SOME ASPECTS OF CENTRAL NERVOUS PHARMACOLOGY[1,2,3]

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Introduction

The literature on central nervous effects of drugs continues to grow exponentially. For the year 1961, *Psychopharmacology Abstracts* alone shows more than 3,000 titles, and several hundred of these may be classified as reviews. It would be impossible to digest all the literature pertinent to the present annual review or even to list the titles within the available space. Instead, we shall have to be satisfied with a representative sample sufficient to serve as a basis for discussion of some current trends. The samples selected will not necessarily be the best work in the field, and the valuable contributions of many authors will be neglected, also some potentially reviewable fields will receive scant or no mention. For this spotty treatment we will offer no excuse except our limitations of time, space, inclination, and competence to do justice to this broad and important field of research.

Theoretical Considerations on Drug Actions Upon Central Neurones and Synapses

Classes of junctional transmission.—It is an aphorism that drug actions involving neurones or effectors occur primarily at synaptic regions rather than upon the unapposed membranes of cells. Drugs have served as valuable tools in unscrambling the complexities of synaptic interactions of the central and autonomic nervous systems, and the theories derived from drug studies have led to further rationalizations concerning the mechanisms of action underlying the overt behavioral effects of particular drugs. It seems appropriate to begin this review with some current neurophysiological theories of synaptic action based in considerable part on the use of drugs.

Grundfest (1), in one of his more recent exhaustive reviews, has again analyzed the physiology and pharmacology of junctional transmission, based in part on his own considerable work in various central and peripheral systems, and in invertebrate as well as vertebrate material. Grundfest distinguishes between ephapses in which direct electrical excitation occurs, as in many invertebrate giant fiber junctions and vertebrate cardiac cells, and

[1] The survey of the literature pertaining to this review was concluded in May 1962.

[2] Abbreviations used in this chapter include: ATP (adenosinetriphosphate); DMI (desmethylimipramine); DOPA (dihydroxyphenylalanine); GABA (γ-aminobutyric); 5-HTP (5-hydroxytryptophane); LSD (lysergic acid diethylamide); MAO (monoamine oxidase); W12⁰6 (2-ethylamino-3-phenyl-norcamphane)

[3] Some previously unpublished observations are the result of investigations supported by U.S.P.H.S. M-4545 and M-5503.

synapses—a term he reserves for all electrically inexcitable junctions, which by exclusion implies chemical transmission.

The pharmacological properties of ephapses are presumed to be comparatively simple and related to those of the unapposed membranes of the corresponding cells. One may object to Grundfest's restriction of the definition of synapses, since there are no obvious anatomical differences indicating electrical excitability or its absence at a junction. Where chemical transmission is assumed, and this would include the vast majority of all junctions including those of the vertebrate central system, it is implied that arriving impulses release transmitter substances from submicroscopic vesicles upon specialized postsynaptic chemoreceptive areas across the synaptic cleft, although the responsive area is not necessarily restricted to the zone of synaptic apposition.

Grundfest also assumes that a transformation can occur between electrically excitable and inexcitable membrane, based on the previous work of Axelsson and Thesleff on denervated muscle and of Miledi on fetal muscle, plus some observations of Grundfest on skeletal muscle and electroplaques. Of chemically sensitive electrically inexcitable membranes there appear to be several types based on the changes in specific ion permeability caused by chemical mediators. However, they can be roughly classified as depolarizing (excitatory) and hyperpolarizing (inhibitory).

With this background of synaptic types, a partial classification of synaptically active drugs is possible. In Grundfest's classification acetylcholine may be an activator of either type of synapse, depending on the tissue. Pentylenetetrazol (Metrazol) is predominantly a depolarizing activator, while λ-aminobutyric acid (GABA) is a hyperpolarizing activator for certain crustacean cells, but serves as an inactivator of hyperpolarization at axodendritic synapses of mammalian brain. (Note however the direct hyperpolarizing effect of GABA shown by Curtis for cells of the spinal cord. Whichever the mechanism, the end result of GABA would be inhibition.) Curare is classed by Grundfest as an inactivator of either type of synapse, but with the net effect of inhibition (presumably he still considers curariform action to be present in cells of the mammalian cerebral cortex, although we have previously questioned this conclusion in the case of rabbits and man). Strychnine is classed as an inactivator of hyperpolarizing inhibitory synapses, with the net effect of excitation. Picrotoxin is pictured as a sort of opposite of GABA, causing inactivation of inhibitory synapses in the lobster neuromyal junction, but activation of excitatory synapses in the axodendritic cortical synapses of the cat. (As described later, Eccles would now classify picrotoxin essentially as an inactivator of inhibitory depolarizing axo-axonal presynaptic synapses, without direct effect on cell bodies.) Carnitine is proposed as another kind of antithesis to GABA, causing activation of depolarization in the lobster and inactivation of hyperpolarization in the cat cortex. As to other variations of the role of GABA, Grundfest attributes the effects de-

scribed by Curtis on spinal neurones to a direct action on nonsynaptic membranes.

Classification of drug effects on the above basis is certainly not simple, as can be seen from the examples already given. Furthermore, the picture is complicated by the possible occurrence of more than one type of synaptic membrane in the same cell, by differential effects on synaptic and non-synaptic membrane of the same cell, and by the interplay of cells with different properties even in simple systems, not to mention the transcendental complexities of the mammalian central nervous system. The recent addition of a presynaptic inhibitory junction system of Eccles adds further variations (see later).

Furthermore, there may be a number of actions of drugs directly on the ultimate release of mediators by synaptic vesicles. Thus, at least in the lobster neuromyal junction, where miniature endplate potentials referrable to quantal discharge of vesicles may be studied, serotonin and the ammonium ion seem to have a direct releasing action, while picrotoxin and phenylethylamine act further upstream on the conductile component of synaptic membrane, initiating spikes. The dinoflagellate "clam poison" previously described by Shantz appears to block the conduction process in nerve terminals of the lobster neuromyal junction.

This somewhat lengthy review of a review by Grundfest serves to outline the most systematic approach yet devised for a universal classification of synaptic mechanisms. How far it is applicable to the mammalian central nervous system remains to be seen.

A number of questions regarding mammalian central synapses can be raised. As indicated later, Curtis questions whether naturally occurring substances shown to affect nerve cells need necessarily be true transmitters in the sense that they can be released only by input impulses. The occurrence of spontaneous miniature endplate potentials (2), without the necessity of input impulses, illustrates the possibility of a different type of modulator action even if the quantal spontaneous discharges are those of the true transmitter substance, which need not always be the case. It is evident that any number of naturally occurring substances in the environment of target cells might modify their behavior, and that these effects might in turn be modified by exogenous drugs, without direct involvement in the transmitter process. In short, the actions of drugs do not imply the occurrence of a relevant impulse-dependent transmitter process. Rigorous proofs are required to justify the acceptance of a particular transmitter at a particular site.

Finally it is not inconceivable that tandem transmitter systems may occur. Koelle and other investigators have previously raised the question whether impulse-liberated acetylcholine in a nerve terminal might in turn liberate other transmitters from the ultimate vesicles of the same cell, for example, in certain autonomic neurones. Thus, the term "transmitter" need not apply solely to interaction across a synaptic cleft. Although this may

seem like a concession to the views of Nachmansohn (3), who continues to postulate a cholinergic transmitter step for the conduction process in axones, the conduction-transmitter step seems to be an unnecessary feature for axones with a large factor of safety in their direct electrical excitability, and has never been supported by the kind of rigorous proof required for demonstration of transmitters elsewhere.

Central inhibition.—The physiology and pharmacology of central nervous inhibition have particularly intrigued leading neurophysiologists for several decades. It had been evident for some time that there must be at least two types of inhibition, possibly more. In the 1940's, Lloyd had demonstrated what appeared to be a direct monosynaptic inhibition of motoneurones; while Renshaw, in addition to his elucidation of a system of inhibitory interneurones fed by recurrent collaterals of motoneurones, had also found evidence for what appeared to be presynaptic inhibitory interaction. The exhaustive exploitation of the intracellular microelectrode method by Eccles and colleagues led to the recognition of hyperpolarizing inhibition, which Eccles earlier explained as a direct electrotonic effect but subsequently found it necessary to explain as a transmitter action, since polarization experiments revealed it to be a selective change in ion permeability. There still remained a demonstrable type of non-hyperpolarizing inhibition which required different explanation. The recent work of Eccles and others (4 to 11) leads to a conception which will now be summarized.

Two types of inhibitory mechanism are distinguishable in the spinal cord. One may be called postsynaptic, the other presynaptic. They differ radically in mechanism, in their measurable electrical signs, in their response to drugs, and in their distribution in the cerebrospinal axis.

Postsynaptic inhibition is the type long championed by Eccles. The mechanism is inferred to be the liberation of an inhibitory mediator by presynaptic terminals upon a postsynaptic special receptor zone, which is made more premeable to K and Cl but not to Na. The electrical resultant is hyperpolarization of the target postsynaptic zone. Strychnine is the prototype par excellence of blocking agents against this type of inhibition. Tetanus toxin may act specifically to prevent the liberation of the still unknown inhibitory substance.

Presynaptic inhibition depends upon terminals synapsing with the ultimate excitatory presynaptic terminals. These axo-axonal terminals are histologically demonstrable by electron microscopy. It is inferred that they act by pre-emption, partially depolarizing the excitatory terminal to the extent that an excitatory impulse cannot be transmitted. The mediator responsible for this effect may be GABA. The change in the membrane of the presynaptic excitatory terminal is essentially increased permeability to all ions (as with the presumed mechanism of the excitatory synapse itself), thus essentially a shunt block of impulse transmission. Picrotoxin is a relatively specific blocking agent for this type of transmission. Pentobarbital in smaller doses in-

creases and prolongs this type of block, but in larger doses also blocks excitatory transmission.

The principal ways in which presynaptic inhibition differs from postsynaptic are evident in electrical recordings from selected spinal sites. Postsynaptic inhibition is evidenced primarily by hyperpolarization of the target motoneurone. Presynaptic inhibition is evidenced primarily by a relatively prolonged negative dorsal root potential and a correlated positive cord dorsum potential, indicating depolarization of the afferent input.

Geographically, the presynaptic type is most evident in the cord, while the postsynaptic type increases in prominence up the cerebrospinal axis until it is the exclusive form in the cortex itself.

In brief summary of Eccles' views, presynaptic inhibition is prolonged, primitive, implies a shunt mechanism, and is blocked by picrotoxin. Postsynaptic inhibition is shortlived, more prominent in cerebrum, implies a hyperpolarization mechanism, and is blocked by strychnine.

Of convulsant agents, pentylenetetrazole affects neither of these systems and by exclusion may be considered a stimulator of excitatory synapses. Mephenesin does not enhance either inhibitory system. Cholinergic and anticholinergic agents do not affect these inhibitory systems.

