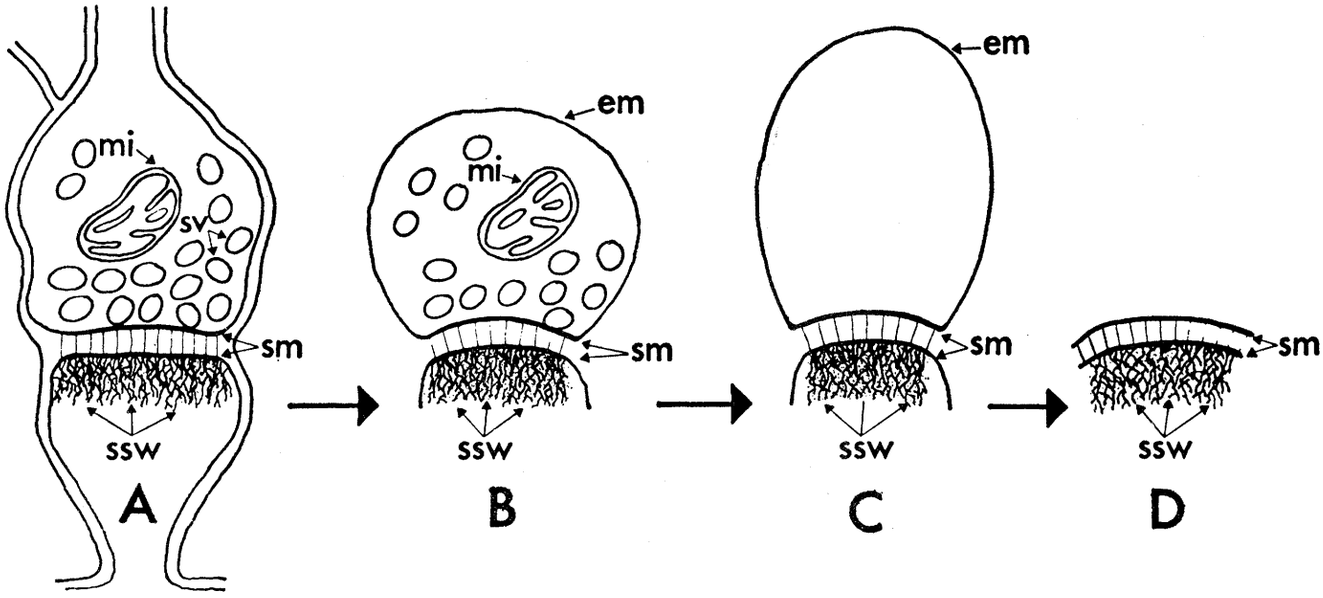

Fig. 1. Diagram of the systematic dissection of a synaptic ending from the cerebral cortex. Synaptic ending as observed (A) *in situ*, (B) after isolation, and (C) after the osmotic shock. (D) The junctional complex left after treatment with a detergent. Ending membrane, *em*; mitochondria, *mi*; synaptic vesicles, *sv*; synaptic membranes, joined by the intersynaptic filaments, *sm*; subsynaptic web, *ssw*.

inducing a bioelectric potential. Such a mechanism is found both in excitatory and inhibitory synapses, and the end result depends on the chemical nature of the transmitter and on the molecular structure of the receptor.

Quantal Nature of Synaptic Transmission and Synaptic Vesicles

In 1952, Fatt and Katz (*3*) made the important discovery that the transmitter is released in multimolecular packets, or quanta, which are remarkably uniform in size. This quantal release was evidenced by minute, transient fluctuations in voltage recorded from the myoneural region; these were called the miniature end-plate potentials. A quantum may correspond to several thousand acetylcholine molecules simultaneously released at a definite locus of the synapse. Katz (*4*) postulated that under the action of the nerve impulse there would be a synchronous discharge of a certain number of quantal units which give rise to the much larger synaptic potential.

At that time the quantal release did not have any morphological correlate. However, Bennett and I found that chemical synapses contained a specific component composed of vesicles about 500 Å wide and remarkably uniform in size; these we designated the synaptic vesicles (*5*). We interpreted these vesicles as the site of storage of the transmitters, and I tried by different experiments to demonstrate their relation to synaptic transmission (*6*). By electrically stimulating a peripheral synapse, I was able to demonstrate a significant change in the number of vesicles which was related to the frequency of the stimulus (*7*). Del Castillo and Katz (*8*) made the correlation between the presence of synaptic vesicles and the quantal release in synaptic transmission.

Our studies and those of others (*9*) demonstrated further complexities in the organization of the synaptic region. Of particular interest for this review are the thickenings of the synaptic membranes; the presence of the intersynaptic filaments, which join the two membranes across the cleft; and a system of filaments, the subsynaptic web, projecting into the postsynaptic region (Fig. 1A) (*10*). Such components constitute what may be called the junctional complex of the synapse (Fig. 1D).

Other studies led to the finding of special granulated vesicles in adrenergic peripheral endings (*11*) and in the anterior hypothalamus. In this region of the brain we also found elliptical and complex vesicles in addition to the round-shaped and granulated ones (*12*). The similarity, with respect to ultrastructure, between the synaptic vesicles and the neurosecretory vesicles (or granules) found in the hypothalamic neurohypophyseal system and in other neurosecretory structures led me to postulate that there is a basic correspondence between the two structures and that within the nerve endings there occurs a localized process of neurosecretion by which the transmitters are synthesized and released (*13*). I also postulated that the synaptic vesicles were formed at the endings by fragmentation of the neurotubules observed in isolated axoplasm from myelinated nerve fibers (*14*). With Pellegrino de Iraldi we have recently observed that neurotubules are rapidly transformed into vesicles at the end of a regenerating axon.

Isolation of Various Types of Synaptic Endings

The early work on cell fractionation, initiated in Cambridge, England (*15*), by 1960 resulted in the isolation of the nerve endings from brain. This advance permitted us to introduce methods of quantitative cytochemistry and to correlate the neurochemical results with the submicroscopic organization of the synaptic region. Independently, Gray and Whittaker (*16*) and our group (*17*) were able to demonstrate that the mitochondrial fraction of brain contained isolated nerve endings, which could be separated by gradient centrifugation. These investigations provided the foundation for the neurochemical and neuropharmacological studies that since then have been actively carried

out in both of these laboratories and in others (*18*).

