BIOCHEMICAL MECHANISMS OF DRUG ACTION[1]

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In this review an attempt is made to focus upon the biochemical effects of certain drugs which, according to the authors' prejudices, may reasonably correlate with pharmacological activity and therapeutic usefulness; upon some drug-induced alterations in biochemical processes which have been attempted in order to produce interesting and, hopefully, useful changes in physiological function; and upon studies in areas which are judged pertinent to the investigation and eventual understanding of biochemical mechanisms.

Chemical Identity of Receptors[2]

An understanding of the chemical nature of receptors is of utmost importance in elucidating the mode of action of many drugs, and yet progress in this area has been slow. Woolley & Gommi (185), extending their investigations into the phospholipid nature of serotonin receptors, destroyed the responsiveness of rat uterus horn or strips of rat stomach by treating the tissue with neuraminidase and EDTA. Sensitivity of the tissue to serotonin was restored partly by the addition of gangliosides to the bath. Larger concentrations of the inactivating enzyme solution decreased also the responsiveness of the tissue to acetylcholine. Woolley & Gommi (186, 187) have designed two types of assay for materials they term "receptor lipids." In one procedure, rats were fed large quantities of galactose, on the thesis that the sugar would inhibit the enzymes responsible for the synthesis of serotonin receptors. However tenuous this assumption may be, strips of stomach from these animals were less sensitive than normal to serotonin but fully responsive to acetylcholine. Addition of graded concentrations of "serotonin receptor lipid" to the stomach strips from galactose-fed rats, followed by measurement of the response to serotonin, allowed estimation of the efficiency of the lipid in restoring tissue sensitivity. The second type of assay is similar to the first, but stomach strips from normal rats were treated with neuraminidase and EDTA to destroy sensitivity to serotonin prior to restoration of the

[1] The survey of the literature pertaining to this review was concluded in July 1966.

[2] Use of the term "receptor" in both biochemical and physiological senses is unfortunate but apparently unavoidable. It should be borne in mind that, while physiological receptors indeed may also be biochemical receptors, all biochemical receptors do not fall into the former category in the traditional sense of the word. To cite one case in point, Barrett (8) has stated, " . . . it would appear that neither this (hyperglycaemic response) nor the fat mobilizing effect of the catecholamines can be defined as exactly as the cardiovascular responses in terms of receptor types." Here, indeed, a physiological receptor is probably not involved, but in the following the physiological connotation will be adhered to. Discussions of the semantic ambiguities encountered in the use of the term "receptor" have appeared in recent reviews (1, 46, 95).

sensitivity by the addition of lipids. While the approach of Woolley & Gommi to the problem is of considerable interest, it is premature definitely to identify materials capable of restoring the sensitivity of the tissue to serotonin with "receptor substances." In this connection, it is pertinent to note that, although stomach strips from galactose-fed rats lost sensitivity to serotonin, uteri from these animals were reasonably responsive to the amine. No doubt, the receptor is a complex structure, the composition and organization of which can hardly be revealed by a single, relatively simple technique.

Dikstein & Sulman (35) have also attempted to identify materials having "receptor-like" activity by an approach somewhat similar to that of Woolley and co-workers. They washed rectus abdominis muscle from the toad, *Bufo viridis*, with 25 per cent acetone, which was found to destroy the tissue's responsiveness to acetylcholine. The slow return of sensitivity of the tissue was greatly accelerated by the addition of phosphatidylserine or phosphatidylethanolamine to the bath. Moderate success was achieved also in similar experiments with rabbit uterus and its responsiveness to norepinephrine. Unfortunately, the specificity of the effect of acetone treatment on phosphatidylserine and phosphatidylethanolamine was not examined.

These same investigators (36), working with rabbit aorta *in vitro*, found competition between Dibenamine and epinephrine in binding to tissue cephalins. While these experiments do not prove that the chemoreceptors involved are composed either entirely or even partly of phospholipids, it seems probable that the lipids play an important part in neuromuscular function, and this should be considered whenever the mechanism of drug action is sought.

Beychok (13) re-examined the so-called acetylcholine receptor which has been prepared from the electric organ of *Electrophorus electricus*. The material was found to be quite inhomogenous; and this, coupled with the fact that the substance had no greater affinity for *d*-tubocurarine than did several other tissue proteins, makes it unlikely that the preparation is indeed the cholinergic receptor substance.

Although the conclusions reached may not be entirely justified, the work reported by Takagi et al. (168) provides another interesting approach to the study of receptors. Canine intestinal smooth muscle was exposed to atropine to mask acetylcholine receptors, and then with unlabeled Dibenamine to mask other receptors and nonspecific binding sites. The atropine was washed out, and then H^3-Dibenamine was added to the bath, thus labeling the acetylcholine receptors. Particulate fractions, rich in tritium, were obtained from homogenates of the tissue, and the suggestion was made that the receptor may be protein-like. Further work will be needed to demonstrate this conclusively, however.

Drug-Receptor Interactions

A number of papers have appeared in which physical measurements and mathematical treatment were applied in predicting drug-receptor inter-

actions and, thus, pharmacological activity. Although the material may be more appropriate in the realm of structure-activity relationships, a sampling of these papers will be included here because of their bearing upon the subject of biochemical mechanisms. Hansch and co-workers (45, 67–71, 82) have correlated partition coefficients in series of compounds with biological activity, and then calculated the partition coefficient of the hypothetical compound of a given series which would influence optimally the biological function under scrutiny. This treatment, which will become more sophisticated as ionic interaction effects are explored more thoroughly, may provide a valuable guide in the design of more effective drugs. Perhaps of more interest, however, is the principle implicit in the cited work, that compounds must cross diverse barriers to elicit a physiological response. Obviously, complex mathematical treatment will be necessary to describe adequately the overall action of drugs.

Free & Wilson (42) developed a mathematical model for assigning numbers to the influence of molecule substituents in drug series upon biological potency. Purcell (133) applied the principles set forth to a series of 3-carbamoylpiperidines and found the method useful in predicting anticholinesterase activity. Using data selected from the literature, Portoghese (132) showed that the analgesic activity of N-substituted phenylpiperidine derivatives of different series (i.e., one substituent different) changed in parallel fashion when common substitutions were made at another position. Logarithmic plots of potencies yielded straight lines with slopes essentially unity, which may correlate with similar modes of binding of the different series. This approach might prove of value in uncovering common modes of action among drugs.

