BIOCHEMICAL MECHANISM OF DRUG ACTION[1,2]

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In the introduction to a review article it is traditional for the author to state that it is impossible to cover all aspects of this topic because of lack of space. It is not intended to set a precedent in this article, but additional reasons exist for limiting this review. For example, one of the more popular research areas of activity reported on this past year involved measurements of the levels in the brain of ATP, the catecholamines, 5HT, ACh, GABA, or histamine as affected by various drugs. It might be asked, however, whether changes in the levels of these neurohumors or metabolites in the brain necessarily have any *primary* relevance to the mechanism of action of a drug? Granting the validity of this approach as a first approximation of the biochemical sequellae that result from drug treatment (1), nevertheless, when one realizes the paucity of our knowledge of control mechanisms in cells, a serious interpretation of such data obtained after the administration of a single drug, essentially an inhibitor, or after the sequential administration of several drugs, is difficult. In these instances the investigator may well be measuring an effect that is completely unrelated to either the therapeutic or toxic action of the inhibitor. To rephrase an aphorism, only the uninhibited attempt to interpret the effects of inhibitors. Thus, a complete coverage of the biochemical effect that results from the administration of a drug not only may be impractical because of the enormous volume of data that has resulted from this approach, but also its utility in formulating an explanation of drug action may be dubious. On the other hand, of course, if the title of this review were to be adhered to strictly and only biochemical mechanisms *reponsible* for drug action were to be covered, there would be very little to write about. Obviously, a compromise is necessary and this has been attempted.

A further problem relating to the subject material covered in this review

[1] The survey of the literature pertaining to this review was concluded in July 1963.

[2] Abbreviations used in the text are as follows: ATP (adenosine triphosphate); ADP (adenosine diphosphate); AMP (adenosine monophosphate); 5HT (5-hydroxytryptamine); 5HTP (5-hydroxytryptophan); ACh (acetylcholine); GABA (γ-aminobutyric acid); DFP (diisopropyl fluorophosphonate); ACTH (adrenal corticotrophic hormone); RNA (ribonucleic acid); DCI (dichloroisoproterenol); DNP (dinitrophenol); NAD (nicotinamide adenine dinucleotide, DPN); NADH (reduced nicotinamide adenine dinucleotide, DPNH); NADP (nicotinamide adenine dinucleotide phosphate, TPN); NADPH (reduced adenine dinucleotide phosphate; TPNH); TEA (Tetraethylammonium).

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concerns the questionable value of recording data that result from the study of drugs in concentrations that are nonpharmacological. This situation applies most strikingly to chlorpromazine, a drug that at a concentration of about 10^{-3} *M* has been shown to affect almost every biochemical system in which it has been tested. Since a pharmacological effect of the tranquilizer can be noted at a concentration in the body tissues of about 10^{-5} *M*, the biochemical effects obtained when the agent is used at a level 100-fold higher are of doubtful significance. Accordingly, another limitation on the subject matter of this review is that, in general, experiments in which grossly nonpharmacological concentrations of drugs were used will not be discussed.

A final note in this prelude is that in this paper drugs are regarded as chemicals foreign to animals; thus no attempt will be made to record biochemical events elicited by a naturally occurring metabolite in the body, e.g., hormones or vitamins.

Drugs Affecting the Autonomic Nervous System

Adrenergic nervous system.—During the past year, as in previous years, the interest in the "autonomic drugs," as reflected by the quantity of papers on this subject, far outweighed the therapeutic value of these agents. Regardless of the historical reasons that may have accounted for this situation in past years, the current attention given to drugs that affect the adrenergic nervous system, appears to be due to the imaginative use of tritiated norepinephrine, particularly by Axelrod and his colleagues.

The availability of the neurotransmitter with high specific radioactivity has enabled these investigators to use physiological concentrations of norepinephrine (at least in more recent experiments), and to measure its uptake and release by tissues as influenced by a variety of drugs and experimental procedures. The validity of these experiments rests on the postulate that the exogenously administered amine becomes localized at the identical site where the endogenous norepinephrine is found. That this situation appears to obtain is shown by the observations that (*a*) decentralization of the superior cervical ganglion of a cat inhibited the spontaneous release of previously administered H^3-norepinephrine from organs that are innervated by postganglionic fibers derived from this ganglion (2); (*b*) chronic denervation of the superior cervical ganglion caused an inhibition of the uptake of a subsequent injection of H^3-norepinephrine by these organs (3); (*c*) in subcellular distribution studies both endogenous and exogenously administered labeled amine are found in the same particulate fraction in the heart (4, 5); (*d*) radioautographic techniques indicate that the bulk of H^3-norepinephrine is located at sympathetic nerve endings (6); (*e*) stimulation of the splenic nerve caused the release of H^3-norepinephrine from the innervated organ (7); and (*f*) a brief stimulation of the postganglionic fibers of the stellate ganglion resulted in an increased uptake of the catecholamine by the atria of the cat (8). It should be kept in mind however, that in these and subsequent studies to be discussed, the labeled norepinephrine is a mixture of the *d*- and *l*-forms; thus until more

information is available concerning the localization and fate of *d*-norepinephrine, some caution must be applied in the interpretation of the data obtained with this valuable tool (9). In addition, in many investigations of this type, the meaning of "uptake" is not clear; sometimes it means exchange and sometimes it refers to net accumulation.

