SUPERSENSITIVITY AND SUBSENSITIVITY TO SYMPATHOMIMETIC AMINES

ULLRICH TRENDELENBURG

Department of Pharmacology, Harvard Medical School, Boston, Massachusetts

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INTRODUCTION

More than a hundred years ago Budge (22) described the development after sympathetic denervation of the iris of a phenomenon which later became known as "paradoxical pupillary dilatation" (89). In 1899 Lewandowsky (92) reported that the response of the previously denervated nictitating membrane of the cat to intravenous injections of adrenal extracts was "noch schöner" (even more beautiful) than that of the normal side; Anderson (4) then carried out the first experimental analysis of these phenomena and proved them to be due to supersensitivity of the denervated muscle to epinephrine. Since these early beginnings, a vast amount of information has been accumulated, and numerous hypotheses have been advanced to account for the mechanisms involved in the development of supersensitivity to norepinephrine. No attempt will be made to cover even a considerable part of this material. The aims of this review are twofold: 1) to draw attention to the complexity of the nature of supersensitivity to sympathomimetic amines and to point out the many problems which must be solved before we reach some understanding of the mechanisms involved, and 2) to present the ideas and concepts which are the result of about five years of work in this field. Emphasis is placed not so much on results, which can be found in the relevant publications, but rather on concepts which may help to orient and stimulate interest in this subject.

Many difficulties in design of experiments and in interpretation of results stem from the fact that pharmacologists perform two different kinds of experiments: 1) those in which a new compound is studied under experimental conditions so well understood that conclusions can be reached as to the mechanism of action of the new compound; or 2) those in which the pharmacological properties of a well-known agent are used as a tool for the exploration of physiological mechanisms. In reality, our knowledge of the normal physiology and of the mechanism of action of drugs is so limited that there is no clear borderline between the two approaches. Hence, experiments contain elements of both 1) and 2). Reserpine, for instance, is used as a pharmacological tool for depleting the norepinephrine stores of tissues; depletion of the norepinephrine stores is used as a tool in the study of the mechanism of action of tyramine; tyramine, in turn, is used as a pharmacological tool for obtaining rough estimates of the degree of depletion of norepinephrine stores; and all these experimental findings are taken to clarify both the mechanism of action of the agents employed and the normal physiology of the transmitter of adrenergic nerve terminals.

The fact that we are facing so many unknown factors makes it very difficult to build up a coherent discussion step by step; every advance in one direction has led us to new problems which usually extend into the foundations from which the advance has been made. The review has been arranged as follows. The first section deals with problems relating to the measurement of sensitivity. These are important because the field suffers from a lack of quantitative evidence. The second section deals with the physiology and pharmacology of the nictitating membrane, the organ which has provided much of the evidence bearing on supersensitivity and subsensitivity to sympathomimetic amines. The next two sections deal mainly with descriptive aspects: the changes and modifications of dose-response curves of sympathomimetic amines by various drugs or procedures known to cause super- and subsensitivity to this group of substances. The final two sections deal with the mechanism of action and with hypotheses put forward as explanations for the phenomena of super- and subsensitivity.

The following procedures and agents will receive special attention: denervation, decentralization, pretreatment with reserpine, and the administration of cocaine. In the context of this article, "denervation" stands for chronic postganglionic denervation (in the case of the nictitating membrane, experiments are carried out 7 to 14 days after surgical removal of the superior cervical ganglion). "Decentralization" stands for chronic preganglionic denervation (experiments performed 7 to 14 days after section of the cervical sympathetic chain). Whenever the term "pretreatment" is used, it refers to the application of the agent *before* the beginning of the experiment (usually 24 hours prior to the experiment); "administration" refers to an injection of the agent *during* the actual experiment. Because of the division of this article, reference will be made in the first part to "direct" and "indirect" actions of sympathomimetic amines, although the actual mechanism of this "indirect" action will be discussed only in the second part.