In a brief review article, Schnaare & Martin (143) discussed the potential value of applying quantum mechanical calculations of the electronic characteristics of molecules in the prediction of drug-receptor interactions, an approach which may develop into one of considerable value in the study of biochemical mechanisms and in the design of new drugs. Neely (124), using molecular orbital calculations, estimated the sum of electron deficiencies at the C-O position of a number of phenols, values which are proportional to pK numbers and stretching vibration frequencies. All of these measure the phenolic dissociation tendency and correlate with the affinity of the N-methyl carbamates and phosphoramidates of the phenols for the esteratic site of cholinesterase. Thus, the numbers are a measure of the potencies of these derivatives as enzyme inhibitors. On the basis of data derived from nuclear magnetic resonance studies, Jardetzky & Wade-Jardetzky (83) concluded that the *p*-aminobenzenesulfonamide moiety of simple N-1 derivatives is the primary binding site of the compounds with bovine serum albumin. The suggestion is that nuclear magnetic resonance measurements may be of utility in elucidating the structure and function of drug-receptor complexes.

Several years ago Paton (128) suggested that agonist action may not be a function of the concentration of the drug-receptor complex, but of the turn-

over rate of the complex, a concept which has received adequate discussion elsewhere (20, 116). Burgen (19) has proposed a theoretical treatment for drug-receptor kinetics, the utility of which cannot be assessed until wider application of the developed equations has been attempted.

Bebbington et al. (12) studied the muscarinic activity of compounds related to oxotremorine and of choline esters, using as criteria of potency the contraction of isolated guinea pig ileum and hypotension in anesthetized cats. From the structure-activity relationships observed, it was concluded that activity of this type depends upon a three-point interaction between the compound and the acetylcholine receptor.

Using radioactive compounds, Paton & Rang (129) studied the kinetics of uptake and washout of atropine in guinea pig intestinal smooth muscle. The rates observed did not correlate with those for receptor blockade if a single binding site were postulated. Computer analogue studies suggested that four binding sites in series, only one of which is in contact with the surrounding medium, would explain the data.

According to Rang (135), the rate-limiting step in recovery of smooth muscle sensitivity to acetylcholine after treatment with atropine or hyoscine is dissociation of the receptor-inhibitor complex, rather than diffusion.

Acetylcholine

Triethylcholine and troxonium tosylate, like hemicholinium No. 3, inhibit the synthesis of acetylcholine without interfering with the acetylation of choline (15). Bull & Hemsworth (17) also found triethylcholine to inhibit the synthesis of acetylcholine *in vitro* (brain homogenate or slices); they favor the explanation that the drug prevents access of choline to subcellular particles which contain choline acetyltransferase, a mechanism which has been proposed earlier for the similar effects of hemicholinium (109), and is considered as a possible mode of action for triethylcholine and troxonium tosylate by Bhatnagar et al. (15). Hemicholinium, triethylcholine, tetraethylammonium, and troxonium depress the elaboration of acetylcholine by the stimulated superior cervical ganglion of the cat, although none prevented release of the neurotransmitter (111), and therefore exert their presynaptic ganglionic effect by inhibition of acetylcholine synthesis. The observation is of interest that the ability of the quaternary ammonium compounds to interfere with presynaptic acetylcholine turnover does not parallel their ability to interfere with the action of acetylcholine postsynaptically; i.e., that the structural requirements differ for these two functions.

Holan (75) attempted to correlate anticholinergic (versus Ditran) activity in dogs with the ability of some compounds to inhibit cholineacetylase *in vitro*. The active substances, viz., *p*-phenylmandelic acid, 3-phenanthrylglycolic acid, and benzilic acid, were not very potent, concentrations of 1 mg/ml or higher being required for effective enzyme inhibition. However, the principle seems to be valid and may prove useful in further studies. In this connection, it should be noted that lipid-soluble cholinolytic and cholino-

mimetic compounds have been shown to have greater central nervous system effects than water-soluble substances (72, 73).

The nonidentity of cholinergic receptors and cholinesterase (4, 34) has been confirmed (90) by the results of studies on the comparative activities, as anticholinergics and as acetylcholinesterase inhibitors, of compounds acting at the anionic site, at the esteratic site, and at both sites of the enzyme.

The structural similarity between acetylcholine and local anesthetics (2) was reconsidered by Bartels (9) who employed the monocellular electroplax preparation to determine the effects of compounds upon cell potentials. In the series of compounds studied, a gradation of properties was noted; i.e., from acetylcholine, purely a synaptic activator, to tetracaine methiodide, a synaptic inhibitor which blocks excitable membranes without depolarization. Benzoylcholine, intermediate in the series, possessed both properties. It appears that the structural modification of acetylcholine to yield compounds with local anesthetic activity involves changes which permit access of the molecules to the conducting membrane (10). The tertiary amine analogue of benzoylcholine, 2-dimethylaminoethylbenzoate, is purely a blocking agent, i.e., a receptor inhibitor. The relative order of activity of this compound and its sulfur and selenium isologues were the same upon squid axon and the electroplax junction. Thus, the receptor for acetylcholine seems to be similar in the axon and at the nerve junction, which suggests that it may be a specific entity (136).

Kinetic studies on the hydrolysis of acetylcholine and other acetic acid esters in the presence of acetylcholinesterase led Krupka (96) to the conclusion that a common intermediate, an enzyme-acetate combination, is formed during hydrolysis. Deacetylation of the complex apparently limited the rate of reaction with acetylcholine, acetylthiocholine, and phenylacetate. Triethylammonium, a noncompetitive inhibitor, formed inactive complexes with both the enzyme and the enzyme-acetate complex. Kellett & Hite (89) examined the ability of quaternary ammonium compounds to inhibit acetylcholinesterase *in vitro*. By using not only straight chain alkyl compounds, but also the comparatively rigid molecules derived from 1-azabicyclo(2,2,2) octane, and comparing K_i values with calculated ionic volumes, it was concluded that the affinity of quaternary ammonium ions for the anionic site of the enzyme is enhanced by symmetry and compactness of form, by the presence of *n*-propyl groups, and by maximal ionic volumes (i.e., ca.110–150 Å). However, the forces which operate in determining the affinity of the inhibitor for the enzyme are interdependent, and at present only gross criteria for size and shape of the quaternary ion can be stipulated to predict potency (88).