Localized chemical stimulation at synapses.—Most of the evidence concerning chemical receptivity of neurones has been indirect in the sense that the test drugs were applied systemically or at least diffusely, leading to possible interactions or indirect effects which would be reflected even in records from single impaled neurones. Recently there has been increasing use of highly localized microinjections into or upon the surface of central neurones, exemplified by the work of Curtis and collaborators (12 to 14), an outgrowth of earlier investigations with Eccles.

Curtis (15), as co-author of a recent monograph on chemical mediators, reviews succinctly the present state of our knowledge concerning transmitter substances of the central nervous system, based in part on his own investigations with microelectrode recording from single cells during microinjection or local application of various amines and amino acids. With this procedure, only the Renshaw cells appear to be responsive to cholinesters, many other cell types having been tried. Neither norepinephrine nor serotonin has yet been shown active by local injection. Histamine and adenosinetriphosphate (ATP) have likewise failed to show local actions. However, many monocarboxylic acids including GABA are shown to depress spinal neuron function, while several common dicarboxylic acids are depolarizing and excitatory. Curtis argues that these normally occurring intracellular amino acids cannot be true impulse-dependent transmitters, and concludes that the transmitters at most central synapses are simply not known.

Evidence from electron microscopy.—DeRobertis, who has already contributed in a major way to the ultramicroscopic anatomy of neurones through electron microscopy, has now extended his studies to ultracentrifuged par-

ticulate fractions of brain (9, 16 to 18). Prehaps the most interesting fraction is that formed of synaptic terminals still attached to subsynaptic membrane. These paired structures are recognizable by the peculiar vesicular plate on the presynaptic side of the double membrane. The endings form a considerable fraction, about one sixth of brain tissue. Further fractionation of the endings can be achieved, and it appears possible to separate fractions both rich and poor in acetylcholine and cholinesterase, suggesting both cholinergic and noncholinergic endings. Elsewhere adrenergic terminals have been described, and other granule-containing structures seen in the pineal gland. The possiblities for study of the ultramicropharmacology of specific synapses opens a rich field of research where formerly there was merely conjecture.

Incidentally, in recent unpublished material DeRobertis (9) has demonstrated the frequent occurrence of synaptic fragments with well-defined interneuronal filaments showing continuity of the pre- and postsynaptic membranes. Although their occurrence raises the possibility of direct membrane conduction across synapses, they do not appear to present sufficient cross-sectional area to serve this purpose. Neither would they serve as channels for the injection of transmitters, since they are not necessarily in relation to the vesicular plaques which presumably contain the mediator stores, and there is considerable interpenetration of the apposed membranes in the absence of any filaments. We would guess that the connecting filaments are primarily of embryological significance—the relics of cell division.

Another phenomenon revealed in terminals by electron microscopy is the occurrence of terminals overlaid upon other terminals, found in all except the highest levels of the cerebrospinal axis, and identified by the arrangement of the vesicular plaques. Eccles (9) has used these as supporting evidence for a system of presynpatic inhibitory synapses, postulated from his pharmacological studies on electrical records of single impaled cells.

It is evident that a subtle revolution has occurred in criteria of anatomical verification of pharmacological findings, with the increasing availability of electron microscopy. But the positive identification of transmitters will evidently be a laborious process (19).

INDIRECT PHARMACOLOGICAL EVIDENCE CONCERNING CENTRAL MEDIATORS AND MODULATORS

General.—During the last decade much energy has been expended in the attempt to find proof of central synaptic transmission by mediators already recognized in peripheral autonomic pharmacology. Although earlier the primary interest was in acetylcholine as a universal interneuronal mediator, by analogy with its demonstrated essential role in autonomic ganglia, more recently there has been an increasing emphasis on other naturally occurring substances, including the catecholamines, the serotonin amine series, various other amines and amino acids including GABA by analogy with its role in certain invertebrate synapses, and to a limited extent histamine and various

polypeptides. Underlying this search is the basic belief, for which there is some evidence especially from the observations of Eccles and many collaborators from microelectrode studies on spinal motoneurones, that central excitation and inhibition require chemical mediator steps.

To this pessimistic reviewer it appears that there is still no proof positive of the essential role of any particular chemical mediator for any particular central synaptic site. The positive evidence, not proof, is usually of three types: first, the demonstrated actions of various blocking or facilitating agents relevant to one or another peripheral mediator such as acetylcholine; second, the demonstration of the natural occurrence of such substances locally at certain central sites, together with enzyme systems known to be involved in their synthesis and destruction; third, the argument by exclusion that certain synaptic phenomena cannot be explained without invoking some unknown special mediator substances, as opposed to an explanation based on more general electrophysiological principles derived largely from peripheral axonology. An additional type of evidence is provided by histological studies, particularly electron microscopy, illustrating special features of presynaptic terminals and postsynaptic zones which would suggest chemical specialization.

The best evidence for central synaptic mediator action is probably that of Eccles and collaborators for cholinergic activation of Renshaw inhibitory cells by recurrent collaterals of spinal motoneurones. Evidence for an unknown inhibitory mediator from the Renshaw cells is also strongly suggestive. Beyond these, a strong argument for a variety of amine and amino acid mediators at cerebral cortical synapses has been made by Grundfest and Purpura in numerous articles over many years.

Of the various types of evidence, all are incomplete and presumptive. Ideally, the proof of a particular mediator would be somewhat as follows: the suspect mediator is shown to be released by presynaptic nerve impulses; the presumed synaptic effect is obtainable by exogenous administration of the suspect mediator; the amount of mediator released is more than enough to account for the postsynaptic effect; agents which block the postsynaptic effect of the exogenous test substance also block the same effect of presynaptic impulses. These steps in proof are the neuropharmacological equivalents of Koch's postulates.

Clearly this complete sequence is yet to be proved for any particular mediator at any particular site. Yet even the most jaundiced reviewer cannot dismiss the mountain of reported observations on empirical central effects of drugs presumed related to peripheral mediator systems. What synthesis can be made of these discrepancies between theory and practice?

It has been evident to us for some time that the trouble lies in the concept of the "essential" mediator. Is it not possible that many naturally occurring substances, including those liberated by nerve impulses, are able to modify the activity of target neurones, although themselves not essential for the

process of synaptic transmission? To such a substance we would give the name, "modulator," rather than "mediator," since the latter term has come to imply an essential role in central synaptic transmission.

Starting from this concept of modulator action, we have attempted to schematize the various ways in which naturally occurring substances may modulate transmitter action of synapses. Especially through the work of Sabelli in our laboratories (20, 21), using a large battery of tests of central functions and a barrage of various autonomic agents and their combinations, it has been possible to recognize the following situations, with regard to either cholinergic or catecholaminic modulation:

(*a*) Functions modifiable by exogenous suspect modulators but not by relevant blocking agents except against the administered modulator. It is presumed that the target neurones are sensitive but do not normally encounter the suspect modulator.

(*b*) Functions modifiable by the suspect modulator exogenously administered, and also altered in a more or less consistent way by various blocking agents and enzyme inhibitors. Here the evidence would be compatible with true mediator action, but proof is lacking that the suspect modulator is indeed the only possible mediator.

(*c*) Functions modifiable only by various blocking agents relevant to a suspect modulator system. It is presumed that the synaptic system is normally saturated with the modulator substance in question.

(*d*) Functions modifiable by both blocking and facilitating agents of the same suspect modulator, but in the same direction by both.

(*e*) As far as any balance is concerned between cholinergic and adrenergic-modulated effects on a given function, examples are found where one or the other modulator system is ineffective, others where both act in the same direction, and some showing classical antagonism.

Without detailing these and other interesting varieties of presumed modulator action, it can be said that the brain shows a surprising variety of pharmacologic arrangements involving what seem to be cholinergic and adrenergic systems as deduced from their responses to exogenous drugs. The profiles are even more intricate if muscarinic and nicotinic cholinergic effects are separately considered, if the alpha and beta adrenergic systems are also differentiated, and if the serotonin system is included. Certainly there does not appear to be any simple pharmacological pattern in the central nervous system corresponding to that in the autonomic outflow and peripheral target cells. Furthermore, none of the functions studied behaves as if it depended on an essential synaptic transmitter step involving any of the usual peripheral transmitter substances.

With this background, let us return to a review of the current literature on central effects of autonomic-related drugs.

The aromatic alkylamines.—The central pharmacology of the amines derived from tyrosine and tryptophane has been given considerable importance within the past decade in large part because of the explosive develop-

ment of the field of pharmacotherapy of mental illness. The new drugs of principal interest have properties which can be roughly binarily classified as anti-adrenergic for the major anti-schizophrenics and pro-adrenergic for the major anti-depressives, although this is a very poor first approximation to their actual pharmacological spectra, which usually includes blocking or facilitating actions on various test systems with presumed receptors for acetylcholine, alpha and beta adrenergic transmitters, serotonin or histamine.

It is generally assumed, but not so easily demonstrated, that mental illness involves pathological alterations in blood and urine chemistry which have some diagnostic potential value, and that drugs can be used to imitate or ameliorate the chemical distortions as well as the behavioral changes. Sourkes (22) has briefly summarized some of the pertinent biochemical literature. In passing, it might be noted that Sourkes' research has shown more impressive correlations for neurological disorders without notable behavioral pathology than for purely psychiatric conditions. Barbeau, Murphy & Sourkes (23) showed unusual variations of urinary catecholamine excretion in patients with various basal ganglion disorders. For example, dopamine excretion was unusually low in Parkinsonism, particularly post-encephalitic, and unusually high in Wilson's Disease. Sourkes (24) has also written a long and detailed epitome of chemical methodology in the study of pharmacologically active substances in the nervous system.

A number of general reviews and summaries should be mentioned:

Von Euler (25) has written an authoritative and detailed account of the chemistry of epinephrine and norepinephrine. Karki *et al.* (26), have summarized the storage, synthesis and metabolism of monoamines in the developing brain. Histamine and histamine-liberators have been reviewed by Halpern (27). Walascek (28) has surveyed the pharmacological and biochemical properties of serotonin, as a background for a discussion of its possible role in neurological and psychiatric disorders. The chemistry of various psychotomimetic drugs is reviewed by Downing (29). The action of drugs on various aspects of brain metabolism but particularly intermediate carbohydrate and amine metabolism has been reviewed by Quastel (30). In a theoretical discussion of possible alternative modes of action of psychotherapeutic drugs, Brodie *et al.* (31) have restated their views that these agents must be explained on the basis of alteration of synaptic transmission, rather than by more general non-synaptic effects on cerebral metabolism, etc.

(*a*) *Serotonin.*—The role of serotonin in brain function has been explored indirectly for a number of years by means of drugs presumed to alter its cerebral concentration, but in recent years with an increasing number of actual correlations between behavior and brain chemistry. The group associated with Brodie have particularly championed a functional role of serotonin. Unfortunately no pure serotonin antagonists have been available for study.

