STRUCTURE-ACTIVITY RELATIONSHIPS OF AMIDINE DERIVATIVES

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I. INTRODUCTION

A common type of pharmacological investigation takes the form of studying chemical structures which yield the specific action of a particular drug. Studies along these lines have sometimes been of great value in that they have led to the discovery of a more useful drug than the prototype. However, they have often been disappointing in that the structure-action relationships noted could not be satisfactorily explained.

Some of the difficulties are obvious. Thus, if the prototype is a drug of complex structure, it may prove impossible to obtain enough compounds related to it to permit the systematic modification of the chemical properties of the prototype. The type of pharmacological activity displayed may be difficult to characterize or to measure conveniently. Again, if some of the compounds of a group are not stable chemically, it may be difficult to distinguish their own pharmacological effects from those of metabolites.

Certain other difficulties were clearly recognized by Barger and Dale (22) in their pioneer investigation of sympathomimetic amines. Some of their comments are so apposite to this review that they bear repeating fifty years later.

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Barger and Dale found that approximation to adrenaline in structure is, on the whole, attended with increasing intensity of sympathomimetic activity, and with increasing specificity of the action. Now, purely quantitative differences could be explained plausibly in several ways, *e.g.*, by attributing them to differences in the ability of the amines to reach the site of action. But how could one explain why motor and inhibitor activity vary to some extent independently? Although Barger and Dale found it desirable to postulate two distinct types of "receptor," they did not consider that differences in the pharmacological properties of the amines could be accounted for merely by postulating different sets of drug-receptors for different amines.

Since the most striking feature of the pharmacological activity of sympathomimetic amines is its more or less strict localization to cells innervated by the sympathetic nervous system, Barger and Dale argued that "there must evidently be something in those cells, or connected with them and them only, which has a strong affinity for these amines." However, they added a qualification: "But the property on which this affinity depends is by no means necessarily or even probably that which confers stimulant activity on the amines . . . the stimulant action may depend on one property, the distribution of the action on quite a different property."

Barger and Dale considered at length whether the sympathomimetic activity of the amines they tested was due to a "chemical" or to a purely "physical" action, but they were unable to reach a firm decision. Indeed, it can be seen that many of their difficulties stemmed from lack of knowledge about what happens at the sub-cellular level. As they themselves realized, merely postulating the existence of "drug-receptors" throws little light on the situation. To produce a productive hypothesis, one must make certain assumptions about the interaction between a drug and its receptor.

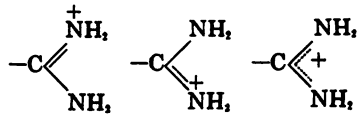
With amidine derivatives, as with sympathomimetic amines and many other groups of drugs, attempts to interpret structure-action relationships have led experimenters to consider such questions as the following: Do drugs of a particular type differ in their ability to reach the hypothetical receptors? May the effectiveness of the drug-receptor interaction vary from one drug to another? Is the interaction between drug and receptor a chemical process, involving activation of the receptor by electron transfer? Are the receptors which respond to chemicals that are foreign to the body activated normally by chemicals of physiological importance?

These questions have invited attention in the case of amidine derivatives because some of the common difficulties in the way of interpreting structure-action relationships (*e.g.*, those attending the use of drugs which are of complex structure or which are subject to metabolic change) may be obviated through using compounds of this type. The structure-action relationship for amidine derivatives which receive special attention in this review are those which are thought to provide clues to the understanding of drug-receptor interaction.

II. CHEMICAL FEATURES OF AMIDINE DERIVATIVES

A. Structure and nomenclature

Amidine derivatives possess the strongly basic group which is conventionally represented as $-C(NH_2):\overset{+}{N}H_2$. This structural formula is misleading insofar as it suggests that the two nitrogen atoms are differently bonded. The structures (I) and (II) resemble each other so closely that resonance occurs (262). Consequently, the structure of the amidinium ion is neither (I) nor (II); it cannot be represented accurately with the classical single and double bonds. Unfortunately, the use of such a formula as (III) imposes typographical difficulties. The conventional formula will therefore be employed except when special attention is being given to resonance.



(I) (II) (III)

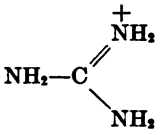
The compounds with which this review is especially concerned are those of formula $R—X—C(NH_2):\overset{+}{N}H_2$, where R is an alkyl group joined to the amidinium group either directly (as in ordinary amidines) or through an oxygen atom (alkyl *iso*ureas, also known as *O*-alkyl-uroniums), a sulphur atom (alkyl *iso*thioureas, also known as *S*-alkyl-thiouroniums), or an imino group (alkylguanidines).

It is important not to confuse alkyl *iso*thioureas with ordinary (*N*-substituted) alkyl thioureas. Transposition of the alkyl group from the nitrogen to the sulphur atom effects *inter alia* a great increase in basic strength. Whereas thiourea and its *N*-substituted derivatives exist normally as neutral (unionized) molecules or zwitterions, (*S*-)methyl *iso*thiourea and its homologues exist almost entirely as cations in the pH range likely to be found in pharmacological experiments. To emphasize this difference, the pharmacological effects obtained with the sulphate and other salts of methyl *iso*thiourea will be referred to as those of *S*-methyl-thiouronium (I.U.P.A.C. nomenclature). Similarly, the pharmacological effects obtained with alkyl *iso*ureas will be referred to as those of *O*-alkyl-uroniums. It is already customary with certain other types of drug to describe the properties of the drug with reference to the appropriate cation, *e.g.*, potassium, hexamethonium. One assumes in such cases that, although the drug is being tested as a salt, it is the cation which is the molecular species of pharmacological importance (6).

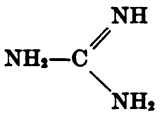
B. Basic strength

Some amidine derivatives are very strong bases because of resonance (262). A good example is provided by guanidine. The carbon atom of the guanidinium ion (IV) has attached to it three groups which resemble each other closely

enough to permit extensive hybridization. With the neutral molecule (V), the groups attached to the carbon atom differ sufficiently to make conditions far less favourable for resonance (262). Consequently, the ionization of guanidine is difficult to repress. The pKa of guanidine is no less than 13.6, which means that guanidine is almost as strong a base as sodium hydroxide. The basic strength of guanidine is but little affected by methylation (17, 198, *cf.* 58).



(IV)



(V)

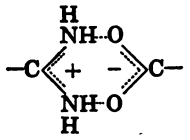
Acetamidine, $CH_3—C(NH_2):\overset{+}{N}H_2$, has fewer equivalent electronic structures than guanidine; its cation is doubly but not triply degenerate. Probably for this reason, it is not so strong a base, having a pKa of 12.4 (226). *O*-methyl-uronium, $CH_3O—C(NH_2):\overset{+}{N}H_2$, and *S*-methyl-thiouronium, $CH_3S—C(NH_2):\overset{+}{N}H_2$, are still weaker bases (7, 86). Even so, their recorded pKa values show that the pH of a solution containing any one of them would have to be raised to almost 10 to reduce ionization to 50%. At the pH of blood, only about 1% would be in the unionized form. At this pH, only about 0.001% of acetamidine, and hardly any guanidine or methyl-guanidine, would be in the unionized form. The pKa of diguanide and its mono- and di-alkyl derivatives is about 11.5; phenyl-diguanide is slightly less basic (219).

C. Affinity for anions

Because of their positive charge, amidinium ions will be attracted to negatively charged groups. They will be attracted in a biological medium not only to small anions, but also to the anionic groups of macromolecules, *e.g.*, the carboxyl and phosphatic groups of proteins. However, since an amidinium ion will be constantly bombarded by other molecules, the duration of contact between an amidinium ion and a particular anion is likely to be short unless the electrostatic attraction between oppositely charged groups is supplemented by some other type of bonding.

The narrow range of melting points found for many of the carboxylates of *S*-benzyl-thiouronium and several other thiouronium derivatives suggested to Walker (256) "that the forces which bind crystals of this type together are comparable in strength throughout the series and that the radicals attached to the $—S\cdot C(NH_2):\overset{+}{N}H_2$ and $—C(:O)\cdot\overset{-}{O}$ groups respectively make little or no contribution to the stability of the crystal." Walker has therefore postulated a structure (VI) for amidinium carboxylates in which "ionic bonds are formed *simultaneously* between the two oxygen atoms sharing the negative charge in the carboxylate ion and the two nitrogen atoms sharing the positive charge in the amidinium group." All the atoms shown in (VI) would be coplanar and assume a relatively rigid configuration of minimum potential energy, the implicit hydrogen

bonds being short and relatively strong. Evidence in favour of such a structure has since been provided by the technique of X-ray diffraction (154). A similar union would be expected to occur between amidinium ions and anions other than carboxylates, in which a negative charge is shared between oxygen atoms and which have a resonance energy similar to that of the amidinium group (256). On the other hand, this type of union (VI) would be made difficult by the attachment of an alkyl group to one of the nitrogen atoms of the amidinium group.



(VI)

With amidine derivatives, such as acetamidine and its homologues, which contain only a hydrocarbon chain in addition to the amidine group, it is improbable that any *strong* bond other than the reinforced ionic bond just described could be formed between cation and anion. However, weak bonds of the van der Waals type would be formed between the atoms of the cation and those of the anion. The van der Waals attraction between molecules can be substantial provided that both molecules are large and that a substantial proportion of the two sets of atoms can come close together (for the van der Waals attraction between two atoms is inversely proportional to the seventh power of the distance between them).

D. Chemical potential

From what has just been said, it would be expected that an amidinium ion with a long hydrocarbon "tail" would be more tightly bound to an anionic receptor than would one with only a short hydrocarbon "tail," supposing that close contact between drug and receptor were possible. However, this is not the only way in which chain-length could affect pharmacological activity.

Irrespective of whether the site of action of a drug is on a cell surface or within a cell, the concentration of the drug at the site of action is likely to be different from its concentration in the medium surrounding the cell. A detailed explanation of this phenomenon has been given by Ferguson (105), who introduced the term "biophase" to distinguish the phase in which a drug exerts its action from that in which its concentration is normally measured, *e.g.*, blood, inhaled air, the Ringer solution surrounding an isolated organ.

Some solutes become concentrated in a surface layer. Those that do are substances which have less affinity for water molecules than these have for each other. Such "hydrophobic" solutes tend to be squeezed out of the bulk aqueous phase. They will readily enter a phase consisting mainly of non-polar molecules. However, this process will be counteracted to some extent by thermal agitation. The thermodynamic activity ("chemical potential") of a solute measures its tendency to escape from the bulk phase; it is roughly proportional to the actual concentration of the solute divided by its concentration in the saturated solution.

The more nearly saturated the solution, the greater will be the chemical potential of the solute.

Ferguson (105) showed that the intensity of the pharmacological effects of certain types of drug can be predicted with considerable accuracy from estimates of their chemical potentials under the experimental conditions employed. It would appear in such cases that one drug may be more potent than another, not necessarily because it is more effective at the site of action, but because it can achieve a higher concentration in the biophase for a given molar concentration in the phase in which the drugs are administered.

We would therefore expect that lengthening the alkyl chain of an amidinium ion would affect pharmacological activity in that the distribution of a higher homologue between the bulk phase and the biophase would be different from that of a lower homologue. However, when chain-length is much increased, a stage will be reached at which water-solubility is so slight that too few molecules of the homologue can reach the site of action for them to produce much effect there. This explains the occurrence of a "cut-off" (section IVC-5).

It is not easy to gauge whether structural changes influence activity more by modifying the distribution of a drug between bulk phase and biophase than by modifying its affinity for receptors. One has to consider such points as whether the drug can rapidly make contact with the receptors under the experimental conditions employed (28, 99, 143, 205) and whether the drug can fit the binding site so closely that the van der Waals attraction can become strong. A categorical answer cannot be given.

III. PHARMACOLOGICAL ACTIONS OF THE PROTOTYPES

In this section evidence is presented concerning the mechanisms by which certain amidine derivatives produce their chief pharmacological effects. The compounds mentioned are not necessarily the most potent of their type; they happen to be those amidine derivatives of fairly simple structure which, for such reasons as availability and known activity, have received most attention from experimenters. Some of their more characteristic actions are described in detail to provide criteria for comparing the effects of these prototypes with those of other compounds.

A. S-Methyl-thiouronium

The pressor action of *S*-methyl-thiouronium was discovered by Smirk (233), who tested numerous substances related chemically to urea in the hope of finding among them drugs with useful circulatory properties. Hueper and Ichniowski (145) showed it to be more effective in dogs than ephedrine, tyramine, or pituitrin for the treatment of histamine shock. *S*-Methyl-thiouronium has been found useful for maintaining blood pressure at a safe level during spinal anaesthesia (234). However, it is probably no longer employed clinically. The pressor action of *S*-methyl-thiouronium remains of interest chiefly because it appears to be of radically different type from that of typical sympathomimetic amines.

1. Effects on the cardiovascular system. An *S*-methyl-thiouronium salt given

intravenously in doses of 1 to 10 mg/kg produces large persistent rises of blood pressure in anaesthetized dogs, cats, and rats (87, 91, 100, 159, 171, 211). It may produce only a small rise, or even a fall, of blood pressure in the rabbit (171, 211), an animal which is insensitive also to certain other pressor agents (12). The pressor action of *S*-methyl-thiouronium is much less conspicuous in healthy unanaesthetized humans than in patients with low blood pressures.

Large pressor responses can be obtained with *S*-methyl-thiouronium in spinal cats (211) and in animals which have been decerebrated and pithed (171). Its pressor action is therefore not dependent on the integrity of the central nervous system. As *S*-methyl-thiouronium is able to constrict perfused blood vessels (88, 99, 100, 101), its pressor action would appear to be due in part at least to a vasoconstrictor action of peripheral origin. Strong evidence for this has come from experiments in which it was arranged that an animal's blood entered a limb by way of a pump and a delaying circuit. Under these conditions a dose of *S*-methyl-thiouronium which produced a substantial rise of blood pressure in the main portion of the animal did not affect the vascular tone of the perfused innervated (or denervated) limb until the blood containing it reached the limb (89).

S-Methyl-thiouronium generally slows the heart rate, even in vagotomized animals (100, 159, 171, 233, 234). In healthy human subjects, slowing of the heart rate is more evident than elevation of the blood pressure (233). The isolated perfused heart is also slowed (171). Both positive and negative inotropic actions have been noted (171, 211).

One interesting feature of the cardiovascular action of *S*-methyl-thiouronium is the occurrence of tachyphylaxis, *i.e.*, a diminishing pressor response to successive equal doses (100, 159, 171). Animals which have been rendered insensitive to the pressor action of *S*-methyl-thiouronium by repeated injection of the drug are not rendered equally insensitive to the pressor actions of amphetamine or ephedrine. Conversely, animals which have been rendered insensitive to the pressor actions of ephedrine or amphetamine by repeated injections of the amine still respond normally to *S*-methyl-thiouronium (91). Neither the pressor action nor the vasoconstrictor action of *S*-methyl-thiouronium is antagonized by antiadrenaline drugs (88, 101, 171) or by drugs which deplete tissues of catecholamines (91). The site of the pressor action of *S*-methyl-thiouronium is therefore likely to be different from either that of ephedrine or that of adrenaline. The pressor action of *S*-methyl-thiouronium is antagonized by papaverine but not by hexamethonium, lysergic acid diethylamide, or atropine (66, 91, 171).

In the presence of *S*-methyl-thiouronium certain other drugs produce greater cardiovascular effects than usual. The pressor action of adrenaline in anaesthetized dogs and cats is enhanced (66, 100, 213), as is the vasoconstrictor action of adrenaline on perfused rat blood vessels (97, 100) and the vasoconstrictor action of acetylcholine on perfused rabbit pulmonary vessels (99). In cats, electrical stimulation of the vagus produces greater cardiac slowing after treatment with *S*-methyl-thiouronium than before (211). The depressor action of acetylcholine is not potentiated, however (211).