Solter (153), also working with rigid-structure analogues of acetylcholine and choline, found certain quinuclidine derivatives to bind more tightly than substrate to cholinesterase. Presumably, these compounds must undergo less conformational change than acetylcholine in binding to the enzyme and thus form a complex with it more easily.

Neither enzyme inhibitor complex formation nor enzyme carbamoylation

by itself satisfactorily explains certain aspects of the inhibition of cholinesterase by carbamates. O'Brien (126) has proposed a dual mechanism in which complex formation is followed by carbamoylation of the enzyme, and then decarbamoylation to regenerate the enzyme. The process seems to be quite similar to that proposed for esterase inhibition by organophosphates (110). Spontaneous dealkylation of cholinesterase-organophosphorus inhibitor complexes proceeds according to first order kinetics (31, 41), and seems to be associated closely with, if not identical to, the "aging" process. Coult & Marsh (31) pointed out that any allylmethylphosphonofluoridate in which the allyl group easily forms carbonium ion would produce a rapidly dealkylating inhibitor-enzyme complex; thus, poisoning with such a compound would be resistant to therapeutic treatment with oximes and atropine.

The possibility of cholinergic mechanisms being involved in reserpine-induced tissue catecholamine depletion was considered by Stitzel et al. (161). Hemicholinium No. 3 was found not to affect the depletion of brain and heart norepinephrine following reserpine administration to rabbits. However, amine depletion of the adrenal glands, after not only reserpine but also insulin or morphine, was significantly inhibited by the administration of hemicholinium, and this inhibition was reversed by choline. This, together with the fact that none of the depleting agents influenced catecholamine concentrations in the acutely denervated adrenal, shows the reserpine effect upon the gland to be mediated through central cholinergic activation, a conclusion in agreement with the earlier work of Holzbauer & Vogt (76).

Anti-inflammatory

Whitehouse and his collaborators have continued their vigorous attack upon the problem of the biochemical mechanism of anti-inflammatory drugs. Whitehouse (179) has presented an exhaustive review of the biochemical and pharmacological properties of anti-inflammatory substances, and also discussed biochemical misfunctions associated with rheumatic disease, the influencing of which might correlate with therapeutic effects. Skidmore & Whitehouse (149), Whitehouse & Dean (182), and Whitehouse & Boström (180) reported studies on the ability of a variety of compounds to uncouple oxidative phosphorylation, compounds which have anti-inflammatory activity per se or through metabolic conversion to other substances. Whitehouse & Skidmore (183) related three apparently diverse biochemical properties of acidic anti-inflammatory agents to their ability to bind lysyl-epsilon-amino groups of enzymes involved. They proposed the use of reversal of aldehyde binding to protein as a correlate of anti-inflammatory activity (150). Preliminary data have been published (181) suggesting that some basic anti-inflammatory drugs, like acidic ones, may inhibit certain proteases and thus protect connective tissue from degradation during the inflammatory process. Mizushima et al. (113–115) also suggested that the effects of nonsteroid anti-inflammatory agents upon enzymes may, at least in part, depend upon their interaction with proteins.

In the aggregate, the work of Whitehouse, Skidmore and their co-workers

nicely illustrates the process of gleaning a reasonable mechanism from the chaff of apparently unrelated biochemical facts. Whether or not the proposed mechanism proves to be correct, the deductive approach used is a valuable one in general, and may ultimately disclose the mechanism of action of many drugs. As stated by Giarman & Freedman (49), "An approach which focuses on the biochemical sequelae of drug administration provides an initial grip upon the problem and a tangible path to possible basic control mechanisms."

Wallace (176), working with leukocytes *in vitro* and with human subjects, showed that, while colchicine and trimethylcolchicinic acid are similar in therapeutic effectiveness, only colchicine inhibited the production of acid (presumably lactic) by leukocytes. However, Howell (78) found that colchicine inhibited phagocytosis of labeled urate microcrystals, and that the utilization of glucose, stimulated during the process, was inhibited by colchicine. The suggestion was made that colchicine may exert its therapeutic effect, at least in part, by interrupting the inflammatory response-urate deposition cycle, a process considered operational in gout (52, 145). Rajan (134) arrived at a similar conclusion, having observed that colchicine had a stabilizing effect upon lysosomes, thus preventing the release of inflammation-producing hydrolases. Allen et al. (3) studied the rate of growth of sodium urate crystals *in vitro*, with the objective of discovering agents which would modify axial growth and thus the formation of pain-producing needles. Although the two inhibitors which they found, bismark brown and methylene blue, seem to promise little therapeutically, the approach may prove rewarding.

A series of papers summarizing the clinical use of allopurinol, a xanthine oxidase inhibitor, was published in the October, 1965, issue of *Arthritis and Rheumatism* (77, 92, 94, 152, 188, 189). The compound is capable of lowering plasma uric acid concentrations and, although concern has been expressed that xanthine, which is no more soluble than uric acid, might accumulate and crystalize out *in vivo*, such an event has not been observed. Increases in urine xanthine and hypoxanthine excretion caused by the drug generally did not match the decrease in uric acid excretion (188). The explanation for this is not available, but it seems possible that correlary metabolic routes or feedback inhibition of pathways to hypoxanthine could be involved [see McCollister et al. (108)].

In gouty patients characterized by overproduction of uric acid, the administration of azathiopurine reduced uric acid excretion, plasma urate concentrations, and C^{14}-glycine incorporation into uric acid to normal levels (154). Since the drug does not influence purine metabolism in normal subjects, it was suggested that it inhibits a shunt mechanism which is operative in hyperuricemic gout.

Tranquilizing Agents

The effect of chlorpromazine upon body temperature has been known for a long time, and Kollias & Bullard (93) obtained evidence that "chlorpromazine abolishes all mechanisms of temperature regulation for both heat

and cold." It is becoming more and more apparent that many of the previously reported effects of chlorpromazine *in vivo* were the result of hypothermia rather than a direct effect of the drug. For example, the increased incorporation of labeled amino acids into tissues, and the increased concentrations of NAD and nicotinamide which have been observed in chlorpromazine-treated animals are related to hypothermia (146), as is also the prevention by chlorpromazine of iproniazid acceleration of serotonin accumulation in the brain (11). Fortunately, animals which have received chlorpromazine can be maintained in an isothermic condition if they are kept in an environment of elevated temperature, and most *in vivo* work is now done with animals so maintained.