In 1958 Burn & Rand (10) suggested that tyramine and certain other sympathomimetic amines act by releasing norepinephrine from intracellular stores. Both von Euler & Lishajko (11) and Schümann (12), using chromaffin granules from the splenic nerve, showed that tyramine releases the bound catecholamine, and subsequently Schümann & Philippu (13) observed that norepinephrine is released almost stoichiometrically by either tyramine or amphetamine from granules isolated from the adrenal medulla. Utilizing an isolated rat heart preparation that had been perfused with H^3-norepinephrine, Axelrod et al. (14) showed that successive injections of tyramine caused a progressively smaller release of the labeled catecholamine and this decrease paralleled the progressive fall both in the increased amplitude of contraction and in the heart rate that resulted from the administration of tyramine. This releasing action of tyramine on the catecholamines of the heart also has been observed recently by Weiner et al. (15) in rats and by Iversen & Whitby (16) in mice. In this latter report it was shown also that only part of the norepinephrine in the heart is released by tyramine, despite the fact that the subcellular localization of the catecholamine in the heart is the same in untreated and tyramine-treated animal. On this point, Potter & Axelrod (17) found that H^3-norepinephrine that is bound for a short time in the heart is more easily released by tyramine than is catecholamine that had been present in the heart for a long time. This finding led these workers to postulate that there are two pools of norepinephrine in the heart, one with a half life of several hours and the other with a half life of about one day. This suggestion has been made by other investigators (15). This observation may be compared to that of Blaschko & Welch (18) who reported in 1953 that of the catecholamines present in granules of the bovine adrenal medulla, about 20 per cent was released almost instantaneously upon intravenous injection of the granules into cats, (as determined by the rise in blood pressure) while the residual catecholamines were more gradually released.

The situation, then, with respect to the mechanism of action of tyramine, appears to be that the tachyphylaxis observed with this amine is at least partially explainable on the assumption that tyramine can release certain sources of norepinephrine so that it can react with its receptor site, but not necessarily more stable reserve stores of the catecholamine. That tachyphylaxis persists in spite of the presence of norepinephrine in a depot may be explained by the possibility that tyramine becomes attached to the receptor site and until it is removed, either by diffusion or by the action of monoamine oxidase, the nonspecific binding site of norepinephrine cannot function to replenish the receptor locale with the neurohumor.

The mechanism of action of the two antihypertensive agents, guanethidine

and bretylium, usually considered together because of similarity of effects, still remains unclear. Unquestionably, both agents inhibit the response to postganglionic stimulation (18) but attempts to focus on the mechanism of this action have led to contradictory data that are only partially explainable on the basis of dosage regimen or species or organ differences [for background material on this subject the reader is referred to Fawaz (20)]. Matsumoto & Horita (21) have shown that although guanethidine depletes the catecholamine content of rabbit heart, pretreatment of the animal with methamphetamine prevented this depletion. This action may be explained by the finding of these authors that methamphetamine prevents the uptake of guanethidine by the heart. However, since the authors did not study the dose-response relationship with respect to the depleting action of guanethidine, it is not known whether the level of guanethidine in the heart, after pretreatment with methamphetamine, is too low to discharge the catecholamine or another explanation is required.

In confirmation of earlier work (22, 23), Inesi et al. (24) have shown that the prior administration of bretylium will prevent the depletion of catecholamines in the heart by reserpine. Also it was observed in this study that bretylium antagonizes both the increase in blood pressure and phosphorylase *a* activity that was promoted by a monoamine oxidase inhibitor, McNeilB-343.[4] McCoubrey (25) found that bretylium given to cats daily for two weeks in doses of 10 mg/kg did not deplete the catecholamine content of sympathetic ganglia; in fact, the slight rise that was observed, suggests an impairment of the release mechanism. When the daily dose of bretylium was raised to 30 mg/kg, however, the ganglia were depleted of their catecholamine content.

The norepinephrine-releasing action of α-methyl-metatyrosine was investigated by Udenfriend & Zaltzman-Nirenberg (26). The metabolism of this compound involves first a decarboxylation to form α-methyl-metatyramine followed by β-oxidation to yield β-hydroxy-α methyl-metatyramine (metaraminol, Aramine). These workers have found that the action of α-methyl metatyrosine is mediated through α-methyl metatyramine and metaraminol. This conclusion was reached by using α-methyl dopa hydrazine to inhibit the decarboxylation of α-methyl metatyrosine and benzyloxyamine to inhibit the β-oxidation of α-methyl metatyramine to metaraminol. The depletion was measured in the guinea pig heart. In this study it was demonstrated that (*a*) metaraminol is 5 to 10 times more potent than α-methyl metatyramine; (*b*) less than stoichiometric amounts are needed to release the norepinephrine; and, (*c*) as with reserpine, the depletion continues even when no measurable quantity of the releasing agent could be found. Hess (27) has shown that in guinea pigs, α-methyl metatyrosine not only releases previously bound H^3-norepinephrine but also it prevents the binding of the catecholamine. Another inhibitor of amino acid decarboxylase that has received a

[4] 4-(*m*-chlorophenylcarbamoyloxy)-2-butynyl trimethylammonium chloride.

great deal of attention is α-methyl dopa [for a general review see Clark (28)]. Sharman & Smith (29) observed that injection of α-methyl dopa into rats causes a fall in the level of 5HT and its derivatives in brain; no evidence for the release of 5HT was obtained. The possibility that the action of α-methyl dopa is attributable in part to its inhibitory action on the uptake of 5HTP has recently been ruled out according to Schanberg (30). In addition to its obvious action in preventing the synthesis of 5HT, however, α-methyl dopa may owe its long-lasting properties to its proclivity for attachment to binding sites for 5HT and to its resistance to attack by monamine oxidase. Porter et al. (31) employed a hydrazino analog of α-methyl dopa and found an inhibition of the formation of 5HT from 5HTP when the analog was administered to rats; in addition, the compound inhibited the production of $C^{14}O_2$ when rats were given carboxyl-labeled tyrosine, phenylalanine, or α-methyl dopa. The drug did not reduce the elimination of labeled CO_2 by rats injected with glutamic acid $C^{14}OOH$.