2. Effects on nerve-muscle preparations. The tone and motility of isolated intestinal strips are increased by *S*-methyl-thiouronium in a concentration of 10^{-4} M or higher (66, 171, 211). This action is partly antagonized by atropine in approximately equimolar amounts (66, 88, 171). An increase in intestinal tone and motility can also be demonstrated *in vivo* (211). Similar actions are exerted on the bladder and uterus, but *S*-methyl-thiouronium does not constrict bronchial muscle to any noteworthy extent, though it can enhance the bronchoconstrictor action of histamine (88, 171).

The action of *S*-methyl-thiouronium on skeletal muscle has been studied chiefly through the use of Bülbring's rat diaphragm preparation (43, 88, 214). Contractions of the diaphragm evoked by maximal electrical stimuli applied to the phrenic nerve are increased by *S*-methyl-thiouronium. Contractions evoked by direct stimulation of the muscle are also increased, even when enough tubocurarine is present to make the muscle quite insensitive to nervous stimulation (88). The effect of tubocurarine in lower concentrations is sharply antagonized by *S*-methyl-thiouronium (88, 214). The potentiating action of *S*-methyl-thiouronium on muscle contraction is not much affected by doubling or quadrupling the calcium content of the bath, but it is reduced—sometimes even prevented—when the calcium content is reduced sufficiently to reduce the amplitude of the twitch (88). *S*-Methyl-thiouronium produced no further increase in the amplitude of the muscle twitch when this had already been increased by raising the potassium ion content of the bath; nor could the amplitude of the twitch be further increased by giving potassium if it had already been increased by giving *S*-methyl-thiouronium (88).

S-Methyl-thiouronium does not itself cause the contraction of frog rectus abdominis muscle, but it enhances the contractions of the muscle produced by acetylcholine (88, *cf.* 211). It has also been shown to enhance the action of neostigmine on a cat sciatic nerve-gastrocnemius muscle preparation (88). An anti-curare action has been demonstrated *in vivo* as well as with isolated preparations (214). The action of mephenesin is antagonized by *S*-methyl-thiouronium, but not strongly (214).

3. Other effects. Pulmonary ventilation is increased temporarily by *S*-methyl-thiouronium (100, 213). It has been tried as an analeptic in experimental barbiturate poisoning, but with unimpressive results (213). Even large doses do not produce subjective sensations in unanaesthetized subjects (233).

In large doses it affects metabolism in several ways (211, 215). The blood sugar level is decreased, also the level of liver lipids. With toxic doses, the growth of young rats is severely checked. The LD50 of *S*-methyl-thiouronium sulphate is about 300 to 350 mg/kg for rats and mice, about 100 to 200 mg/kg for dogs and cats, but only about 50 mg/kg for rabbits. The effects of poisonous doses have been described in detail by Hueper and Ichniowski (146). It would seem that *S*-methyl-thiouronium, in contrast to ordinary (*N*-substituted) thioureas, has little antithyroid activity.

B. 4-Methyl-2-amino-pyridine

Like *S*-methyl-thiouronium, 4-methyl-2-amino-pyridine (W-45, Ascensil) gained notice initially on account of its ability to raise blood pressure and stimulate respiration (134). Further work has drawn attention to certain of its central actions.

1. Effects on the central nervous system. Although clinical evidence is lacking as yet, various laboratory studies have suggested that 4-methyl-2-amino-pyridine possesses strong analgesic activity. Von Haxthausen (135) concluded from tests involving the application of graded thermal or electrical stimuli to rats, mice, and rabbits that 4-methyl-2-amino-pyridine is about 60 to 70% as potent as morphine. Fastier and McDowall (94) concluded from a different battery of tests that 4-methyl-2-amino-pyridine is much less potent than this. A reason for the discrepancy may be found in the observation that 4-methyl-2-amino-pyridine has a more rapid and less persistent action than morphine. A comparison of the analgesic effects of the drugs made within 10 to 15 minutes after their injection would suggest that 4-methyl-2-amino-pyridine acts almost as strongly as morphine. If, however, the maximum effect of 4-methyl-2-amino-pyridine is compared with the maximum effect of morphine (obtained later in the experiment), then it would appear that the amino-pyridine is only about one-tenth as active as morphine on a dosage basis (94). Nevertheless, even this degree of analgesic activity is remarkable in a compound of fairly simple structure (XII).

If 4-methyl-2-amino-pyridine and morphine acted at the same site, then one might expect "therapeutic interference" (5) to occur when the drugs are given together, with the weaker antagonizing the action of the stronger. However, 4-methyl-2-amino-pyridine does not antagonize the analgesic action of morphine (132) though it antagonizes the depressant action of morphine on respiration (135). Antagonism of the analgesic action of 4-methyl-2-amino-pyridine by *N*-allyl-normorphine (Nalorphine), was observed by von Haxthausen (135) but not by Grimmett, Neame, and Fastier (132).

Experimental cough is inhibited by 4-methyl-2-amino-pyridine (135). It elicits the Straub phenomenon in mice and, in large doses, produces catatonia in rats. However, it shows little resemblance to morphine in certain other respects (94, 135). Thus, it stimulates respiration, raises blood pressure, and has little constipating effect. Under some experimental conditions (134), 4-methyl-2-amino-pyridine has a pronounced analeptic action. However, it was not found to reduce the proportion of mice dying as a result of their having been given a dose of pentobarbitone sodium approximate to the LD50 5 to 15 minutes beforehand (1). The LD50 of 4-methyl-2-amino-pyridine in mice is about 80 to 100 mg/kg. Convulsions are produced with about half this dose, and strong analgesia with about one-tenth of it (94).

2. Effects on the cardiovascular system. The pressor action of 4-methyl-2-amino-pyridine is comparable in magnitude and duration to that of ephedrine (134). However, there is no "cross-tachyphylaxis"; an animal which has become insensitive to the pressor action of ephedrine still responds well to 4-methyl-

2-amino-pyridine (91). Doses of anti-adrenaline drugs which suppress almost completely the pressor action of ephedrine inhibit that of 4-methyl-2-amino-pyridine to the extent of 40 to 50% (134).

Schoepke and Shideman (225b) have shown recently that 4-methyl-2-amino-pyridine has a positive inotropic action and a positive chronotropic action on the isolated auricles and on the papillary muscle of the cat. Effective doses restored competence in the failing heart-lung preparation of the dog for periods of 1 hour or longer. When preparations of auricles or papillary muscle were obtained from cats which had been given sufficient reserpine to reduce markedly the catecholamine content of the myocardium, the positive inotropic action of 4-methyl-2-amino-pyridine was abolished. Hence it would seem that this action is due to the release of catecholamines from storage sites.

Doses of 4-methyl-2-amino-pyridine which raise arterial blood pressure generally slow the heart (127). In dogs to which the drug was given by slow intravenous infusion, there was a marked increase in peripheral resistance accompanied by an increase in right atrial pressure and a reduction in both cardiac output and myocardial oxygen usage; however, the calculated cardiac efficiency, cardiac respiratory quotient, and total body oxygen consumption and respiratory quotient were unchanged (217). The effect on coronary flow is variable (127). The intraarterial injection of 4-methyl-2-amino-pyridine causes narrowing of the vessels of the skin and resting muscles; it increases blood flow in working muscles (127). Perfused rat blood vessels are constricted (94).

3. Other effects. Experimental bronchial spasm in the cat produced by pilocarpine or by hyperventilation is alleviated slightly by 4-methyl-2-amino-pyridine (135). It does not contract the cat's nictitating membrane and has little or no action on the seminal vesicle. However, it temporarily decreases intestinal movement in the anaesthetized cat and renders isolated intestinal strips less sensitive to such agents as histamine and barium (135).

The compound potentiates the maximal contractions of the rat diaphragm preparation elicited by electrical shocks applied either to the phrenic nerve or directly to the muscle (94). It has slight local anaesthetic activity (94).

C. Guanidine

The fibrillary twitching produced in frogs by large doses of guanidine was first described more than eighty years ago (122, 210). Interest in other properties of guanidine was stimulated by reports (2, 84, 157, 163) that simple guanidine bases occur naturally.

It is not unlikely that the guanidine and methyl-guanidine alleged to be present in the whole blood and urine are derived from certain guanidine derivatives of undoubted physiological importance. The basic amino acid, arginine, is a guanidine derivative; so is creatine. The methyl-guanidine "isolated" by Koch (157, 158) and others who used mercuric salts as precipitants was probably an artifact, since creatine readily undergoes atmospheric oxidation in the presence of mercuric salts, yielding creatone; the latter is soon hydrolyzed to oxalic acid and methyl-guanidine (85, 130). Evidence based on the isolation of picrates is

also suspect (131). In more recent procedures (15, 16, 207, 221, 222, 260, 261), protein is removed by the Folin-Wu procedure. Guanidine-like substances remaining are obtained in more concentrated solution through the use of blood charcoal or some such agent; they are then estimated colorimetrically (81, 179, 182, 189, 207, 260, 261). One important interfering substance is creatine. It can be estimated separately and the value obtained subtracted from the blood or urinary "guanidine," or it can be removed by autoclaving the final extract with hydrochloric acid. So determined, blood "guanidine" ranges normally between 2 and 3 mg/l (13, 14, 16, 179, 180, 207, 260, 261). Although it is possible to separate guanidine and its simple derivatives from other compounds which give similar colour reactions, this has seldom been done. According to Van Pilsum *et al.* (254), whole blood contains less than 0.4 mg/l of guanidine itself and less than 0.2 mg/l of methyl-guanidine. Their evidence makes one wonder whether whole blood or urine contains any guanidine or methyl-guanidine. At least it would seem that no more than traces of these particular guanidines are present.

Retention of guanidine bases has been blamed for several of the ills that flesh is heir to, including parathyroid tetany (206) and essential hypertension (173, 174). Blood "guanidine" may reach an abnormally high level in hypertension, but this appears due to associated kidney damage and not to the hypertension *per se*. The increase in cases of renal insufficiency parallels that of other non-protein nitrogen components: it does not seem to have any special significance (51, 69).

If the researches to which these speculations gave rise have contributed little to our knowledge of any disease, they have at any rate provided a rich assortment of pharmacological information. Properties of guanidine and its alkyl derivatives have been described in many papers published during the last fifty years. Much effort has gone into elucidating the mechanisms by which guanidine affects neuromuscular transmission.

1. Effects on the cardiovascular system. Guanidine in large doses (100 mg/kg or more) raises the blood pressure of anaesthetized dogs and cats (68, 178, 197). Its pressor action is less evident in the rabbit (10, 68, 72). There may be a sharp but transient fall of blood pressure immediately after the intravenous injection of guanidine, even in atropinized or vagotomized animals. This fall is attributed to cardiac depression (10). The rise of blood pressure is often accompanied by slowing the heart (173, 178).

In dogs, guanidine produces larger and more persistent rises of blood pressure when it is given intracisternally than when it is given intravenously in the same dose (216). This and other evidence (197) suggests that the pressor action of guanidine may have a central component. However, the rises of blood pressure produced by the intravenous injection of guanidine are believed to be due in large part to a direct constrictor action on blood vessels (68, 101, 175). De Waele and Bulcke (68) have demonstrated by Nolf's 3-manometer technique that guanidine has a vasoconstrictor action which is largely of peripheral origin, the response of a denervated limb being but little less than that of an innervated limb. The ear vessels of rabbits are narrowed and the capillaries may constrict so much as to become almost invisible (175). Isolated perfused blood vessels

are constricted by guanidine (47, 101, 245). Various effects on isolated heart preparations have been reported (23, 47, 113, 210). Whether the beat is accelerated or slowed would seem to depend partly on dosage.

Nicotine has been found to abolish or even reverse the constrictor action of guanidine on perfused frog blood vessels (47). This finding suggests that the vasoconstrictor action of guanidine, like those of nicotine and ephedrine (45), may be due in large measure to liberation of catecholamines from peripheral depots. Sugawara and Tada (240) have shown that guanidine can cause a large and sustained increase in the output of adrenaline from the suprarenal medulla. However, if guanidine had an indirect sympathomimetic action, one would expect its vasoconstrictor action to be antagonized by ergotoxine (a mixture of ergocornine, ergocristine, and ergocryptine); but this was not observed in experiments with the pithed rat hindquarters preparation (101). Lack of ergotoxine antagonism is reasonable evidence that noradrenaline release is not involved. The pressor action of guanidine is unaffected by treatment with atropine (10, 175), but it can be opposed by giving calcium (175).

2. Effects on smooth muscle preparations. Guanidine has not a powerful action on smooth muscle preparations. In a concentration of 10^{-3} M or higher it increases the tone of rabbit intestinal strips, which effect is reduced but normally not prevented by atropinization (101, 239). Guanidine also increases the tone of uteri taken from rats or from virgin guinea pigs (239). The rise in tone produced by guanidine is reduced by liver extracts (239), perhaps merely because of adsorption of guanidine on the protein. The melanophores of frogs are contracted (201).

3. Effects on nerve-muscle preparations. The action of guanidine on skeletal muscle preparations has been studied in much greater detail. The muscle twitches evoked by guanidine have fascinated several generations of experimenters.

With amphibian muscle, stimulation is most pronounced with concentrations of 0.002 M to 0.005 M; higher concentrations bring about a rapid onset of a curare-like paralysis (123, 129, 210). The contractions are characterized by rhythmicity and by uniformity of size over long periods (224). Grouping is observed in certain phases and the size of the contractions varies from one group to another. In frogs, at the height of poisoning, contractions are no longer mainly fascicular; entire muscles are contracted in regular sequence (111, 122).

The early workers (121, 210) assumed that guanidine causes muscle twitches by stimulating nerve-endings, since they found that the twitching can be prevented by treatment with curare. Führer (115), who performed experiments with various excised frog muscle preparations, considered the site of action to be the myoneural junction because guanidine had no obvious effect either on the nerve-free end of the sartorius muscle or on denervated preparations. Langley (164) criticized some of Führer's findings trenchantly. Camis (49), at Langley's instigation, carried out experiments which indicated that twitching can still be obtained after nerve section and complete degeneration of the fibres. This Führer denied in later papers (116, 119). Paton and Findlay (206) concluded: "These results (of Camis), along with Führer's admission that twitching of the muscle may

occur after degeneration of the nerve, seem to indicate that although guanidine may act chiefly upon nerve-endings, in sufficient concentration it certainly acts also at some point beyond them." Their own results were held to support this view, but it did not find general acceptance.

Frank and his collaborators (108, 111, 112) broke fresh ground. They found that small doses of guanidine, which produced no detectable effects, nevertheless increased the response to nervous impulses. They observed that guanidine in small doses augmented the action of parasympathomimetic drugs such as acetylcholine and physostigmine. Frank *et al.* (108) cut the hypoglossal nerve in dogs and obtained subsequently the Vulpian-Heidenhain phenomenon (*i.e.*, contracture of the tongue muscles upon stimulation of the chorda tympani fibres in the lingual nerve). Guanidine and methyl-guanidine were then given in amounts sufficient to provide evidence of intoxication. No effect on the paralyzed musculature was seen, but if an intraarterial injection of acetylcholine was given the paralyzed portion of the tongue was thrown into a prolonged tonic contraction. Only about one-twentieth of the customary amount of acetylcholine was needed to demonstrate this effect. Similar responses were obtained after section of the lingual and sciatic nerves. Thus, the authors came to the conclusion that guanidine acts by increasing the sensitivity of skeletal muscle to agents like acetylcholine.