An excellent systematic review by Gyermek (32) on serotonin antagonists has appeared. Knowledge of serotonin receptors and blocking agents is

based almost entirely on studies in such simple organs as rat uterus and guinea pig ileum, less on such neuroneural systems as sympathetic ganglia, and hardly at all on central nervous functions. There are apparently several classes of serotonin receptors overlapping in particular systems with sensitivity to such better-known transmitters as acetylcholine, histamine and the catecholamines. The classes of drugs showing antiserotonin action encompass a considerable part of the pharmacopoeia: ergot alkaloids including lysergic acid diethylamide (LSD) and derivatives, various indoles and congeners, antihistamines, phenothiazines, antiadrenergic and adrenergic-depleting drugs, atropinics, local anesthetics, morphine-like analgesics, some catecholamines but particularly isoproterenol, acetylcholine, GABA, and a number of other agents. It is interesting to note that nicotinic cholinergics and anticholinergics were not effective, nor were various amphetaminics.

Although many of the above mentioned agents have interesting central nervous effects, it is obvious that their mode of action is not limited to serotonin receptors. Of the newer and relatively specific synthetic serotonin antagonists which have been used clinically for peripheral actions (antipruritic, antidermatitic, etc.), neither cyproheptadine nor methysergid has obvious actions on the central nervous system. This and the well-known lack of central symptoms in patients with serotonin-rich carcinoid tumors tends to cast doubt on an important role of serotonin in normal or pathological functions of the human brain, despite the widely accepted importance attached to serotonin.

The usual type of evidence in support of a central role of serotonin is indicated by the following examples.

Brune *et al.* (33) conclude from studies with reserpine and isocarboxazid that a moderate rise in brain serotonin is associated with tranquilization, a larger increase with excitation.

A disruptive effect of increasing levels of brain serotonin on operant pecking behavior of pigeons is implied in the work of Aprison & Ferster (34, 35), who injected 5-hydroxytryptophane (5-HTP) after administration of iproniazid. From the correlation of behavior with measurement of brain monoamine oxidase activity, they assume an equivalent alteration of brain serotonin, which however was not measured directly. The results, although interesting, illustrate the ambiguity of this type of correlation, since it cannot be verified that the behavioral effect was not due to the direct action of the precursor itself. The necessity for a truly multifactor analysis in any behavioral-chemical correlation study is obvious.

Costa and the Himwiches (36) find a correlation of serotonin level in various brain centers with aggressive behavior and severity of neurological signs in dogs treated with tranylcypromine followed by 5-HTP. In a related report, these same authors found that prior treatment with imipramine caused sleep after small doses of 5-HTP. They also found a reduction of 5-HTP decarboxylase in brains of chronic imipramine treated dogs, and

showed that imipramine *in vitro* in low concentration inhibited this enzyme. It occurs to us that in this, as in other studies conducted with 5-HTP, one may be observing a direct action of the administered amino acid rather than of serotonin.

Costa & Brodie (37) contend that depletion of brain catecholamines is not responsible for sedation by reserpine, since norepinephrine and dopamine could be selectively depleted without sedation by alpha-methylmetahydroxytyrosine, while impairment of serotonin binding by other drugs is invariably associated with sedation. On the other hand the excitement caused by monoamine oxidase (MAO) inhibitors may be mediated by catecholamines. Their discussion also indicates that in addition to absolute levels of biogenic amines in brain it is necessary to know their state of binding, which may be differentially affected by drugs.

The current knowledge of binding of biogenic amines in tissues has been summarized recently by Green (38).

For serotonin the most complete evidence for its state of binding comes from mast cells rather than neurones, as exemplified by the detailed studies of Hagen (39).

It is possible that serotonin is not the only tryptophane derivative to be considered with regard to a central function role. Vane *et al.* (40) have presented evidence for specific tryptamine receptors of predominantly excitatory type, both in brain and spinal cord of the cat. These can be blocked by various serotonin antagonists.

(*b*) *Catecholamines.*—In opposition to the serotonin enthusiasts are a number of investigators who find evidence for a role of naturally occurring catecholamines in brain function. Carlsson and collaborators have turned their attention in recent years particularly to the role of dopamine.

As an example, Carlsson (41) reports some experiments with mice and rabbits on high doses of nialamide tending to confirm his view that brain catecholamines, rather than serotonin, are involved in the control of alertness. By means of reserpine the catecholamine and serotonin brain levels could be manipulated somewhat independently for purposes of correlation with behavior.

In accord with previous findings in other species, Bertler (42) reports an unusually high level of dopamine in contrast to other catecholamines in the putamen and caudate nucleus of the human brain, and restates the possible relation of dopamine to extrapyramidal motor functions.

Everett & Wiegand (43) have reported some data on the correlation between brain levels of various amines and behavior in mice, using the previously reported methods of Wiegand's group for measurement of dihydroxyphenylalanine (DOPA), dopamine, norepinephrine, epinephrine and serotonin, for correlation with the behavioral profiles developed by Everett and co-workers. DOPA, deserpidine, or the monoamine oxidase inhibitor pargylline were administered alone or in a combination sequence. Taking

their entire range of results, from marked catecholamine depletion by deserpidine to marked increase by administration of DOPA following pargylline, there is an excellent correlation between brain levels of dopamine and degree of central excitement. This correlation is not reflected by the other amines, although norepinephrine may correlate secondarily. The results would tend to corroborate the views of Carlsson concerning the importance of dopamine in behavior.

The determinations of Wiegand & Perry (44) on amines in the brains of mice would suggest a relative lack of coupling between dopamine and other catecholamines and serotonin. DOPA injection alone primarily elevates DOPA, while the MAO inhibitor pargylline primarily elevates norepinephrine and serotonin. The combination primarily elevates DOPA and dopamine respectively. With the further use of the amine depleter deserpidine, as in the experiments described by Everett, it is possible to separate the various amine profiles for correlation with behavior. In the variations of these studies which have been seen by the reviewer, it is hard to avoid the conclusion that DOPA itself has a direct excitatory action on the central nervous system.

Much of the research on the central role of serotonin and catecholamines has depended on the use of reserpine and related alkaloids, which are known to cause universal and long lasting depletion of these amines. The mechanism of depletion is therefore of interest, but has usually been studied in tissues other than brain.

Recently there has been increasing attention to reserpine and other drug actions on catecholamine granule suspensions. In a symposium monograph on adrenergic mechanisms (45), which is one of the most extensive compendia on all aspects of catecholamine metabolism and function heretofore assembled, there are a number of reports and comments on the mechanism of catecholamine release from storage sites (46 to 54).

Many publications could be mentioned, including a brief summary by Whittaker (55) of this relatively new field of pharmacological studies with subcellular fractions. Such preparations are obtained by ultracentrifugation methods and verified by electron microscopy to achieve particulate suspensions rich in storage granules, vesicles or mitochondria which can be chemically analyzed for their content of biogenic amines or of relevant enzyme systems, after drug treatment of the animal *in vivo* or of the suspensions themselves *in vitro*. Judging from the current experience of our own colleagues (56), the data obtained from *in vitro* drug treatment of catecholamine granule suspensions gives results considerably different from that obtained by ordinary *in vivo* methods, and leads to the supposition that the subcellular inclusions may not be directly attacked by such drugs as acetylcholine or reserpine, but rather are depleted or discharged by indirect effects exerted normally on the intact cell.

It is noteworthy that Von Euler & Lishajko (57) found that reserpine actually inhibited the spontaneous release of norephinephrine from nerve

granules and prevented the releasing action of tyramine, with somewhat less prominent effects on granules of the adrenal medulla. These results in cell fractionates are opposite to the well known depleting action of reserpine on the same granules *in vivo* and illustrate the importance of the integrity of the cell for drug actions.

Weil-Malherbe *et al.* (58) have determined the effects of a number of agents on brain catecholamines in the rabbit, keeping separate account of the particulate and soluble fractions. Although the results are somewhat complex, it is noteworthy that the depletion of both dopamine and norepinephrine was more rapid from the soluble than the particulate fraction. Taking this finding into consideration with the lack of direct depleting effect of reserpine on granule suspensions from other neural tissues, it is hard to avoid the conclusion that reserpine does not act directly on tightly bound amine storage depots, but acts to free the catecholamines from less tightly bound fractions, the stores then slowly depleting by mass action.

Although epinephrine is well-known to have more marked cerebral actions than norepinephrine, its role as a possible central transmitter would be placed in doubt by its relatively low and stable concentration. Furthermore, Masuoka *et al.* (59) have recently shown that epinephrine is not synthesized *in vitro* in rat brain slices with l-tyrosine as substrate, in contrast to dopamine and norepinephrine. It must be assumed that brain epinephrine originates primarily in the adrenal medulla.

Thus, in summary of this section, it can be said that there is much positive support for a central role of serotonin, catecholamines and their respective precursors and naturally occurring congeners, but that an exclusive role of any one of these agents in a particular function is difficult to prove, in part because the drugs used as blocking or depleting agents or enzyme inhibitors have rather broad spectra of action. Furthermore, biochemical correlations present a confusing picture. It is hard to factor out possible interactions between the amine effects, or to identify functions with the brain samples upon which determinations are made. Also the physical state of the amine rather than its total concentration may be of importance. The mechanisms of normal and drug-induced release become more complicated with recent studies on subcellular fractions. In some instances, as with epinephrine, it is almost certain that the amine cannot function as a true central transmitter, and such a role is dubious for the other known amines.

Cholinergic drugs.—At one time there was some enthusiasm for the concept of acetylcholine as a universal interneuronal transmitter, but as noted earlier the only convincing proof from the studies of Eccles and of Curtis is limited to the special case of recurrent motoneurone collaterals terminating on Renshaw cells of the spinal cord. We also noted from the work of De Robertis the occurrence of acetylcholine-rich nerve terminals, which does not in itself prove the role of the amine as a transmitter at this type of terminal. Hebb (60) has recently discussed the problem of cholinergic neurones.

Regardless of the role of acetylcholine, it has long been evident that

cholinergic drugs can cause a number of central actions, often unpleasant, and that atropinic drugs have been found useful for many years in parkinsonian tremor and rigidity, more recently as antidepressive agents in psychiatry. They are known to cause confusion and hallucinations in high dosage.

Of the many possible aspects of cholinergic drug action on brain, we will confine our discussion largely to the problem of tremor and its prevention, since this subject has stimulated considerable recent reserach.