It would be preferable to abandon the term "potentiation" and to use more

precise terms. However, the literature contains numerous reports of substances and procedures which enhance responses to sympathomimetic amines with no indication of the mechanism by which such an increase in response is brought about; in such situations, the author feels entitled to use the term "potentiation" to point out that we are not yet able to substitute a better defined term.

I. PROBLEMS IN MEASURING CHANGES IN SENSITIVITY

On kymograph or polygraph records, and graphs derived from them, responses are represented in a vertical direction. Blocking or potentiating agents diminish or increase these vertical representations. Much of the literature suggests that persons studying changes in sensitivity are so preoccupied with vertical responses or changes of response as to suffer from "vertical bias."

This form of intellectual distortion has been eliminated in the field of drug antagonism. The statement "the response to 10 μg acetylcholine was reduced by 83 % in the presence of a certain amount of atropine" may be true but remains rather meaningless as long as the relative position of the 10-μg dose of acetylcholine within the dose-response curve is not known. Nowadays the effectiveness of antagonistic agents is defined by the "dose ratio" (62), by the "pA_x value" (119), or by the relative potency, *i.e.*, they are defined by the horizontal distance between dose-response curves. Such horizontal measurements enable us to compare results obtained under different experimental conditions and on different test organs.

However, in the field of supersensitivity the error introduced by "vertical bias" is still widespread and not generally recognized. This can lead to serious misinterpretations of otherwise good results. One commonly used index of supersensitivity is the so-called "potentiation factor," *i.e.*, the ratio "response after"/"response before" the introduction of a potentiating agent or procedure. When such a potentiating agent or procedure causes a parallel shift of the dose-response curve of the agonist to the left (fig. 1), the "potentiating factor" can attain any value between unity and infinity, depending on the particular test dose of the agonist. Usually the relative position of the test dose within the dose-response curve was not known, and therefore one cannot measure the magnitude of the sensitization produced by the agent or procedure. For example, it is customary to compare the responses of the nictitating membrane to injections of equal doses of epinephrine and norepinephrine; since epinephrine is about five times more potent than norepinephrine, the response to epinephrine is greater than that to norepinephrine. From Figure 1 it is obvious that a potentiation of equal magnitude for both amines must result in a greater "potentiation factor" for the less potent norepinephrine than for epinephrine; in spite of this obvious relationship, preferential potentiation of norepinephrine has been postulated from such results. A meaningful measurement of the sensitizing effect of a drug or procedure can be obtained only by the determination of the horizontal shift of the dose-response curve.

In another study, the response of an organ to norepinephrine was determined before and after the administration of cocaine to normal preparations, and a

"potentiation factor" was calculated. This procedure was repeated (with the same test dose of norepinephrine) in preparations which had been pretreated with reserpine, and the "potentiation factor" was found to be smaller. This diminution was taken as evidence that pretreatment with reserpine reduced the sensitizing action of cocaine. However, the schedule of pretreatment with reserpine which was employed in this series of experiments resulted in supersensitivity to norepinephrine; consequently the test dose of norepinephrine was now closer to the maximally effective dose. From Figure 1 it is evident that such a shift must result in a diminution of the "potentiation factor." Hence, the con-

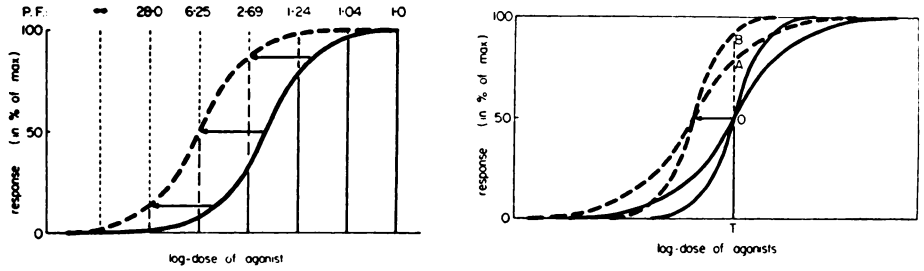


FIG. 1 (*left*). Effect of the relative position of the test dose of the agonist on the "potentiation factor" (P. F.).