Since guanidine has negligible anticholinesterase activity (188, 246), its ability to enhance the contractions of skeletal muscle preparations produced by indirect (*via* the motor nerve) stimulation (88, 104b, 129, 153) or by adding acetylcholine to the Ringer solution bathing the muscle (88, 133) cannot be attributed to a physostigmine-like action. Furthermore, guanidine has been shown to enhance the contractions produced by such other stimulants as potassium and barium (118, 124, 133, 153). Probably because of this "potentiating" activity, guanidine has been found to be of some use in the treatment of myasthenia gravis (67, 190, 191), although its therapeutic value is probably slight (55a). Large doses of guanidine produce salivation, defecation, and various other parasympathomimetic effects (68, 72, 74, 191).

It has been shown by Feng and other investigators (75, 104b) that in the presence of guanidine a single orthodromic nerve impulse may lead to a persistent after-discharge from the muscle end-plate. Guanidine increases the end-plate potential (185, 202). According to Sato (220): "A single centrifugal nerve impulse produces a large and persistent transmitter action at the guanidine-treated region which causes a repetitive series of large e.p.p., and when each of these attains a certain critical level it sets up not only a conducted muscle impulse but also stimulates the nerve ending and backfires the recurrent discharge centripetally."

Otsuka and Endo (202) have shown recently that guanidine does not change the sensitivity of the end-plate to acetylcholine. They concluded that guanidine increases the amplitude of the end-plate potential by increasing the quantity of acetylcholine released from the nerve-endings by a single nerve impulse. They found that when neuromuscular transmission was blocked by such drugs as dec-

amethonium and succinylcholine (suxamethonium), guanidine increased the amplitude of the end-plate potential to the point of eliciting a propagated action potential and muscle contraction.

Several investigators have studied the antagonism between guanidine and curare-like drugs. Guanidine does not initiate twitching after muscles have been curarized (104b, 111, 112, 202, 210). Conversely, the neuromuscular paralysis caused by tubocurarine is lessened by guanidine unless enough tubocurarine has been given to produce complete block (88). Even when the response to nervous stimulation of the muscle cannot be restored to any degree, the response to direct electrical stimulation of the muscle is usually increased by guanidine (88). Guanidine antagonizes the action of gallamine (Flaxedil) more strongly than that of tubocurarine, but it is only variably effective against decamethonium (54). The anti-curare action of guanidine develops much more quickly than the action which results in an increase in the amplitude of the maximal contractions produced by direct or indirect stimulation of non-curarized muscle (54). Presumably the two actions are not directly related.

It has been shown that guanidine twitching can be prevented by treatment with local anaesthetics or with magnesium (111), agents which are believed to interfere with the liberation of acetylcholine at nerve-endings.

4. Effects on the central nervous system. One of the first pharmacological observations to be made with guanidine (122) was that it produced tonic extensor spasms in frogs in addition to fibrillary twitches. These spasms persisted after decapitation but disappeared on destruction of the spinal cord. With dogs and rabbits the tonic spasms were even more marked than the fibrillary muscular twitches, which were attributed by Gergens and Baumann to a peripheral action of guanidine.

The convulsive effects of guanidine are not unlike those seen in the tetany which follows removal of the parathyroid glands (156, 206, 247). In guanidine tetany, however, the calcium concentration of the blood is not significantly affected (24, 53, 74, 189, 199, 247, 259) and the condition is not much relieved by the administration of calcium (156, 259). Various other differences between guanidine tetany and parathyroid tetany have been noted (24, 80, 243, 247).

It is clear that both central and peripheral actions have to be invoked to explain such features of guanidine tetany as the contractions of entire muscles in regular sequence and the fibrillary twitches of individual muscles. Effects of guanidine on the cat's spinal cord have been studied by Koizumi (41, 161), who found that a 100 to 200 mg/kg dose given intravenously greatly augmented the ventral root reflexes and dorsal root antidromic discharges ("dorsal root reflex"). Soon afterwards a spontaneous dorsal root discharge began which was not accompanied by any perceptible ventral root activity. At a still later stage of the tetanus the ventral roots also showed spontaneous activity, and antidromic potentials produced by stimulation of ventral roots were similarly augmented.

Another site of action of guanidine on the nervous system has been demonstrated by Sato (220). He has shown that in the presence of guanidine, afferent impulses from nerve terminals in the skin of the frog are initiated spontaneously.

Also, antidromic stimulation of the corresponding sensory nerves results in a reverberation of discharges from these terminals.

5. Effects on metabolism. Guanidine in large doses lowers the blood glucose concentration of laboratory animals (19, 31, 55b, 258). The hypoglycaemia may be sufficiently severe to give rise to convulsions. The onset of convulsions after a dose of guanidine can be postponed but not prevented by administering glucose (19, 55b).

Guanidine decreased the hyperglycaemia produced in rabbits by glucose or adrenaline (110). However, guanidine itself often produces hyperglycaemia initially (150, 244). Since a hyperglycaemic effect is not obtained in animals from which the suprarenal medulla has been removed, it is probably due to the ability of guanidine to liberate adrenaline *in vivo* (55b, 244).

Storage of glycogen is prevented by guanidine (31, 150, 186). The striking loss of glycogen from the muscles and liver of dogs poisoned with guanidine was considered by Junkersdorf and Weinand (150) to be the primary toxic effect. Fatty degeneration of the liver is another prominent feature of guanidine poisoning (80).

According to Minot (186), the chief effect of guanidine on carbohydrate utilization is interference with oxidative processes in the tissues, causing an abnormal accumulation of lactic acid in the blood. The citric acid content of the serum also rises; after toxic doses of guanidine it may be 5 to 10 times the normal value (183, 184). The accumulation of metabolites such as citrates which would reduce the concentration of calcium ions in the blood and elsewhere may explain why, under some (71, 187) but not all (114, 156, 259) experimental conditions, the toxicity of guanidine can be counteracted to a noteworthy extent by calcium.

A significant depression of the oxygen uptake of isolated brain, liver, and muscle tissues has been produced by 0.005 M guanidine (21). Dickens (70) found that in rat brain slices 0.001 M guanidine produced a complete and reversible inactivation of the Pasteur effect (which is inhibition of glycolysis during aerobic metabolism). This indicates that in the presence of guanidine, an organism is less able than usual to conserve carbohydrate by utilization of the aerobic as distinct from the less efficient anaerobic process of obtaining energy.

D. Decamethylene-diguanidine

Watanabe's discovery (258) of the hypoglycaemic action of guanidine led to a search for compounds of the same type which might be of value in the treatment of diabetes mellitus. Decamethylene-diguanidine (Synthalin) was introduced for this purpose in 1926, four years after the discovery of insulin. Dodecamethylene-diguanidine (Synthalin B) became available for clinical use shortly afterwards. Unlike insulin, these compounds could lower blood sugar when taken by mouth. They did not find clinical employment for long, however, since it soon became clear that they could produce severe liver damage. Furthermore, as Duncan and Baird (76) have pointed out in their recent review on oral hypoglycaemic agents, the synthalins were introduced at an inopportune time. One of the hypoglycaemic

agents in current use, 2-phenylethyl-diguanide (DBI, Phenformin), probably acts in essentially the same way as the synthalins (76).

1. Effects on metabolism. The hypoglycaemic action of decamethylene-diguanidine is slower and more persistent than that of insulin (40, 55b, 57). Decamethylene-diguanidine is much more potent than guanidine, a 3 mg/kg dose of the former being sufficient to produce hypoglycaemic convulsions in dogs (40, 57, 107). As with guanidine, a rise in blood glucose concentration usually precedes the fall. The hyperglycaemic phase can be lessened or prevented by giving ergotamine (236). During the hypoglycaemic phase, the administration of adrenaline or glucagon does not raise the blood glucose level, probably because of the depletion of liver glycogen (40).

The mode of action of decamethylene-diguanidine is quite different from that of such oral hypoglycaemic agents as the sulphonylureas (55b, 76). It does not seem to involve insulin or the pancreas. Thus, Bodo and Marks (40) were able to demonstrate a hypoglycaemic effect in eviscerated decerebrate animals. Experiments on perfused muscle preparations have provided further support for the idea that carbohydrate metabolism in various tissues is directly affected in one or more stages by decamethylene-diguanidine (40). Tissue respiration is diminished and lactic acid production increased at the expense of glycogen and glucose, and the work capacity of the muscles is decreased.

Evidence has gradually accumulated indicating that the essential feature of poisoning with decamethylene-diguanidine is a disturbance of oxidation in the tissues (38, 40, 55b, 76, 121, 230). Mårtensson (183, 184) showed that decamethylene-diguanidine, like guanidine, can cause a large increase in the serum citrate level. This observation suggested that the biochemical lesion might occur at some stage of the Krebs' cycle. The toxic effects of fluoroacetate, attributable in part to calcium deprivation resulting from excessive production of citrate, have been explained in similar fashion (192). Dickens (70) observed that decamethylene-diguanidine acts like guanidine in inhibiting oxidative metabolism in isolated tissues. The former inhibited the Pasteur effect in concentrations as low as 10^{-6} to 10^{-5} M when tested on slices of rat cerebral cortex. Other tissues were less sensitive to amidine derivatives. That the reduction in oxidative activity results from a primary inhibition of phosphorylation has been suggested by Hollunger (142) and by Ungar *et al.* (249). The ATP-ase activity of aged mitochondria is inhibited by amidine derivatives to the same extent as is their oxygen uptake (148, 208, 249).

2. Antimicrobial effects. Decamethylene-diguanidine was tested for trypanocidal activity as a result of the observation that cultures of trypanosomes have a high demand for glucose and oxygen (169, 225); the von Jancsós (149) tested various hypoglycaemic agents in the hope of discovering a good trypanocide. They observed that decamethylene-diguanidine has strong trypanocidal activity, as did Schern and Artagaveytia-Allende (223). However, it soon became clear that decamethylene-diguanidine does not kill trypanosomes by producing an environment poor in glucose (168). As so often happens, an unsound hypothesis proved fruitful, because the investigation of the antimicrobial activity of

decamethylene-diguanidine led on directly to the discovery of pentamidine, propamidine, and other useful chemotherapeutic agents.

3. Other effects. Decamethylene-diguanidine lowers blood pressure. Its depressor action in the cat appears due in large measure to the liberation of histamine (172). It relaxes isolated strips of rabbit intestine but stimulates uterine muscle and reverses the response of the latter to adrenaline (264). In frogs it produces a curare-like paralysis (236).

E. Phenyl-diguanide

Phenyl-diguanide was one of many organic bases tested by MacIntosh and Paton (172) for the property of liberating histamine *in vivo*. It was observed that several diguanides have an action which superficially resembles that of certain veratrum alkaloids in that the fall of blood pressure which these compounds ordinarily produce in cats is not obtained in vagotomized animals. The mechanism of action was elucidated chiefly through the work of Dawes and his collaborators (61, 62, 63). Phenyl-diguanide is now proving of value as an experimental tool for the investigation of chemoreflexes, being almost as potent as 5-hydroxytryptamine in its effect on some types of sensory receptor, yet lacking other forms of pharmacological activity exhibited by 5-hydroxytryptamine.

1. Effects on the cardiovascular system. The response to phenyl-diguanide depends to a remarkable extent upon such experimental conditions as the species of animal used and the depth of anaesthesia (60, 61, 62).

In lightly anaesthetized cats the intravenous injection of phenyl-diguanide is likely to produce a sharp fall of blood pressure and heart rate even with doses as small as 20 to 25 μg/kg, so long as the vagi are intact (61, 62, 96). The fall of blood pressure is due to vasodilatation as well as to slowing of the heart rate, both effects being produced reflexly (61, 89). Phenyl-diguanide hardly affects the blood pressure of vagotomized cats, whether given in small or quite large (1 to 10 mg/kg) doses. Therefore, it appears to have little direct action on the cardiovascular system (96).

The sensory receptors upon which phenyl-diguanide acts chiefly (in cats) have been located approximately by injecting a small fixed dose of the drug at various sites, *e.g.*, into the pulmonary artery, the arch of the aorta, the left coronary artery, or one of the chambers of the heart. It has been found in this way that the sensory receptors which respond to phenyl-diguanide lie chiefly in the heart and lungs (60, 62, 63). Further evidence for an action involving afferent vagal fibres has come from electrophysiological investigations (63). In contrast to the veratrum alkaloids, which act mainly on receptors situated in the cat's left ventricle, phenyl-diguanide acts mainly on receptors situated in the pulmonary bed (60, 63, 204). It can elicit at least three distinct chemoreflexes, *viz.*, the coronary chemoreflex, the pulmonary depressor reflex, and the pulmonary respiratory chemoreflex (60, 63).

There are other ways in which the reflex effects of phenyl-diguanide differ from those of such veratrum alkaloids as elicit the composite "Bezold-Jarisch reflex."

If a differential vagal block is produced by cooling the vagi, the reflex depressor actions of the veratrum alkaloids are blocked at a considerably higher temperature than those of phenyl-diguanide (63). Unlike veratrum alkaloids, phenyl-diguanide does not ordinarily produce reflex falls of blood pressure in dogs; rather, it tends to raise blood pressure and increase respiratory movements in dogs by stimulating sensory receptors (64, 73, 138). Tachyphylaxis is much less evident with phenyl-diguanide than with veratrum alkaloids. Even when the interval between successive small doses of phenyl-diguanide is no more than 5 minutes, reproducible depressor effects can be obtained (61, 96).

2. Other effects. In cats a transient apnoea accompanies the reflex hypotension and bradycardia (61, 63). This triad of effects is not obtained in dogs (64, 138). In other species, too, hyperpnoea may be more prominent than apnoea after the intravenous injection of phenyl-diguanide (96). These effects on respiration are much reduced but not as a rule entirely prevented by vagotomy (60, 62). This is to be expected, since there is evidence of non-vagal afferent pathways for a number of reflexes from the heart, lungs, and great vessels (60).

Certain other types of sensory nerve-endings are stimulated (perhaps not directly) by phenyl-diguanide, *e.g.*, some cutaneous pain receptors (96) and several types of gastrointestinal receptor (44, 204). When phenyl-diguanide is injected intravenously in small doses (50 to 100 μg/kg), it causes in rabbits an increase in peristaltic activity which is obvious on direct inspection of the abdominal viscera. This effect is less evident in cats (96). Bülbring and Lin (44) have shown that phenyl-diguanide evokes the peristaltic reflex in isolated intestinal strips.

In its effects on sensory nerve-endings, phenyl-diguanide closely resembles 5-hydroxytryptamine (96, 193, 204). In various other respects, however, there is little pharmacological resemblance between these two compounds. Thus, phenyl-diguanide appears devoid of vasoconstrictor activity (96) and antidiuretic activity (102). It does not lower body temperature or prolong chloral hydrate sleeping time in mice (96), nor does it increase the tone of Vane's rat fundal strip preparation, which is extremely sensitive to 5-hydroxytryptamine (251). In summary, the only noteworthy effects which phenyl-diguanide has been reported to produce are those elicited by reflex actions.

IV. STRUCTURE-ACTIVITY RELATIONSHIPS

A. Pressor activity

S-Methyl-thiouronium, $CH_3—S—C(NH_2):\overset{+}{N}H_2$, has served as the prototype for several studies of the distribution of pressor activity amongst amidine derivatives and related compounds. The compounds tested may be grouped conveniently as follows:

1. Compounds of the type, $CH_3(CH_2)_n S—C(NH_2):\overset{+}{N}H_2$. Twelve of these homologues of *S*-methyl-thiouronium have been studied, *viz.*, those for which n = 1 to 9, 11, 13, and 15. The last three have little effect on the blood pressure of anaesthetized dogs and cats, probably because of their insolubility in water (87).