Pyrogallol is another enzyme inhibitor that has been used to change the level of catecholamines in the brain; this compound has been shown to inhibit catechol-O-methyl transferase (32). Crout et al. (33) concluded that catechol-O-methyl transferase is of little importance in the inactivation of norepinephrine in the brain since an intraperitoneal injection of pyrogallol had no effect on the concentration of the catecholamine in the brain. Masami et al. (34), however, using both an intracarotid and intracisternal injection of pyrogallol, have recently observed that whereas the former route of administration caused only a slight rise in the level of norepinephrine in the brain, administration of the inhibitor by the latter route resulted in a marked increase in the catecholamine level. In addition, the intracisternal injection of pyrogallol gave rise to behavioral effects that closely mimicked the effects seen after the central administration of epinephrine and norepinephrine. The authors conclude that pyrogallol does not pass the blood-brain barrier. At this point, then, it still remains to be decided what mechanism is responsible for terminating the action of norepinephrine. Regardless of the ultimate fate of the neurohumor, the three choices are (*a*) destruction by monoamine oxidase, (*b*) destruction by catechol-O-methyl transferase, and (*c*) diffusion from the receptor site. Since the K_m of norepinephrine for either enzyme is rather high and since neither enzyme has a high turnover number, the most attractive possibility at the present time appears to be choice (*c*).

Cholinergic Nervous System

In an intriguing study using rat liver preparations of nuclei, mitochondria, microsomes, and the soluble fraction of the cell incubated with DFP^{32}, Ramachandran et al. (35) fractionated the P^{32}-labeled proteins using column chromatography and found over twenty separate peaks of radioactivity. In all these areas the label was found attached to serine. In parallel experiments with liver preparations incubated without DFP, fractions were isolated that

corresponded with the peaks obtained in the experiment with DFP and these were tested for esterase activity using *p*-nitrophenyl acetate as substrate. Esterase activity was found in all fractions that corresponded to the DFP-reactive protein fractions.

Dettbarn & Rosenberg (36), in a continuing effort to implicate ACh in axonal conduction, found that DFP at a concentration of 5×10^{-2} *M* blocked conduction in a desheathed vagus nerve of a rabbit. At this concentration, however, DFP inhibits a variety of enzymes including the tricarboxylic acid cycle (37) so that attempts by this group to use this approach in support of the involvement of ACh in conduction are of questionable value.

Heilbronn (38) has investigated the reaction of tabun-inhibited erythrocyte and plasma cholinesterase using P2S (N-methyl-pyridinium-2 aldoxime methane sulfonate) and TMB4 (N,N,'-trimethylene bis (pyridinium-4 aldoxime) dibromide). TMB4 reactivated both erythrocyte and plasma cholinesterase more rapidly then did P2S: the maximum reactivation obtained was about 75 per cent.

Kirpekar et al. (39) studied bis- and poly-onium neuromuscular blocking agents for anticholinesterase activity. Anticholinesterase activity was noted in both depolarizing and nondepolarizing types of blocking agents, i.e., there was no correlation between the type of neuromuscular blocking drug and the ability to inhibit cholinesterase. In this paper the authors also discussed structure-activity relationships among the various onium derivatives that were tested. Waser & Lüthi (40) described a modification of their original technique in which C^{14}-labeled curare was injected into a mouse and the isolated end plate region of the mouse diaphragm was subjected to radioautography. These investigators calculate that 4×10^{6} molecules of curare will saturate this area.

O'Neill et al. (41) have described the inhibition of respiration of electrically-stimulated cerebral cortical slices by cholinergic blocking drugs. Six cholinolytic drugs of the Ditran (JB329) type inhibited the stimulated respiration of guinea pig cerebral cortical slices at a concentration of 10^{-5} to $5\times10^{-4}M$. No effect was observed at these concentrations on unstimulated respiration. The sensitivity to drugs of stimulated (either electrically or by means of a high concentration of K^{+} in the medium) respiration of cerebral cortical slices has been well-documented by McIlwain (42) and by Quastel (43); unfortunately, there is as yet no direct experimental evidence to explain this phenomenon.

Psychotropic Drugs

Antidepressants.—It is possible that a grave injustice has been inflicted on the science of pharmacology by the classification of certain compounds as monoamine oxidase inhibitors. In fact, unless the evidence is overwhelmingly compelling, it is dangerous to "pigeon-hole" any drug; this is not only for the obvious reason that foreign compounds usually interfere with a variety of

biochemical mechanisms, but also because a premature classification of a mechanism of action of a drug tends to produce myopic experimental procedures with interpretation of data in such manner as to fit them into the one prevailing classification. The monamine oxidase inhibitors are referred to as antidepressant drugs: the causal relationship, however, is less than clear. For example (*a*) the index of monoamine oxidase inhibition has been regarded as a reflection of an increase in the concentration of 5HT and the catecholamines in brain. The peak concentration of the amines may be noted from 2 to 10 hours after medication, depending on the monoamine oxidase inhibitor that is employed, yet the therapeutic effect of these drugs usually is not observed until after at least a week of daily medication. (*b*) Imipramine is regarded as an antidepressant and yet it does not inhibit monoamine oxidase. This point might be argued on the assumption that imipramine has a different mechanism of action, but it is difficult to explain the finding that in rats this agent produces a rise in the level of 5HT in the brain (44). (*c*) Tranylcypramine exhibits antidepressant activity before any inhibition of monoamine oxidase is observed. (*d*) Recently, Pscheidt & Himwich (45) investigated two monoamine oxidase inhibitors, nialamide and isocarboxazid: both agents when given to monkeys produced a rise in the level of amines in the brain. In separate experiments in which the two inhibitors were given together with reserpine, nialamide caused an increased concentration of 5HT in the brain whereas a decrease resulted from the administration of isocarboxazid. How can one explain these findings on the basis of inhibition of monoamine oxidase? Clearly the situation is complex. It is possible that part of the antidepressant activity of these agents is attributable to an inhibition of monoamine oxidase and an increased level of brain amines but until some solid evidence is obtained, wide-angle vision is required.