The potentiating agent (or procedure) is assumed to have caused a parallel shift of the dose-response curve of the agonist to the left (as indicated by the arrows). Vertical bars: response before (solid lines) and after potentiation (broken lines).

FIG. 2 (*right*). Effect of slope of dose-response curve on the magnitude of the response observed after potentiation.

Two dose-response curves of different steepness are shown. They are drawn so that they cross at the test dose (T). The potentiating agent or procedure is assumed to cause a parallel shift of the dose-response curves to the left; the magnitude of this shift is equal for both agonists (arrow). Note that response after potentiation is greater the steeper the dose-response curve.

clusion that pretreatment with reserpine impairs the sensitizing action of cocaine was wrong.

These considerations are not intended to imply that all measurements of "potentiation factor" should be discarded as meaningless. Fleckenstein and Bass (55), for instance, used this ratio to demonstrate the similarity of the sensitizing actions of cocaine and of denervation.

The determination of threshold doses of the agonist before and after potentiation is often used to obtain a horizontal measurement of the magnitude of the sensitizing effect. Figure 1 shows that very small changes in the resting conditions (the position of the zero-line) can lead to gross errors in such determinations. Horizontal shifts of dose-response curves should always be measured in the steep range of the dose-response curve. Moreover, determinations of threshold doses cannot distinguish between true supersensitivity (*i.e.*, a shift of the whole curve) and additive phenomena (see below).

It is usually realized that one should use equieffective doses when one studies,

for example, the potentiation by cocaine of the effects of two different sympathomimetic amines. Yet this safeguard is often not enough. Figure 2 illustrates the dose-response curves of two agonists (solid lines), one being very steep, the other somewhat less steep. In this hypothetical experiment the response to the test dose is indicated by the vertical solid line (T to O). If cocaine shifts both dose-response curves to the left by the same distance (as indicated by the horizontal arrow), then the magnitude of the response obtained after the administration of cocaine is obviously dependent not only on the sensitizing effect of cocaine, but also on the slope of the dose-response curve of the agonist: the steeper the dose-response curve, the greater the increase in response. This is no idle theoretical consideration; the slope of the dose-response curve for some amines is much steeper than that for norepinephrine (nictitating membrane: 151). Furthermore, what has been depicted here for two different dose-response curves is equally true for the flat and the steep part of one and the same dose-response curve; this is also evident from Figure 1.

This relation should be borne in mind when experiments are interpreted in which equal doses of epinephrine and norepinephrine are used. Since epinephrine is more potent than norepinephrine (on the nictitating membrane), the test dose of epinephrine usually falls into a steeper part of the dose-response curve than the dose of norepinephrine. The statement in the literature that "supersensitivity after denervation develops much more slowly for norepinephrine than for epinephrine" is based on experiments of this design; the validity of the statement may therefore be questioned.

Sometimes the determination of a considerable part of the dose-response curve helps to detect and then to analyze different types of "potentiation." The agonists epinephrine (A) and acetylcholine (B) cause contraction of the nictitating membrane, although they react with different receptors; the shape of their respective dose-response curves is similar (103). When equieffective amounts of A and B (for convenience to be described as "1A" and "1B") are injected simultaneously, they have, of course, an additive effect. However, it is important to realize that the response to such a simultaneous injection of equieffective doses (*i.e.*, the response to "1A + 1B") is not twice as large as that to either "1A" or "1B" alone. Rather, the resulting response is equal to that observed after the injection of either "2A" or "2B"; this is analogous to the fact that a car with two engines will not be twice as fast as its normal version but just as fast as a car with twice the horsepower. In other words, the doses of the two injected agonists add rather than their responses: the addition has to be performed in a horizontal and not in a vertical direction.