The first ten members of the series (for which n = 0 to 9) show a steady grada-

tion in activity (87). Only the first three have a predominantly pressor action when given in doses of 1 to 10 mg/kg. As the series is ascended, a dual action on blood pressure becomes increasingly apparent; both pressor and depressor effects can be obtained by giving the *S*-alkyl-thiouronium salt in suitable doses. Tachyphylaxis occurs. Whereas *S*-methyl-thiouronium and its nearer homologues produce strong and well-maintained rises of blood pressure when given for the first time, later doses of these drugs produce much smaller pressor effects. The pressor effect may even be eliminated and a pure depressor effect remain. When an animal no longer responds to the one compound with a rise of blood pressure, its response to other "pressor" amidine derivatives is altered similarly, in contrast to its response to adrenaline and certain other sympathomimetic amines (100). With such homologues as *S*-*n*-butyl-thiouronium and *S*-*n*-amyl-thiouronium (n = 3,4), falls of blood pressure are readily obtained with repeated doses. A predominantly depressor response may be obtained even with initial doses of higher homologues (n = 5 to 9) unless the amount given is small (87).

2. Compounds of the type, CH_3—Y—$C(NH_2){:}\overset{+}{N}H_2$. Three compounds have been tested in which the sulphur atom of *S*-methyl-thiouronium has been replaced by some other small atom or group, *viz.*, *O*-methyl-uronium, methyl-guanidine, and propionamidine (for which Y = O, NH, and CH_2, respectively). All three are pressor agents (101, 177).

3. Other compounds of the type, X—$C(NH_2){:}\overset{+}{N}H_2$. These have been studied less systematically. Predominantly pressor effects have been obtained with amidine derivatives for which X is such a group as CH_3—, $CH_3(CH_2)_2$—, C_2H_5O—, $(CH_3)_2N$—, C_2H_5NH—, CH_3OCH_2S—, $HO(CH_2)_2S$—, or $CH_2{:}CH\cdot CH_2S$— (10, 86, 101). It can be seen that all these substituents contain only a few atoms. Amidine derivatives which have a larger substituent group, *e.g.*, benzyl-guanidine, *S*-2-phenylethyl-thiouronium, do not produce sustained pressor effects; rather, large doses of these compounds are likely to have predominantly depressor effects (50, 101). If the substituent is one which greatly decreases basic strength, the compound does not behave like a typical amidine derivative. Pressor effects have not been obtained with such compounds as *S*-acetyl-thiouronium and *S*-carbomethoxythiouronium (86).

4. Di-amidine derivatives. Eight members of the series of formula, $H_2\overset{+}{N}{:}(H_2N)C—S—(CH_2)_n—S—C(NH_2){:}\overset{+}{N}H_2$, have been tested, *viz.*, those for which n = 0 to 6 and 10. Formamidine disulphide (for which n = 0) has little effect on the blood pressure of anaesthetized dogs and cats (86). Only the *S*,*S*′-methylene derivative has considerable pressor activity. With higher homologues, depressor activity becomes increasingly pronounced (87).

5. N-substituted amidine derivatives. Only a few *N*-substituted amidine derivatives have been found to possess pressor activity like that displayed by *S*-methyl-thiouronium, *e.g.*, *S*,*N*-ethylene-thiouronium (VII), iminazole (VIII), and 2-amino-pyridine (IX). *S*-Methyl-*N*,*N*′-diphenyl-thiouronium is exceptional in displaying considerable pressor activity although its molecule is far from small. It produces large but transient rises of blood pressure in anaesthetized cats (86).

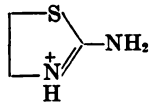
6. Amides and thioamides. Several dozen ureas and thioureas have been tested

for pressor activity, and so have various carbamates, thiocarbamates and thiohydantoins which also possess a —$C(NH_2):O$ or a —$C(NH_2):S$ group. These groups are much less basic than the structurally similar amidine group. None of the amides or thioamides tested has circulatory properties like those of *S*-methyl-thiouronium (86, 100).

7. Alkyl-amines. A number of investigators (11, 22, 77, 196) have studied the circulatory properties of amines belonging to the homologous series of structure, $CH_3(CH_2)_n—\overset{+}{N}H_3$. The pKa of these amines is about 11 (6, 11); they are thus slightly more basic than the *S*-alkyl-thiouroniums. The first three or four members of this series show little resemblance in their pharmacological properties to *S*-methyl-thiouronium, but a much closer resemblance is shown by those with 5 to 7 carbon atoms in the alkyl chain. Some of the latter show potent pressor activity (11, 22, 77).

8. Amino-pyridines. Of the three isomers, only the 2-substituted derivative can be regarded as an amidine derivative. Nevertheless, according to the principle of vinylogy, 4-amino-pyridine (X) should resemble 2-amino-pyridine (IX) closely in many chemical respects, and this is known to be so (79). Even 3-amino-pyridine (XI) is a considerably stronger base than is pyridine itself, though it is a weaker base than either 1-amino-pyridine or 4-amino-pyridine. The pKa of 2-amino-pyridine is 6.9, that of 3-amino-pyridine is 6.1, and that of 4-amino-pyridine is no less than 9.3 (7, 94). All three amino-pyridines raise the blood pressure of anaesthetized cats when injected intravenously in a dose of 1 mg/kg or more (93, 134), the 4-substituted derivative showing most activity and the 3-substituted derivative the least.

The circulatory properties of 4-methyl-2-amino-pyridine (XII) have already been described (section IIIB). Considerable pressor activity is displayed also by the 3-methyl, 5-methyl, and 6-methyl derivatives of 2-amino-pyridine, but not by the *N*-methyl derivatives of 2- and 4-amino-pyridine or by pyridine itself (95).



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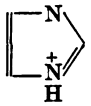

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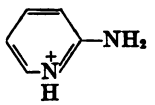

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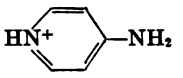

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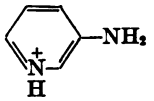

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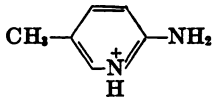


(XII)

9. Localization of pressor actions. So far, it has been tacitly assumed that short-chain amidine derivatives such as *S*-methyl-thiouronium, propionamidine, methyl-guanidine, and 2-amino-pyridine all raise blood pressure by the same mechanisms. The mode of action of pressor analogues of *S*-methyl-thiouronium must now be considered.

As indicated in sections IIIB and IIIC, the pressor effects of 4-methyl-2-amino-pyridine and guanidine are attributable mainly to a direct vasoconstrictor action. Goldblatt and Karsner (125) have summarized evidence indicating that the pressor action of methyl-guanidine and of *asym.*-dimethyl-guanidine "is due, in part at least, to their vasoconstrictor action brought about by stimulating the neuro-muscular apparatus of arterioles." Fastier and Smirk (101) concluded that the pressor actions of *O*-methyl-uronium, acetamidine, and several other short-chain amidine derivatives are due in part at least to a peripheral vasoconstrictor action. All the compounds they tested were found capable of constricting the perfused blood vessels of the pithed rat hindquarters preparation, and representative compounds were shown to produce in decerebrated and pithed cats pressor effects which were at least as great as those obtained in animals with an intact central nervous system. Most of the compounds slowed the heart rate of anaesthetized animals.

Goldblatt and Karsner (125) observed that the rises of blood pressure elicited by *asym.*-dimethyl-guanidine in anaesthetized dogs were accompanied by a definite decrease of limb volume. Since this decrease could be prevented by giving a large dose of ergotoxine (though not by such procedures as denervation of the limb, vagotomy, section of the cord at the second cervical vertebra, or destruction of the medulla and of the entire brain), they considered the vasoconstrictor action of *asym.*-dimethyl-guanidine to be similar to that of adrenaline. The pressor action of 4-methyl-2-amino-pyridine has also been attributed to a sympathomimetic action, chiefly on the basis of its partial suppression by anti-adrenaline drugs (134).

Fastier and Smirk (101) reached a different conclusion. They found in experiments on perfused rat blood vessels that concentrations of ergotoxine which were sufficient to reverse the action of adrenaline did not impair to any noteworthy extent the vasoconstrictor action of *asym.*-dimethyl-guanidine or of any of the other short-chain amidine derivatives tested. Moreover, these compounds increased the tone of isolated strips of intestine. Were their action sympathomimetic, they would be expected to relax the muscle.

The pressor action of *S*-methyl-thiouronium and of 4-methyl-2-amino-pyridine has been distinguished from that of ephedrine-like drugs in several ways (91). It has been shown that when either *S*-methyl-thiouronium or 4-methyl-2-amino-pyridine is injected into cats or rats which have been rendered insensitive to the pressor action of ephedrine, amphetamine, or 2-aminoheptane (Tuamine) by the repeated injection of one of these amines, the amidine derivative produces a rise of blood pressure comparable to that obtained in normal animals. The pressor response to these amidine derivatives, in contrast to the response to ephedrine-like drugs, is unaffected by pretreatment with reserpine. It has also been shown that a dose of methyl phenidate (Ritalin) which completely abolishes the pressor response to amphetamine in most animals has no obvious effect on the pressor response to *S*-methyl-thiouronium, 4-methyl-2-amino-pyridine, 4-amino-pyridine, or 2-aminoheptane (91).

These anomalous findings suggest that short-chain amidine derivatives may

have both a sympathomimetic vasoconstrictor action (due perhaps to an ability to liberate catecholamines *in vivo*) and a vasoconstrictor action of a fundamentally different type (due to an ability—like that of potassium or barium—to contract smooth muscle irrespective of the effect of adrenergic stimulation).

Similar difficulties have arisen in attempts to localize the pressor action of such aliphatic amines as *n*-hexylamine and 2-aminopheptane. These compounds are distinctly more sympathomimetic than short-chain amidine derivatives, judging by the results of Barger and Dale (22), Nakamura (196), Alles (11), Proetz (209), Jackson (148), Lewis (166), and Swanson and Chen (242). Nevertheless they, too, may cause the contraction of ileal strips and of several other smooth muscle preparations which are relaxed by adrenaline. For such reasons Alles (11) has argued that these amines resemble potassium and acetylcholine rather than adrenaline in their pharmacological properties. Alles considers the effects of aliphatic primary amines to be comparable to those of the alkyltrimethylammoniums and other bases, the chemical resemblance of which to acetylcholine is far closer. However, the lack of inhibitory potency can be explained in another way. Aliphatic primary amines might be regarded as sympathomimetic amines which have an affinity for the "α-receptors" postulated by Ahlquist (3) but not for the "β-receptors." This would not be surprising if, as Ariëns and Simonis (18) have suggested, the amino group of adrenaline is of primary importance for stimulating α-receptors but the catechol portion of the molecule for stimulating β-receptors.

These difficulties in characterizing the pressor actions of short-chain amidine derivatives and certain other aliphatic bases emphasize the need for testing compounds under strictly comparable conditions when structure-activity relationships are being investigated. The use of labels such as "sympathomimetic" and "nicotinic" may be quite misleading.

10. Comments. Possession of pressor activity would seem to be restricted to those chemical relatives of *S*-methyl-thiouronium which are sufficiently basic to exist mainly as cations at the pH of the blood. Since the pressor activity of methyl-guanidine, which is so strongly basic that it must exist almost exclusively in the ionized form throughout the pH range of biological media, is approximately equal to that of *S*-methyl-thiouronium, it may be deduced that the cation is the pharmacologically active molecular species, supposing that these two amidine derivatives have the same site of action.

Nearly all the pressor analogues of *S*-methyl-thiouronium are compounds with small cations. The few compounds which have fairly large cations and yet which possess considerable pressor activity (*e.g.*, *S*-methyl-*N*,*N'*-diphenyl-thiouronium) may have a mode of action which is different from that of short-chain amidine derivatives. Unfortunately, evidence on this point is lacking.

Two explanations for the inactivity of short-chain amines deserve consideration: 1) these compounds may show little resemblance to short-chain amidinium derivatives because of a specific affinity of the amidine group for certain tissue receptors; and 2) the much greater pharmacological activity of short-chain amidine derivatives might be due merely to their being adsorbed more strongly

than the corresponding amines. The second explanation is supported by the fact that aliphatic amines with 5 to 7 carbon atoms in the alkyl chain resemble short-chain amidine derivatives far more closely in their pharmacological properties than do amines with short side-chains; for it can be argued that the extra CH_2 groups in the alkyl chain of the higher homologues compensate for the smaller affinity of the ammonium (as compared with the amidinium) group for receptors.

Stronger evidence on this point has been obtained by comparing the circulatory and other pharmacological properties of the isomeric amino-pyridines (IX, X, XI). Since the pharmacological resemblance between 2-amino-pyridine and 4-amino-pyridine has been found to be close (93), it may be concluded that the spatial relationship of the two nitrogen atoms of the amino-pyridines is not of fundamental importance for conferring pressor activity and allied pharmacological properties.

B. Muscle-stimulating activity

Although a number of preparations of both smooth and skeletal muscle have been used for analyzing the pressor effects of certain amidine derivatives (sections IIIA to IIIC), only a few of these preparations have been used for studies of structure-activity relationships. Hence not many results are available for discussion.

1. Effects on perfused blood vessels. The vasoconstrictor action of *S*-methyl-thiouronium on pithed rat hindquarters perfused at a constant rate is much less intense but more persistent than that of adrenaline. Several minutes elapse before the perfusion pressure increase—resulting from the vasoconstriction—reaches its maximum, and the return to the initial pressure is even slower (99). Similar responses have been obtained with *S*-ethyl-thiouronium, *O*-methyl-uronium, *O*-ethyl-uronium, methyl-guanidine, ethyl-guanidine, *asym.*-dimethyl-guanidine, acetamidine, propionamidine, *S*,*N*-ethylene-thiouronium, 2-amino-pyridine, and iminazole (87, 93, 101). The vasoconstrictor actions of these short-chain amidine derivatives are not antagonized appreciably by ergotoxine.

When tested under the same conditions, homologues of *S*-methyl-thiouronium produce vasoconstriction initially; but, if the dose is large (0.1 ml of a 0.1 M or more concentrated solution) or if the *S*-alkyl chain is long, the perfusion pressure soon begins to fall (99). This creates a distinct "dip" in the perfusion pressure tracing. The dip coincides with a period of reduced sensitivity to adrenaline. This is followed by a second vasoconstrictor phase.

The effects of long-chain homologues of *S*-methyl-thiouronium are much more persistent than those of lower homologues. Whereas the effects of *S*-*n*-nonyl-thiouronium and *S*-*n*-decyl-thiouronium may be apparent for upwards of an hour after their injection, the effects of *S*-methyl-thiouronium and its nearer homologues do not persist for more than 10 to 20 minutes under the same conditions (99). Presumably the long-chain derivatives are better able to remain attached to the receptors in the face of continuous washing with the perfusing Ringer solution.

Numerous amidine derivatives have been found capable of producing, accord-

ing to the experimental conditions, either an increased or a decreased response to a test dose of adrenaline (97, 101). Particular attention has been given to the compounds of formula $CH_3(CH_2)_nS—C(NH_2):\overset{+}{N}H_2$. The first three members of the series decrease sensitivity to adrenaline only when they are present in very high concentration; otherwise, they usually have a potentiating effect. With the compounds for which $n = 3$ to 7, a dual effect on sensitivity can readily be demonstrated. The usual effect of *S-n*-nonyl- or *S-n*-decyl-thiouronium is to decrease sensitivity to adrenaline; an enhanced effect is observed only when the concentration of the amidine derivative is low and falls within a very narrow range (97). The influence of chain-length on this property of *S*-alkyl-thiouroniums is indicated graphically in Figure 1.

The concentration range over which an amidine derivative potentiates the vasoconstrictor action of adrenaline is not very different from that over which it produces strong vasoconstriction. However, the changes in sensitivity to adrenaline are not the result of the perfusion pressure changes, for the response to adrenaline can be altered greatly at fixed perfusion pressure level (99). It would seem that distinctly higher concentrations of an amidine derivative are needed for maximal potentiation of adrenaline than for maximal vasoconstriction. Similarly, in experiments on anaesthetized dogs and cats, enhancement of the pressor action of adrenaline is usually most evident at the stage of the experiment at which tachyphylaxis to the direct pressor action has become evident.