Lewis & van Petten (46) studied the possible relationship among antidepressants, inhibition of monoamine oxidase, and increase in the brain level of ATP; iproniazid, isoniazid, phenelzine, pheniprazine, tranylcypramine, harmine, imipramine, amitryptyline, orphinedrine, diphenhydramine, and cocaine were studied. All these agents with the exception of harmine and diphenhydramine produced an increased concentration of ATP in the brain. Since both iproniazed and isoniazid were equally effective in raising the level of ATP while isoniazid is an ineffectual monoamine oxidase inhibitor, and since harmine did not raise the concentration of ATP in the brain while this drug is an efficient monoamine oxidase inhibitor, these findings do not suggest a relationship between inhibition of monoamine oxidase and the ability to produce an increase in the concentration of ATP in the brain.

A variety of monoamine oxidase inhibitors, e.g., tranylcypramine, phenylisopropylhydrazine, ethyl tryptamine, MO 911, harmaline, amphetamine and methamphetamine, were shown by Anderson & Ammann (47) to produce a reversible block of neuromuscular transmission in an isolated rat-diaphragm preparation. Since some of these agents are irreversible

monoamine oxidase inhibitors, the authors concluded that the inhibition of this enzyme has no relevance to neuromuscular blockade.

Fumagalli et al. (48), using C^{14} acetate and P^{32}-phosphate, have studied the effect of imipramine and desmethylimipramine on lipid biosynthesis in brain. Both agents at a concentration of $10^{-4}M$ increased fatty acid synthesis of phospholipids. No effect was observed on acetate oxidation, incorporation of acetate into cholesterol, P^{32}-phosphate incorporation into phospholipids or synthesis of the fatty acids of neutral fat. In experiments *in vivo*, desmethylimipramine stimulated fatty acid synthesis of neutral fat and phospholipid, whereas imipramine had no effect in this short time experiment (1 hr).

Smith et al. (49) reported that iproniazid is cleaved nonenzymatically to form isopropylhydrazine. The authors state that this compound, or a further oxidation product of it, represents the true inhibitor of monoamine oxidase.

In a study of the effects of CNS stimulants on the concentration of norepinephrine in the rat brain, Baird & Lewis (50) using *d* and *l* isomers of of amphetamine and ephedrine, found a correlation between CNS stimulation and the ability of these agents to lower the level of norepinephrine in the brain.

Tranquilizers.—An enormous amount of work has been performed on the relationship of reserpine and chlorpromazine to brain amines, oxidative metabolism, ACTH, and cell membranes. What the findings mean in terms of an explanation of the therapeutic action of these agents and their derivatives, is still unclear. Some of these studies reflect a genuinely creative attempt to gain some insight into the mechanism of action of the tranquilizers but unfortunately there are also many pedestrian investigations in this area that, at best, only corroborate well-documented findings.

The effect of reserpine and its analogs on the adenine nucleotides and creatine phosphate in rat brain has been studied by Kaul & Lewis (51). A correlation was observed between reserpine-like compounds that produce sedation and a decreased ATP/ADP ratio (mediated by a fall in the ATP level). Only a moderate and unconvincing correlation, however, was obtained in the time course of reserpine sedation with changes in the ATP level in the brain. There were no significant changes in the creatine phosphate or total adenine nucleotide pool.

A single dose of reserpine of 5 mg/kg, injected into a rat, raised the level of liver-ketoglutaric transaminase by about 100 per cent; the effect lasted for at least 72 hours (52). This finding may reflect the action of reserpine on the adrenal cortex. Westermann et al. (53) found that reserpine and its active derivatives caused the release of ACTH. This secretion was correlated with a reduction in the level of brain amines by more than 50 per cent, i.e., if the depletion of the amines was less than 50 per cent no ACTH was released. Ashford & Shapero (54) noted that a single injection of reserpine, chlorpromazine, benzactyzine, or phenobarbital promoted the release of ACTH; a tolerance to these agents developed after pretreatment for five days but

ether still caused the release of ACTH. There was no effect of reserpine and the other agents noted above when the animal was hypophysectomized or pretreated with hydrocortisone. Smith et al. (55) also observed secretion of ACTH after the administration of phenothiazine tranquilizers such as chlorpromazine, promazine, or trifluoperazine; the ACTH content of the pituitary declined to about 30 per cent of normal. In studies on the effect of reserpine on the uptake of norepinephrine by a particulate preparation of brain, Mirkin et al. (56) showed that pretreatment of a rat with reserpine diminished the uptake in the particulate preparation when it was incubated with norepinephrine. In experiments *in vivo*, Weiner & Trendelenburg (57) found that reserpine did not block the immediate uptake of labeled epinephrine into the heart. Michaelson & Whittaker (58) demonstrated that neither reserpine nor iproniazid affected the distribution of 5HT in the guinea pig brain in terms of the ratio of the amine found in the soluble fraction to that in the particulate fraction of the cell.