Incorrect conclusions may be drawn from biological data with chlorpromazine if the drug's well-known effect upon membranes [see (60) and (159) for references to earlier work] is ignored. Guth & Spirtes (62), in a review entitled "The Phenothiazine Tranquilizers: Biochemical and Biophysical Actions," pointed out that enzyme inhibition observed with membrane-endowed systems (i.e., mitochondria, tissue slices, and homogenates) might well be due to the effect of chlorpromazine on permeability rather than on the enzyme per se. The membrane effect of chlorpromazine may also explain its favorable action in certain types of shock, according to Guth et al. (61). It was found that chlorpromazine inhibited the spontaneous liberation of acid phosphatase and protein from incubated liver lysosomes, and *in vivo* prevented the loss of liver acid phosphatase after the administration of vitamin A or *Escherichia coli* endotoxin, both of which labilize lysosomes. Presumably, the effect of chlorpromazine in shock would depend upon its ability to prevent the liberation of hydrolytic enzymes from lysosomes. Medina et al. (112) observed structural changes in mitochondria (guinea pig heart sarcosomes) after treatment with chlorpromazine as well as with other phenothiazines. They suggested that the observed alterations may explain, at least in part, the effects of phenothiazines upon mitochondrial oxidative processes.

The involvement of chlorpromazine in adrenergic mechanisms seems to be established beyond reasonable doubt. Increased concentrations of catecholamine metabolites have been found in the brains of animals treated with chlorpromazine. Using the phenothiazine in addition to a monoamine oxidase (MAO) inhibitor (nialamide), Carlsson & Lindqvist (23) found increases in concentrations of 3-methoxytyramine and normetanephrine to be greater when the drug combination was used than when the MAO inhibitor alone was administered. Gey & Pletscher (48) demonstrated increased amounts of catechol C^{14}-carboxylic acids in chlorpromazine-treated rats which had been given 2-C^{14}-DOPA.

Andén et al. (6) found increased concentrations of 3,4-dihydroxyphenylacetic acid and homovanillic acid in the corpus striatum of rabbits which had received chlorpromazine. Laverty & Sharman (101), although unable to demonstrate a correlation between brain area levels of norepinephrine,

serotonin, and metabolites of these with behavioral effects of certain excitants and phenothiazine tranquilizers, did observe an effect of the phenothiazines on DOPAmine metabolism. It is of interest that thioridazine, a phenothiazine which has been reported to produce little Parkinson-like sequelae in man, differed from the other three compounds tested. A single dose of thioridazine, unlike chlorpromazine, did not deplete the caudate nucleus of DOPAmine and, after repeated administration of thioridazine, changes in homovanillic acid, seen initially, disappeared, again in contradistinction to the three other phenothiazines. Also, thioridazine caused depletion of hypothalamic norepinephrine, while chlorpromazine did not. Although the exact mechanism for this effect of chlorpromazine is not known yet, the findings may be attributed to a compensatory activation of monoaminergic neurons after blockade of receptors, as proposed by Carlsson & Lindqvist (23), or to decreased storage of newly formed amines, as suggested by Gey & Pletscher (48). Reduced elimination rate and increased synthesis of the acid metabolites, as proposed by Andén et al. (6) may also be involved. More distinctive data are needed to determine whether one or more of these mechanisms are responsible for the observed effects.

Thoenen et al. (173) interpreted pharmacological evidence to explain peripheral effects of varying doses of chlorpromazine. At low doses, the drug not only inhibits the uptake of norepinephrine into nerve endings but also blocks α-adrenergic receptors. These two effects may balance one another so that norepinephrine potentiation is not produced. At higher doses of chlorpromazine, α-adrenergic blockade predominates, but also a third factor enters, namely a depression of norepinephrine release following electrical stimulation of the nerve. Thus, a distinction can be made between chlorpromazine on the one hand and compounds exemplified by cocaine and imipramine on the other. With the latter drugs, α-adrenergic blockade occurs only at high doses and, thus, potentiation of the peripheral effects of sympathetic nerve stimulation can be observed readily (63, 170) at lower doses. The latter potentiation, which is discussed more fully elsewhere in this review, is probably due to interference with reuptake of norepinephrine at sympathetic nerve endings, which is a function of both chlorpromazine and the antidepressants.

Antidepressants

The mode of action of antidepressants, and their clinical pharmacology, was discussed by Klerman & Cole in 1965 (91). Decsi (33) presented an exhaustive review of the "Biochemical Effects of Drugs Acting on the Central Nervous System." This title is an understatement of the scope of the paper, for the author not only searched the literature thoroughly, but also made an admirable attempt to disclose common biochemical effects of compounds with similar physiological activities in order to arrive at reasonable hypotheses concerning modes of action. Decsi's review, written in English, is highly recommended to those interested in the field.

The biochemical basis for antidepressant action remains elusive, and it seems that only when the phenomena of membrane transport and mental function are both thoroughly understood can the problem be resolved successfully. However, the store of knowledge impinging on the main theme continues to increase rapidly.

Further evidence has appeared refuting the idea that imipramine-like compounds function as anticholinergic agents (65, 158), but their involvement in adrenergic mechanisms has received additional support. Bunney & Davis, in a review entitled "Norepinephrine in Depressive Reactions" (18), cited data, published through 1964, favorable to the view that these drugs function centrally by blocking norepinephrine uptake at adrenergic nerve endings. It is probable that interference with uptake extends to substances, other than the naturally occurring catecholamines, which are also transported into central as well as peripheral adrenergic neurons. This concept has been invoked to explain certain actions of imipramine-like compounds [e.g., see Stone et al. (163)].

Working with spinal cats, Haefely et al. (64) were able to correlate clinical antidepressant effects of imipramine and related compounds with their influence upon the peripheral effects of norepinephrine, liberated by cervical sympathetic nerve stimulation or administered to the animals. Potentiation phenomena observed with low doses of the compounds were attributed to their affinity for and blockade of the access of norepinephrine to its binding sites, while the antagonism seen with higher doses was presumed to be related to affinity for receptors, e.g., α-adrenergic blocking actions. Evidence from experiments with the perfused cat spleen (170) dealing with the effect of imipramine and a related substance (Ro 4-6011) upon sympathetic nerve stimulation-induced release of norepinephrine, and upon uptake of the catecholamine from the perfusion fluid, contributed to this interpretation. The observations on the cardiovascular effects of desmethylimipramine in the dog (86, 87) are consistent with these views.