2. Effects on isolated intestinal strips. *S*-Methyl-thiouronium, propionamidine, *asym.*-dimethyl-guanidine, 2-amino-pyridine, iminazole, and several other short-chain amidine derivatives have been shown to increase the tone of isolated strips

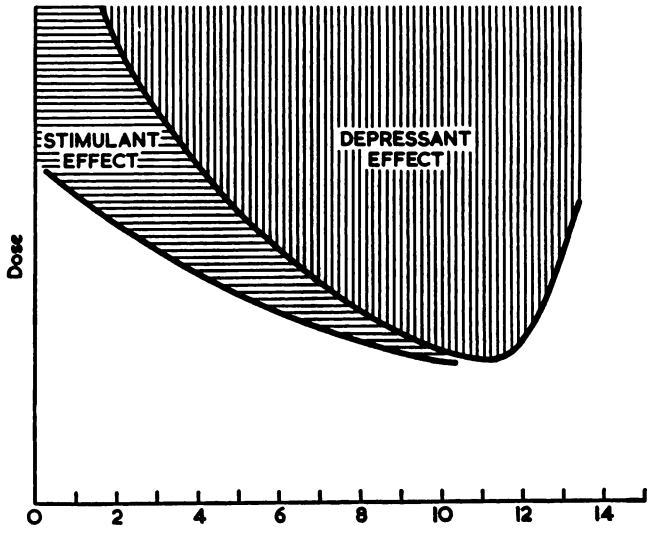


FIG. 1

of rabbit intestine when tested in concentrations of 10^{-4} M to 10^{-3} M. Their action is weakly antagonized by atropine (66, 87, 101). A few long-chain amidine derivatives also have a mild stimulant action, possibly due to histamine release (264). However, a strongly depressant action seems more typical of long-chain amidine derivatives.

The influence of chain-length has been studied in detail in the case of the thiouroniums of formula, $CH_3(CH_2)_nS—C(NH_2):\overset{+}{N}H_2$. When tested in a concentration of 10^{-4} M, only the first four members of the series increased tone regularly (99). *S-n*-Amyl- and *S-n*-hexyl-thiouronium produced irregular effects; sometimes they increased tone but more often they lowered it. Purely depressant effects were obtained with homologues for which $n = 6$ to 9. Still higher members of the series are not sufficiently soluble for their effects to be compared with those of lower homologues. Similar results were obtained (99) with a test concentration of 10^{-5} M. As the series was ascended, it became increasingly difficult to reverse the depressant action of an *S*-alkyl-thiouronium by repeatedly washing the muscle with Ringer solution. A dual action on muscle tone was demonstrated convincingly in the case of *S-n*-amyl-thiouronium and its near homologues through the use of a "constant flow" technique (98, 99), but there was no indication of stimulation preceding depression with any of the doses of *S-n*-nonyl- or *S-n*-decyl-thiouronium tried (99).

Attempts to discover whether amidine derivatives can enhance the sensitivity of intestinal strips to other stimulants (*e.g.*, acetylcholine, histamine) have not yielded convincing results (66, 99).

3. Effects on rat diaphragms. *S*-Methyl-thiouronium, *S*-ethyl-thiouronium, guanidine, methyl-guanidine, *asym.*-dimethyl-guanidine, 2-amino-pyridine, and 4-methyl-2-amino-pyridine have been shown to potentiate the effect of maximal electrical shocks applied indirectly to Bülbring's rat diaphragm preparation (43) when tested in concentrations of about 10^{-4} M to 10^{-3} M (88, 93, 94, 211). *S-n*-Butyl- and *S-n*-hexyl-thiouronium reduced the amplitude of the twitch when tested in the same range of doses. Transitional behaviour was displayed by *S-n*-propyl-thiouronium: whereas moderate concentrations (M/2,000 to M/500) somewhat increased the amplitude of the twitch, higher concentrations decreased it.

4. Comments. The amidine derivatives which have been found to resemble *S*-methyl-thiouronium and guanidine most closely in their effects on perfused blood vessels, intestinal strips, and rat diaphragms are short-chain amidine derivatives which display strong pressor activity. In the case of the *S*-alkyl-thiouroniums, at any rate, it is clear that lengthening the chain attached to the amidine group results in the masking of stimulant activity by depressant activity. Reasons for this will be advanced after the depressant actions of amidine derivatives on nerve-muscle preparations have been described in more detail.

C. Muscle-depressing activity

As very few compounds apart from certain amidine derivatives and sympathomimetic amines are able to produce large rises of blood pressure in experimental

animals, it is informative to study the influence of chemical structure on pressor activity. Depressor activity, on the other hand, is displayed by many compounds. Even with amidine derivatives, possession of depressor activity does not demarcate with considerable accuracy compounds which have a strong direct action on blood vessels from those which do not. Therefore, little need be said about the distribution of this property amongst amidine derivatives.

1. Depressor activity. Amidine derivatives have been shown to lower blood pressure by several mechanisms, depending on the compound and the experimental conditions.

In cats, numerous amidine derivatives lower blood pressure mainly by evoking the coronary and certain other chemoreflexes. The influence of chemical structure on reflex depressor activity is described in section IVD.

Most of the amidine derivatives examined by MacIntosh and Paton (172) produced in cats a sudden fall of arterial blood pressure, the beginning of which was delayed for some 20 to 25 seconds after the injection. Examination of blood or plasma obtained during the period of lowered blood pressure showed that it contained a depressor substance which acted within a few seconds after its injection. This substance was identified pharmacologically as histamine. It was obtained in amounts sufficient to account for the vascular effects of the bases which had the delayed depressor action. The most potent of the 11 di-amidine derivatives tested were propamidine and stilbamidine—compounds of therapeutic importance (225a). Octamethylene-diamidine and decamethylene-diguanidine were almost as active.

Of the various mono-amidine derivatives tested by MacIntosh and Paton, the only ones with the delayed depressor action were certain benzamidine derivatives. Simple aliphatic amidines, guanidines, and thiouroniums were inactive as histamine-releasers (172). Yet some of these compounds possess strong depressor activity (87, 126). For example, with the homologues of *S*-methyl-thiouronium, depressor activity increases steadily as the series is ascended up to the tenth member, beyond which point water-insolubility precludes high activity (87). Since vagotomy scarcely affects the depressor actions of long-chain mono-amidine derivatives, the falls of blood pressure they produce cannot be attributed to the coronary and similar chemoreflexes. At least one other type of depressor action must be postulated. With certain di-amidines, too, it would seem that depressor activity is not always due largely to histamine release or to reflex actions (136).

2. Vasodilator activity. *S*-Methyl-thiouronium and several of its homologues have been tested on cat hindlimbs perfused *in vivo*. The blood vessels of this preparation, unlike those of the pithed rat hindquarters, have their innervation intact and have normal tone. Whereas such compounds as *S*-*n*-amyl-thiouronium and *S*-*n*-nonyl-thiouronium produce only a slight dip in the perfusion pressure of pithed rat hindquarters (section IVB-1), they produce large falls of perfusion pressure when injected into perfused cat hindlimbs even in doses as small as 1 μM, potency increasing with chain-length (89, 99). If the tone of the perfused rat blood vessels is increased by adding adrenaline to the perfusing Ringer solu-

tion, then vasodilator effects which closely resemble those obtained in the cat hindlimb with *S*-alkyl and *S*-aralkyl-thiouroniums can be obtained in the rat hindquarters also (99). Yet if barium or ergotoxine is used in place of adrenaline for increasing vascular tone, even such bases as *S*-*n*-nonyl-thiouronium produce effects which are still predominantly vasoconstrictor (99). This finding, together with the observation that the dip in perfusion pressure tracings coincides with a period of reduced sensitivity to adrenaline, suggests that the dilator effects of *S*-alkyl-thiouroniums on blood vessels which are under nervous control depend in part at least upon a reduced response to adrenergic or sympathomimetic stimuli.

S-2-(1′-Naphthyl-)ethyl-thiouronium, *S*-3-phenyl-*n*-propyl-thiouronium, and certain other *S*-alkyl-thiouroniums dilate the vessels of perfused cat hindlimbs at least as strongly as long-chain *S*-alkyl-thiouroniums (61, 89). Some of the compounds tested possess strong reflex depressor activity (section IVD). However, direct vasodilator activity and reflex depressor activity do not run parallel. Thus, phenyl-diguanide and *cis*-*S*-*m*-bromocyclohexyl-thiouronium have but feeble vasodilator actions. Conversely, strong vasodilator activity is exhibited by several thiouronium derivatives in which reflex depressor activity is inconspicuous (89).

The only di-amidine derivatives tested on perfused cat hindlimbs, *S*,*S*′-tetramethylene- and *S*,*S*′-hexamethylene-dithiouronium, do not closely resemble long-chain mono-thiouronium derivatives (89). Some preparations are insensitive to them, and others soon become so, possibly because of the loss of histamine from binding sites. A preparation which has become insensitive to these di-thiouronium derivatives still responds normally to long-chain mono-thiouronium derivatives.

3. Anti-adrenaline activity. This can be studied conveniently with the rat hindquarters preparation. It has been found with the *S*-alkyl-thiouroniums that as the alkyl chain is lengthened, anti-adrenaline activity is intensified up to the *S*-*n*-decyl derivative (97). The highly-insoluble *S*-*n*-dodecyl, *S*-*n*-tetradecyl, and *S*-*n*-hexadecyl derivatives show little activity. As the *S*-*n*-undecyl derivative has not been tested, it is not clear whether the "cut-off" in activity occurs after the *S*-*n*-decyl or the *S*-*n*-undecyl derivative. There is a 10-fold increase in potency on going from *S*-*n*-propyl- to *S*-*n*-hexyl-thiouronium, and a comparable increase on going from *S*-*n*-hexyl- to *S*-*n*-nonyl-thiouronium. The anti-adrenaline effects of the long-chain derivatives are not only far more intensive but also far more persistent than those of lower homologues given in equimolar amounts (99).

Anti-adrenaline effects have also been obtained with *S*,*S*′-alkylene-dithiouroniums and certain other amidine derivatives (59, 97, 264), but structure-activity relationships for these other compounds have not been studied systematically. About all that can be said is that anti-adrenaline activity seems to be more pronounced with long-chain derivatives than with compounds having small molecules.

4. Intestine-relaxing activity. Here, again, the *S*-alkyl-thiouroniums have received special attention (99). Up to the *S*-*n*-decyl derivative there is a steady increase in depressant activity. When the concentration of an alkyl-thiouronium

is sufficiently high for it to decrease intestinal tone, the strip is rendered less sensitive to acetylcholine and also to histamine, nicotine, and potassium. Measurement of the concentrations of different *S*-alkyl-thiouroniums needed to inhibit to a given extent the response to a fixed dose of acetylcholine or histamine shows that potency increases by a factor of 2 to 3 with the addition of each methylene group up to the *S*-*n*-decyl derivative.

Various other amidine derivatives have been reported to decrease intestinal motility (126). None of the compounds which act strongly has a small molecule; short-chain amidine derivatives tend to increase tone except when present in high concentration.

5. Comments. Differences between *S*-alkyl-thiouroniums in their depressant effects on perfused blood vessels and on isolated intestinal strips appear to be quantitative rather than qualitative. Since the compounds have almost identical structures, it is probable that they combine with the same receptors in producing these depressant effects. Increase in duration of effect with increasing chain-length (up to the "cut-off" imposed by extreme water-insolubility) is to be expected if van der Waals forces make a substantial contribution to the binding of the drug to the receptor (section IIC). A 2- to 3-fold increase in the intensity of depressant effects is also to be expected if the receptors do not lie within the same bulk phase as that in which the drug is administered and its concentration measured, for chain-length affects to this degree such physical properties of homologues as depend upon a distribution between two phases (section IID).

Although fewer observations have been made with other amidine derivatives, it would seem that with these, too, the size of the cation is of major importance in determining the intensity of muscle-depressant activity.

Next, we may inquire whether muscle-stimulating and muscle-depressing actions are closely linked. How can we explain such findings as those depicted in Figure 1? It would seem that those very properties of amidine derivatives which make for strong depressant activity preclude possession of strong stimulant activity. Now, if depressant activity is related to the ability of an amidine derivative to remain attached to the appropriate receptors, might not stimulant activity be related to the opposite attribute, *viz.*, the ability to interact with these receptors for brief intervals?

One hypothesis to account for the main effects of amidine derivatives on muscle is based upon the work of Albert and his colleagues (4, 8, 9, 218) on the mode of action of the acridine antiseptics. The distribution of antibacterial activity amongst acridines and related bases resembles the distribution of muscle-depressant activity amongst amidine derivatives. With both sets of compounds high activity is restricted to those bases which ionize freely to yield large cations. Presumably, the cation must be large in order that the van der Waals attraction between it and the receptor can be strong. Such findings suggested that acridine antibacterial agents might act by competing with some cation of physiological importance. Strong evidence was soon obtained to support the view that there is competition between acridinium ions and hydrogen ions for anionic receptor groups of bacteria (9). As Albert (4) concluded: "Kationic

chemotherapeutic agents seem to act through their kations competing with hydrogen ions for the same positions (anions) on a vital enzyme. This competition could be successful (*i.e.*, toxic to the parasite) only when the molecular complexity of the drug is such that sufficient adsorption occurs significantly to favour retention of the drug in the face of continual assaults by hydrogen ions."

The similarity in the structure-activity relationships for acridines and for amidine derivatives prompted the suggestion (86) that amidine derivatives, too, might act by competing with some simple cation of physiological importance. To account for the influence of chemical structure on "stimulant" actions, it was found desirable to make another major assumption, *viz.*, that corresponding stimulant and depressant actions of amidine derivatives, such as their direct vasoconstrictor and vasodilator actions, are exerted at the same site (89). It was reasoned that if long-chain amidine derivatives depress muscle tone by blocking the action of some natural stimulant, short-chain amidine derivatives might be able to raise muscle tone by simulating the action of this hypothetical stimulant. Now, since it has been found with numerous amidine derivatives that both stimulant and depressant actions can be obtained with one and the same compound, one is driven by the preceding hypothesis to conclude that these compounds with dual actions can act *either* in the same way as the natural stimulant *or* as its antagonist. Which type of action is obtained seems to depend, as indicated in Figure 1, upon dosage and upon the size of the cation. Depressant effects are obtained under conditions in which amidine derivatives would be expected to achieve a high concentration at the site of action, stimulant effects under conditions in which they would achieve a lower concentration.

Paton (205) has recently put forward a general theory of drug action based on the assumption that the most important feature of the interaction between a drug and a receptor is the *rate* at which combination occurs. According to this view, stimulation is brought about by the act of combination as distinct from the continuous occupation of a receptor (which would be expected to cause depression, since the occupying drug is blocking further combinations). Results like those shown in Figure 1 can be explained readily on this basis (205).

As already indicated, pressor analogues of *S*-methyl-thiouronium have few structural features in common. Provided that the cation is kept small, its structure can be altered in a variety of ways without loss of characteristic properties. Hence one does not get any obvious clues from the structural features of active compounds as to what the "natural stimulant" of the receptors, supposing that there is one, might be. Among the naturally occurring bases or cations which might be considered are adrenaline, acetylcholine, histamine, 5-hydroxytryptamine, sodium, and potassium.

It is easy to overlook sodium and potassium unless one is aware that their ions have water molecules attached more or less firmly to them and that these ions are therefore much larger than would appear from their usual representation (12). It is not unlikely that the solvated sodium and potassium ions are comparable in size to the ions of short-chain amidine derivatives like guanidine and acetamidine, for we would not expect the latter to be solvated since water mole-

cules could not approach the charged core of the molecule sufficiently closely to be strongly affected by it. We can narrow the field substantially—virtually to acetylcholine, potassium, and sodium—if we assume that the "natural stimulant" is comparable in size to short-chain amidine derivatives.