In experiments on the uptake of H^3-norepinephrine in the heart, Rosell & Axelrod (59) attempted to relate the inhibition of uptake with various properties of phenothiazine drugs and derivatives. The authors used chlorpromazine, promethazine, promazine, and selenopromazine as examples of drugs exhibiting primarily antiepinephrine, antihistamine or sedative actions. The inhibition of uptake of norepinephrine in the heart was correlated only with drugs that exercised a strong antiepinephrine action. Davis et al. (60) noted an increase in the catecholamine content of adrenal glands and aortic walls of rats after treatment with chlorpromazine (5 mg/kg) daily for four days. Similar results were obtained with mecamylamine, TEA, and hexamethonium.

Chlorpromazine, at a concentration of $10^{-4}M$, was shown by Pritchard & Quastel (61) to stimulate the incorporation of glycerol, glycine, and serine into the phospholipids of rat cerebral cortical slices. Incorporation occurred primarily in phosphatidic acid and phosphatidyl serine. In similar experiments using slices from guinea pig brain, Magee et al. (62) showed that chlorpromazine at a concentration of $10^{-4}M$ and also azacyclonal ($10^{-3}M$) increased incorporation of P^{32}-phosphate into phosphatidic acid, phosphatidyl inositol, and phosphotidyl serine. These drugs had no effect on oxygen uptake, lactate production or the specific radioactivity of ATP. Although ACh and eserine also stimulated the incorporation in a similar manner, an additive effect was observed when either azacyclonal or chlorpromazine was combined with ACh-eserine. In addition, chlorpromazine and azacyclonal were additive in their ability to stimulate the incorporation of P^{32}-phosphate. Although from these observations the authors attempt to implicate three distinct mechanisms that are operative in this system, the omission of graded dose-response curves for each of the agents preclude a definitive statement on this point at this time.

In a study that is somewhat difficult to interpret, Carver (63) found that

a wide variety of phenothiazines, and also imipramine, at a concentration of $5\times10^{-4}M$ inhibited both beef adrenal and rat brain glucose-6-phosphate dehydrogenase. None of these drugs inhibited 6-phosphogluconic dehydrogenase of rat brain while, with the exception of imipramine, all inhibited this enzyme in the beef adrenal.

Aghajanian (64) observed that chlorpromazine ($5\times10^{-5}M$) stimulated the respiration of brain mitochondria in a medium with a low concentration of ADP and a high oxygen tension. When the level of ADP was raised, no effect of chlorpromazine was evident.

In companion pieces, Spirtes & Guth (65), and Freedman & Guth (66) described the effect of chlorpromazine on membranes. These investigations are particularly noteworthy because of the demonstration of an effect by chlorpromazine at a concentration well within the pharmacological range. Thus, at a concentration of $5\times10^{-6}M$ the tranquilizer prevented the swelling that occurs when rat liver mitochondria are suspended in isotonic sucrose; chlorpromazine sulfoxide at $5\times10^{-4}M$ had no effect. In addition, when mitochondria were suspended in isotonic KCl, chlorpromazine did not prevent the swelling phenomenon. In studies of the inhibitory effect of chlorpromazine on the induced (urea, glycerol or hypotonicity) hemolysis of human red blood cells, chlorpromazine ($7.5\times10^{-6}M$) and trifluoperazine ($1\times10^{-6}M$) prevented hypotonic hemolysis. Higher concentrations were required to prevent the hemolysis of erythrocytes in the presence of urea or glycerol. Chlorpromazine sulfoxide gave no protection even at a concentration of $10^{-3}M$. Although limited in number, these studies suggest a correlation between the clinical potency of these phenothiazine derivatives and their effect on membranes. A correlation also exists between the effect on the erythrocytes and the uptake of the drug by the cell.

Both benzquinamide, an acetate derivative of benzoquinolizine, and its parent alcohol were found to disrupt avoidance behavior in rats. An estimation of the levels of catecholamines and 5HT in the brain showed that the alcohol depressed the level of amines, but the acetate derivative did not. In parallel studies in monkeys, both compounds disrupted avoidance behavior but neither compound lowered the concentration of the amines in the brain. The authors concluded that there is no relationship between disruption of avoidance behavior and lowered brain amine levels. (67).

For a thorough compilation of agents that affect the binding and release of all the biogenic amines, the review by Green is recommended (68). Shore (69) has recently reviewed the release of 5HT and the catecholamines by drugs.

Narcotics and Analgesics

Brassard & Quastel (70) observed that morphine ($5\times10^{-3}M$) stimulates the incorporation of P^{32}-phosphate into phospholipids of rat brain slices; the radioactivity was located primarily in phosphatidic acid, phosphoinositol,

and a compound that was identified tentatively as a diphosphoinositide. There was no effect of morphine on the oxidative metabolism of the slice and no effect on the incorporation of P^{32}-phosphate into phosphotidyl choline or ethanolamine. Similar results were obtained using imipramine in a concentration of $10^{-4}M$. Simon (71) has found that morphine and some analogues inhibited the synthesis of RNA in *E. coli;* inhibition was obtained at a concentration of 10^{-4} to $10^{-3}M$ and recovery was complete after removal of the drug.

It has been known for some time that salicylates are capable of uncoupling oxidative phosphorylation (72, 73, 74). In a recent paper on this subject Falconi et al. (75) found that salicylate (1 to $5\times10^{-3}M$) inhibited the P_i^{32}-ATP exchange reaction, and the P_i-H_2O^{18} exchange and that these reactions are accompanied by a stimulation of ATPase activity. These results are qualitatively similar to those obtained with DNP but the latter is 100 times more potent. The relationship of the uncoupling activity of salicylates to the analgesic, antipyretic, and antirheumatic properties of the drug are still unknown.