Tremorine® (1,4-pyrrolidino-2,3-butyne) is a useful laboratory drug originally shown by Everett and co-workers to produce a parkinson-like syndrome in various species. It has subsequently been widely used as a test agent for the study of tremors and of antiparkinsonism drugs. Until recently there have been some perplexing problems about the ultimate site of action of this drug, which produces a variety of parasympathetic signs as well as central nervous effects when administered systemically, but has relatively poor or questionable actions on topical application or in various isolated tissues. We originally interpreted this pattern as a centrogenic parasympathetic action. Now it appears that this discrepancy may be explained on the basis of an active metabolite, Oxotremorine, to which the original drug is slowly converted in the body. Welch (61) has reported on his studies with the metabolite obtained by incubation of Tremorine with liver slices. The activated form produced prompt tremors after intravenous injection in mice, in contrast to the long latency with Tremorine itself. Even diluted urine from previously Tremorine-treated mice caused prompt effects on intravenous injection into other mice. The activated form showed marked potency as a vasodepressor and in stimulation of isolated intestinal strips, in contrast to the inactivity of Tremorine itself. As with Tremorine, the Oxotremorine is antagonizable be atropine. Thus it appears that the active principle of Tremorine can be classed as a potent muscarinic metabolite with strong central actions in addition to direct peripheral effects, thus resolving most of the earlier discrepancies in the mode of action of the parent drug.

Everett (62) has briefly reviewed the history and present problems regarding Tremorine, emphasizing again the marked antagonism of all peripheral and central actions by atropinic drugs, and the evidence for an intermediate metabolite. A most extensive review and investigation of Tremorine is to be found in the doctoral thesis of Friedman (63). Kaelber *et al.* (64) have been active in the study of the localization of Tremorine action in the cat.

In our experience (65), Tremorine does not produce true tremors in the cat, but causes a marked rage-like response, in addition to the typical parasympathetic signs seen in all species. Sabelli, the reviewer and colleagues have studied the rage reaction produced in cats by injection of Tremorine, seen also with arecoline, morphine and LSD. Tremorine rage can be prevented by a number of drugs having demonstrable atropinic action, including atropine itself, n-ethyl-3-piperidylcyclopentenylphenyl glycolate (Ditran), perphenazine, amitriptyline and imipramine, the latter at toxic dose levels.

Proadrenergic drugs such as etryptamine and nialamide failed to alter the rage. Neither did reserpine, which incidentally together with Tremorine, caused a type of head tremor never seen in cats with either drug alone. Our interpretation of the rage effect is that Tremorine activates a normally inactive muscarinic modulator linkage. The tremor mechanism, however, requires in addition, the depletion or blocking of a serotonin or catecholaminic linkage, at least in the cat. As to the locus of action of tremorogenic action of Tremorine, we had previously found in rats that fairly complete bilateral destruction of caudate nuclei gave some quantitative protection against tremors, which however occurred with their usual vigor at three or four times the usual dosage. Compared with other centers, the caudate seemed to be an important but not exclusive Tremorine-sensitive center.

In connection with Tremorine action, it was previously noted by Everett and us that there are marked species differences in the tremor response, which is absent or questionable in cats and rabbits but prominent in mice, rats, dogs and monkeys, the latter species showing a remarkable imitation of human parkinsonism, including the rigidity, posture, facies and poverty of movement, as well as a resting tremor modified by dynamic postural changes. Since all species are sensitive to the parasympathetic-like effects of Tremorine, it is apparently the tremor mechanism itself which shows species variation in drug sensitivity.

Other tremorigens.—Various other drugs are known to cause tremor in animals. Nicotine was particularly investigated some years ago by Bovet and Longo, and histamine has been variously reported to cause tremors, but in our experience they are not dramatic. Harmine is another reported tremorigenic agent, of interest because of its structural resemblance to serotonin. Adrenergic drugs as a rule do not cause tremor, but epinephrine in man is known to cause a type of fine tremor, not of parkinson type. According to Ernst (66), *p*-methoxydopamine can cause a type of hypokinetic rigidity, presumably of extrapyramidal type. Although cats are not in general a favorable species for production of tremor by systemic drug administration, Domer (67) has studied tremor induced in cats by the intraventricular administration of such agents as chlorpromazine, pentobarbital and serotonin.

The above discussion suggests that tremor is not strictly a cholinergic phenomenon, and that other possible modulators may be involved in the facilitation or prevention of postural tremor. From a variety of drug interaction studies in mice, Stern (68) concludes that an increase in endogenous serotonin is involved in the pharmacological prevention of tremors induced by harmine or Tremorine, and that dopamine is not involved.

Veratramine is another drug producing tremors of a sort in rats, as well as convulsons in higher dosage. A report by Schoetensack & Hallman (69) illustrates the value of veratramine in differentiating between central depressant drugs. Both tremors and convulsions were blocked by centrally-acting

muscle relaxants of the relatively non-sedative type, such as mephenesin, but not by meprobamate, and others which appear to operate at higher levels of the cerebrospinal axis. Also ineffective were most of the common hypnotics and anticonvulsants, as well as various ganglionic blockers, other anticholinergic agents and antiadrenergics. Some central depressants of simple structure, such as urethane and chloral hydrate, blocked convulsions but not tremor. It was proved by transection that lower brainstem and cerebellum were necessary for the tremors. The veratramine tremors are thus quite different in origin and pharmacology from those produced by Tremorine, harmine, etc., and may be of special interest for defining the locus and mechanism of various central depressants.

Iatrogenic parkinsonism.—The pharmacology of drug-induced tremor has assumed particular importance because of the wide-spread occurrence of parkinsonian signs and other extrapyramidal signs in patients receiving typical phenothiazine tranquilizers and, to a lesser extent, reserpine. For the most part these side-effects are controllable by atropine and various other atropinic antiparkinson drugs, leading to the impression that they are of central cholinergic origin, as is also assumed with other spontaneous forms of parkinsonism. Since the offending drugs are in part classifiable as central catecholamine or serotonin blockers, the further assumption is allowable that the extrapyramidal signs arise from some central imbalance in favor of cholinergic systems. This is all the more interesting in view of the fact that the offending drugs are used for the control of schizophrenia, ostensibly a disorder involving a central modulator imbalance in favor of the catecholamines or serotonin. Furthermore, the atropinic drugs are notable for their ability to produce hallucinatory effects in nonschizophrenics, although it is noteworthy that the control of schizophrenia by phenothiazines does not seem to be impaired by the concurrent administration of atropinic antiparkinson drugs. It is also noteworthy that the typical phenothiazine drugs themselves can be shown to have moderate central atropinic actions, at least in animal EEG studies.

Putting all these considerations together, it would seem that drug-induced parkinsonism might be more likely accounted for by a relative deficiency of catecholamines or serotonin than by an excess of cholinergic action. As previously noted, Sourkes and co-workers found a low excretion of dopamine metabolites in clinical parkinsonism. Barbeau (70) had discussed the chemical evidence for a role of dopamine in basal ganglia disease in man.

By implication, also, the particular central imbalance if any in schizophrenia would be the relative excess of the catecholamine-serotonin system rather than a deficit in cholinergic systems.

These propositions are difficult to test in animals because typical iatrogenic parkinsonism is not seen in laboratory species on administration of phenothiazines or reserpine, although other extrapyramidal features, such as reserpine flexor rigidity and chlorpromazine-induced choreiform jumping,

may be demonstrated. Hallucinatory behavior is difficult to verify in animals, and is more suggestive with such agents as morphine than with atropinics.

To complicate the problem further, the atropinic agents do not exert blocking actions on acetylcholine alone, but have a rather wide range of mediator blocking effects. Furthermore, their well-known alerting action in some species does not seem from our studies to be completely explainable as an antimuscarinic effect. Aspects of the biological actions and clinical usage of centrally acting atropinic drugs were summarized by Anichkov (71), in a previous review.

Whether in man there is some more subtle pharmacological aspect of the relation between schizophrenia and parkinsonism remains to be seen. The frequent occurrence of disorders of the extrapyramidal system in patients receiving high doses of reserpinoid and phenothiazine tranquilizers has led Kline & Mettler (72) to reopen the question of a possible relation between predisposition to both schizophrenia and parkinsonism. The converse phenomenon is the development of psychotic episodes in occasional parkinson patients receiving atropinic drugs. Despite some interesting analogies, the evidence is poor for predisposition in individual patients. It is commonly assumed that the conditions are somewhat opposite with regard to a supposed cholinergic imbalance.

Reviewing statistics on the incidence of extrapyramidal effects in patients treated with various phenothiazines, Cole & Clyde (73) conclude that there is no relation between clinical efficacy in schizophrenia and the appearance of extrapyramidal signs. Phenothiazines differ considerably in the reported incidence of such side-effects, fluphenazine for example having a higher percentage than thioridazine for equivalent antischizophrenic effect. Where the largest mass of data is available, in the case of chlorpromazine, analysis shows that high dosage with the appearance of disorders of basal ganglia is not essential to the improvement of schizophrenia.

Kruse (74) has remarked on the correlation between the antihallucinatory effects and extrapyramidal symptoms induced by fluphenazine. Myrian thopoulos *et al.* (75) have discussed evidence for a hereditary predisposition toward drug-induced parkinsonism. Various authors have noted the lack of hereditary features in true clinical parkinsonism. In fact the largest population with this disorder has heretofore been older people with a suggestive history of involvement in several epidemics of encephalitis approximately forty years ago. It does not seem that there is any necessary predisposition of overt clinical parkinson patients to exacerbation by the antischizophrenic drugs.

Thus more questions have been raised than answered by a consideration of the possible role of cholinergic mechanisms in human schizophrenia and parkinsonism. The only universal finding appears to be the ability of atropinics to control human parkinsonism and variously-induced tremors in animals, not in itself a basis for a cholinergic mechanism of tremors.

Cholinergic systems may be involved in other central functions. Metz (76) has summarized evidence for a role of a cholinergic system in respiratory control. Also for some time acetylcholine has been implicated in the mediation of discharge of the cells of the hypothalamic postpituitary tract. But to return to our original point of departure, except for the excitation of Renshaw cells of the cord there are no convincing cases of cholinergic transmission in the central nervous system. Whatever cholinergic phenomena have been studed appear to represent either modulator functions or perhaps even adventitious effects not relevant to normal synaptic function.

Other mediators and modulators.—Aside from acetylcholine, catecholamines and serotonin, there is no lack of naturally-occurring candidate substances for a role in central functions. We noted earlier the findings of Curtis on excitatory and inhibitory effects of various amino acids studied by microapplication. The distribution, metabolism and possible functional significance of GABA in the mammalian brain has been summarized and discussed by Roberts (77). Although GABA has been the most discussed of potential transmitter amino acids in recent years, a number of naturally occurring amino and guanidino acids and congeners have more recently been studied, following the pioneer efforts of Hayashi. As an example, Takahashi and coworkers (78) have found guanidinoacetic acid to resemble GABA in its local inhibitory effects on initial cortical evoked electrical responses, while gamma-guanidinobutyric acid and related acids and esters augmented the dentritic response.