Desmethylimipramine was shown to block the pressor responses to tyramine, amphetamine, and guanethidine, and to reverse the effect of bretylium (32). This, together with other evidence in the literature, was taken to show that the antidepressant may prevent the release of norepinephrine at nerve endings caused by indirectly acting sympathomimetic amines, but not the release initiated by nerve impulses. Of course, the data can be explained equally well if it is assumed, as discussed above, that desmethylimipramine blocks the entry of the various agents into the neurons, rather than affecting the egress of norepinephrine (26, 47, 137). Direct evidence for this is provided by the report that, in the reserpinized dog, desmethylimipramine reduced the restorative effect of norepinephrine on the response to tyramine, and the small uptake of norepinephrine by the hearts of reserpinized animals was prevented by the drug (86).

The effects of protriptyline upon peripheral autonomic function closely resemble those of imipramine and other related compounds (22, 163). The

data of Cairncross (22) showed protriptyline to potentiate the peripheral actions of nerve impulse-released norepinephrine, as well as those of administered norepinephrine, and to antagonize the effects of guanethidine and of tyramine. Since cocaine antagonized tyramine but not guanethidine, these workers postulated that protriptyline has a dual action, acting both intracellularly, like guanethidine, and on the cell membrane, like cocaine, in preventing the passage of catecholamine. Costa et al. (30), quoting then (July 1965) unpublished work of Tozer, Neft, Hammer & Brodie, stated that desmethylimipramine affects the permeability of the granule membrane. However, Carlsson & Waldeck (25) and Ross & Renyi (138) interpreted their data to show that desmethylimipramine inhibits the passage of amines across the cell membrane. Perhaps different experimental conditions provided divergent answers in this instance, and further work is needed to clarify the point. Dresse & DeMayer (37), in a series of neuroleptics including butyrophenone and phenothiazine derivatives, found some compounds to prevent the passage of norepinephrine from the cytoplasm into intracellular granules in rat brain. They distinguished between two classes of compounds, viz., compounds acting primarily at the intracellular granule membrane level and compounds acting primarily at the cell membrane level. Perhaps it is not premature to conjecture at this point that the preponderance of one type of activity for a compound might depend upon many factors and that, at this early stage of knowledge, substances should not be categorized without reservation.

There is general agreement, nonetheless, that imipramine and similar compounds block the entrance of norepinephrine into neurons somewhere between the exterior of the cells and the interior of the granules; and, as already mentioned, other compounds, i.e., catecholamine depletors, likewise probably are denied ingress, thus explaining a number of the actions of the antidepressants.

Santi & Fassina (142) showed that, in the rat, rather large doses (25–50 mg/kg) of imipramine, desmethylimipramine, and amitriptyline produced 60 to 80 per cent increases in plasma free fatty acid concentrations, the effect being a delayed one, showing a peak some 150 minutes after drug administration. Presumably, an accumulation of catecholamines, resulting from release from the adrenal medulla, the sympathetic nerve endings, or both and prevented from re-entry into the tissues by the drugs, would explain this observation.

Some experiments upon hyperpyrexia resulting from the administration of monoamine oxidase inhibitors followed by antidepressants (105) should be mentioned, since follow-up studies may be expected to yield interesting data upon mechanisms. Imipramine, given after tranylcypromine, phenelzine, or nialamide, consistently produced fatal hyperpyrexia. Amitriptyline interacted in this way with phenelzine and nialamide, but not with tranylcypromine; and trimipramine, a side-chain-methylated derivative of imipramine, did not produce hyperpyrexia when administered in conjunction

with any of the three monoamine oxidase inhibitors. However, the three imipramine-like drugs had similar potencies in reversing reserpine- or chlorpromazine-induced ptosis. While it is true that the monoamine oxidase inhibitors may have other types of activity in addition, nonetheless there seems to have been revealed a subtle difference among the closely related antidepressants. The complexities and uncertainties of interpreting experiments of this sort can be emphasized by citing, in addition, the work of Loew & Taeschler (103). Imipramine, desmethylimipramine, noramitriptyline, and dibenzepine, given to rabbits 30 minutes prior to the administration of 5-hydroxytryptophan, potentiated the hyperthermia caused by the latter compound. Amitriptyline was ineffective under similar conditions, but did potentiate 5-hydroxytryptophan hyperpyrexia if given to the animals 18 hours earlier, presumably because of its metabolism to noramitriptyline.

In this connection, it is pertinent to note that Bickel & Brodie (16), studying a large series of compounds, concluded that the difference in structure between tranquilizing and antidepressant compounds may be only a change from a tertiary to a secondary amine. Interestingly, this conversion can be accomplished *in vivo*. Also of importance is the observation (50) that clinically useful antidepressants partially blocked the uptake of H^3-norepinephrine by the animal brain after intraventricular injection of the catecholamine, whereas neither chlorpromazine nor inactive structures related to the antidepressants did so.

At present, the diametrically opposite clinical utility of the chlorpromazine-related tranquilizers and the imipramine-like antidepressant compounds seems to depend upon quantitative differences in pharmacological effects of compounds which are qualitatively quite similar in action. A plausible statement of the circumstances, which is attributable to Haefely, Hürlimann & Thoenen (63), was set down in the last paragraph of the section on tranquilizers.

Biosynthesis of Catecholamines

Although no attempt can be made to treat the subject adequately here, work in the field has been extensive and cannot be ignored, particularly since two large areas of pharmacology are involved, namely cardiovascular and central nervous system function.

The second step in the metabolic conversion of tyrosine to norepinephrine, i.e., the decarboxylation of dihydroxyphenylalanine to yield dihydroxyphenethylamine, is generally thought to be not rate-limiting. Therefore, attention is focused upon the two oxidation, or hydroxylation, steps in the series. Of these two, the *m*-ring hydroxylation of tyrosine to yield dihydroxyphenylalanine seems to be the more important (rate-limiting) one, at least in the adrenal medulla and in the heart (102). However, the β-hydroxylation of dihydroxyphenethylamine to yield norepinephrine is also of interest, particularly because inhibition of this conversion can lead to lower concentrations of norepinephrine, but unchanged or higher concentrations of dihy-

droxyphenethylamine in the brain, where the β-hydroxylation step may be rate-limiting (85). Musacchio et al. (119) have suggested that although β-hydroxylation may not be rate-limiting normally, it may become rate-limiting under certain conditions, i.e., of drug administration.