Cardiotonic Drugs

Phosphorylase activation.—The activation of phosphorylase as an explanation for the positive inotropic effect of the catecholamines, theophylline, and aminophylline is currently a strongly contested issue. Belford & Feinleib (76) observed that epinephrine, norepinephrine, isoproterenol, Ca^{++}, and aminophylline all produce an increase in the glucose-6-phosphate level in the cat ventricle. The authors explain these effects in the usual manner, i.e., an increased production of cyclic AMP (3′, 5′, AMP) which in turn increases the synthesis of active phosphorylase (phosphorylase *a*) and this activation results in glycogenolysis and subsequent rise in the glucose-6-phosphate concentration. Aminophylline inhibits the destruction of the cyclic AMP. The authors conclude that a parallelism exists between the positive inotropic effect and the increased level of glucose-6-phosphate. Rall & West (77) found that theophylline and aminophylline but not caffeine, cause an increased inotropic response to norepinephrine; theophylline also potentiated the inotropic effect of tyramine. Hess et al. (78) concluded that a relationship exists between activation of phosphorylase by epinephrine and increase in isometric tension of the heart. A close inspection of their data, however, reveals that, at low concentrations of the catecholamine, it is rather difficult to see this correlation between activation of phosphorylase and increased isometric tension. Vincent & Ellis (79) studied the effect of ACh, DCI, and theophylline on glycogenolysis in the isolated rat heart. When ACh was infused into the heart the glycogenolytic effect of epinephrine and theophylline was completely counteracted. ACh has been shown in previous investigations to inhibit the snythesis of cyclic AMP in the heart (80) and to decrease the concentration of active phosphorylase (81). Dichloroisoproterenol also

counteracted the effect of epinephrine and this activity also has been recorded previously (82). Mayer et al. (83) and Mayer (84) could find no correlation between active phosphorylase of a dog heart *in situ* and the increase in contractile force when small doses of eprinephrine were employed. In addition, with a small dose of the catecholamine, no increase in the glucose-6-phosphate level was noted in the heart, although an increase in the contractile force was observed.

To the observer who is admittedly unfamiliar with this terrain, it would appear that at the present time the proponents of the phosphorylase activation theory are on shaky ground. Most of these investigators are somewhat overzealous in administering catecholamines to their preparation in view of the fact that 0.1 μg/kg of the amine is enough to elicit increased contractility. Keeping this fact in mind, it would appear that the investigators in this area should be able to arrive at a decisive answer to this problem in the very near future.

Ouabain and ATPase.—Currently one of the most energetically investigated areas in experimental biology, the inhibition of a Na-K-activated ATPase by ouabain and other cardiac glycosides and the similarity between this ATPase and the active transport of Na^+ has been reviewed so extensively during the past few years (85, 86) that a lengthy discussion of the problem is unwarranted; accordingly only recent reports will be noted. It may be worthwhile, however, to summarize the main characteristics that are common to both the sodium pump mechanism and the Na-K activated ATPase: (*a*) localization in the cell membrane; (*b*) activation by $Na^+ + K^+$; (*c*) substitution of NH_4^+ for K^+; (*d*) substrate specificity (ATP); (*e*) pH sensitivity; (*f*) inhibition by ouabain, Ca^{++}, Cu^{++} and F^-; (*g*) no inhibitory effect of iodoacetate, DNP, NaN_3, $ASO_4^{\equiv}$, CN^-, or I; (*h*) the rate of $Na^+ + K^+$ transport correlates with the rate of ATPase activity in various tissues.

Wheeler & Whittam (87) found that at $2 \times 10^{-5} M$, ouabain caused a 50 percent inhibition of the cation activated ATPase of kidney cortex. This paper is also recommended for a well-written summary of previous work. Rummel et al. (88) studying the K^+ flux in human erythrocytes postulate that Ca^{++} and ouabain act competitively on the inside of the cell membrane. Auditore & Murray (89) isolated the Na-K-activated ATPase from rabbit ventricles and observed that ouabain produces a 50 per cent inhibition of this activity at $6 \times 10^{-4} M$. Gonda & Quastel (90) showed that incubation of ouabain, at $1 \times 10^{-5} M$, with brain cortical slices (*a*) increases the efflux of amino acids; (*b*) inhibits the formation of labeled glutamine from glucose or glutamate; (*c*) decreases the influx of creatine and glutamate; and (*d*) has no effect on respiration regardless of the concentration of K^+ in the medium. Schwartz et al. (91) prepared the cation-activated ATPase from guinea pig brain and by differential centrifugation techniques found that this microsomal enzyme is associated with membranes rather than RNA-containing granules of the microsomes. Inhibition of the ATPase by ouabain could be

discerned at a concentration of $10^{-7}M$. Dengler et al. (92) reported an active uptake of norepinephrine by the pineal gland of the cat; this uptake was inhibited by $10^{-5}M$ ouabain and also by $10^{-6}M$ reserpine. Bonting et al. (93) found a Na-K-ATPase in each of twenty-one tissues obtained from ten different species; the highest activity was observed in guinea pig brain. In eight tissues the authors found a correlation between the rate of transport of Na^+ and K^+ and the ATPase activity. In the other tissues that were tested the rates were too low to allow accurate estimations. The authors made the point that different tissues have differing sensitivities to ouabain. In an earlier study Bonting et al. (94) had described the presence of Na-K-ATPase in ciliary epithelium and postulated that this enzyme may play a role in aqueous humor dynamics. This hypothesis has recently been supported by Becker (95), who found that the intravitreous injection of ouabain will lower the intraocular pressure. Jones et al. (96) prepared a Na-K-ATPase from rat kidney endoplasmic reticulum and observed that when this preparation was added to a glycolyzing system derived from the cytoplasm of the cell, glycolytic activity was stimulated by over 50 per cent. These investigators localized the site of stimulation at the level of phosphoglyceric kinase and postulate that the ATP produced in this reaction, which is then utilized by the ATPase, represents a channeling of metabolic energy from glycolysis into the sodium pump.