Figure 2 shows three methods of cell fractionation that have been developed to produce: (i) the separation of different types of nerve endings (Figs. 1B and 3A), (ii) the isolation of synaptic vesicles (Fig. 3B), and (iii) the separation of different types of nerve-ending membranes (Fig. 1C). A fourth and more recent method for the separation of the junctional complex (Fig. 1D) will be mentioned later.

Table 1 shows a summary of the distribution, in the various submitochondrial fractions of brain, of the biogenic amines acetylcholine (*19*), 5-hydroxytryptamine (*20*), noradrenaline, dopamine (*21*), and histamine (*22*). These findings, together with the results on the enzymes cholineacetylase, cholinesterase (*19*), 5-hydroxytryptophane decarboxylase (*23*), and catechol-*O*-methyltransferase (*24*) demonstrate that the nerve endings separated in two subfractions are essentially aminergic (that is, they contain biogenic amines), while another large subfraction (Fig. 2) of nerve endings is essentially nonaminergic and lacks these active biogenic amines. Recently a fraction of very small nerve endings rich in histamine has been isolated from the microsomal fraction of the cerebral cortex (*22*).

Isolation of Inhibitory Nerve Endings

The literature on γ-aminobutyric acid and inhibition in the nervous system is too vast to be reviewed here (*25*). I shall only say that there is an increasing amount of evidence indicating that this amino acid plays an essential role in inhibitory synapses of brain.

The two main enzymes of the γ-aminobutyric acid system, glutamic acid decarboxylase and γ-aminobutyric acid aminotransferase, have different localizations in the submitochondrial fractions (*26*). Glutamic acid decarboxylase, the enzyme that irreversibly catalyzes the formation of γ-aminobutyric acid from L-glutamic acid, was found concentrated in the fraction containing nonaminergic endings. On the contrary, γ-aminobutyric acid aminotransferase, the enzyme that catalyzes the reversible transamination of γ-aminobutyric acid with α-ketoglutarate to succinic semialdehyde, is preferentially localized in neuronal mitochondria and has a distribution similar to that of succinic dehydrogenase (Table 1). Because of the strict correlation existing between glutamic acid decarboxylase and γ-aminobutyric acid (*27*), it was postulated that the nonaminergic nerve endings rich in glutamic acid decarboxylase also contained γ-aminobutyric acid. Evidences were given that the enzyme could be attached to the outer surface of the synaptic vesicles by Ca^{2+}, and it was postulated that γ-aminobutyric acid could be collected into the synaptic vesicles, thus participating at the synapse as a true transmitter (*26*) (Fig. 4).

More recently it was found that the convulsant drug methionine sulfoximine, which acts on several enzymes related to the systems of glutamate, glutamine, and γ-aminobutyric acid, binds to nerve endings and produces structural alterations in the nonaminergic type of nerve endings (*28*). Among these nerve endings there is also a larger proportion having elongated or elliptical synaptic vesicles (*29*), which were related by Uchizono (*30*) to the inhibitory synapses. Recently, I have suggested that the nonaminergic nerve endings contain the bulk of the inhibitory synapses of the cerebral cortex (*29*).

Isolation of Synaptic Vesicles

Isolation of synaptic vesicles is the most direct approach to the elucidation of the postulate that they were the site of storage of transmitter substances (*5*). The method for their isolation (*31*) starts with the treatment of the mitochondrial fraction with a hypotonic so-

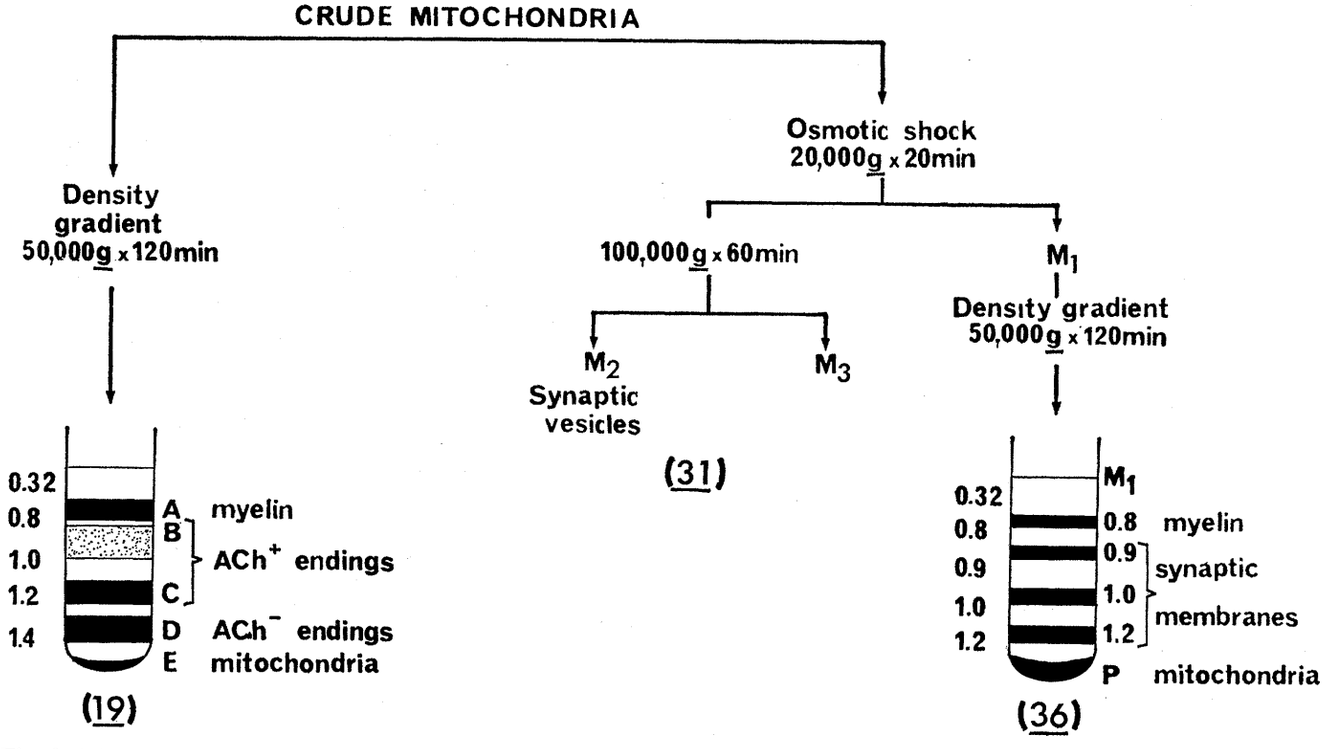

Fig. 2. Diagram showing three methods of cell fractionation developed in our laboratory for the isolation of nerve endings, synaptic vesicles, and nerve-ending membranes; all start with the crude mitochondrial fraction.

lution (Fig. 2). This causes the swelling of the isolated nerve endings (Fig. 3A), the rupture of the limiting membrane, and the release of the enclosed vesicles, mitochondria, and axoplasm. These can then be separated by differential centrifugation. The synaptic vesicles are preferentially found in the M_2 subfraction (Fig. 3B), while the membranous portion of the endings goes to the much bulkier fraction M_1.