This empirical fact is of importance for all those conditions where either the local concentration of norepinephrine in close proximity to the receptor is increased, or where there is an increased sensitivity to the normal local concentration of norepinephrine. When under such conditions a dose-response curve is determined for acetylcholine (B), a distortion of the dose-response curve is observed, since the amount of norepinephrine already present (for convenience described as "1A") increases the response to an equieffective dose of acetyl-

choline ("1B") to what would have been expected with "2B." However, with higher doses of B the contribution of "1A" becomes negligible, since the response to "30B" is increased by the addition of "1A" to "30B + 1A" = "31B" which, on a log-scale, is hardly different from "30B." As a result, only the lower part of the dose-response curve of acetylcholine is affected by the potentiating agent or procedure (145). Whenever such a change in the shape of the dose-response curve is observed, an additive phenomenon of this kind may be involved. It would be misleading to describe this as true supersensitivity, since this term implies a parallel shift of the whole dose-response curve. Furthermore, the determination of threshold doses alone may lead to serious misinterpretation.

It is well known that small amounts of many sympathomimetic amines (*e.g.*, ephedrine (63, 77)) increase the response to a subsequent injection of norepinephrine or epinephrine, whereas larger amounts depress the effect of norepinephrine. Unfortunately, in many studies of potentiating agents or procedures, ephedrine-like amines are included in the series of test substances, *i.e.*, ephedrine-like compounds are injected at various times prior to the injection of norepinephrine; consequently the results become uninterpretable, since (in the absence of a rather involved statistical analysis) it must remain obscure whether the reported increase in response to norepinephrine was actually due to the potentiating agent under test or to the preceding injection(s) of an ephedrine-like "potentiating" sympathomimetic amine.

Blood pressure is the variable most easily recorded in an anesthetized animal. However, blood pressure changes are also the most difficult to analyze. For example, it is well known that pretreatment with reserpine enhances the effects of various anesthetics (20). In pretreated animals the response to test doses of sympathomimetic amines might be augmented because of any of the following factors: 1) the use of less anesthetic might enhance the sensitivity of the cardiovascular system, 2) the use of the usual amount of anesthetic might result in very deep anesthesia which depresses the normal reflex control of the blood pressure, and 3) depletion of the catecholamine stores might enhance pressor responses either by lowering the blood pressure or by interfering with reflexes. To establish a sensitization of vascular smooth muscle to sympathomimetic agents is most difficult under these circumstances. Most of these complications can be avoided by the use of spinal animals prepared under ether. Since they are essentially unanesthetized (during the actual experiment) and free of cardiovascular reflex control, conclusions as to the development of changes in sensitivity of vascular smooth muscle and of cardiac tissues can be made with greater confidence.

II. INNERVATION AND RECEPTORS OF THE NICTITATING MEMBRANE

The nictitating membrane of the cat lacks many of the complications just noted in the cardiovascular system. Since a large amount of the work concerned with the problems of super- and subsensitivity has been carried out on the nictitating membrane, a short review of its innervation and its pharmacological receptors is indicated.

A. Innervation of the nictitating membrane

The visible part of the nictitating membrane consists of cartilage. The smooth muscle extends from this cartilage into the depth of the orbit; it consists of two parts, the medial and the inferior smooth muscle (1). The nictitating membrane is innervated by sympathetic fibers only. The majority of the postganglionic fibers originate in the superior cervical ganglion, enter the cranial cavity and join the trigeminal nerve. The sympathetic supply to the medial smooth muscle accompanies the ophthalmic division and then its nasociliary and infratrochlear branches; the supply to the inferior smooth muscle accompanies the maxillary division and then its zygomatic branch. A detailed anatomical and functional study of the nerve supply has been reported by Thompson (136).