Coronary dilators.—Krantz et al. (99) found that when glyceryl trinitrate was perfused through coronary vessels, the amount of nitrite that formed was too small to account for coronary vasodilatation. In addition, isosorbide dinitrate produced coronary dilatation and yet was not reduced to nitrite. For these reasons the authors conclude that the nitrate ion has a vasodilating activity without the necessity of prior reduction to nitrite.

Anticoagulants.—The effect of anticoagulants on the high energy phosphate of rat auricles was investigated by Wosilait (100). A large variety of anticoagulants, including both coumarin and phenindione derivatives, caused a decrease in the amplitude and rate of contraction. The creatine phosphate concentration was lowered, but the adenine nucleotide pool did not change in concentration. The lack of effect on ATP may be explained on the basis that the creatine phosphate-ATP equilibrium is far in favor of ATP.

Alcohol Deterrents

Two interesting investigations on the mechanism of action of tetraethylthiuram disulfide (Disulfiram, Antabuse) have been reported by Strömme (97, 98). Diethyldithiocarbamate inhibited both brain and yeast hexokinase only when the agent was oxidized to disulfiram; this oxidation is assumed by Strömme to be catalyzed by cytochrome C in tissues and by methemoglobin in erythrocytes. The disulfide form is thought to be the active inhibitor, since glutathione, which will reduce the disulfide, also prevents the inhibition of hexokinase. The major actions of disulfiram, however, can still be ascribed

to the inhibition of aldehyde dehydrogenase, aldehyde oxidase, and xanthine oxidase.

Diuretics

Using kidney cell debris (850×g sediment) as a source of Na-K-ATPase, Taylor (101) has shown that organic mercurials such as Mersalyl, Merchloran, Esidrone, and *p*-chloromercuribenzoate inhibit the enzyme in a manner similar to that of ouabain. The concentration of the mercurials used in this study was $3\times10^{-4}M$ and since mercurials concentrate in the kidney this level of the drugs is pharmacologically reasonable. Iodoacetate, iodoacetamide, theophylline, theobromine, caffeine, and chlorthiazide did not inhibit the ATPase of this preparation. Landon & Morris (102) used a carefully prepared ATPase from rat kidney microsomal membranes and showed that mercaptomerin and meralluride inhibited the enzyme at 0.7 mM. These authors suggest that this inhibitory effect on ATPase may represent the therapeutic action of the mercurials.

Maren (103, 104) has made a thorough study, both *in vivo* and *in vitro*, of the kinetics of carbonic anhydrase. He has shown that at least 99 per cent of the enzyme must be inhibited before a physiological effect can be demonstrated; the optimum therapeutic effect occurred when 99.9 per cent of the enzyme was inhibited. He has also demonstrated that carbonic anhydrase is present in about 1000-fold excess and is not rate-limiting in acid-base balance.

Anticonvulsants

Gray et al. (105) postulate that catecholamines are involved in the anticonvulsant activity of the carbonic anhydrase inhibitors, but not in the action of diphenylhydantoin and phenobarbital. This conclusion arises from the observation that pretreatment of animals with reserpine, tetrabenazine, or α-methyl dopa antagonizes the anticonvulsant activity of carbonic anhydrase inhibitors. Reserpine had no effect on the carbonic anhydrase activity of the brain. In addition, iproniazid, dopa, and tranylcypromine prevent the antagonistic action of reserpine in inhibiting the anticonvulsant action of acetazolamide (Diamox). Although the reserpine-pretreatment also abolishes the anticonvulsant activity of diphenylhydantoin and phenobarbital, the authors state that a direct relationship exists between brain excitability and anticonvulsant potency of diphenylhydantoin and phenobarbital and that these drugs are not dependent on the presence of catecholamines.

Steroids

Humber et al. (106) found that 16,16-difluoromethyl ethers of equilin and equilenin inhibited the incorporation of mevalonic acid into cholesterol by a rat liver homogenate; the natural estrogens, estrone, equiline and equilenin, were not inhibitory. The concentration of the inhibitors used in this study was $10^{-4}M$. Also, at a concentration of $4\times10^{-4}M$, Gomez-Puyon

et al. (107) observed that triamcinolone inhibited mitochondrial swelling, uncoupled oxidative phosphorylation, inhibited the exchange between P^{32}-phosphate and ATP and increased the latent ATPase. In these two studies the high concentration of drugs that were employed preclude discussions of the relevance of these findings to the mechanism of action of the drugs.

In a rapidly expanding area of interest, Kimberg & Yielding (108) have studied the structural and functional changes in pyruvic kinase that are induced by diethystilbestrol. The drug ($8\times10^{-5}M$) reversibly inhibited pyruvic kinase of rabbit muscle. Inhibition could be reversed by dilution or prevented by NADH or NAD; NADP or NADPH had no effect. The molecular weight of the enzyme was not altered by treatment with diethylstilbestrol, but the electrophoretic mobility and viscosity of the enzyme were changed. Another intriguing paper on control mechanisms is that of McGuire & Pesch (109), who isolated a pyridine nucleotide transhydrogenase from anterior pituitary glands, and showed that the enzyme is stimulated by estradiol, 5HT, epinephrine, norepinephrine, and phenylacetaldehyde. The authors suggest that glucose oxidation via the hexose monophosphate shunt is regulated by this hormonally sensitive enzyme.