Comparison of the pharmacological effects of naturally occurring bases with those of typical short-chain amidine derivatives also helps to eliminate some of the possibilities (88). Thus, there are important differences between typical short-chain amidine derivatives and sympathomimetic amines (section IVA-9). The pharmacological properties of the potassium ion come closest to those expected of the "natural stimulant" as judged by the effects of short-chain amidine derivatives (88).

The well-known effects of guanidine on nerve-muscle preparations (section IIIC-3) deserve further comment at this stage. Long ago Führer (115) compared the action of guanidine to that of a univalent alkali cation. He pointed out that the actions of both are antagonized by calcium ions and by certain other divalent cations. The ability of calcium to antagonize some, though by no means all, of the pharmacological actions of guanidine (46, 104b, 115, 119, 133, 178, 186, 201, 216) has been considered by several investigators to provide a clue to the mode of action of guanidine. Harvey (133) noted that the addition of guanidine to the Ringer solution bathing a frog rectus abdominis preparation increased the response to potassium or to acetylcholine in the same way as did a reduction in the calcium content of the bath. These potentiating effects of guanidine could be reduced by raising the calcium content of the bath. Harvey considered the "spontaneous" activity seen after the addition of guanidine to be little different from that caused by calcium lack—a view disproved by Sato (220). However, so closely linked are the physiological actions of calcium and potassium that Harvey and others have been content to attribute the pharmacological effects of guanidine to changes in ionic balance rather than to anything so specific as induced calcium lack or potassium excess. Minot, Dodd and Riven (190) expressed what was probably a widely held view when they wrote: "It is possible that guanidine exerts its action on muscles through changes in the effect of inorganic salts."

Now, as has just been indicated, the chemical relatives of guanidine which resemble it closely in their effects on nerve-muscle preparations are those, the salts of which ionize freely to yield small cations. It is much easier to suppose that these cations can simulate the physiological action of a univalent alkali ion than that they can bring about a reduction in the effective concentration of a divalent ion such as calcium. As explained in section IIC, amidinium ions are likely to compete with other cations for various anionic sites. One of these base-exchange processes might well constitute the "trigger" action of pharmacological importance.

It is of interest that Paton (205) has, for different reasons, assigned to potassium the chief function in the model which he has proposed to account for the stimulant actions of various organic bases. He suggests that "the drug exchanges with potassium at the receptor, and is then released from the receptor in exchange

for potassium derived intracellularly, so that the chemoceptive action involves an extraction of potassium from the tissue."

It should be emphasized that conclusive evidence of competition between amidinium ions and potassium ions has yet to be obtained. The possibility that competition between amidinium ions and cations other than potassium can be of pharmacological importance must not be overlooked. Thus, the results of Otsuka and Endo (section IIIC-3) would seem to implicate acetylcholine; they can be explained most easily by supposing that guanidine causes a gross leakage of acetylcholine from nerve endings by the process of base-exchange. It is less likely that competition between amidinium ions and sodium ions is of major pharmacological importance, judging by the results of studies (65, 144, 165, 167, 170, 241) undertaken to see whether loss of activity resulting from sodium deficiency can be restored by guanidine and related organic cations.

D. Reflex depressor activity

The compounds regarded in this section as having reflex depressor activity are those chemical relatives of phenyl-diguanide which produce in lightly anaesthetized cats a sharp fall of blood pressure and heart rate preventable by vagotomy. The further characterization of these compounds will be dealt with later (section IVD-6).

It is relatively easy to compare the reflex depressor effects of potent compounds since there is seldom any hint of interaction between them, or of cumulative effects, even when the interval between successive injections is no more than 5 minutes, probably because large effects can be elicited with quite small doses (10 to 100 μg/kg). Potency has generally been assessed by finding what dose of a compound is needed to match the reflex depressor effect of a 100 μg dose of phenyl-diguanide. The "relative molar activities" given in this section have been calculated from published data, taking phenyl-diguanide as 1. If, for example, a compound has a molecular weight twice that of phenyl-diguanide and it has to be given in a dose of about 500 μg to produce a reflex depressor effect equal to that produced by a 100-μg dose of phenyl-diguanide, its relative molar activity is given as 0.4.

The data used are those of Dawes and Mott (62), Dawes and Fastier (61), Fastier (90), Fastier, Waal and Wong (103), and Fastier, McDowall and Waal (96).

1. Compounds of the type, C_6H_5—X—$C(NH_2):\overset{+}{N}H_2$. Several compounds of this type apart from phenyl-diguanide, for which X is NH—C(:NH)NH, are highly active. The relative molar activities of other compounds tested to date are:

1.0 for X = $CH_2CH_2CH_2S$
0.4 for X = CH_2CH_2S
0.25 for X = CH_2S
0.65 for X = CH_2CH_2NH
0.15 for X = CH_2NH
0.1 for X = NH
0.2 for X = $CH_2CH_2CH_2$
0.3 for X = CH_2CH_2
0.05 for X = CH_2

These results show that certain other connecting groups can be substituted for the NH—C(:NH)NH group of phenyl-diguanide without much loss of activity.

2. Compounds of the type, X—$(CH_2)_nS$—$C(\overset{+}{N}H_2):NH_2$. The relative molar activities of compounds tested to date are:

0.02 for X =	methyl	and n = 0 or 1
0.05 for X =	methyl	and n = 2
0.1 for X =	methyl	and n = 3
0.15 for X =	methyl	and n = 4
0.1 for X =	methyl	and n = 5
0.05 for X =	methyl	and n = 6
0.02 for X =	methyl	and n = 7–11
0.15 for X =	cyclohexyl	and n = 0
0.03 for X =	cyclohexyl	and n = 2
0.02 for X =	cyclohexyl	and n = 5
0.15 for X =	thienyl	and n = 1
0.25 for X =	phenyl	and n = 1
0.4 for X =	phenyl	and n = 2
1.0 for X =	phenyl	and n = 3
0.6 for X =	1-naphthyl	and n = 1
3.3 for X =	1-naphthyl	and n = 2

These results show that it is not essential for possession of reflex depressor activity that a compound should have a phenyl group attached to the amidine group by a short side-chain. Some purely aliphatic amidine derivatives possess considerable activity. Nevertheless, the most potent compounds obtained as yet have a terminal aromatic group.

3. Other compounds of the type, Y—$C(\overset{+}{N}H_2):NH_2$. Amidine derivatives for which Y is small (*e.g.*, CH_3, C_2H_5, C_2H_2O, CH_3NH, $NH_2C(:NH)NH$, NH_2) possess little or no reflex depressor activity. Nor do those for which Y is very long or bulky, *e.g.*, *S*-*n*-decyl-thiouronium, *S*-5-cyclohexyl-*n*-amyl-thiouronium, *S*-4-(1′-tetrahydronaphthyl)-*n*-butyl-thiouronium, dibenzyl-guanidine, and di-*o*-tolyl-guanidine.

The effect of introducing certain substituents into the benzene ring of phenyl-diguanide or of *S*-benzyl-thiouronium is indicated by the relative molar activities of the following compounds:

(Substituent)	Phenyl-diguanide	S-Benzyl-thiouronium
o-Cl	2.5	0.3
m-Cl	3.5	1.4
p-Cl	1.4	0.2
o-CH_3	2.2	0.05
m-CH_3	0.7	0.15
p-CH_3	0.3	0.02
o-Br	—	0.2
m-Br	—	1.4
p-Br	—	0.05

These results show that the position of the substituent strongly influences activity. Hence the effects of particular substituents can be compared directly

only when they occupy the same positions in the benzene ring. With *o*-substituted phenyl-diguanides, the relative molar activities of the Cl, CH_3, CH_3O, HO, and NO_2 derivatives are 2.5, 2.2, 0.2, 0.02, and 0.02, respectively. With *m*-substituted phenyl-diguanides, the relative molar activities of the Cl, CH_3, HO, and NO_2 derivatives are 3.5, 0.7, 0.3, and 0.02, respectively. With *p*-substituted phenyl-diguanides the relative molar activities of the Cl, Br, CH_3O, CH_3, C_2H_5, I, HO, and CH_3COO derivatives are 1.4, 1.1, 0.6, 0.3, 0.3, 0.15, 0.05, and 0.02, respectively. These results show that a Cl group enhances the activity of phenyl-diguanide irrespective of its position in the ring. Compounds containing a CH_3 in place of a Cl group are less active. Most other substituents, the effects of which have been studied, decrease activity substantially. Likewise with substituted *S*-benzyl-thiouroniums, the Cl derivatives are more active than the corresponding CH_3 derivatives, with Br derivatives exhibiting intermediate activity.

It is possible to introduce at least 2 nuclear substituents into the molecule of phenyl-diguanide without losing potent reflex depressor activity. The relative molar activities of the compounds tested to date are 1.6 for the 2-CH_3,4-Cl derivative, 1.6 for the 2-CH_3,4-Br derivative, 1.3 for the 2-CH_3,4-CH_3 derivative, 1.6 for the 4-CH_3,2-Br derivative, 0.9 for the 3-CH_3,4-CH_3 derivative, 0.7 for the 3-CH_3,5-CH_3 derivative, 1.8 for the 3-CH_3O,5-Cl derivative, and 4.4 for the 2-CH_3O,5-Cl derivative.

Few other compounds in which an amidine group is joined by a short aliphatic chain to an aromatic group have been examined. Several of those which contain a naphthyl group are highly active, *e.g.*, *S*-2-(1′-naphthyl-)ethyl-thiouronium, 2-naphthyl-guanidine, 1-naphthyl-diguanide. Much less activity is shown by compounds with a terminal alicyclic group, such as *S*-2-cyclohexylethyl-thiouronium; but an exception is provided by *cis*-*S*-*m*-bromocyclohexyl-thiouronium, the relative molar activity of which is about 1.5.

The *S*,*S*′-alkylene di-thiouroniums and other di-amidine derivatives tested were almost or completely inactive.

4. N-substituted amidine derivatives. Results obtained to date indicate that the introduction of substituents into the amidine group of an active compound is likely to diminish or even abolish activity. Thus, no reflex depressor effects were obtained with the *N*-amino, *N*-ethyl, *N*-allyl, *N*-acetyl, *N*,*N*′-ethylene, and *N*,*N*′-diethyl derivatives of *S*-benzyl-thiouronium. None of the *N*-substituted amidine derivatives tested to date has shown more than slight reflex depressor activity (a relative molar activity in the range 0.02 to 0.1). Among the most active are *S*,*N*-(1-bromomethyl-)ethylene-thiouronium and *S*-methyl-*N*-phenyl-thiouronium. The former compound has been wrongly referred to previously (61) as *S*,*N*-(2-bromo-)trimethylene-thiouronium (104a). The compounds found to have little or no activity include *S*-methyl-*N*,*N*′-diphenyl-thiouronium, *S*,*N*-trimethylene-thiouronium, 2-amino-pyridine, and 2-amino-quinoline.

5. Aralkylamines and other compounds. Benzylamine and 2-phenylethylamine are inactive (in contrast to such amidine derivatives as benzyl-guanidine, *S*-2-phenylethyl-thiouronium, and phenyl-propionamidine); so is *N*-benzyl-thiourea. Nevertheless, there is at least one compound outside the amidine group which

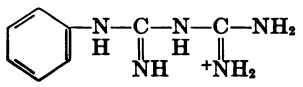
possesses strong reflex depressor activity and which resembles potent amidine derivatives sufficiently closely in chemical structure to suggest that it acts on the same receptors, *viz.*, 5-hydroxytryptamine. Tryptamine itself is almost inactive.

6. Localization of reflex depressor actions. It is clear from the evidence reviewed by Dawes and Comroe (60) and by Heymans and Neil (139) that there are several ways in which a reflex fall of blood pressure and reduction of heart rate may be produced by drugs. Some of the compounds which share this property with phenyl-diguanide are so unlike it in chemical structure that they would hardly be expected to have an affinity for the same receptor patches of cells. Hence it is not surprising that detailed comparisons of the reflex actions of (say) veratridine and phenyl-diguanide (63) or of lobeline and phenyl-diguanide (29) have revealed major differences. But no such differences have been found when the reflex actions of potent thiouronium derivatives (61) or of 5-hydroxytryptamine (96, 193) have been compared with those of phenyl-diguanide. Thus, the reflex depressor action of 5-hydroxytryptamine resembles that of phenyl-diguanide in that it is not prevented—like that of veratridine—by cooling the vagi to about 10°C. Neither 5-hydroxytryptamine nor phenyl-diguanide elicits the coronary and allied chemoreflexes in the dog, in contrast to such drugs as veratridine and lobeline, which are active in both the dog and cat. And the reflex action of 5-hydroxytryptamine, like that of phenyl-diguanide but unlike that of lobeline, can be temporarily inhibited by giving 2-naphthyl-guanidine, an amidine derivative which itself produces reflex falls of blood pressure but which acts as a blocking agent when given in larger doses than those which stimulate the sensory receptors.

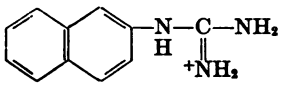
7. Comments. The resemblance between the reflex effects of phenyl-diguanide and those of 5-hydroxytryptamine has been stressed because it is considered to provide a clue to the understanding of the structure-activity relationships described in this section. If some of the chemoreflexes initiated by 5-hydroxytryptamine are of physiological importance, then it is not unlikely that parts of certain sensory nerve endings are "tailored" for receiving molecules of 5-hydroxytryptamine. If we suppose further that receptors of this type can respond to molecules other than 5-hydroxytryptamine, we are tempted to conclude that reflex activity will increase the more nearly the analogue approximates 5-hydroxytryptamine in chemical structure.

Now, almost all the amidine derivatives which have been found to be highly active are compounds of the type: aromatic group—short aliphatic chain—amidine group. Compounds such as phenyl-diguanide (XIII) and 2-naphthyl-guanidine (XIV) are not very different from 5-hydroxytryptamine (XV) in shape and size. It is thought significant that less activity is displayed by benzyl-guanidine and *S*-benzyl-thiouronium than by homologues of these amidine derivatives which are nearer in size to 5-hydroxytryptamine; also, that little or no activity is displayed by amidine derivatives which differ to a much greater extent from 5-hydroxytryptamine in molecular size. The high activity of certain amidine derivatives with a naphthyl substituent is to be noted in this regard.

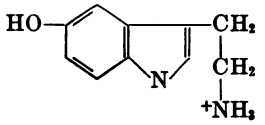
If a good "fit" between drug and receptor is needed for high activity, then the large, flat naphthyl group should correspond more closely to the 5-hydroxyindolyl group of 5-hydroxytryptamine than a phenyl group.



(XIII)



(XIV)



(XV)

As there are several ways in which nuclear substituents might affect activity, the effect on reflex depressor activity of introducing one or more substituents into the aromatic ring of phenyl-diguanide or of *S*-benzyl-thiouronium is not easy to account for.

A substituent might by virtue of its own electrical charge affect the charge distribution of the whole molecule. With phenyl-diguanide derivatives the effect of a nucleophilic or electrophilic substituent would be relayed right along the side-chain, because the atoms of the benzene ring and those of the diguanide chain are part of the same resonating system; but with *S*-benzyl-thiouronium derivatives the ionization of the amidine group should scarcely be affected by nuclear substituents because of the intervening —CH_2S— group. Yet the reflex depressor activity of *S*-benzyl-thiouronium is influenced in much the same way as that of phenyl-diguanide by nuclear substitution. For several substituents the order of activity has been found to be *m*- > *o*- > *p*-.