Although the majority of the ganglion cells are located in the superior cervical ganglion, an abnormal location of a few ganglion cells has been described for the preganglionic (46) as well as for the postganglionic nerve trunk (89a). Histological studies of the nerve terminals innervating the nictitating membrane provide further evidence for the view that some ganglion cells are located within the postganglionic nerve trunk; although the large majority of these terminals degenerate within 4 days after surgical removal of the superior cervical ganglion, a small proportion of the terminals show no signs of degeneration even after 12 days (89a). The cell bodies of these resistant fibers are not located in the contralateral ganglion, since ganglionectomy never caused any degenerative changes in the contralateral nerve terminals. Hence, it must be concluded that the nictitating membrane is innervated by a small number of abnormally located ganglion cells which escape the usual procedure of denervation (*i.e.*, surgical removal of the ipsilateral superior cervical ganglion). The degenerative changes appear in ascending order: the first histological signs of degeneration are detected earlier in the nerve terminals than in the nerve fibers.

The preganglionic fibers probably originate in the "centrum cilio-spinale (Budge)" which is located in the lateral column of the spinal cord at the junction of its dorsal and cervical region (162). According to Claude Bernard (16) the preganglionic fibers pass through spinal roots different from those which contain the vasomotor fibers to the orbital region; their conduction velocity is higher than that of vasomotor fibers (17, 47).

Stimulation of the sympathetic nerve supply causes retraction of the nictitating membrane. A movement in the opposite direction (protrusion) can be elicited by stimulation of sensory receptors of the cornea; this reflex involves the 5th (afferent) and 6th (efferent) nerve and results in contraction of the external rectus muscle (117). This external ocular muscle sends some muscle fibers to the nictitating membrane; it is interesting that in this structure a striated muscle is the functional antagonist of a smooth muscle.

Both norepinephrine and acetylcholine cause contraction of the smooth muscle of the nictitating membrane. The postganglionic sympathetic fibers innervating the nictitating membrane are generally believed to be adrenergic, but an admixture of cholinergic fibers has repeatedly been postulated. Instillation of physo-

stigmine into the conjunctival sac potentiates the response of the nictitating membrane to postganglionic stimulation, and the intravenous injection of atropine antagonizes this effect (12, 34). However, such experiments do not prove the presence of cholinergic fibers, since the response of the nictitating membrane to intravenous injections of epinephrine is similarly modified by physostigmine and atropine; this has been observed on the normal and also on the chronically denervated nictitating membrane (27, 125). Moreover, similar observations with epinephrine have been made on the isolated nictitating membrane *in vitro* (40). As physostigmine and atropine are able to modify the response of the nictitating membrane to epinephrine, there is no need to postulate the presence of cholinergic fibers in the postganglionic nerve supply.

When electrical records were obtained *in situ* with surface electrodes applied to the inferior smooth muscle of the nictitating membrane, two action potentials were observed, one of which was potentiated by physostigmine and antagonized by atropine (111a). However, this observation does not prove a cholinergic innervation of the smooth muscle, since Gardiner *et al.* (64a) reported that the nerve supplying the Harderian gland and the blood vessels of the nictitating membrane stain for cholinesterase, while those innervating the smooth muscle do not. The authors concluded that the sympathetic fibers innervating the gland and the blood vessels are cholinergic, and those innervating the smooth muscle are adrenergic. Their study of the effect of various anticholinesterases on the *in vivo* and the *in vitro* preparation of the nictitating membranes supports this view.

Burn and Rand (31) recently put forward the interesting hypothesis that many of the adrenergic fibers of the sympathetic system are of cholinergic nature, the acetylcholine released at the nerve terminals then being responsible for the release of norepinephrine from the peripheral storage site. However, as far as the nictitating membrane is concerned, the postganglionic fibers seem to be truly adrenergic in the traditional meaning of the word. Hemicholinium (which prevents the synthesis of acetylcholine (95)) does not impair the response of the nictitating membrane to postganglionic nerve stimulation either *in vivo* (65, 161) or *in vitro* (65). Furthermore, the stimulant action of acetylcholine on the nictitating membrane is not mediated through the release of norepinephrine, since it persists after depletion of the norepinephrine stores by pretreatment with reserpine (26, 145) and is not blocked by doses of choline 2,6-xylyl ether bromide (TM 10) which block the response of the nictitating membrane to nerve stimulation (145).