Table 2 shows that synaptic vesicles contain the highest concentration of bound acetylcholine (*31*), noradrenaline, dopamine (*21*), and histamine (*22*). 5-Hydroxytryptamine has also been found to be concentrated in the vesicular fraction (*32*). In the rat, cholineacetylase is tightly bound to the synaptic vesicles (*31*); however, in other species, particularly in the pigeon, the enzyme is more soluble in the axoplasm (*33*). Vesicles isolated from the anterior hypothalamus contain a concentration of noradrenaline five to six times greater than that of a similar fraction from total hemispheres. Numerous granulated vesicles are observed in these preparations, and from this and other data one could conclude that the granulated vesicles contain the adrenergic transmitter (*34*). Such studies, confirmed by others (*35*), provide support for the postulate (*5*) that the synaptic vesicles are truly the quantal units for the storage of transmitter substances and that they probably function in chemical transmission.

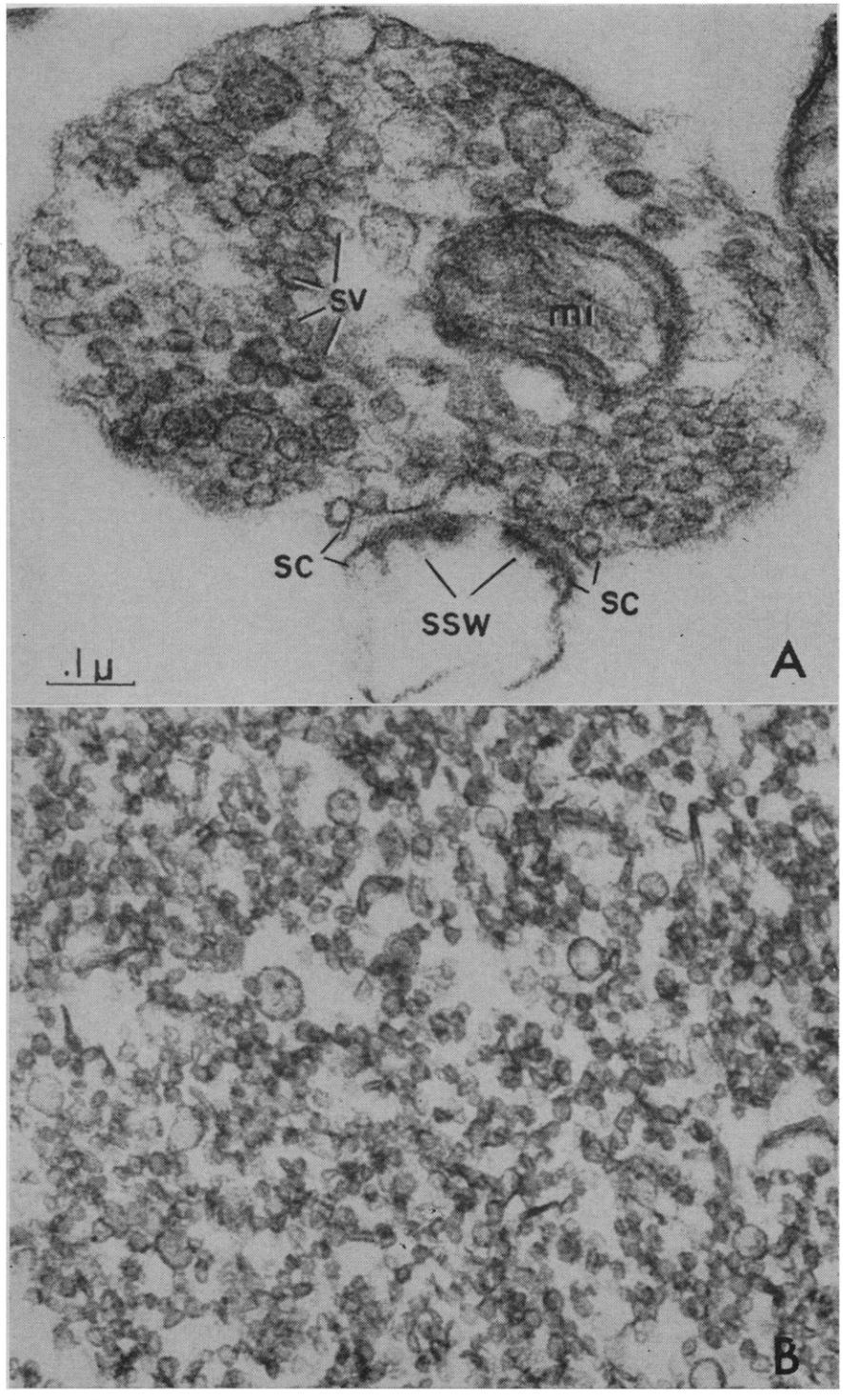

Fig. 3. Electron micrographs of (A) isolated nerve ending from the rat brain (*31*) [mitochondria, *mi*; synaptic cleft, *sc*; synaptic vesicles, *sv*; subsynaptic web, *ssw* (× 85,000)] and (B) isolated synaptic vesicles contained in subfraction M_2 from the cortex of rat brain (× 70,000).