This last finding suggests that steric factors may be very important. Although a bulky substituent might prevent effective contact between drug and receptor, a suitably placed small substituent might increase the affinity of the drug for the receptor by van der Waals or even stronger bonding. The increase in activity obtained by introducing certain substituents into the *m*-position of phenyl-diguanide or of *S*-benzyl-thiouronium invites comparison with the increase in activity obtained by introducing a hydroxyl group into the 5-position of tryptamine.

However, there is another explanation for the inactivity of tryptamine. Vane (252) has shown that, whereas monoamine oxidase inhibitors potentiate the action of tryptamine and many of its analogues on the isolated rat fundus preparation, they do not potentiate the action of 5-hydroxytryptamine or of other hydroxytryptamines. He explains these results by supposing that the polar hydroxyl-group prevents amines like 5-hydroxytryptamine from entering cells and so being destroyed by coming in contact with intracellular amine oxidases. Woolley (267), too, has suggested that the 5-hydroxyl group of 5-hydroxytryptamine limits its ability to penetrate cells. Perhaps the feeble reflex activity of tryptamine is to be explained along these lines. If the hydroxyl group of 5-

hydroxytryptamine were of importance for interaction with the receptors, then one would expect the introduction of a nuclear hydroxyl group into such molecules as phenyl-diguanide to increase their reflex activity, but this has not been observed.

The chief difficulty in the way of accepting the view that amidine derivatives like phenyl-diguanide stimulate certain sensory receptors by virtue of their chemical resemblance to 5-hydroxytryptamine is provided by the lack of activity of amines of similar structure. Such compounds as benzylamine, benzyl-dimethylamine, and 2-phenylethylamine were found to be inactive when tested in doses of up to 300 μg/kg; certain other amines have been shown to possess slight activity, *e.g.*, tyramine, 2-pentachlorophenoxyethylamine (96, 253). As this problem of accounting for pharmacological differences between corresponding amine and amidine derivatives is discussed elsewhere (section IVA-10), no more need be said here.

E. Other properties

As in other sections, attention will be restricted mainly to results obtained with simple aliphatic mono- and di-amidine derivatives. Fortunately, several investigators (36, 120, 155, 172) have made good use of the large collection of compounds assembled by King.

1. Hypoglycaemic activity. Numerous amidine derivatives have been found capable of lowering blood sugar. However, a substantial proportion of the "active" compounds lower blood sugar to a noteworthy extent only when they are given in lethal or near-lethal doses (32, 42, 55b). Assessing hypoglycaemic activity has therefore proved expensive in animals as well as in time. To make the comparison of compounds still more difficult, hypoglycaemia is generally preceded by hyperglycaemia (42, 55b). Some amidine derivatives produce only hyperglycaemia (42).

The members of homologous series which are most active in this respect are those with fairly long hydrocarbon chains (32, 42, 229). It does not appear, however, that there is always a steady increase in potency up to a maximum when a homologous series is ascended. Shikinami *et al.* (229) found that *S*,*S*′-pentamethylene-dithiouronium had as potent a hypoglycaemic action as the homologues with 6, 8, and 10 methylene groups. It was their impression that derivatives with an uneven number of methylene groups may have a greater effect than those with an even number. Broom (42) found with the polymethylene-diamidines that the compounds with 3, 5, and 11 methylene groups produced only hyperglycaemia in rabbits, whereas those with 7, 8, and 10 methylene groups produced hypoglycaemia after transient hyperglycaemia. He observed similar anomalies with homologous mono-amidines.

Decamethylene-diguanidine (Synthalin) and its near homologues are the most potent of the amidine derivatives tested to date. The corresponding polymethylene-diamidines and polymethylene-dithiouroniums are distinctly less potent (42, 229). Some mono-amidine derivatives possess appreciable activity, *e.g.*, *iso*amyl-guanidine, *iso*amyl-diguanide, 2-phenylethyl-diguanide (Phenformin)

4-aminobutyl-guanidine (Agmatine), *N*-piperidino-guanidine, and *n*-amyl-guanidine (20, 32, 137, 266). These produce severe hypoglycaemia in rabbits when given intravenously in doses of about 50 to 100 mg/kg. Under comparable conditions, decamethylene-diguanidine would lower blood sugar to the same extent in a dose of about 5 mg/kg, while guanidine and other short-chain amidine derivatives would be ineffective unless given in doses well in excess of 100 mg/kg (10, 32, 55b, 211, 258). Amongst inactive compounds may be mentioned creatine, creatinine, arginine, guanidino-acetic acid, guanine, and dicyandiamide (32).

Studies of the mode of action of 2-phenylethyl-diguanide indicate that amidine derivatives of this type lower blood sugar by the same mechanism as guanidine (section IIIC-4) and decamethylene-diguanidine (section IIID-1). Thus, a hypoglycaemic response can be obtained in eviscerated animals; the fall in blood sugar is preceded by an increase in blood lactate, citrate, and phosphate; the fall is accompanied by decreased synthesis and storage of glycogen; and the symptoms are but poorly relieved by the administration of adrenaline (52, 76, 106, 162, 228, 238, 249, 263, 265).

The mode of action of other hypoglycaemic amidine derivatives has received much less attention. It has been noted with several series of compounds that hypoglycaemic activity runs parallel with toxicity (32, 42). As a rule the glycogen reserve of the body is almost depleted before hypoglycaemia occurs (32). Broom (42) observed the same signs of liver toxicity with the aliphatic amidines which produced hyperglycaemia followed by hypoglycaemia as he did with those which produced only prolonged hyperglycaemia.

2. Trypanocidal activity. King, Lourie, and Yorke (155, 168, 169) made an extensive survey of the occurrence of trypanocidal activity amongst aliphatic amines, amidines, guanidines, and thiouroniums. They found potent compounds amongst all four types. Compounds of the type $CH_3(CH_2)_n-\overset{+}{N}(CH_3)_3$, or of the type, $(CH_3)_3\overset{+}{N}-(CH_2)_n-\overset{+}{N}(CH_3)_3$, were much less active than the corresponding primary amines. One of the polymethylene-diamidines tested, undecane-diamidine, killed trypanosomes *in vitro* in as high a dilution as the most potent of the polymethylene-diguanidines, which were those with 10, 12, or 14 methylene groups in the hydrocarbon chain. Peak activity was less in the polymethylene-dithiouronium, polymethylene-diamine, alkyl-guanidine, and alkyl-amine series. Although the homologous series were far from complete, the results as a whole suggest that activity increases steadily with lengthening of the hydrocarbon chain until there are at least 10 methylene groups in it. Little or no trypanocidal activity was observed in experiments with arginine, alacreatine, galegine, and several other guanidine derivatives containing a —COOH, — OH or — NH_2 group in the molecule. However, the inactivity of some of these compounds could be attributed to the small size of the molecule rather than to the presence of a particular chemical group. With all the homologous series it was found that increasing trypanocidal activity was attended by increasing toxicity to mice.

3. Antibacterial activity. The compounds used by King, Lourie, and Yorke were tested by Fuller (120) for the ability to inhibit the growth of various types

of bacteria. He observed that activity gradually increased to a maximum during the ascent of a homologous series and then decreased, except with the polymethylene-dithiouroniums, the behaviour of which was irregular. With di-substituted derivatives, maximum activity was usually displayed when the hydrocarbon contained 14 to 18 methylene groups. With mono-substituted amidine derivatives, maximum activity was usually displayed when the hydrocarbon chain contained 12 to 16 methylene groups. In the di-substituted series the fall in activity upon increasing chain-length beyond the optimum was gradual, but in the mono-substituted series the fall was much sharper, especially against gram-negative bacteria. The di-guanidines reached their maximum activity at a shorter chain-length than did the di-amidines or di-amines, but the converse held in the mono-substituted series. Compounds having fewer than 8 methylene groups in the hydrocarbon chain had very slight activity—except in the di-thiouronium series, where activity increased and decreased sharply as the series was ascended. Fuller concluded that the di-substituted compounds are, on the whole, more satisfactory antibacterial agents than the mono-substituted, the higher members being more active at equal chain-length and far less inhibited by serum, though more toxic.

4. Inhibition of monoamine oxidase. Both Blaschko and Duthie (36) and Fastier and Hawkins (92) have tested numerous amidine derivatives for the ability to inhibit the oxidation of tyramine by a monoamine oxidase obtained from rabbit liver, with the following results.

The degree of inhibition produced by a 10^{-3} M concentration of an *S-n*-alkyl-thiouronium increases steadily as the series is ascended until the *S-n*-nonyl derivative is reached. Higher homologues are not completely soluble in the phosphate buffer used to suspend the enzyme powder, and there is a sharp falling off in activity beyond the *S-n*-decyl derivatives. Lengthening the alkyl chain affects similarly the inhibitory activity of alkyl-amidines and alkyl-guanidines. The introduction of an aromatic or alicyclic group into the side-chain of an *S*-alkyl-thiouronium sometimes increases inhibitory potency considerably. Even the presence of substituents in the amidine group itself does not necessarily result in the loss of inhibitory activity. For instance, the *N,N'*-ethylene derivatives of *S-o*-chlorobenzyl-, *S-p*-methoxybenzyl-, and *S*-2-pyridyl-thiouronium are approximately as active as the parent bases. Several di-amidine derivatives are strong inhibitors of monoamine oxidase, *e.g.*, propamidine and pentamidine. In experiments with homologous series of di-amidines, di-guanidines, and di-thiouroniums it has been found that potency increases with chain-length until about 12 to 14 methylene groups are present in the molecule.

It is possible to obtain from rabbit's liver, by prolonged centrifugation and repeated washing of the acetone-dried powder, a completely insoluble preparation of monoamine oxidase (27). This preparation has been used to study the reversibility of the enzyme inhibition produced by amidine derivatives (36, 92). Numerous washings of the enzyme powder are needed to reduce substantially the degree of inhibition produced by a very potent amidine derivative. For example, in an experiment in which 10^{-5} M pentamidine produced 92% inhibi-

tion of the enzyme, 6 washings were needed to reduce the degree of the inhibition to 83%. In another experiment, *S*-*n*-butyl-, *S*-*n*-hexyl-, *S*-*n*-octyl-, and *S*-*n*-decyl-thiouronium were given in concentrations which produced approximately 80% inhibition of enzymatic activity. (These concentrations were respectively 25×10^{-3} M, 6×10^{-3} M, 1.2×10^{-3} M, and 0.2×10^{-3} M, indicating a somewhat greater than 2-fold increase in activity with the addition of each methylene group.) Whereas 3 washings sufficed to restore the full activity of the sample which had been treated with *S*-*n*-butyl-thiouronium, 6 washings reduced by less than 10% the degree of inhibition produced by *S*-*n*-decyl-thiouronium. There was a clear gradation in persistence of effect between the *S*-*n*-butyl and *S*-*n*-decyl derivatives.

5. Diamine oxidase. Di-amidine derivatives as a class are strong inhibitors of this enzyme. It has been noted with several homologous series that the length of the chain joining the two amidine groups may be altered greatly without much change in activity. For example, *S*,*S*′-trimethylene-, *S*,*S*′-hexamethylene-, and *S*,*S*′-decamethylene-dithiouronium all inhibit diamine oxidase to approximately the same extent when tested in a concentration of 10^{-4} M (37). Similarly with the polymethylene-diamidines, the compounds with 7, 8, 9, 10, 11, 12, 14, and 16 methylene groups are all approximately equi-active (35, 37). Negligible activity was displayed by almost all the mono-amidine derivatives tested. However, *S*-methyl-thiouronium (in contrast to its higher homologues) possesses appreciable activity (37) and so does guanidine (33).

6. Analgesic activity. Both 3-amino-pyridine and 4-amino-pyridine, as well as 2-amino-pyridine and several of its methyl derivatives, possess analgesic activity comparable to that of 4-methyl-2-amino-pyridine, as judged by their ability to allay the squirming or writhing produced by 2-phenyl-1,4-benzoquinone in mice (94). Even *S*-methyl-thiouronium shows slight activity. Structure-activity relationships in this field deserve further exploration.

7. Comments. Certain problems raised in this section cannot be answered satisfactorily if such terms as "drug receptor" are used loosely. Since current usage makes the meaning of some of these terms imprecise, it should be made clear in what sense they are used in this review.

As Albert (5) rightly insisted, it is impossible to make a rigid distinction between the "physical" and the "chemical" attributes of a drug molecule. However, it is possible to distinguish between those drugs which owe their pharmacological activity partly to possession of specific structural attributes and those which do not—Ferguson (105) has shown how this may be done. A *pharmacophore* (to make use of an obsolescent term) may be defined as any atom or group which, by virtue of its presence in a molecule, is responsible for pharmacological behaviour other than that predictable by the Ferguson Principle of Structural Non-Specificity. Like the "chromophore" of a dyestuff, a pharmacophore serves as the basis for a form of activity which is modified by all other parts of the molecule.

It may be inferred that some cells contain atoms or groups corresponding to the pharmacophores of drugs, *e.g.*, —SH groups corresponding to the $—Hg^{+}$

group of the mercurials. These complementary groups will be called *drug acceptors*. It is likely that some of the drug acceptors for a particular drug are merely what Veldstra (255) termed "sites of loss." Interaction between drug and the drug acceptor at a site of loss does not directly evoke a pharmacological response. Nevertheless, as Veldstra has pointed out, the taking up of a drug at sites of loss may lead indirectly to demonstrable pharmacological effects, such as potentiation of the response to another drug which combines with the same type of drug acceptor.

A drug acceptor which is not merely a site of loss will be referred to as a *site of action*. In other words, a site of action is a focus for the initiation of demonstrable pharmacological activity. The term *drug receptor* will be used to refer to the site of action together with all those neighbouring atoms which play a considerable part in binding the drug to the site of action or in restricting its access to the site of action.

The amidinium group is obviously a pharmacophore. Judging by the variety of pharmacological effects produced by the prototypes (section III), it seems likely that the amidinium group has an affinity for numerous drug receptors. For the chemical reasons advanced in section II, we would expect amidine derivatives to be attracted to negatively charged groups in biological media. Some of these drug acceptors are likely to be sites of action.

Di-amidine derivatives. If two amidinium groups are present in the one molecule, it should sometimes happen that both groups interact simultaneously with suitably spaced drug acceptors. At other times only one group may be interacting with a drug acceptor. We can therefore expect di-amidine derivatives to possess two sets of pharmacological properties, only one set of which is shared with mono-amidine derivatives.

This is known to be the case. Di-amidine derivatives do not share with mono-amidine derivatives as a class such properties as histamine-liberating activity (section IVC-1) and the ability to inhibit diamine oxidase (section IVE-5). It would seem that if these actions are to be strongly exerted there must be a 2-point attachment of the molecule to the receptor. This does not necessarily imply that a 2-point attachment is needed for interaction to take place. Such an attachment may be important simply on account of the firmness of the anchoring of the drug to the receptor. It must be remembered that, whereas a compound may need to have more than one chemical group in common with an agent like acetylcholine or histamine for it to activate the same receptor (147), possession of but one of these groups may suffice for it to be able to combine with part of the receptor and so deny access to the natural stimulant, as Myers (195) has shown for certain inhibitors of cholinesterases.

Di-amidine derivatives share numerous properties with mono-amidine derivatives. Presumably, when such an action is exerted by a di-amidine derivative, only one amidinium group of the molecule "triggers" the site of action. However, the presence of a second amidinium group in the molecule often modifies activity substantially. To explain why a di-amidine derivative is likely to have stronger depressant actions than a mono-amidine derivative of the

same size, we must consider ways in which the "free" amidinium group of a di-amidine derivative could help to keep the molecule in contact with a receptor.

Now, if there were a site of loss near a site of action, this would serve to anchor the molecule. The 2-point attachment would provide a much firmer binding than a 1-point attachment (supplemented by van der Waals attraction). Even if there were not a 2-point attachment, a "free" cationic group would (because of its electrical charge) repel other cations competing with the drug for the same receptor, an effect which would be especially pronounced if the site of action lay in a pore (33) or a pit (257).