The variety of possible local hormones and potential transmitters or modulator substances which have already been found in mammals or other forms is reviewed by Erspamer (79), the discoverer of a number of these substances, in a previous volume of this annual review. In addition to serotonin and numerous other amines, amino acids and acidic substances, many polypeptides with biological actions have been isolated. Though most actions have been demonstrated on peripheral systems, some are of possible interest in central nervous function, and doubtless many others are still to be isolated and characterized.

If the problem of evaluation is difficult with ordinary amines and amino acids, it becomes even more so for the polypeptides and proteins, because of the lack of specific blocking or facilitating agents and the impossibility of direct chemical estimation, even when the structures are known for a few of the polypeptides. Of those which occur in brain, the oxytocic and antidiuretic hormones have been stated in the past to have central actions, and substance P and others have been suspected of a functional role. Heparin, insulin, antigenic proteins and other large molecules have been studied. The field is still too diffuse to justify a creative review.

Partly because of previous clinical preoccupation with therapeutic insulin shock in psychiatry, there has been some interest in the possibility of direct insulin action on brain. Rafaelson (80) has reviewed this problem. Reviewing his own earlier work and the world literature, Genes (81) concludes that there

is no evidence for a direct action of insulin on the functions of the brain, and that apparent direct effects can be satisfactorily explained on the basis of metabolic changes exerted secondarily on blood sugar levels and the tissue uptake and usage of glucose.

A rather remarkable action on nervous tissue is shown by a nonenzymatic protein factor found in particularly high concentration in mouse salivary gland. It is capable of causing proliferation in embryonic tissue culture of spinal ganglia, but *in vivo* leads to marked overgrowth of sympathetic nervous tissue. Recent studies of a highly purified preparation of this protein by Crain & Wiegand (82) seem to indicate rather minor alterations in catecholamine levels despite the sympathetic hypertrophy. The adrenals appear histologically normal, and there are no unusual behavioral changes. An antibody to this factor can be developed in the rabbit which causes atrophy of the sympathetic in mice. The work of Crain stems from earlier observations of Levy-Montalcini and Cohen on salivary growth factors from various species.

As other examples of protein effects, Capek (83) has reported a variety of excitatory motor and autonomic phenomena resulting from intraventricular administration of Bradykinin in cats, with potentiation of convulsive drugs. Milhailovic (84) has studied EEG effects of intraventricular anticaudate antibody injection in the cat. In some of our own earlier published work with J. Goldberg (85), we found no notable direct effects on the rabbit nervous system of spinal cord antigenic preparations capable of eventually causing allergic encephalomyelitis; the ultimate neuropathology and dysfunction seemed to depend primarily on vascular changes and ischemia.

Other Centrally Acting Drugs

General.—Recently we have attempted (86) a classification of nonspecific drug actions based on some assumptions about the properties of the generalized nerve membrane, as distinct from chemically specialized synaptic zones. The principal novelty of this concept is the presentation of the protein portion of the membrane as a folded lattice with the folds normally tightened by cross-linkages of amino acid residues, while the lattice is woven of typical peptide linkages cross-bounded by hydrogen bonds to give a maximally impermeable structure. It is assumed that ion exchange in the resting state takes place across the entire thickness of folded membrane, but that the activation process leads to breaking of linkages across folds, leading to sodium influx primarily only into the folds, thus generating the spike potential. Since the portions of structure determining resting and spike potential are thus different, these functions are differently affected by drugs. The simplest kinds of nonspecific drugs, represented by various alkyl ethers and alcohols, are assumed to be monopolar substances competing for hydrogen bonds indiscriminately in the lattice and thus increasing the effective lattice aperture, leading to a depolarizing type of block. As the number of polar sites on the drug capable of hydrogen bond formation are increased, the possibility

of multiple attachment to the membrane and stabilization of the resting potential is increased. With certain constellations of two or more hydrogen bonding points plus an aromatic group which can associate with similar residues of the amino acid side chains, stabilization of the folds can occur, leading to threshold increase and a nondepolarizing type of block, as with the typical local anesthetics and many structurally related blocking agents. Other types of multifocal stabilization can lead to slow refolding of the membrane and delayed recovery, as with typical barbiturates, or prevent slow creeping of fold relations during continuous repetitive stimulation, which appears to be a possible mechanism of anticonvulsant action.

In this schema, the apposed membrane faces at synapses and in other special cases lose to some extent their labile sodium-fold mechanism, and the properties of the basic lattice become most important. Some naturally occurring transmitters are seen as competitors with water for lattice hydrogen bonds, leading to alterations in ion permeability of the lattice which can be depolarizing or hyperpolarizing dependent on the initial aperture size. Blocking agents need not compete with the same lattice sites, but may even act to tighten the otherwise functionless folds at the non-spike-generating synaptic sites.

Although this system gives some plausible consistency to data on centrally-acting drugs, it is highly speculative and based on assumptions about membrane structure which are difficult to prove. Furthermore, it avoids the generally accepted role of lipid constituents of the membrane, and is probably not physically sound with regard to the role of water in drug-membrane interaction. However, it does give some comfort to those who do not wish to see all central drug actions as dependent on chemically specialized synapses, and leaves room for some crucial nonsynaptic drug actions on cell soma and other sites with a poor factor of safety for conduction.

A more sophisticated approach to the special properties of nerve membranes is provided by the recent monograph of Tower, Luse & Grundfest (87) with particular attention to mechanisms of transport of amino acids and other organic molecules across neural membranes, the origin and role of the myelin sheath, and the nature of ion transport—the latter by Grundfest, whose views on drug action are discussed elsewhere in the present review.

There has recently appeared a monumental work by Ling (88), which represents the outgrowth of his long-pursued studies regarding the fixed charge hypothesis of interaction between inorganic ions and cell protoplasm, and differs radically from the usual current views on ion penetrance through living membranes. The work devotes some limited attention to drug effects.

Anesthetics.—The simplest drugs affecting the central nervous system are the general anesthetics—halogenated hydrocarbons, partially unsaturated or ring-stressed hydrocarbons, ethers, alcohols, and other structures of low molecular weight. Most of the volatile anesthetics of interest are character-

ized by their lack of chemical reactivity with tissue constituents, and by their relatively high oil: water solubility ratio. This has led to a number of theories in the past involving essentially the incorporation of these molecules into the nonaqueous phase of the cell membrane, and the consequences for neuronal function.

Recently Pauling (89) has come forward with a concept that emphasizes anew the aqueous phase of the living cell. Other authors, including Szent-Gyorgi and Klotz, have been concerned with the organized state of water within the cell and wherever it conforms to protein structure. That water is not a random solution of single H_2O molecules has been long known to physical chemists. Even in the absence of cells or proteins, the state or organization of water is modified by ions and various ionizable drugs, a subject currently under study by Paul Gordon in our own laboratories. From another direction, the petroleum industry has been concerned with the problem of pseudo-ice formation by water in association with typical hydrocarbons, the so-called clathrate structure. Starting from a knowledge of hydrate crystal structure of the clathrate type, Pauling has calculated the state of organization of water imposed by typical general anesthetics. From this he arrives at a theory of general anesthesia based on the distortion of water structure associated with the cell membrane. The concept at this time fails to account for the ultimate consequences of clathrate formation on the ion movements which are the basis of excitation, but this is also true of all previous theories concerned only with the cell lipid phase. Perhaps the most important aspect of Pauling's contribution is that it raises the possibility of actual calculations for the total complex of protein-ion-drug-water, which would be the beginning of a real theory of pharmacology at the molecular level.

Mary Brazier (90) has written a review on the physiological basis of anesthetic actions on the brain, with particular regard to electrical evidence of the roles of reticular formation and cerebral cortex. The monumental work of Adriani (91) on physics and chemistry of anesthesia remains the principal reference on volatile and intravenous anesthetics and narcotics.

Other depressants.—In a preceding review of this series, Domino (92) dealt with the site of action of various nervous system depressants. Shideman (93) has recently reviewed the clinical pharmacology of hypnotics and sedatives.

A careful electrophysiological analysis of sensory pathways in the curarized cat by Sinitsyn (94), using stimulation and recording from a variety of levels in the cerebrospinal axis, suggests a plausible mechanism for the analgesic actions of trimeperidine, methadone and morphine. The classical conduction pathways and thalamocortical projection systems were not altered at doses which effectively reduced the evoked potentials in several associative and nonspecific projection systems both in brainstem and thalamus. These findings lend support to the view that the morphine type of analgesic causes some kind of uncoupling between specific sensory perception

and its generalized spread to affective systems, and may be of interest also for the psychic actions of analgesics. It has long been evident that morphine is not a general depressant of the central nervous sytem, but has mixed and highly selective actions, of both inhibitory and excitatory type, suggestive of the brain actions of more autonomically-related drugs.

Anticonvulsants.—Most studies on experimental anticonvulsant action are performed on acute preparations and thus miss to some extent the features of chronic pathology and chronic medication characteristic of clinical epilepsy. For several years Morell and various co-workers have been studying chronic lesions produced by focal ethyl chloride freezing of the cortex, as have previously Kopeloff and Barrera with alumina cream applications. To give proper credit, the focal freezing method should probably be attributed to Speransky approximately thirty years ago. The most interesting aspect of Morell's work has been the demonstration of secondary contralateral "conditioned" EEG spike foci, which show histological and biochemical pathological features and are self-perpetuating, as shown by slab isolation experiments. In a recent pharmacological study, Morell & Baker (95) have shown that chronic phenobarbital administration in guinea pigs could prevent the development of secondary lesions, as well as suppress the discharge of already established lesions. Chlorpromazine prevented secondary lesions during administration but they appeared after withdrawal. Diphenylhydantoin failed to prevent or suppress secondary lesions. The results with this drug are in accord with the principle that diphenylhydantoin does not suppress foci or lower seizure threshold, but prevents generalized spread of the seizure process.

Gunn *et al.* (96) have reviewed the clinical and theoretical pharmacology of anticonvulsant drugs and some adjuncts used in the treatment of epilepsy. An exhaustive monograph on the chemistry of anticonvulsants has been prepared by Close & Spielman (97). The most extensive recent compendium on epilepsy and its treatment is the two-volume work of Lennox (98). Problems of the screening and evaluation of anticonvulsant and other neurological drugs are discussed in a symposium volume edited by Forster (99).

Convulsants.—Bovet & Longo (100) have summarized their own observations on localization of biochemical and EEG findings in the brain associated with the production of experimental seizures by a number of representative convulsant agents. The desynchronizing cholinergic agents such as nicotine and anticholinesterases, as well as some amphetamines, may act primarily as specific excitants of the brainstem reticular system. The slow acting hydrazides and pentylenetetrazole are grouped together as having a common final generalized excitatory effect, although the biochemical mechanisms are different. Strychnine acts in accordance with its known role of inhibitory blockade. Nikethamide is in a different class from any of these. Anoxic convulsions represent escape and hyperexcitability of the posterior brain stem. Morphine convulsions are of cortico-diencephalic origin. Codeine, unlike morphine, has some strychnine-like action.