Isolation of Different Types of Synaptic Membranes

The osmotic disruption of the nerve endings (*31*), used to separate the synaptic vesicles, may also be employed to isolate the limiting membrane of the nerve endings. These structures are continuous with the synaptic membranes forming, with the attached structures, the junctional complex (Fig. 1C). Because most of these membranes go to the M_1 fraction, for their separation gradient centrifugation is indispensable (*36*) (Fig. 2). The pellet M_1p contains about 50 percent of the protein of M_1 and most of the mitochondria of the homogenate, while the nerve-ending membranes are found in layers $M_1 0.9$, $M_1 1.0$, and $M_1 1.2$ (Fig. 1C).

These various membranous populations, which are separated by their different specific gravities (dependent on the ratio of lipid to protein), may also be characterized by: (i) a group of membrane-bound enzymes (*36*), (ii) their capacities to bind to radioactive blocking agents (*37*), and (iii) some differences in chemical composition, particularly in the content of gangliosides (*38*).

Table 3 shows that acetylcholinesterase is concentrated in the membranes of $M_1 0.9$ and $M_1 1.0$, while those of $M_1 1.2$ have small quantities of this enzyme and probably belong to the noncholinergic nerve endings. The localization of Na^+- and K^+-activated adenosine triphosphatase in these M_1 subfractions is of particular interest in view of the activity of this enzyme in transphosphorylations and cation trans-

port (*39*). Several investigations suggest that the final step in the action of adenosine triphosphatase could involve a K^+-activated phosphatase. Albers *et al.* (*40*) found that both enzymes are closely associated with the membranous components of the nerve endings, and Hosie (*41*) made similar observations for adenosine triphosphatase. Table 3 shows that, while Na^+- and K^+-activated adenosine triphosphatase tends to be more closely associated with the membranes rich in acetylcholinesterase, the *p*-nitrophenyl phosphatase, activated by K^+, is more widespread in the M_1 1.2 fraction, which has little cholinesterase. Glutamine synthetase is mainly concentrated in microsomal membranes (*42, 26*); however, there is a definite portion of this enzyme that is associated with the nerve-ending membrane.

Of great interest is the distribution of adenyl cyclase, an enzyme which reaches its highest concentration in the cerebral cortex and which, by catalyzing the production of cyclic adenosine monophosphate, is involved in the regulation of various cell activities (*43*). Another enzyme, cyclic phosphodiesterase may function as a modulator of adenyl cyclase, acting on the intracellular concentration of cyclic adenosine monophosphate. Adenyl cyclase is bound to microsomal membranes but is most highly concentrated in the isolated nerve-ending membranes (*44*). Cyclic phosphodiesterase, while partly free, is also present bound to the same nerve-ending membranes (Table 3). The localization of these two enzymes favors the view that cyclic adenosine monophosphate may be important in the regulation of synaptic endings, but the exact nature of this participation is still unknown.

Receptor Properties of Isolated Nerve-Ending Membranes

A basic postulate of the chemical theory of synaptic transmission is that the subsynaptic membrane should contain the chemical receptors for the various transmitters. Because the junctional complex, with the subsynaptic membrane and other structures, remains attached to the nerve membrane (Fig. 1C), it was expected that these membranes should also contain the chemical receptors. This property has so far been investigated with the use of radioactive cholinergic blocking agents (*37*), but some adrenergic blocking drugs are now being used.

Table 1. Distribution of the biogenic amines and enzymes of the γ-aminobutyric acid system. (A–E) Submitochondrial fractions isolated by gradient centrifugation (Fig. 2). Results are expressed as the relative specific concentrations which are the percentages of amine or enzyme recovered divided by the percentage of protein recovered.

Structure	Submitochondrial fraction				
	Myelin (A)	Small nerve endings (B)	Nerve endings (C)	Nerve endings (D)	Mitochondria (E)
		Biogenic amines			
Acetylcholine (*19*)	0.15	2.24	2.99	0.94	0.58
5-Hydroxytryptamine (*20*)	0.61	0.78	2.17	0.76	0.48
Noradrenaline (*21*)	0.32	2.05	1.66	0.77	0.72
Dopamine (*21*)	0.79	1.85	1.13	0.91	0.71
Histamine (*22*)	0.72	2.70	1.56	0.44	0.70
		Enzymes			
Glutamic acid decarboxylase (*26*)	0.02	0.49	1.22	2.00	0.40
γ-Aminobutyric acid aminotransferase (*26*)	0.15	0.11	0.29	1.10	8.00
Succinic dehydrogenase (*26*)			0.52	2.10	7.60