Antimicrobial activity. Since the toxicity of aliphatic primary amines and amidines for bacteria and trypanosomes is very similar, it would seem that there is no differentiation between a $-\overset{+}{N}H_3$ group and a $-C(NH_2):\overset{+}{N}H_2$ group at the sites of action. It is therefore not surprising that certain poly-amines, which themselves have a slight antibacterial action, can antagonize the antibacterial action of potent di-amidine derivatives (30, 235). Competition between the two for anionic sites would be expected to result in "therapeutic interference" (5, 255). According to Schoenbach and Greenspan (225a), the "theory of cationic competition does not explain the lack of inhibition by many small-molecular compounds containing free cations." However, this criticism is no longer valid, now that the role of molecular complexity in determining the affinity of cations for drug receptors is better understood (4).

It is more difficult to explain the lack of potency of compounds containing $-\overset{+}{N}(CH_3)_3$ groups in place of amidinium groups. King, Lourie, and Yorke (155) suggested that quaternary compounds might be inactive because they cannot be hydrolyzed to free bases. If this were the explanation, we would expect the polymethylene-diguanidines to be almost equally inactive, since the basic strength of guanidine and its alkyl derivatives is comparable to that of sodium hydroxide (17, 198). It seems more likely that the lack of activity of methonium compounds is due to their inability to form hydrogen bonds with anionic receptors.

Bichowsky-Slomnitzki (30) observed that the bacteriostatic action of two highly potent amidine derivatives, stilbamidine and pentamidine, was antagonized through a wide range of concentrations by nucleic acids and by hydrolyzed nucleic acids. The antagonistic action of the mononucleotides tested (adenylic acid and guanylic acid) was about one-twentieth that of the polynucleotides. The nucleosides, guanosine and adenosine, were devoid of activity, as were the purine bases. The inactivity of nucleosides, as distinct from nucleotides, suggests that the phosphate groups of the latter bind amidine derivatives. Since it seemed that a direct chemical reaction between amidine derivatives and nucleic acids led to the formation of insoluble complexes, Bichowsky-Slomnitzki concluded "that the diamidines cause metabolic disturbances of the cell nucleotides by fixation of nuclear substances."

A lowered antibacterial activity of propamidine in the presence of phosphate

ions was demonstrated by Elson (82, 83), who attributed it to direct chemical inactivation of the drug and thought it analogous to the antagonism of amidine derivatives by nucleic acids. Elson observed also that the antibacterial activity of propamidine is influenced by hydrogen ion concentration in the same way as that of the acridines, basic dyes, and cationic detergents. All of these drugs appear to compete with cations of physiological importance for anionic sites on or in micro-organisms, as originally envisaged by the Stearns (4, 237, 250).

While some workers have attempted to explain the antimicrobial activity of amidine derivatives in terms of a general denaturation of nucleo-proteins, others have sought a more specific "metabolic lesion." Much of the evidence has been reviewed by Schoenbach and Greenspan (225a). A hint as to the mechanism of action may be provided by the observation (169, 225a) that the species of trypanosomes which are most sensitive to amidine derivatives are those which have the greatest requirements for glucose and oxygen. Presumably these are the species which are least capable of obtaining energy by means of anaerobic glycolysis. Studies of the antibacterial actions of amidine derivatives likewise suggest that these drugs harm micro-organisms by inhibiting oxidative metabolism (25, 26, 160). The oxidation of certain amino acids is impaired by lower concentrations of amidine derivatives than is the oxidation of carbohydrate substrates (26). By discovering which substrates were most affected, Bernheim (26) concluded that the cytochrome system is not involved.

Inhibition of enzymes. Experiments performed with monoamine oxidase (section IVE-4) are of interest chiefly because the insolubility of the preparation used makes it of value for comparing amidine derivatives as regards ability to remain at a site of action despite attempts to remove them by washing. The results obtained are those anticipated on chemical grounds (section II). It is unlikely that inhibition of monoamine oxidase is largely responsible for the ability of various amidine derivatives to potentiate the vasoconstrictor action of adrenaline (97, 101). Dawes' observation, that amidine derivatives decrease the inactivation of adrenaline by the liver (59), can be readily explained by supposing that the amidine derivatives prevent wastage of adrenaline by occupying "sites of loss."

It is a pity that few amidine derivatives have been tested on enzymes such as those concerned with the transfer of "energy-rich" phosphates, because studies along these lines promise to throw considerable light on the main mechanism of action of amidine derivatives. Due in large measure to renewal of interest in the hypoglycaemic activity of amidine derivatives, effects of amidine derivatives on various enzyme systems are now receiving much attention. Although the results of laboratory experiments hardly encourage its clinical use (265), 2-phenylethyl-diguanide has proved moderately effective in the treatment of diabetes mellitus (39, 52, 76, 248, 266). Even the synthalins may have been unduly maligned (76).

Clarke and Forbath (52) observed that rat diaphragms when incubated in a medium containing 2-phenylethyl-diguanide, showed an increased glucose uptake, increased glycogen breakdown, and an increased lactate and inorganic

phosphate production. They considered it unlikely that the phosphate came from the Embden-Meyerhof esters, for this would have resulted in a decrease rather than an increase in lactate production. Adenosine triphosphate (ATP), ADP, or CP (creatine-phosphate) seemed to be the most likely source.

In contrast to metabolic poisons like dinitrophenol, 2-phenylethyl-diguanide did not increase the volume of distribution of several pentoses, nor did it alter inulin space or water content. Since an increased glucose uptake was obtained without a change in permeability, Clarke and Forbath suggest that 2-phenylethyl-diguanide might stimulate the metabolism of glucose by the "insulin-nonresponsive glycolytic system" postulated by Shaw and Stadie (227). This system, in contrast to the "insulin-responsive glycolytic system," produces lactic acid rather than glycogen and is thought to exist on the surface of the diaphragm. Marsh and Haugaard (181) have demonstrated that the surface of the diaphragm exhibits ATP-ase activity.

It has been suggested (231, 232) that an ATP-ase in this situation may play a vital role in the active transport of sodium and potassium. Dinitrophenol and certain other agents which inhibit oxidative phosphorylation are known to inhibit the active transport of sodium and potassium through cell membranes (48, 141). The possibility that some of the metabolic actions of amidine derivatives may be intimately linked with their actions on nerve and muscle seems worthy of investigation.

Hollunger (142) showed that 0.02 to 0.03 M guanidine inhibits almost completely, through reversibly, the oxygen uptake of isolated rabbit kidney cortex mitochondria, which in the presence of the hexokinase system oxidize glutamate. Guanidine had no effect on the dehydrogenase, cytochrome reductase, or cytochrome oxidase activity of the mitochondria. As little as 15 to 20 $\times$ 10^{-5} M dinitrophenol completely reversed the guanidine inhibition and inhibited the phosphate uptake. The oxidative activity of non-phosphorylating mitochondria was not inhibited by guanidine. Methyl-guanidine and *asym.*-dimethyl-guanidine were less active than guanidine, but decamethylene-diguanidine was much more active. In Hollunger's opinion, "All available data support the view that guanidine inhibits the oxidative activity of mitochondria by inhibiting the mechanism that couples the oxidation at the pyridine nucleotide-cytochrome c level with phosphorylation."

Similar observations have been made by Ungar, Psychoyos and Hall (249), who found that 2-phenylethyl-diguanide inhibited the Krebs cycle when added to tissue homogenates. In concentrations of about 10^{-4} M it acted directly on DPN-dependent processes (oxidation of citrate, fumarate, α-ketoglutarate, oxalacetate, and pyruvate). Considerably higher concentrations of 2-phenylethyl-diguanide were needed to block the succinoxidase and cytochrome oxidase systems. Neither of the latter systems is therefore likely to be the primary site of action of the drug, as has been suggested by other workers (238, 263). To explain why the addition of diphosphopyridine nucleotide (DPN) can reverse the effect of 2-phenylethyl-diguanide, Ungar *et al.* sought spectrophotometric evidence for a "combination or formation of a complex" between the

two compounds, but found none. They therefore favoured the idea of competition between DPN and 2-phenylethyl-diguanide for some enzymatic sites (*cf.* Bichowsky-Slomnitzki, *loc. cit.*).

Ungar, Psychoyos and Hall (249) showed for a series of 16 amidine derivatives (including decamethylene-diguanidine and 2-phenylethyl-diguanide) that the inhibition of oxidation went in parallel with inhibition of phosphorylation. However, they obtained no valid correlation between ability to inhibit oxidative phosphorylation *in vitro* and ability to lower blood sugar *in vivo*. This, and other evidence, indicated that the Krebs cycle block is not essential for the production of hypoglycaemia by amidine derivatives.

Thus it would appear that amidine derivatives exert their metabolic actions, like their neuromuscular actions, at several sites. After reviewing almost all the evidence available to date, Creutzfeldt and Söling (55b) concluded that the mechanism of the hypoglycaemic effect of amidine derivatives has not been clearly established. In their opinion, it is unlikely that the blood sugar decreases produced in human diabetic patients are due to increased anaerobic glycolysis.

V. GENERAL COMMENTS AND CONCLUSIONS

The most easily explained of the structure-action relationships described are those in which a particular form of pharmacological activity increases steadily to a maximum and then falls off sharply when a homologous series of amidine derivatives is ascended. As already indicated (sections IIC, IID, and IVC), this relationship would be expected if we assume: 1) that the amidinium group of the homologues is their only pharmacophore, 2) that the pharmacological response is due to the occupation of the receptors by amidinium ions, and 3) that the interaction between drug and receptor takes place in some phase other than that in which the drug is administered.

Typical of the pharmacological actions which become more intense when a homologous series of aliphatic amidine derivatives is ascended are those resulting in dilatation of blood vessels, relaxation of isolated intestinal strips, a decreased vasoconstrictor response to adrenaline, the killing of trypanosomes, and inhibition of monoamine oxidase.

These effects are produced also by aliphatic di-amidine derivatives, especially those with a long hydrocarbon chain. However, structure-activity relationships for di-amidine derivatives are usually more complex than those for monoamidine derivatives, as would be expected for the reasons given in section IVE-7.

Numerous amidine derivatives have been shown to have a dual action on nerve-muscle preparations. They can both stimulate and depress, according to the experimental conditions. As indicated in section IVC, the influence of chemical structure on the stimulant activity of amidine derivatives may be explained by assuming: 1) that corresponding stimulant and depressant actions involve the same pharmacophore (*i.e.*, the amidinium group) and the same receptors, and 2) that stimulation is a quantal process, brought about by the act of combination of the pharmacophore with the receptor and prevented from recurring for so long as the pharmacophore remains in contact with the receptor. For the

reasons given in section II we would expect the stimulant actions of amidine derivatives to be displayed best by the compounds which have small cations. This expectation is realized for such properties as vasoconstrictor activity.

Reflex depressor activity appears to be an outstanding exception. The results described in section IVD show that the compounds which are most active in producing reflex depressor effects are those in which the amidinium group forms part of a fairly large molecule. It should be noted, however, that this type of activity has not been assessed in the same way as, say, pressor or vasoconstrictor activity. The amidine derivatives regarded as being the most potent reflex depressor agents are not those found to produce the largest and most persistent effects, but those which needed to be given in only very small doses to match a given transitory and far from maximal response. Some of these amidine derivatives have been shown to block the receptors for the chemoreflexes, after initially stimulating them, when given in doses not much greater than those needed for matching the response to a small dose of phenyl-diguanide (96). It is also to be noted that the pathways for the chemoreflexes probably contain a number of synapses, so that the relationship between the stimulus and the cardiovascular response may be far from simple. Since a variety of amidine derivatives, including a few aliphatic compounds, possess appreciable reflex depressor activity, it would seem that the only pharmacophore needed is the amidine group. Nevertheless, the increase in activity noted with approximation to the structure of 5-hydroxytryptamine suggests that the properties of the "receptor" (as defined in section IVE-7), as distinct from those of the "site of action," play a large part in determining the influence of chemical structure on reflex depressor activity.

Walker (256) has invoked resonance to explain why pressor activity is inconspicuous in most of the *N*-substituted amidine derivatives which have been tested to date. He pointed out that the resonance of the unsubstituted amidinium group is greatly impaired by the introduction of substituents which do not permit the formulation of equivalent structures. Such substituents would be expected to decrease the affinity of the compound for the phosphate or carboxylate groups of cells by interfering with the formation of doublet ion-pairs. The deleterious effect of *N*-substitution on reflex depressor activity can also be accounted for in this way.

It is thought significant that the pharmacological properties of 4-amino-pyridine are very similar to those of such amidine derivatives as 2-amino-pyridine and *S*-methyl-thiouronium, because 4-amino-pyridine shares with these other compounds the property of resonance, although it is not an amidine derivative. Because of resonance all the atoms of 4-amino-pyridine will be coplanar. Its molecule will therefore be more compact than that of bases like piperidine or diethylamine which possess approximately the same number of atoms but which do not possess comparable pharmacological activity. Resonance will not enable 4-amino-pyridine to form a carboxylate or phosphate with doublet ion-pairs (as depicted in VI). However, resonance could influence the reaction between 4-amino-pyridine (or an amidine derivative) and an anionic group by stabilizing the cation. The ability of creatine phosphate and arginine

phosphate to provide "high energy bonds" has been explained along these lines (140, 152); it has been suggested that resonance stabilization of the main hydrolysis product, *viz.*, the amidine derivative, accounts for the unusual thermodynamic instability of the phosphate.

One of the major difficulties in accounting for the distribution of such properties as pressor activity and reflex depressor activity amongst amidine derivatives and related compounds is to explain why the amines corresponding to potent amidine derivatives may possess little or no activity. Perhaps the resonance of the amidinium group increases the absorbability of the molecule. However, no confident assertion can be made until more has been learned, not only about the ordinary pharmacological properties of corresponding amines and amidine derivatives, but also about the chemical features of their combination with receptors.

SUMMARY

The pharmacological actions of amidine [$-C(\overset{+}{N}H_2):NH_2$] derivatives given most attention in this review are those affecting blood pressure, smooth and skeletal muscle tone, and metabolism. An account has been given of the distribution of pressor, reflex depressor, vasoconstrictor, hypoglycaemic, antibacterial, and certain other forms of pharmacological activity amongst amidine derivatives and compounds chemically related to them. Attention has been restricted largely to compounds of fairly simple structure, particularly those belonging to aliphatic homologous series, in order to emphasize factors which are believed to be of major importance in determining activity.

Basic strength is one such factor. The characteristic properties of amidine derivatives as a class are those of amidinium cations. These cations are thought to compete with cations of physiological importance for anionic sites on or in cells.

Molecular size is also of major importance. It would be expected on chemical grounds that an amidinium ion with a long hydrocarbon chain would be better able than a lower homologue to become concentrated in the phase in which the drug receptors lie, and also to remain in contact with the receptors for longer periods on the average in the face of competition from other cations. Hence it is not surprising that some, though by no means all, pharmacological actions of amidine derivatives increase in intensity and duration when a homologous series is ascended (up to a "cut-off" imposed by extreme water-insolubility). These actions are probably ones which depend upon the occupation of, as distinct from the act of combination with, drug receptors. The distribution of such properties as pressor activity can be explained most simply by assuming that stimulant actions of amidine derivatives are brought about by a quantal process, occurring as the result of the drug's making contact with the receptor and being prevented from recurring for so long as the two remain in contact.

The characteristics of the drug receptor appear to be highly important for some forms of activity but not for others. Thus, it seems important for pos-

session of strong reflex depressor activity that the size and shape of the molecule should be comparable to that of 5-hydroxytryptamine. On the other hand, the ability of numerous amines and amidine derivatives to exert trypanocidal and antibacterial actions implies that little specificity of structure is needed to permit interaction with certain other receptors.

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