Tyrosine hydroxylase.—Nagatsu et al. (122) purified and characterized a soluble tyrosine hydroxylase from beef adrenal medulla. The enzyme was detected in brain, adrenals, and sympathetically innervated tissues of several species, and tetrahydrofolic acid was shown to stimulate enzyme activity. Other pteridines can act as cofactor, as was reported by Ellenbogen et al. (38). Von Studnitz (164) showed the enzyme to be present in several human tissues, including adrenal, neuroblastoma, liver, and brain. In the rat brain, norepinephrine reappears as the effect of enzyme inhibition (see below) wears off, more rapidly in cell bodies than in nerve terminals, suggesting localization of the enzyme in the cell bodies (5).

Compounds such as α-methyltyrosine, its 3-iodo and 3,5-diodo derivatives, and 3-iodotyrosine are competitive inhibitors of tyrosine hydroxylase *in vitro* (58, 81, 174). *In vivo*, these compounds cause decreases in tissue catecholamine concentrations (54, 157) without affecting brain serotonin; and, pharmacologically, they produce sedation, ptosis, muscle flaccidity, and reduce motor activity (156, 157). α-Methyltyrosine apparently does not influence catecholamine storage (29), but exerts its effects principally if not wholly through the inhibition of tyrosine hydroxylase.

Electrical stimulation of the nerve to the isolated spleen of the cat resulted in the appearance of less norepinephrine in the perfusate, and less contraction of the organ, if the animals were given α-methyltyrosine prior to the experiments (172). α-Methylnorephinephrine was detected in the tissue and in the perfusates, but the amounts were small, and the effects of α-methyltyrosine were considered to be caused mainly by tyrosine hydroxylase inhibition. The amino acid had a similar effect on the nictitating membrane, but only when given in quantities greater than required to affect the spleen, presumably because the turnover rate of norepinephrine differs in the two organs.

α-Methyltyrosine exhibits sedative activity in rats and also blocks the behavioral effects of amphetamine, the block being reversed by monoamine oxidase inhibitors (177). Presumably, amphetamine releases norepinephrine from a labile pool in nerve endings which is filled rapidly by newly synthesized norepinephrine, but only slowly from the stable pool (178). In the dog, α-methyltyrosine reduces the blood pressure response to tyramine or to carotid occlusion (14) and in man the synthesis of catecholamines is depressed by the amino acid (148).

Feedback depression of catecholamine synthesis, presumably by the inhibitory effect of the catecholamines on tyrosine hydroxylase, has been proposed as a mechanism involved in the mode of action of monoamine oxidase inhibitors (125).

For further discussion of tyrosine hydroxylase inhibitors, with emphasis

on the biochemical and adrenergic neuronal effects of α-methyltyrosine, the paper by Spector (155) should be consulted.

Dihydroxyphenethylamine β-hydroxylase.—Kaufman & Friedman (85) reviewed the literature on DOPAmine β-hydroxylase, and Goldstein (53) has discussed, in particular, the effects of disulfiram *in vivo*. Friedman & Kaufman (44) and Goldstein et al. (56) have described procedures for purification of the enzyme, and presented additional evidence that it is a copper protein. Friedman & Kaufman (43, 44) have shown that the copper in the enzyme molecule is reduced by ascorbic acid, which is included in reaction mixtures as a cofactor, and reoxidized stoichiometrically during the hydroxylation of the substrate. The list of chelating agents which will inhibit the enzyme continues to grow (55), although disulfiram (or its reduction product diethyldithiocarbamate) has been used most generally in *in vivo* studies.

Administration of rather large doses of diethyldithiocarbamate to rabbits and rats depressed concentrations of norepinephrine in the intestines and increased DOPAmine concentrations (28). This finding could not be confirmed by Carlsson et al. (24). However, the latter workers reported that in areas of the rat brain which normally contain norephinephrine the drug caused a decrease in norepinephrine concentrations, as judged both by biochemical analysis and by the fluorescence micrographic technique, and an increase in the concentration of DOPAmine, in accord with the findings of others. But in those areas of the brain where DOPAmine is considered to play a role in nerve impulse transmission, no change was observed in DOPAmine content of the neurons. Thus, the psychopharmacological effects of β-hydroxylation inhibitors may suggest a role for the postulated central DOPAminergic mechanism. It is also of interest that a significant increase in adrenal gland DOPAmine content was observed in the diethyldithiocarbamate-treated rats.

Thoenen et al. (171) treated cats with disulfiram and observed decreased contractile responses of the nictitating membrane and isolated perfused spleen to electrical stimulation of sympathetic nerves. Less norepinephrine and more DOPAmine were found in the spleens of the disulfiram-treated cats than in normal animals, and the latter catecholamine, like norepinephrine, was liberated into the perfusate by nerve stimulation. Thus, in the disulfiram-treated cats, DOPAmine may have served in a substitute transmitter capacity.

Musacchio et al. (120), by injecting disulfiram into rats, were able to block the β-hydroxylation not only of DOPAmine but also of tyramine, its α-methyl derivative, and *m*-tyramine. In this most interesting study, it was shown that the firmness of binding of amines by particulate fractions from certain tissues depends upon the number of hydroxy groups in the molecule. *In vivo*, β-hydroxylated amines are retained by the tissues more persistently than are the parent nonhydroxylated compounds (118). Disulfiram prevented restoration of the cardiovascular response in reserpinized animals by

DOPAmine and methylDOPA (117), presumably by preventing the formation of norepinephrine and α-methylnorepinephrine, respectively.

Goldstein & Nakajima (57) reported that the moderate disulfiram-induced depression of heart norepinephrine concentrations in adrenalectomized rats was augmented by exposure of the animals to cold. This is similar to the finding that stress also potentiates the effect of tyrosine hydroxylase inhibitors [see Spector (155)] and may reflect the greater activity of the autonomic nervous system under stressful conditions.

It is too early to predict that compounds which inhibit the biosynthesis of catecholamines will play a useful role in therapeutics. The best inhibitors now known are not extremely potent, and until more active drugs are available clinical results are not likely to be striking. However, the fact that the enzymes are not entirely specific and catalyze the hydroxylation of various phenethylamine derivatives, including of course many sympathomimetic amines, may be of considerable value in explaining certain aspects of drug action. To cite one well-known example, the metabolism of methylDOPA to α-methylnorepinephrine may be a prerequisite for the antihypertensive usefulness of the α-methyl amino acid [see Stone & Porter (162)].