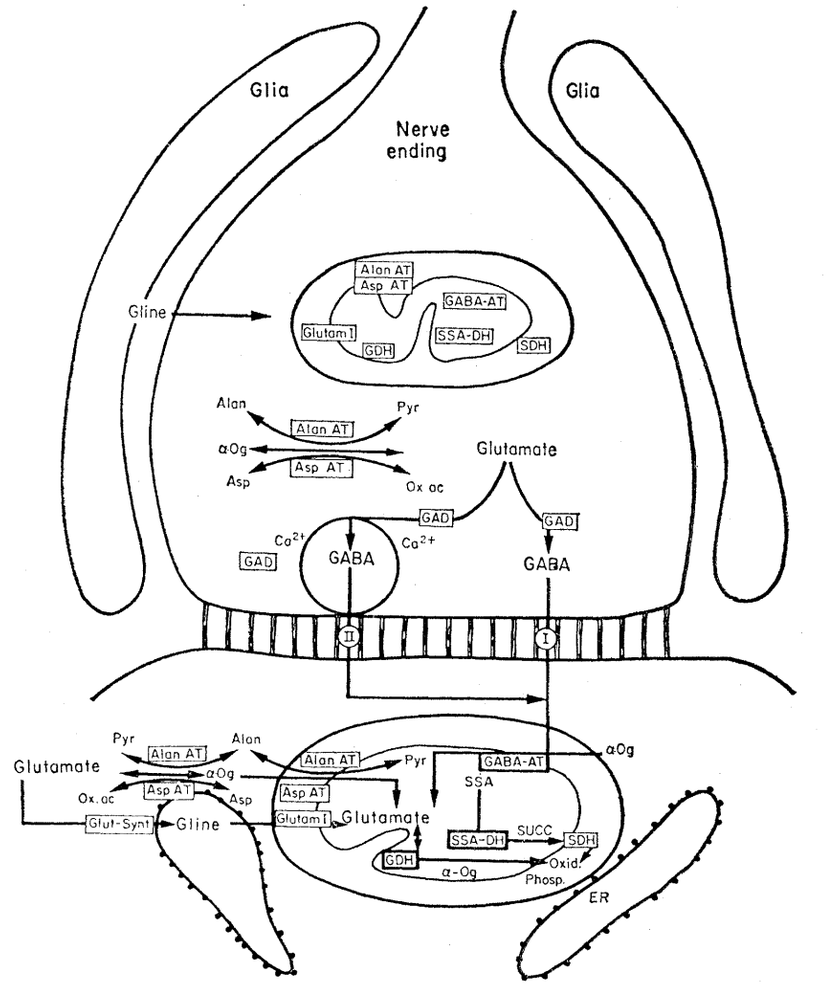

Fig. 4. General diagram of the structural and biochemical organization of a synaptic ending of inhibitory nature. The localization of the enzymes of the glutamate, glutamine, and γ-aminobutyric acid (*GABA*) cycles in the different compartments and subcompartments is indicated. Two possible presynaptic mechanisms for the action of γ-aminobutyric acid are indicated by I and II. In II, γ-aminobutyric acid acts as a transmitter. [For a more complete description, see (*26*).] *Alan*, alanine; *Alan AT*, alanine aminotransferase; *Asp*, aspartate; *Asp AT*, aspartate aminotransferase; *α-Og*, α-oxoglutarate; *ER*, endoplasmic reticulum; *GABA-AT*, γ-aminobutyric acid transferase; *GAD*, glutamate decarboxylase; *GDH*, glutamate dehydrogenase; *Gline*, glutamine; *Glut-Synt*, glutamine synthetase; *Glutam I*, glutaminase I; *Ox. ac*, oxaloacetate; *Oxid. Phosp.*, oxidative phosphorylation; *Pyr*, pyruvate; *SDH*, succinate dehydrogenase; *SSA*, succinate semialdehyde; *SSA-DH*, succinate semialdehyde dehydrogenase; *SUCC*, succinate.

Table 2. Content of biogenic amines in the bulk fraction (M_1), in synaptic vesicles (M_2), and in the soluble fraction (M_3). The crude mitochondrial fraction of the brain was shocked osmotically and then centrifuged as indicated in Fig. 2. The results are expressed in relative specific concentrations as defined in Table 1.

Biogenic amines	Fraction		
	M_1	M_2	M_3
Acetylcholine (*31*)	0.55	2.85	1.20
Noradrenaline (*21*)	0.40	2.56	1.93
Dopamine (*21*)	0.46	2.46	1.72
Histamine (*22*)	0.39	2.24	2.27

Table 4 shows the remarkable correlation that exists between the presence of acetylcholinesterase and the capacity of the membrane to bind to three cholinergic blocking agents. This result indicates that both the enzyme and the cholinergic receptor are associated in the same nerve-ending membrane. The $M_1$1.2 membranes, which have little acetylcholinesterase, also have less binding capacity for the cholinergic blocking agents. In high concentrations atropine, eserine, and the natural transmitter acetylcholine can interfere with the binding of dimethyl-C^{14}-*d*-tubocurarine and with that of methyl-C^{14}-hexamethonium (*45*).

There is also a remarkable correlation between the presence of acetylcholinesterase and the content of gangliosides in the membranes (*38*). Since gangliosides have been frequently implicated in the receptor properties of membranes (*46*), particularly in relation to serotonin (*47*), this distribution may be of special interest in relation to the problem of the receptor. In a recent work (*38*), it is demonstrated not only that the membranes with much acetylcholinesterase have the highest concentration of gangliosides but that the purified synaptic vesicles are practically devoid of these acid glycolipids. This finding does not support the suggestion (*48*) that gangliosides could be involved in the binding of acetylcholine within the synaptic vesicles.

Isolation of the Junctional Complex and the Chemical Receptor

A further step in the dissection of the nerve ending has recently been achieved after a systematic study of the action of the nonionic detergent Triton X-100 on various subcellular fractions from brain (*49*). While the detergent had little or no action on cholineacetylase or on the mitochondrial enzyme monoaminoxidase (*50*), it caused considerable solubilization of acetylcholinesterase and of the other membrane-bound enzymes; in addition, there was loss of protein, especially from the membranes of nerve endings. These and other biochemical findings and the results of corresponding electron microscopic study revealed that, while myelin, mitochondria, and synaptic vesicles were less affected by the detergent, there was a preferential effect on the limiting membrane of the nerve ending (Fig. 1, C and D). Even more interesting was the fact that the junctional complexes, comprising both synaptic membranes (joined by the intersynaptic filaments) and the subsynaptic web, were resistent to the treatment. If a fraction of isolated nerve-ending membranes (such as $M_1$1.0) was treated with Triton, the sediment obtained contained a mass of isolated junctional complexes (*45*).

Although not unexpected, it was interesting to find that the binding capacity of the junctional complexes for the cholinergic blocking agents remained practically unaffected by the treatment with Triton. The ratio of the percentage of uptake to the amount of acetylcholinesterase is in all cases above 1.0, and it may reach as much as 4.2, an indication that there is a considerable concentration of the receptor with respect to the enzyme contained in the original nerve-ending membrane. One such experiment is illustrated in Fig. 5 in which, for the $M_1$1.0 fraction, the value of this ratio is 3.0.

These findings support the interpretation that the receptor properties are localized in the junctional complexes, probably at the subsynaptic membrane, while the membrane-bound enzymes have a wider distribution that includes the limiting membrane of the ending. Furthermore, the binding sites for the cholinergic blocking agents and for acetylcholinesterase are in two separate macromolecular entities.

Antiserums against Nerve Endings and Other Synaptic Structures

Inmunochemical studies may give indirect information on the cytochemical organization of the synaptic region (*51*). A purified fraction of nerve endings (Table 1, fraction C) from rabbit (homologous system) or from cat cerebral cortex (heterologous system) was

Table 3. Distribution of various enzymes in subfractions of M_1 on a density gradient (see Fig. 2). The content of each fraction, as observed under the electron microscope, is indicated. Enzymes: acetylcholinesterase (AChE), Na^+- and K^+-activated adenosine triphosphatase (Na^+- K^+ ATPase), K^+-activated *p*-nitrophenyl phosphatase, glutamine synthetase (GS), adenyl cyclase, cyclic phosphodiesterase, and monoaminoxidase (MAO). The results are expressed as relative specific activities, which are the percentages of activity recovered divided by the percentages of protein recovered. Data from (*36*) and (*44*).