Because of the almost unique role of strychnine as an inhibitor of direct inhibitory synapses, it would be useful to have additional synthetic drugs with strychnine-like properties. Longo & Corrado (101) have investigated strychnine-like synthetics including 4-phenyl-4-formyl-4-methylpiperidine. It acts as an inhibitory blocker on spinal reflexes but may also have some depressant action on excitatory synapses. Another strychnine-like agent previously investigated by these authors is 5,7-diphenyl-diazamantan-6-ol (102), which seems to mimic strychnine exactly by the criteria of Eccles for blockade of postsynaptic inhibition. Corrado & Longo (103) have also shown that the classical convulsant thebaine appears to act by the same inhibitory blockade as strychnine, perhaps in even a more uncomplicated way than strychnine. Brucine and tetanus toxin are other agents considered to be in the same class, but the Bovet group considers that tetanus toxin, presumed to be an inhibitor of synthesis of the inhibitory mediator, must have additional actions, particularly in view of some studies by Naquet.

Lewin *et al.* (104) have analyzed the spinal excitatory action of pentylentetrazole. In recent discussions Eccles (9) has characterized picrotoxin as different from strychnine in having a blocking or possibly slow depleting action on the mediator responsible for the presynaptic type of inhibition, which involves pre-emptive depolarization of presynaptic terminals. Such a mechanism seems to overlook the presumed direct excitatory actions of picrotoxin. Pentylenetetrazole, according to the Grundfest classification, would probably be an activator of excitatory synaptic mediators. Possibly many of these classifications are only first approximations, based on the least detectable action.

Analeptics and convulsants in general have been reviewed at length by Hahn (105), and Adriani (106) has recently briefly reviewed the respiratory stimulants. Of the analeptics, bemegride has received considerable recent attention with regard to treatment of barbiturate poisoning, but whether it is notably superior to or different in action from other convulsant analeptics so used is not certain. Moreover, there is still some controversy as to whether analeptic drugs add any advantage outweighing their risks in treatment of barbiturate poisoning. Clemmesen *et al.* (107) have reviewed the problem.

Tranquilizers.—A new epoch in psychopharmacology began a decade ago with the rediscovery of the antischizophrenic action of Rauwolf's alkaloids by western medicine, and the concurrent promotion of chlorpromazine and later other phenothiazines for the same purpose. But despite much therapeutic use, the rationale of the empirically successful antischizophrenic medications has escaped experimental analysis. In the laboratory reserpine and its congeners continue to behave as universal depleters of catecholamines and serotonin, and the phenothiazines behave as weakly atropinic mixed stimulants and depressants. The two classes produce in animals different central nervous signs which seem to have little if any relevance to their common antischizophrenic usefulness in man.

There have been developed also a variety of weakly general depressant nonbarbiturate sedatives, particularly for the treatment of anxiety in ambulatory patients, typified by the heavily promoted meprobamate and chlordiazepoxide. Again their laboratory analysis has failed to reveal any remarkable features to distinguish them from general depressants of a mildly anticonvulsant type, with some resemblance in effect to the centrally-active sedative-hypnotic muscle relaxants.

A small army of probable placebos, previously unprofitable atropinics and antihistaminics, weakly nicotinic cholinergics and other nondescript pharmaceuticals have paraded in rapid review through the literature and onto the druggist's shelf, from which they dispense pleasure and profit out of any proportion to their biological activity, and lead to despair pharmacologists who attempt to find a laboratory explanation of their dubious therapeutic benefits.

From the vantage point of the Psychopharmacology Service Center, where much clinical data is processed and analyzed, Cole (108) has recently attempted a critical review of typical psychotropic drug studies. After ten years of study of the two earliest drugs, chlorpromazine and reserpine, where comparisons have been made on many thousands of patients drawn from the same populations, it appears that chlorpromazine is the more effective antischizophrenic drug, but more subtle deductions are still lacking in statistical support. With phenothiazines, there appears to be little difference in general efficacy between chlorpromazine and other newer agents (thiorodiazine, flupheniazine, trifluoperazine, perphenazine), but promazine is less impressive. No clear distinction has yet been made among patients who appear to respond better to one or another of these congeners.

Among recent reviews of tranquilizers is one by Parkes (109), covering clinical aspects as well as physiological mechanisms.

Das Gupta (110) has reviewed his own considerable work and that of others on the attempt to localize by neurophysiological methods the site and mode of action of chlorpromazine. The principal depressant action appears to be exerted on posterior hypothalamus and rostral midbrain reticular formation, which act as a functional unit. Caudal to these structures, the depressant effects weaken and practically disappear in the spinal cord. Rostrally, excitatory and even convulsant effects appear. By suppressing preferentially the posterior hypothalamic and rostral mesencephalic assembly, the "ergotropic" centers of Hess, the animal is converted to a "trophotrope" with primary emphasis on manintenance functions of the nervous system. The concept is extended to therapeutic changes in schizophrenic patients, but seems to us more relevant to the prompt action of phenothiazines in quieting agitated patients than to the long term extroverting effects of these drugs and reserpine on withdrawn patients, which is a still unexplained but fascinating clinical phenomenon.

According to the report of Desci (111) there is a high correlation between

the *in vivo* action of a wide variety of phenothiazines and other tranquilizers in their ability to inhibit conditioned reflexes and potentiate hexobarbital narcosis and their *in vitro* ability to inhibit oxidative phosphorylation and adenosine triphosphatase activity.

Among properties of the phenothiazines which have been somewhat overshadowed by their central actions, is nicotinic blockade of the neuromuscular junction. Jindal & Deshpande (112) report partial neuromyal block in dogs with systemic administration of tolerable doses of chlorpromazine, promethazine and prochlorperazine.

The effects of phenothiazines and other psychotropic drugs on oxidative metabolism, with particular attention to crucial steps in the electron transport chain, have been discussed by Richter (113).

Of actions of chlorpromazine and other phenothiazines having possible significance for central nervous function, Pletscher & Gey (114) have emphasized their ability to prevent the binding or uptake of various aromatic amines and amino acids by brain. In tissue studies migration of the aromatic amines between granules and cytoplasm was shown to be inhibited.

Another evidence of the stabilizing effect of chlorpromazine is illustrated by Spirtes (115) in a demonstration that this drug can prevent swelling of liver and brain mitochondria by hypotonic and other agents. One wonders whether this is indeed a membrane stabilization or more likely a protein denaturation sufficient to militate against any osmotic phenomena with the drug concentrations used.

Sandberg (116) has reviewed some untoward actions of chlorpromazine and other central nervous system drugs on the fetus and newborn after maternal administration.

Because of the recent teratological disaster with a widely-circulated bland sedative, thalidomide, numerous authors and agencies have become more aware of the need for rigorous animal chronic toxicity studies, extending to observations on the second generation and in various species (117, 118).

In view of the wide notoriety given to fetal toxicity of thalidomide, attention is called to an article by Kuhn & Van Maanen (119) on an experimental analysis of its central nervous system effects. Judging from their data, thalidomide is evidently not a barbiturate-like sedative, has anticonvulsant action similar to that of diphenylhydantoin, and potentiates various types of depressant drugs. It might be classified as a somnifacient. In view of species differences in response to this drug, and its withdrawal from clinical use because of tragic side-effects, the basis of its brief popularity will probably go unknown.

Antidepressives.—Following the superficially successful therapeutic waves of antischizophrenic and antianxiety drugs in the last decade there has been a recent wave of antidepressants, more properly antidepressives, most improperly psychic energizers (since the concept of psychic energy is a metaphysical psychoanalytic concept with no basis in brain chemistry). The

relevant drugs fall into three general classes: amphetaminic; monoamine-oxidase inhibitors typified by iproniazid; weakly atropinic agents, of which imipramine is representative.

None of the antidepressive drugs is clearly an unmixed central stimulant. The older amphetaminic group preserves wakefullness and focuses attention, but with some loss of exploratory locomotion and total uncommitted vigilance both in animals and man. The monoamineoxidase inhibitors, so-called, frequently are based on amphetaminic structure, but do not always have amphetaminic properties in animals unless these are primed by catecholamine precursors, such as dihydroxyphenylanine. The atropinic group represented by benactyzine, more recently imipramine and amitryptylline, have a complex profile of action that almost defies description, combining the mixed excitation and depression of central functions common to atropine and scopolamine with still other features which escape easy description. Obviously these latter drugs must have comparatively selective central nervous actions. It should be noted also that the typical phenothiazines are weakly atropinic.

Most of the so-called antidepressives have in common the ability to oppose the tranquilizing effects of reserpine, as discussed by Chen *et al.* (120). A little thought suggests that if such a drug is antagonist to reserpine after relatively complete depletion of catecholamines and serotonin, its action must be explained as a substitution for the stimulation by the now-depleted catecholamine or serotonin. In the earlier experiments of Everett *et al.* (43), it was evident that reserpine (or deserpidine) antagonism by antidepressives was best demonstrated when the animal was also loaded with dihydroxyphenylalanine (DOPA), presumably the precursor of dopamine. Retrospectively, it is not easy to justify these synergisms as a proof of the excitatory role of dopamine, since they might also imply a facilitating action on DOPA excitation.

Our colleagues Sabelli *et al.* (121) have reported central atropinic actions and various peripheral effector results with amitriptyline that reflect something more than a simple adrenergic-facilitating action of this drug or its parent, imipramine.

Among recent summaries, Levy (122) has reviewed the hydrazide and hydrazine monoamineoxidase inhibitors. Shepherd & Wing (123) have attempted a review based on chemical, psychological and psychiatric aspects of action on the psychotropic drugs. Zbinden & Randall (124) have reviewed animal methodology for the screening of potential psychotherapeutic drugs, including the use of other centrally-acting drugs as background for evaluation of new agents. Baker (125) has written an extensive annual review covering most categories of drugs currently used in neurology and psychiatry. Brodie and co-workers (126) have reviewed the literature on psychotherapeutic drugs. Killam (9) has recently outlined modern trends in research on psychotropic drugs, with emphasis on the need for biochemical-neurophysiological

correlations, and examples of the use of special computer techniques in psychological-electrophysiological correlations. Irwin (127), a devotee of elaborate profiles in the study of drug action on animal behavior, questions whether these give adequate basis for predicting drug action in man. The recent literature concerning drug effects and methodology in animal psychology with some attention also to human studies, has been expertly reviewed by Dews (128) in a previous volume of this annual review. One direction of psychological research with drugs concerns their effect on sleep-deprived subjects performing fatiguing tasks. An example is the report of Holliday & Devery (129) on a comparison of *d*-amphetamine, meprobamate, amitriptyline and placebo with an amphetamine-like stimulant W12-6 (2-ethnylamino-3-phenyl-norcamphane), using a student population. The two amphetaminics significantly enhanced performance, while meprobamate and amitriptyline retarded performance. The results with amitriptyline illustrate that an effective antidepres*sive* agent need not be antidepres*sant*. A difference between caffeine and amphetamine is noted by Dureman (130), in a study comparing these drugs on a student population with another stimulant (5-phenyl-2-amino-4-oxo-oxazolidine, "stimul"). The latter drug was like amphetamine in improving fine motor coordination, but lacked the cardiovascular effects of amphetamine and also did not promote visual motor persistence phenomena.