Antibiotics

The inhibition of a biological clock by actinomycin has been demonstrated by Karakashian & Hastings (110). A marine dinoflagellate (*Gonyaulax polyedra*) exhibits rhythmic bioluminescence and this rhythm is inhibited by the antibiotic at a concentration of 0.02 μg/ml; neither chlormaphenicol nor amethopterin interfere with the rhythm. The detailed mechanism of action of actinomycin has been investigated by Goldberg et al. (111). It has already been shown that this antibiotic inhibits the DNA-dependent synthesis of RNA in intact cells and isolated enzymes from both mammalian and bacterial species and that the mechanism of action of the antibiotic is related to its binding to DNA (112 to 117). In the report cited (111), it was shown that the extent of binding of actinomycin is directly a function of the guanine residues in DNA. The optimal binding is found with the native helical configuration and is influenced by nucleotide sequences that surround the guanine residues.

Using mitochondria obtained from rat kidney, heart, brain, and spleen, Wadkins & Lehninger (118) observed that oligomycin inhibited the ATP-ADP exchange. It was previously shown that the antibiotic is a specific and potent uncoupler of oxidative phosphorylation (119). Glynn (120) reported that oligomycin inhibited the release of inorganic phosphate from the electric organ of the electric eel. Jöbsis & Vreman (121) observed that 5 to 10 μg of the antibiotic per ml inhibited the Na-K-ATPase of rabbit brain.

The effect of chloramphenicol on protein synthesis in a cell-free extract of *E. coli* has been investigated by Rendi & Ochoa (122, 123). The antibiotic did not inhibit the esterification of amino acids to transfer RNA, but did interfere with the subsequent transfer of the aminoacyl residues to the

ribosomes; oxytetracycline behaved similarly. The *l*-erythro and *l*-threo forms of chloramphenicol were much less active and this finding correlates with their low bacteriostatic activity. In addition, the known selectivity of the antibiotic for bacteria fits in with the observation that chloroamphenicol at pharmacological concentrations does not inhibit mammalian protein synthesis [but see ref. (125)]. Franklin (124) also has observed that chlortetracycline, oxytetracycline, and tetracycline all inhibit the transfer of amino acids to ribosomal protein. No interference with the attachment of the amino acids to transfer RNA was observed. Weisberger et al. (125) found that 0.001 μM chloramphenicol inhibited the incorporation of phenylalanine into reticulocyte ribosomes in the presence of added messenger RNA and that this inhibition could be overcome by added excess polyuridylic acid. The authors believe that chloramphenicol acts either by preventing the attachment of polyuridylic acid on ribosomes or else the antibiotic inactivates uridylic acid residues before they can combine with activated S-RNA.

In 1946 Duguid (126) suggested that the action of penicillin might be mediated by inhibition of cell wall synthesis of bacteria. Subsequently, Park (127) showed that penicillin caused the accumulation of uridine-linked peptides, later identified as precursors of cell wall mucopeptide in staphylococci (128). Although this explanation for the mechanism of action of penicillin is accepted in the case of gram-positive organisms, the action of the antibiotic with gram-negative bacteria is uncertain because of some reports that penicillin did not inhibit the growth of *E. coli* (129, 130). Rogers & Mandelstam (131) have reinvestigated this problem and have shown, by measuring the incorporation of glucose into bound diaminopimelic acid, that both benzylpenicillin and ampicillin inhibit the synthesis of cell wall mucopeptide in *E. coli*. The authors discuss the inability of other investigators to demonstrate the effect and conclude that the mechanism of action of pencillin is the same for gram-negative and gram-positive organisms. Ciak & Hahn (132) correlated morphological and chemical events in *Staphylococcus aureus* exposed to penicillin, and have shown, in five different strains of this organism, that lysis of the cell wall is concomitant with an accumulation of uridine diphosphatemuramic acyl peptides.

Young et al. (133) have studied the inhibition of protein synthesis *in vivo* in mice, rabbits, and dogs by acetoxycycloheximide. At a dose of 5 mg/kg this agent inhibited the incorporation of C^{14}-labeled amino acids into protein. In kidney and liver the drug inhibited the incorporation of P^{32}-phosphate into phosphoprotein, but this inhibition did not occur in the thymus or appendix. The authors claim that acetoxycycloheximide is more potent then puromycin in inhibiting protein synthesis. Flexner et al. (134) injected puromycin into the brains of mice and found that, although protein synthesis was inhibited 83 per cent, there was no effect on learning or memory in the mice. This is an interesting approach that may be worth while pursuing using inhibitors of nucleic acid synthesis that are introduced directly into the brain.

For a complete discussion of the mechanism of action of antibiotics, the recent review by Gale is recommended (135).

Cancer Chemotherapeutic Agents

The plethora of recent reviews (136, 137) of these drugs obviate the need for further discussion in this article.

Concluding Remarks

Considering the impact of biochemistry on other medical sciences, e.g., microbiology, the slow acceptance of this discipline by pharmacology is difficult to understand. Although physiological experimentation is valuable for defining a problem, a fundamental understanding of the mechanism of action of a drug can come only from a biochemical or biophysical approach. At the present time there are not more than about half a dozen drugs whose mechanism of action at a cellular or subcellular level is known with certainty. It is to be hoped that the relative complacency concerning this state of affairs may soon be modified.

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