Subfraction	Structure	Enzymes						
		AChE	Na^+-K^+ ATPase	K^+*p*-Nitrophenyl phosphatase	GS	Adenyl cyclase	Phosphodiesterase	MAO
$M_1$0.8	Myelin	1.68	1.37	0.56	0.66	1.06	1.32	0.44
$M_1$0.9	Synaptic membranes	3.22	2.28	2.41	0.96	2.04	1.77	0.33
$M_1$0.9	Cynaptic membranes	2.13	3.16	1.39	2.04	2.46	2.68	0.23
$M_1$1.2	Synaptic membranes	0.98	1.40	2.53	1.74	1.95	1.03	0.65
M_1p	Mitochondria	0.15	0.17	0.30	0.73	0.31	0.43	1.56

Table 4. Distribution of acetylcholinesterase (AChE), ganglioside content measured as neuraminic acid (NANA); and uptake of dimethyl-C^{14}-*d*-tubocurarine (C^{14}DMTC), methyl-C^{14}-hexamethonium (C^{14}MHM), and H^3-alloferin in subfractions of M_1 on a density gradient (see Fig. 2). Acetylcholinesterase is expressed as the relative specific activity, NANA as the relative specific content, and uptake of radioactive blocking agents as the specific binding ratio (counts per milligram of protein in fraction divided by counts per milligram of protein in the total particulate fraction).

Subfraction	Structure	AChE	NANA	C^{14}DMTC	C^{14}MHM	H^3-Alloferin
$M_1$0.8	Myelin	1.64	1.17	2.14	2.92	3.86
$M_1$0.9	Synaptic membranes	3.40	3.98	4.16	4.44	4.04
$M_1$1.0	Synaptic membranes	3.45	2.92	6.88	4.76	4.37
$M_1$1.2	Synaptic membranes	1.44	1.54	3.00	2.52	2.89
M_1p	Mitochondria	0.38	0.25	1.60	0.72	1.87

resuspended in Freund's adjuvant and injected into rabbits. These antiserums were studied by complement fixation against various subcellular fractions, and also in their effect on a system of isolated nerve endings in vitro, which was then studied under the electron microscope. Also investigated was the effect of topically applied antiserum on the visual cortex of the cat. In the presence of complement, both antiserums had a lytic action on the isolated nerve endings. A focal activity in the form of high-voltage epileptiform discharges was obtained electrophysiologically. A more potent antiserum has been recently produced against a purified fraction $M_1 1.0$ of nerve-ending membranes from cat brain cortex. This antiserum, previously absorbed with brain mitochondria and myelin and in the presence of complement, produced disruption of the nerve endings with liberation of synaptic vesicles; there was practically no effect on mitochondria and myelin.

This type of analysis is of considerable interest if performed on proteins isolated from brain or on other chemically defined constituents. Moore (*52*) has recently separated and purified an extremely acidic protein (the so-called S-100) that is unique to the nervous tissue. An antiserum against S-100 has been produced by Levine (*53*), who has generously made it available to us. The S-100 protein is more concentrated in white than in gray matter and, according to Hyden and McEwen (*54*), is localized in glia and in neuronal nuclei. Experiments performed so far in our laboratory demonstrate that the antiserum to S-100 produces disruption of nerve endings in vitro and, when applied in vivo to mollusc neurons recorded intracellularly, causes destruction of the electrophysiological properties of the neuron, which is accompanied by cytolysis.

Present and Future

The work on isolated synaptic components of the brain is rapidly expanding into several lines of research. One of them involves the determination of the molecular species in the various membranous structures that integrate the synaptic region. Recent studies refer particularly to the structural lipids and proteolipids and to some of the enzymes involved in their metabolism (*55*). Various isolated nerve-ending membranes have a molar proportion

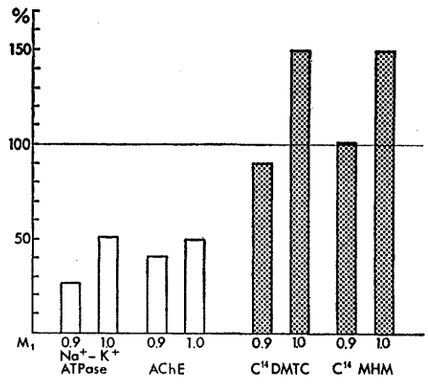

Fig. 5. Diagram showing the percentage of Na^+- and K^+-activated adenosine triphosphatase (Na^+-K^+ ATPase) and acetylcholinesterase (AChE) and of the uptake of dimethyl-C^{14}-*d*-tubocurarine (C^{14}DMTC) and methyl-C^{14}-hexamethonium (C^{14}MHM) remaining after treatment of the M_1 fraction with Triton X-100. In each case the untreated control is 100 percent. From (*45*).

of phospholipids, cholesterol, galactolipids, and amino acids of proteolipid which is similar for all of them, but which differs very significantly from that of the other lipoprotein membranes [that is, those constituting the synaptic vesicles, myelin, mitochondria, or microsomes (*56*)]. These studies demonstrate that there is a plurality of membranous structures in brain, each one having a biochemical specialization.

Future investigations will certainly go into more dynamic approaches and lines of increasing functional interest. Observations have been made on the rate of synthesis of proteins and some enzymes present in the nerve ending (*57*). These studies are related to the now classical theory of axonic flow proposed by Weiss (*58*), and they open the possibility of studying the rate of flow and turnover time of molecular components present at the synapse. The synaptic ending has an active glycolytic metabolism (*59*), and the mitochondria contained in them can synthesize protein (*57*).

By incubating isolated nerve endings, in different osmotic concentrations (*26*), one may be able to learn about the position of the active group of an enzyme or a chemical receptor or about the permeability of a substrate to a certain subcellular compartment. For example, it can be demonstrated that acetylcholinesterase, Na^+- and K^+-activated adenosine triphosphatase, and the cholinergic receptor are located on the outer surface of the synaptic ending; while adenyl cyclase has the active group in the inner surface of the nerve-ending membrane, this site becoming available to the substrate only after the osmotic shock (*44*). Of special interest is the demonstration of some biochemical differences between the mitochondria present within nerve endings and those in neuronal or glial perikarya. These differences are related to the concentrations of enzymes active in the metabolism of important amino acids, to the regulatory action exerted by substrates on some of these enzymes, and to the differential sensitivity of nicotinamide-adenine dinucleotide dehydrogenases (*26*).