Another example of detailed psychological analysis is the study of Nash (131) with *d*-amphetamine, phenobarbital, and an amphetamine-barbiturate combination, performed on penitentiary inmates. The amphetamine results illustrate especially a heightening of attention mechanism, which can improve performance in some intellectual tasks while failing to enhance or even reducing performance on complex tasks demanding shifts of attention. Incidentally, this study also showed some differences between caffeine and amphetamine, notably an impairment of hand steadiness test by the former, enhancement by the latter.

Ross *et el.* (132), in a psychological study of the effects of *d*-amphetamine on mood and psychomotor performance in elderly veterans, have attempted an experimental design in which the placebo and drug effects can easily be sorted out. It is interesting to note that the drug group in this study showed unpleasant affective response and impaired motor performance, while the placebo group showed positive affective response. The study shows the importance of factoring out the role of expectation in clinical drug studies.

Jus (133) has reviewed the action of various psychotropic drugs on conditioned reflexes in animals and man, together with some electrophysiological considerations.

Cook & Kelleher (134) have reviewed recent advances in animal psychological methods of drug testing, concluding that such tests have value for the classification of drugs according to behavioral categories.

Werner (135) has recently published an extensive review of the central

stimulant and antidepressant drugs, including evidence from normative psychological studies and from use in depressed patients, as well as in animal investigations, on which he bases a discussion of mechanism of action. Recognizing that some drugs may have both monoamine oxidase inhibitor (MAOI) action and also a direct central stimulating action of amphetamine type not dependent on MAOI action, he raises the question whether MAO and the neural membrane may not have similar receptor sites, accounting for the similar structural requirements of both amphetaminic and MAOI types of drugs. The argument is further extended to non-amphetaminic nonMAOI antidepressive drugs typified by imipramine and amitriptyline. Werner also points out incidentally some findings suggesting that these drugs or the amines which they presumably alter need not act solely upon neurones, but may affect glia, blood vessels, etc. Thus we must still further extend our categories of hypothetical receptor sites upon which the amines might act to modify behavior.

Another example of the differentiation between antidepressives of somewhat comparable clinical worth is illustrated by a report of Rothman *et al.* (136), on a small sample of freshly-admitted hospitalized depressive patients, treated with imipramine, isocarboxazid or placebo in a double-blind study with psychiatric rating and psychological function tests. Although affective improvement was similar in the two drug groups, there was an improvement in cognitive and psychomotor functioning with isocarboxazid but not with imipramine. Placebo effect was not significant. It may be seen that the more amphetaminic hydrazide drug possessed psychological actions which although useful in themselves were not essential to improvement of the depressive state.

The work of Schallek *et al.* (137) illustrates some of the neurophysiological techniques currently employed to define locus and type of action of psychotropic drugs in the brains of experimental animals. Their criteria include rage behavior in rats with septal lesions, threshold and duration of EEG afterdischarge evoked by electrical stimulation of limbic centers, and the limbic and neocortical EEG. The antidepressive imipramine reduces the effects of limbic stimulation, while other antidepressives such as iproniazid and isocarboxazid have the opposite effect.

As an example of a current mode of neurophysiological exploration of psychotropic drug action, the recent studies of Rubio-Chevannier and colleagues (138) may be cited. In their investigations of imipramine, they used chronic electrode implantations for stimulation and EEG recording in a number of brain centers in freely mobile cats. They found that the EEG of the olfactory bulb gave a better correlation with behavioral alertness than did the cortical EEG. Imipramine had a biphasic action, increasing the direct and sensory excitability of the brain-stem reticular activating system at low dosage, depressing them at higher dosage. Rage and autonomic effects evoked by hypothalamic stimulation likewise showed a biphasic threshold change. On the basis of such experiments the clinical antidepressive action of

imipramine is attributed to its low-dosage hypothalamic effect, but the explanation seems oversimplified. Perhaps the greatest merit of this type of study is its potential use in classifying drugs by electrophysiological patterns of localization, rather than in direct proof of clinical mechanism.

The possibility that the actions of imipramine may be mediated through a metabolite, desmethylimipramine (DMI), has been raised by the discovery and investigation of this substance by Gillette and colleagues (139).

Stein & Seifter (140) have used the self-stimulation method of Olds in rats to demonstrate that imipramine can facilitate the threshold-lowering action of metamphetamine on posterior hypothalamus and midbrain tegmentum. The imipramine effect was in turn antagonized by chlorpromazine.

Epilogue.—There is still a wide gap between theory and practice with regard to drugs active on the central nervous system. A physicochemically-based picture of nonspecific central depressants appears hopeful, and convulsant drugs are becoming more explainable in terms of newer studies in synaptic transmission. The greatest current wave of literature concerns tranquilizers and antidepressives which are superficially relevant to autonomic pharmacology, leading to some disappointment that central analogues of autonomic synapses have not yet been found. But even if they were found, it seems unlikely that they would easily explain the diversity of behavioral effects of the psychotropic drugs. One might as readily attempt to explain religious miracles by chemical analysis and bioassay of the waters of Lourdes.

LITERATURE CITED

1. Grundfest, H., in *Biophysics of Physiological and Pharmacological Actions*, 329–89 (Publ. No. 69, A. M. Shanes, Ed., Amer. Assoc. Adv. Sci., Washington, D. C., 1961).
2. Dudel, J., *et al.*, *J. Physiol.*, **155**, 514–29 (1961)
3. Nachmansohn, D., *Science*, **134**, 1962–68 (1961)
4. Eccles, J. C., *Proc. Roy. Soc. (London), Ser. B* **153**, 445–76 (1961)
5. Eccles, J. C., *et al.*, *J. Physiol.*, **159**, 147–66 (1961)
6. Eccles, J. C., *et al.*, *J. Physiol.*, **161**, 237–57 (1962)
7. Eccles, J. C., *et al. J. Physiol.*, **159**, 461–78 (1961)
8. Eccles, J. C., *et al.*, *J. Physiol.*, **161**, 258–81 (1962)
9. Eccles, J. C., Grundfest, H., Prosser, C. L., Killam, K. R. DeRobertis, E., and others in Symposium monograph: *Unfinished Tasks in the Behavioral Sciences* (Chicago Medical School, Chicago, Ill., to be published, 1963)
10. Andersen, P., *et al.*, *Nature*, **194**, 740–43 (1962)
11. Naess, A. K., *Farmakoterapi (Oslo)*, **18**, 40–51 (1962)
12. Curtis, D. R., *et al.*, *J. Neurochem.*, **6**, 117–41 (1960)
13. Curtis, D. R., *et al.*, *J. Neurophysiol.*, **24**, 80–90 (1961)
14. Curtis, D. R., *et al.*, *Brit., J. Pharmacol.*, **16**, 262–83 (1961)
15. Brooks, C. M., Gilbert, J. L., Levey, H. A., and Curtis, D. R. *Humors, Hormones and Neurosecretions* (University Publishers, New York, 1962)
16. DeRobertis, E., *Biochem. Pharmacol.*, **8**, 29 (1961)
17. DeRobertis, E., *et al.*, *J. Neurochem.*, **9**, 23–25 (1962)
18. DeRobertis, E., *et al.*, *Nature*, **194**, 794–95 (1962)
19. Whittaker, V. P., *Biochem. Pharmacol.*, **5**, 392–98 (1961)
20. Sabelli, H. C., and Toman, J. E. P., *Amer. Zool.*, **1**, 468 (1961)
21. Sabelli, H. C., and Toman, J. E. P., *Proc. Fed. Amer. Soc. Exp. Biol.*, **21**, 331 (1962)
22. Sourkes, T. L., *Can. Med. Assoc. J.*, **85**, 487–90 (1961)