Carbonic Anhydrase

The interesting observation has been made that erythrocyte carbonic anhydrase can catalyze not only the hydration of acetaldehyde (130), but also the hydrolysis of an ester, *p*-nitrophenylacetate (131). Acetazolamide was shown to inhibit the latter reaction noncompetitively, as it does the hydration of carbon dioxide.

Tanimukai et al. (169) reconfirmed the fact that carbonic anhydrase inhibitors which reach the brain exhibit anticonvulsant properties (versus maximal electroshock seizures in rats) and also demonstrated a direct correlation between degree of decrease in brain enzyme activity and anticonvulsant potency. It was suggested tentatively that regional distribution of certain drugs in the brain, as judged by enzyme inhibition, corresponds to their different clinical indications, a proposal which would seem to merit further investigation. One may reasonably wonder at this point whether, counter to most current concepts, the anticonvulsant property of carbonic anhydrase inhibitors may be due to alterations in the state of brain substances other than carbonate, i.e., aldehydes or esters.

Gotoh et al. (59) re-examined the known stimulating effect of acetazolamide upon cerebral blood flow in man. It was concluded that in man, as in the monkey, increased P_{CO_2} in the brain, resulting from inhibition of erythrocyte carbonic anhydrase, directly stimulated blood flow and, therefore, the supply of oxygen available to the brain. Since the increased blood flow is limited to the brain, acetazolamide is considered to be of potential usefulness in the treatment of cerebral ischemia and hypoxia. However, it seems that the pharmacological actions of acetazolamide are probably not mediated

simply through its ability to inhibit carbonic anhydrase. From the fact that catecholamine-depleting agents, including not only reserpine but also α-methyl-*m*-tyrosine and α-methyltyrosine, are antagonistic to the anticonvulsant effect of acetazolamide, and the fact that both α- and β-adrenergic blocking agents also are antagonistic, it is probable that the agent may function via adrenergic mechanisms (141).

Although not dealing directly with carbonic anhydrase, the studies of Butler et al. (21) with trimethadione are pertinent to the subject. A metabolite of the drug, 5,5-dimethyl-2,4-oxazolidinedione, is not metabolized and is excreted slowly. In man, it causes a decrease in plasma bicarbonate equivalent to the amount of its anion present. Thus, the compound may act as a slowly excreted acid which accumulates and alters acid-base equilibria in the tissues which, by a mechanism not yet revealed, alters the incidence of petit mal seizures.

Diazoxide

Rubin and co-workers described the hypotensive effect of the nondiuretic benzothiadiazine diazoxide, and suggested that a direct action on vascular smooth muscle was involved (139, 140). The drug was found to be hyperglycemic in animals and in man (80), and has been considered of possible utility in the treatment of certain hypoglycemic states (39). The adrenal gland is involved in the hyperglycemia produced by diazoxide (160, 166), and it is the cortex, rather than the medulla, which is necessary for the response (97, 160, 184). Potassium ions also play a critical role, inhibiting the response if present in excess. Potentiation of the hyperglycemia can be induced either by feeding animals a low potassium diet or by the administration of kaliuretic agents (84, 97, 184); however, hydrochlorothiazide may have an influence on carbohydrate metabolism independent of its effect upon potassium excretion (147). Since isopropylmethoxyamine decreased the effect of diazoxide upon plasma glucose and free fatty acids, it was thought that catecholamines were involved (167). However, dichloroisoproterenol, which is known to block the metabolic effects of epinephrine but not those of 3′,5′-AMP, does not prevent diazoxide-induced hyperglycemia (144); dihydroergotamine, on the other hand, which blocks both epinephrine and 3′,5′-AMP, does reduce diazoxide hyperglycemia. The agent produces decreases in liver and skeletal muscle glycogen, increases in glucose-1-phosphate, glucose-6-phosphate, and lactate, all of which indicates that the compound increases the rate of glycogenolysis; and it inhibits 3′,5′-AMP phosphodiesterase (144). Therefore, diazoxide, in the presence of corticosteroids, produces hyperglycemia by increasing phosphorylase and decreasing glycogen synthetase.

It is thought by Kaess et al. (84) that potassium deficiency in the cells leads to a compensatory increase in sodium ion concentrations which promotes an inhibitory effect of diazoxide on phosphorylase phosphatase. Losert et al. (104) found that diazoxide also decreases the levels of insulin in plasma,

which may account for the observed depression of glycogen synthetase by the drug. *In vitro*, diazoxide inhibits the glucose-triggered release of insulin from pancreas slices (79).

The data linking 3′,5′-AMP and diazoxide-induced hyperglycemia seem rather convincing and, in view of the many functions of drugs now known or believed to be mediated by 3′,5′-AMP, it would not be surprising if it were responsible also for the hypotension which follows diazoxide administration.

Cardiac Glycosides

Increases in both actomyosin ATPase and mitochondrial enzyme were found in the hearts of rats which had received digoxin intraperitoneally, the peak effect being noted three to four hours after drug administration (66). Although the mechanism of enzyme induction is not known, increased ATPase activity may be associated with greater myofibrillar contractile strength.

The reversal of digitalis-induced ventricular arrhythmias by pronethalol does not depend upon the adrenergic β-blocking activity of the latter compound (7, 106), but possibly upon a guanethidine-like action. This should not be construed to argue against the potential usefulness of β-blockade in all types of arrhythmia, however.

Sodium- plus potassium-activated ATPase (transport ATPase) is inhibited reversibly by cardiotonic steroids, e.g., acetylstrophanthidine. Hokin et al. (74) prepared the 3-iodo- and 3-bromoacetyl derivatives of strophanthidine and found their inhibition of the enzyme was mainly irreversible. Presumably, these compounds react at the cardiotonic steroid site, and the reaction may be of help in characterizing the site and in isolating the transport ATPase protein.

Godfraind & Godfraind-DeBecker (51) studied the conditions under which the biphasic effects of ouabain upon cardiac muscle can be observed. Initial potentiation of the response to catecholamines was most prominent at low concentrations of the glycoside and at low temperature, while inhibition was more prominent at higher concentrations. *In vitro*, transport ATPase was reported to be stimulated by low concentrations of ouabain and inhibited at higher concentrations (127).