Several of these concepts are summarized in the diagram of Fig. 4, in which I have tried to give an integrated view of the structural and biochemical organization of an inhibitory synaptic complex as a self-regulatory unit in brain. In addition to consideration of the morphological compartments and subcompartments indicated, account should be taken of the existence of pools of free amino acids, of the relative concentrations and absolute speed of reaction of the various enzymes, the permeability of the compartmental membranes, and of the localization of the systems furnishing the substrates within the living cell. Further complexities are introduced by the relationship between the various enzymes and the glycolitic and citric acid cycles, by the existence of regulatory mechanisms controlling enzyme activity, by the competition for substrates, or by the specific inhibition of isoenzymes in the various compartments (*60*).

It is hoped that in the future these types of studies will fulfill the aim of cytochemistry as defined by Bensley: "that of outlining within the exiguous confines of the cell the elusive and mysterious chemical pattern which is the basis of life" (*61*).

References and Notes

1. J. C. Eccles, *The Physiology of Synapses* (Springer-Verlag, Berlin, 1964).
2. E. Du Bois Reymond, *Ges. Abhandl. Allg. Muskel Nervenphysok* **2**, 700 (1877).
3. P. Fatt and B. Katz, *J. Physiol.* **117**, 109 (1952).
4. B. Katz, *Bull. Johns Hopkins Hosp.* **102**, 275 (1958); *Rev. Mod. Phys.* **3**, 524 (1959).
5. E. De Robertis and H. S. Bennett, *Fed. Proc.* **13**, 35 (1954); *J. Biophys. Biochem. Cytol.* **1**, 47 (1955).
6. E. De Robertis, *ibid.* **2**, 503 (1956).
7. ——— and A. Vaz Ferreira, *ibid.* **3**, 611 (1957).
8. J. Del Castillo and B. Katz, *Progr. Biophys. Biochem. Chem.* **6**, 121 (1956).
9. S. L. Palay, *Exp. Cell Res.* **5**, 275 (1958); E. G. Gray, *J. Anat.* **93**, 420 (1959).
10. E. De Robertis, A. Pellegrino de Iraldi, G. Rodríguez de Lores Arnaiz, L. Salganicoff, *Anat. Rec.* **139**, 220 (1961).
11. E. De Robertis and A. Pellegrino de Iraldi, *ibid.*, p. 298; *J. Biophys. Biochem. Cytol.* **10**, 361 (1961); K. C. Richardson, *J. Anat.* **96**,