23. Barbeau, A., Murphy, G. F., and Sourkes, T. L., *Science*, **133**, 1706–07 (1961)
24. Sourkes, T. L., *Methods in Med. Res.*, **9**, 121–254 (1961)
25. Von Euler, U. S., in *Hormones in Blood*, 515–82 (Academic Press, London, 1961)
26. Karki, N. *et al*, *J. Neurochem.*, **9**, 53–58 (1962)
27. Halpern, B. N., *Actualites Pharmacol.*, **13**, 109–29 (1960)
28. Walascek, E. J., *World Neurol.*, **2**, 602–17 (1961)
29. Downing, D. F., *Quart. Rev.*, **16**, 133–62 (1962)
30. Quastel, J. H., in *Reports, VII Internat. Cong. Neurol.*, 245–80 (Rome 1961)
31. Brodie, B. B., Sulser, F., and Costa, E., *Rev. Canad. Biol.*, **20**, 279–85 (1961)
32. Gyermek, L., *Pharmacol. Rev.*, **13**, 399–439 (1961)
33. Brune, G. G. *et al.*, *Science*, **133**, 190–92 (1961)
34. Aprison, M. H., and Ferster, C. B., *J. Neurochem.*, **6**, 350–57 (1961)
35. Aprison, M. H. and Ferster, C. B., *J. Pharmacol. Exp. Therap.*, **131**, 100–07 (1961)
36. Costa, E., Himwich, W. A., and Himwich, H. E., in *Neuro-Psychopharmacology*, **2**, 475–78 (Elsevier Publ. Co., Amsterdam, 1961)
37. Costa, E., and Brodie, B. B., *Biochem. Pharmacol.*, **8**, 81–82 (1961)
38. Green, J. P., *Advances in Pharmacology*, **1**, 349–422 (1962)
39. Hagen, P., *Canad. J. Biochem.*, **39**, 639–42 (1961)
40. Vane, J. R., Collier, H. O. J., Corne, S. J., Marley, E., and Bradley, P. B., *Nature*, **191**, 1068–69 (1961)
41. Carlsson, A., in *Neuro-Psychopharmacology*, **2**, 417–21 (Elsevier Publ-Co., Amsterdam, 1961)
42. Bertler, A., *Acta Physiol. Scand.*, **51**, 97 (107 (1961)
43. Everett, G. M., and Wiegand, R. G., *Biochem. Pharmacol.*, **8**, 85–92 (1962)
44. Wiegand, R. G., and Perry, J. E., *Biochem. Pharmacol.*, **7**, 181–86 (1961)
45. Wolstenholme, G. E. W., O'Connor, M., and Vane, J. R. (Eds.) *Adrenergic Mechanisms* (Little, Brown & Co., Boston, Mass., 1961)
46. Blaschko, H., 496 (in ref. 45)
47. Boyd, J. D., 490 (in ref. 45)
48. Brown, L., 512 (in ref. 45)
49. Eränkö, O., 490 (in ref. 45)
50. Furchgott, R. F., 498, 511 (in ref. 45)
51. Hagen, P. B., 489 (in ref. 45)
52. Hillarp, N. A., 481 (in ref. 45)
53. Schümann H. J., **10**, 487 (in ref. 45)
54. von Euler, U. S., 324 (in ref. 45)
55. Whittaker, V. P., *Biochem. Pharmacol.*, **8**, 30 (1961)
56. Greenberg, R., and Sabelli, H. C., *J. Pharm. Exp. Therap.* (submitted, 1962)
57. von Euler, U. S., and Lishajko, F., *Acta Physiol. Scand.*, **52**, 137–45 (1961)
58. Weil-Malherbe, H., Posner, H. S., and Bowles, G. R., *J. Pharm. Exp. Therap.*, **132**, 278–86 (1961)
59. Masuoka, D. T., Clark, W. G., and Schott, H. F., *Rev. Can. Biol.*, **20**, 1–5 (1961)
60. Hebb, C., *Nature*, **192**, 527–29 (1961)
61. Welch, R. M., *Proc. Soc. Exp. Biol. Med.*, **107**, 731–34 (1961)
62. Everett, G. M., *Rev. Can. Biol.*, **20**, 278 (1961)
63. Friedman, A. H., *Sites of Action of "Tremorine,"* 152 pp. (Doctoral thesis, Univ. of Illinois, 1959)
64. Kaelber, W. W., *et al.*, *J. Neuropathol. Exp. Neurol.*, **20**, 263–74 (1961)
65. Sabelli, H. C., and Toman, J. E. P., *Excerpta Medica, Intern. Congr. Series*, 48, 1229 (1962)
66. Ernst, A. M., *Nature*, **193**, 178–79 (1962)
67. Domer, F. R., *J. Physiol.*, **154**, 34–35(P) (1960)
68. Stern, P., *Biochem. Pharmacol.*, **8**, 26–27 (1961)
69. Schoetensack, W., and Hallman, G., *Biochem. Pharmacol.*, **8**, 26 (1961)
70. Barbeau, A., *Arch. Neurol.*, **4**, 97–102 (1961)
71. Anichkov, S. V., *Ann. Rev. Pharmacol.*, **1**, 21–28 (1961)
72. Kline, N. S., and Mettler, F. A., *Rev. Can. Biol.*, **20**, 583–87 (1961)
73. Cole, J. O., and Clyde, D. J., *Rev. Can. Biol.*, **20**, 565–74 (1961)
74. Kruse, W., *Current Therap. Res.*, **3**, 19–22 (1961)
75. Myrianthopoulos, N. C., *et al.*, *Arch. Neurol.*, **6**, 5–9 (1962)
76. Metz, B., *Neurology*, **11**, 37–45 (1961)
77. Roberts, E., in *Regional Neurochemistry*, 324–39 (Pergamon Press, New York, 1961)

78. Takahashi, H., Arai, B., and Koshino, C., *Japan J. Physiol.*, **11**, 403–9 (1961)
79. Erspamer, V., *Ann. Rev. Pharmacol.*, **1**, 175–218 (1961)
80. Rafaelson, O. J., *Metabolism*, **10**, 99–114 (1961)
81. Genes, S. G., *Uspekhi Sovrem. Biol.*, **51**, 188–302 (1961)*
82. Crain, S. M., and Wiegand, R. G., *Proc. Soc. Exper. Biol. Med.*, **107**, 663–65 (1961)
83. Capek, R., *Biochem. Pharmacol.*, **8**, 178 (1961)
84. Mihailovic, L., *Nature*, **192**, 665–66 (1961)
85. Goldberg, J., and Toman, J. E. P., Neurology, **10**, 1015–19 (1960)
86. Toman, J. E. P., in *Neurochemistry*, 728–65, (Elliott, K. A. C., Ed., Charles C Thomas, Publ., Springfield, Ill., 2nd. Ed. 1962)
87. Tower, D. B., Luse, S. A., and Grundfest, H., *Properties of Membranes and Diseases of the Nervous System*, (Springer Publishing Co., Inc., New York, 1962)
88. Ling, G. N., *A Physical Theory of the Living State* (Blaisdell Publ. Co., New York, 1962)
89. Pauling, L., *Science*, **134**, 15–21 (1961)
90. Brazier, M. A. B., *Brit. J. Anesthesia*, **33**, 194–204 (1961)
91. Adriani, J., *Chemistry and Physics of Anesthesia*, 2nd. Ed. (Charles C Thomas, Springfield, Ill., 1960)
92. Domino, E. F., *Ann. Rev. Pharmacol.*, **2**, 215–50 (1962)
93. Shideman, F. E., *Clin. Pharmacol. Therap.*, **2**, 313–44 (1961)
94. Sinitsyn, L. N., *Farmakol. i. Toksikol. USSR*, **24**, 259–67 (1961)*
95. Morell, F., and Baker, L., *Neurology*, **11**, 651–64 (1961)
96. Gunn, C. G., Gogerty, J., and Wolf, S., *Clin. Pharm. Therap.*, **2**, 733–49 (1961)
97. Close, W. J. and Spielman, M. A., *Medicinal Chem.*, **5**, 1–349 (1961)
98. Lennox, W. G., and Lennox, M. A., *Epilepsy and Related Disorders*, 2 Volumes (Little, Brown & Co. Boston, Mass., 1960)
99. Forster, F. M., (Ed.) *Evaluation of Drug Therapy in Neurological and Sensory Diseases* (Univ. Wisconsin Press, Madison, Wisconsin, 1961)
100. Bovet, D., and Longo, V. G., in *Regional Neurochemistry*, 456–64 (Pergamon Press, New York, 1961)
101. Longo, V. G., and Corrado, A. P., *Proc. Soc. Exp. Biol. Med.*, **107**, 272–74, 1961)
102. Longo, V. G., *J. Pharmacol. Exp. Therap.*, **132**, 240–44, (1961)
103. Corrado, A. P., and Longo, V. G., *Arch. Intern. Pharmacodyn.*, **132**, 255–69 (1961)
104. Lewin, J., *et al. J. Pharmacol. Exp. Therap.*, **132**, 245–50 (1961)
105. Hahn, F., *Pharmacol. Rev.*, **12**, 447–530 (1960).
106. Adriani, J., *Conn. Med. J.*, **26**, 320–23 (1962)
107. Clemmesen, C.,*et al. Clin. Pharmacol. Therap.*, **2**, 220–29 (1961)
108. Cole, J. O., *Psychopharmacol. Serv. Center Bull.*, **2**, 28–38 (1962)
109. Parkes, M. W., **1**, 72–131, in *Progress in Medicinal Chemistry* (Butterworth, Washington, D. C., 1961)
110. Das Gupta, S. R., *Indian J. Physiol. Pharmacol.*, **5**, 5–18 (1961)
111. Desci, L., *Psychopharmacologia*, **2**, 224–42 (1961)
112. Jindal, M. N. and Deshpande, V. R., *Arch. Intern. Pharmacodynam. Therap.*, **132**, 322–30 (1961)
113. Richter, D., *Neuro-Psychopharmacology*, **2**, 422–34 (Elsevier Publ. Co., Amsterdam, 1961)
114. Pletscher, A. and Gey, K. F., *Biochem. Pharmacol.*, **8**, 82 (1961)
115. Spirtes, M. A., *Nature*, **190**, 274–75 (1961)
116. Sandberg, D. H., *Calif. Med.*, **94**, 287–91 (1961)
117. MacGregor, A. G., and Perry, W. L. M., *Lancet*, **I**, 1233–34 (1962)
118. Beck, F., *Brit. Med. J.*, **II**, 544 (1962)
119. Kuhn, W. L., and Van Maanen, E. F., *J. Pharmacol. Exp. Therap.*, **134**, 60–68 (1961)
120. Chen, G., *et al.*, *J. Pharmacol. Exp. Therap.*, **131**, 179–84 (1961)
121. Sabelli, H. C., Levin, J. J., Seigler, L. O., and Toman, J. E. P., *The Pharmacologist*, **3**, 68 (1961)
122. Levy, J., *Actualites Pharm.*, **14**, 125–96 (1961)
123. Shepherd, M., and Wing, L., *Advan. Pharmacol.*, **1**, 227–76 (1962)
124. Zbinden, G., and Randall, L. O., *Rev. Can. Biol.*, **20**, 251–59 (1961)
125. Baker, W. B., *Progr. Neurol. Psychiat.*, **16**, 95–123 (1961)
126. Brodie, B. B., Sulser, F., and Costa, E., *Ann. Rev. Med.*, **12**, 349–68 (1961)
127. Irwin, S., *Science*, **136**, 123–28 (1962)

128. Dews, P. B., *Ann. Rev. Pharmacol.*, **1**, 145–74 (1961)
129. Holliday, A. R., and Devery, W. J., *Clin. Pharmacol. Therap.*, **3**, 5–15 (1962)
130. Dureman, E. I., *Clin. Pharmacol. Therap.*, **3**, 29–33 (1962)
131. Nash, H., *J. Nervous Mental Diseases*, **134**, 203–17 (1961)
132. Ross, S., Krugmen, A. D., Lyerly, S. B. and Clyde, D. J., *Psychol. Repts.*, **10**, 383–92 (1962)
133. Jus, A., *Neurol. Neurochir. Psychiat. Polska*, **11**, 479–87 (1961)
134. Cook, L., and Kelleher, R. T., in *Neuro-Psychopharmacology*, **2**, 77–92 (Elsevier Publ. Co., Amsterdam, 1961)
135. Werner, G., *Clin. Pharm. Therap.*, **3**, 59–96 (1962)
136. Rothman, T., Grayson, H., and Ferguson, J., *J. Neuropsychiat.*, **2**, 158–62 (1961)
137. Schallek, W., Kuehn, A., and Naitan, J., *Ann. N. Y. Acad. Sci.*, **96**, 303–12 (1962)
138. Rubio-Chevannier, H., Bach-y-Rita, G., Penaloza-Rojas, J., and Hernandez-Peon, R., *Exptl. Neurol.*, **4**, 214–20 (1961)
139. Gillette, J. R., Dingell, J. V., Sulser, F., Kuntzman, R., and Brodie, B. B., *Experientia.*, **17**, 417–18 (1961)
140. Stein, L., and Seifter, J., *Science*, **134**, 286–87 (1961)

* English translation will be announced in *Technical Translations*, issued by the Office of Technical Services, U. S. Department of Commerce, and will be made available by the Photoduplication Service, Library of Congress, and by the SLA Translation Center at the John Crerar Library, Chicago, Illinois.

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