General

Smith & Williams (151), in a two-part review, adequately discussed chemical mechanisms involved in diverse biochemical reactions, including enzyme catalysis, hormone action, and receptor phenomena. These authors speculate that some drugs, rather than reacting with receptors, may alter biochemical processes in the vicinity of the receptors, with consequent changes in physiological function. The biochemical effect need not be at the enzyme level; many drugs, because of their ionic nature, might themselves act as catalysts in biochemical reactions. Although purely conjectural, this

concept is based firmly upon well-known chemical reaction mechanisms and may serve to explain drug action when currently more popular lines of interpretation fail.

The idea that drugs with various types of activity exert their effects by altering membrane permeability is not new. The case generally has been difficult to prove, however, and any approach to the problem is worthy of note. Feinstein (40) found local anesthetics to prevent the extraction of Ca^{++} from aqueous methanol solutions into chloroform in the presence of phospholipids and of lipids extracted from muscle and nervous tissue. A reaction of the drugs with cell membrane lipids may underlie an effect upon cellular ion flux and thus provide a chemical explanation of local anesthetic activity. Nayler (123) reported similar experiments with lipids extracted from cardiac musle membrane. Quinidine, in reasonably low concentrations, prevented the extraction of Ca^{++} into chloroform-lipid, and *in vivo* might act by preventing the transport of Ca^{++} across the membranes.

Colburn & Maas (27) assayed synaptosomes from rat brain and found concentrations of metal ions, such as Cu^{++}, Fe^{+++}, Mg^{++}, and Zn^{++}, sufficient to chelate the catecholamines held therein. From potentiometric titration data and water-solvent partition data, support was gained for the idea that catecholamines are bound in synaptosomes in an amine-ATP-metal (probably Cu^{++}, Fe^{+++}, or both) complex. It should be noted that the strength of binding of amines in the complex is directly proportional to the number of hydroxyl groups in the molecule, which correlates well with *in vivo* data (121).

Phosphatidylserine, as well as phospholipid extracted from synaptosomes, was found to form ternary complexes with metal ions and ATP and with metal ions and norepinephrine. It was suggested that attack of the resting membrane metal ion-ATP-phospholipid complex by ATPase permits catecholamine to enter the complex and move across the membrane. The proposed membrane model can be invoked to explain the transport of Na^{+}, K^{+}, and other materials; the original papers (27, 107) should be consulted for details.

Vogel et al. (175) made the interesting observation that certain enzymes are inhibited by various compounds which have nothing in common structurally, except aromaticity. In the experiments reported, multi-ring compounds were more potent than single-ring substances. These observations call to mind the many reports of enzyme inhibition, usually with quite large concentrations of the inhibitors, which have little bearing upon therapeutic mechanisms.

Medina et al. (112) studied the effect of phenothiazine and six of its derivatives upon the respiration of isolated guinea pig heart sarcosomes, and found the compounds, like thyroxine, to depress active state respiration. Like thyroxine also, the phenothiazines affected the structure of the microsomes, as judged from turbidimetric and electron microscopic observations. Although the authors point out that the evidence does not prove a direct causal relationship between structural changes in the microsomes and their

respiration, it seems possible that in this and in many other instances such structural changes, rather than specific or quasispecific interference in the respiratory chain, may underlie certain biochemical effects of drugs. It is perhaps premature to implicate a similar mechanism in other effects of drugs; however, since lack of therapeutic specificity may depend in some instances upon phenomena of this sort, it would be only prudent to consider it a real possibility.

Laborit (98) thinks that a tissue's reaction to drugs depends upon its "metabolic structure"; i.e., upon whether the Embden-Meyerhof-Krebs (ADP⇌ATP; DPN⇌DPNH) system or the pentose pathway (TPN⇌TPNH) is predominant. In essence, the concept is that drugs which block, directly or indirectly, any stage in the Embden-Meyerhof-Krebs system will cause depression of those tissues or organs in which this system operates; and conversely, stimulation of the system leads to activation (contraction, secretion, etc.). The pentose pathway, on the other hand, is not an energy-yielding pathway, and drugs which influence it change principally the interconversion between TPN and TPNH. Recently, the hypothesis has been elaborated upon (99), and the mode of action of nicotine has been fitted into the theoretical framework. Laborit & Weber (100) interpreted the neurotropic actions of strychnine, 4-hydroxybutyric acid, thiopental, ether and chloroform, and of pentetrazole on the basis of the theory.

Two papers by Sulman and his co-workers, extending studies on the effect of drugs upon endocrine function, will be mentioned here, not because they bear directly upon the present subject, but because they pertain to an approach which is ignored too often in attempts to uncover biochemical mechanisms. Zor et al. (190) related the effect of pargyline in decreasing the rate of cartilage growth in rats to its potentiating effect upon corticosteroids. Also, a number of monoamine oxidase inhibitors were found to depress growth hormone release. Superstine & Sulman (165) found that chlordiazepoxide and diazepam do not influence the output of pituitary hormones and, therefore, do not exert their tranquilizing influence via the hypothalamus. However, guanethidine suppressed the secretion of follicle-stimulating hormone, luteinizing hormone, and somatotropin, while stimulating the secretion of the luteotropic, adrenocorticotropic, and antidiuretic hormones. Thus, although the altered hormone secretion may not bear directly upon the therapeutic mode of action of guanethidine, the suggestion is that the hypothalamus may be intermediary in the drug's useful physiological effects. Obviously, an effect of a drug upon endocrine function or hormone action may either account, at least partly, for its pharmacological effects, or may alter the physiological function in such a way that its primary action is masked.

Conclusions

Of the various areas of biochemistry which have been considered important to the study of drug action mechanisms, certain ones, according to current thought, seem most fundamental. First, knowledge of the chemical

composition of membranes is critically important. All drugs must pass biological barriers, and many, probably more than now suspected, alter membrane function. Second, understanding of the chemical nature of receptors—here used in the broadest sense—is of basic importance. Whether a drug reacts with a physiological receptor, an enzyme, or a membrane, obviously it will attack only those sites with suitable chemical configurations. Third, the sequences of both chemical and physiological events initiated by a primary interaction must be recognized and correlated with drug action. Information of this kind can be useful in deducing fundamental mechanisms, although erroneous conclusions may be drawn.

It would have been desirable to categorize reports in the literature on the basis of their importance to the above objectives. However, few papers can be found which fit neatly into such a schema. Another approach was used, therefore, one which regrettably viewed the topic from a distant point of vantage. Nevertheless, it is clearly seen, even from afar, that fundamental aspects of drug action are under investigation, and that more rapid progress in the field can be anticipated.

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