427 (1962); M. A. Grillo and S. L. Palay, *Proc. Int. Cong. Electron Microscopy 5th* **2**, u-1 (1962).
12. A. Pellegrino de Iraldi, H. Farini Duggan, E. De Robertis, *Anat. Rec.* **145**, 521 (1963).
13. E. De Robertis, *Histophysiology of Synapses and Neurosecretion* (Pergamon Press, New York, 1964).
14. ——— and C. M. Franchi, *J. Exp. Med.* **98**, 269 (1953).
15. C. O. Hebb and V. P. Whittaker, *J. Physiol.* **142**, 187 (1958); V. P. Whittaker, *Biochem. J.* **72**, 694 (1959).
16. E. G. Gray and V. P. Whittaker, *J. Physiol.* **153**, 2 (1960).
17. E. De Robertis *et al.*, *Sesiones Científicas de Biología, Mendoza, Argentina, 1960*, p. 24; *J. Biophys. Biochem. Cytol.* **9**, 229 (1961).
18. A. Inouye, K. Kataoka, J. Shigawa, *Nature* **194**, 286 (1962); H. Weinsten, E. Roberts, T. Kakefuda, *Biochem. Pharmacol.* **12**, 503 (1963); R. W. Ryall, *J. Neurochem.* **11**, 131 (1964); M. Kurakawa, T. Sakamoto, M. Kato, *Biochem. Biophys. Acta* **94**, 307 (1965).
19. E. De Robertis, A. Pellegrino de Iraldi, G. Rodríguez de Lores Arnaiz, L. Salganicoff, *J. Neurochem.* **9**, 23 (1962); E. De Robertis, G. Rodríguez de Lores Arnaiz, L. Salganicoff, A. Pellegrino de Iraldi, L. M. Zieher, *ibid.* **10** (1963).
20. L. M. Zieher and E. De Robertis, *Biochem. Pharmacol.* **12**, 596 (1963).
21. ———, *Congr. Asoc. Latinoamer. Ciencias Fisiológicas 6th Viña del Mar, Chile* (Editorial Universitaria, S.A., Santiago de Chile. 1964), p. 150.
22. A. Kataoka and E. De Robertis, *J. Pharmacol. Exp. Therap.*, in press.
23. G. Rodríguez de Lores Arnaiz and E. De Robertis, *J. Neurochem.* **11**, 213 (1964).
24. M. Alberici, G. Rodríguez de Lores Arnaiz, E. De Robertis, *Life Sci.* **4**, 1951 (1964).
25. E. Roberts, Ed., *Inhibition in the Nervous System and Gamma-Aminobutyric Acid* (Pergamon Press, New York, 1960); E. Florey, Ed., *Nervous Inhibition* (Pergamon Press, New York, 1961); in *Symposium on Structure and Function of Neuronal Inhibitory Mechanisms, Inst. Meet. Neurobiol. 4th Stockholm* (1966).
26. L. Salganicoff and E. De Robertis, *Life Sci.* **2**, 85 (1963); *J. Neurochem.* **12**, 287 (1965).
27. B. Sisken, E. Roberts, C. F. Baxter, in *Inhibition in the Nervous System and Gamma-Aminobutyric Acid*, E. Roberts, Ed. (Pergamon Press, New York, 1960), p. 219.
28. E. De Robertis, G. Rodríguez de Lores Arnaiz, O. Z. Sellinger, *Nature* **212**, 537 (1966); E. De Robertis, O. Z. Sellinger, G. Rodríguez de Lores Arnaiz, M. Alberici, L. M. Zieher, *J. Neurochem.* **14**, 81 (1967).
29. E. De Robertis, *Int. Meet. Neurobiol. 4th Stockholm*, in press.
30. K. Uchizono, *Nature* **207**, 642 (1965).
31. E. De Robertis, G. Rodríguez de Lores Arnaiz, A. Pellegrino de Iraldi, *Nature* **194**, 794 (1962); E. De Robertis, G. Rodríguez de Lores Arnaiz, L. Salganicoff, A. Pellegrino de Iraldi, L. M. Zieher, *J. Neurochem.* **10**, 225 (1963).
32. E. W. Maynert, R. Levi, A. J. De Lorenzo, *J. Pharmacol. Exp. Therap.* **144**, 385 (1964).
33. R. E. McCaman, G. Rodríguez de Lores Arnaiz, E. De Robertis, *J. Neurochem.* **12**, 927 (1965).
34. E. De Robertis, A. Pellegrino de Iraldi, G. Rodríguez de Lores Arnaiz, L. M. Zieher, *Life Sci.* **4**, 193 (1965); E. De Robertis, *Pharmacol. Rev.* **18**, 413 (1966).
35. V. P. Whittaker, I. A. Michaelson, R. J. Kirkland, *Biochem. J.* **90**, 293 (1964); V. P. Whittaker and M. N. Sheridan, *J. Neurochem.* **12**, 363 (1965).
36. E. De Robertis, M. Alberici, G. Rodríguez de Lores Arnaiz, J. M. Azcurra, *Life Sci.* **5**, 577 (1966).
37. J. M. Azcurra and E. De Robertis, *Int. J. Neuropharmacol.* **6**, 15 (1967).
38. E. G. Lapetina, E. F. Soto, E. De Robertis, *Biochim. Biophys. Acta* **135**, 33 (1967).
39. J. C. Skou, *ibid.* **42**, 6 (1960); R. W. Albers, S. Fahn, G. S. Kowal, *Proc. Nat. Acad. Sci. U.S.* **50**, 474 (1963).
40. R. W. Albers, G. Rodríguez de Lores Arnaiz, E. De Robertis, *Proc. Nat. Acad. Sci. U.S.* **53**, 557 (1965).
41. R. J. A. Hosie, *Biochem. J.* **96**, 404 (1965).
42. O. Z. Sellinger and I. De Balbian Verster, *J. Biol. Chem.* **237**, 2836 (1963).
43. E. W. Sutherland and T. W. Rall, *Pharmacol. Rev.* **12**, 265 (1960); E. W. Sutherland, I. Øye, R. W. Butcher, *Recent Progr. Hormone Res.* **21**, 632 (1965).
44. E. De Robertis, G. Rodríguez de Lores Arnaiz, M. Alberici, E. W. Sutherland, R. W. Butcher, in preparation.
45. E. De Robertis, J. M. Azcurra, S. Fiszer, *Brain Res.*, in press.
46. W. E. Van Heyningen, *J. Gen. Microbiol.* **20**, 291, 301, 310 (1959).
47. D. W. Wooley and B. W. Gommi, *Nature* **202**, 1074 (1964).
48. R. M. Burton, R. E. Howard, S. Baer, I. M. Balfour, *Biochim. Biophys. Acta* **84**, 441 (1964).
49. S. Fiszer and E. De Robertis, *Brain Res.*, in press.
50. G. Rodríguez de Lores Arnaiz and E. De Robertis, *J. Neurochem.* **9**, 503 (1962).
51. E. De Robertis, E. Lapetina, J. Pecci Saavedra, E. F. Soto, *Life Sci.* **5**, 1979 (1966).
52. B. W. Moore and D. McGregor, *J. Biol. Chem.* **240**, 1647 (1965).
53. L. Levine and B. W. Moore, *Neurosciences Rev. Bull.* **3**, 18 (1965).
54. H. Hyden and B. McEwen, *Proc. Nat. Acad. Sci. U.S.* **55**, 354 (1966).
55. L. M. Seminario, N. Hren, C. J. Gómez, *J. Neurochem.* **11**, 197 (1964); J. Eichberg, Jr., V. P. Whittaker, R. M. C. Dawson, *Biochem. J.* **92**, 91 (1964); A. A. Abdel-Latif and L. G. Abood, *J. Neurochem.* **12**, 157 (1965); J. Durell and M. A. Sodd, *ibid.* **13**, 487 (1966).
56. E. G. Lapetina, E. F. Soto, E. De Robertis, in preparation.
57. S. H. Barondes, *Science* **146**, 779 (1964); *J. Neurochem.* **13**, 721 (1966).
58. P. Weiss and H. B. Hiscoe, *J. Exp. Zool.* **107**, 315 (1948).
59. R. Tanaka and L. G. Abood, *J. Neurochem.* **10**, 571 (1963).
60. E. De Robertis, in *Nerve as a Tissue*, K. Rodahl and B. Issekutz, Eds. (McGraw-Hill, New York, 1966), p. 88.
61. The recent original research contained in this paper has been supported by grants from the Consejo Nacional de Investigaciones Científicas y Técnicas and NIH (NB 06953-01).

"The Man-Made World," A New Course for High Schools

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E. E. David, Jr., and J. G. Truxal

The Engineering Concepts Curriculum Project is the outgrowth of a 1963 meeting, sponsored by the National Science Foundation, held to consider the teaching of physics. The project is developing a high-school cultural course with technical and scientific content. It is designed to contribute to technical literacy for high school graduates who will not necessarily follow a career in engineering or science. They should have a knowledge of science and technology sufficient to enable them to think rationally about technologically based issues affecting society.

This view is widely held by educators, and yet one still hears the obverse argument that, to *use* and to *benefit* from the fruits of technology, a person does not require technical knowledge but needs only a modicum of skill or familiarity with technique. According to this argument, the driver of an automobile need not understand the mechanism of the internal combustion engine and the television viewer does not need to understand the superheterodyne principle and Maxwell's equations. The answer is linked to the increasing influence of science and technology on the shape and quality of life in our society. Today, citizens must be knowledgeable in these areas if they are to be effective in guiding technology in directions that suit man's purposes without allowing it to impair his liberty, ruin his environment, or destroy his privacy.

There is a more immediate and, some think, a more compelling reason for educating citizens in technical matters. Modes of thought and action inspired by science and engineering are being increasingly used in business, goverment, economics, education, and medicine. The precision of thought and language gained through mathematics, through manipulation of symbols, and

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