B. Pharmacological receptors of the nictitating membrane

Barger and Dale (14) studied a series of sympathomimetic amines and observed differences between their excitatory and inhibitory effects; the authors postulated two different receptors, the activation of which led to either an excitatory or an inhibitory response, respectively. This concept was later reinvestigated by Ahlquist (3) who defined two types of receptor, alpha and beta, not according to the resulting response but on the basis of the relative potencies

of a few selected sympathomimetic amines. Alpha-receptors are characterized by the sequence of potencies (in descending order): epinephrine ≧ norepinephrine ≫ isoproterenol; beta-receptors, on the other hand, are characterized by the sequence: isoproterenol > epinephrine ≧ norepinephrine (≧ stands for "more potent than or equipotent with"). The norepinephrine receptors of the nictitating membrane are predominantly of the alpha-type (3). However, it is possible that inhibitory beta-receptors are also present, since isoproterenol under certain experimental conditions causes a relaxation of the isolated preparation *in vitro* (135).[1]

Activation of the atropine-sensitive acetylcholine receptors results in contraction of the smooth muscle (103, 135, 145). The nictitating membrane also responds to 5-hydroxytryptamine (139); the receptor seems to be of the D-type (according to the definition of Gaddum and Picarelli (64)) since it is blocked by D-lysergic acid diethylamide and by phenoxybenzamine (135) but is not affected by morphine (143). Histamine has no direct action on this smooth muscle (*in vivo*, 137; *in vitro*, 135). Surprisingly, the isolated nictitating membrane responds to ganglion-stimulating substances like nicotine and tetramethylammonium chloride; this response is reduced by hexamethonium (135), by pretreatment with reserpine, or by chronic denervation (26). The response to nicotine thus seems to be mediated through the release of norepinephrine from a storage site within or in close proximity to the postganglionic nerve terminals.

Although histamine has no direct action on the smooth muscle of the nictitating membrane, intravenous injections of this substance cause a contraction of the membrane. Both humoral and nervous factors contribute to this response, since histamine stimulates the adrenal medulla (137), the superior cervical ganglion (137), and the higher centers in the spinal cord and in the brain (142). Histamine also facilitates transmission through the superior cervical ganglion (138, 140, 141). This example illustrates the various factors which can contribute to a response of the nictitating membrane in an intact anesthetized cat.

III. CLASSIFICATION OF SYMPATHOMIMETIC AMINES

The classification under discussion deals with the "direct" and "indirect" actions of sympathomimetic amines. The mechanism of this "indirect" action will be discussed later (Section V). In this section the term "indirect" will be used as it developed historically.

Tainter and Chang (133) were the first who observed that the pressor response of several species to tyramine is antagonized by amounts of cocaine which cause supersensitivity to epinephrine. Soon afterwards Tainter (132) observed that the pressor response to ephedrine is similarly antagonized by cocaine. The next important step was the report of Burn and Tainter (33) that denervation of the

[1] Evidence for beta-receptors has been obtained recently. When the nictitating membrane of the spinal cat was in a state of contraction after nerve stimulation, after acetylcholine or after ergotoxine, isoproterenol caused relaxation. In this action isoproterenol was more potent than epinephrine or norepinephrine; dichloroisoproterenol antagonized this action while phenoxybenzamine did not (Smith, submitted to J. Physiol.)

iris of the cat has the same effect as the administration of cocaine; that is, after degeneration of the postganglionic nerve fibers, intravenous injections of tyramine and ephedrine cause smaller responses of the denervated than of the innervated structures, although the denervated iris is supersensitive to epinephrine. The authors concluded that, while epinephrine has a "direct" action on smooth muscle, tyramine exerts its sympathomimetic effects through an action on the sympathetic nerve endings; hence, it has an "indirect" action. A second important finding was that supersensitivity to epinephrine after either denervation or the administration of cocaine is accompanied by subsensitivity to tyramine.

Fleckenstein and Burn (56) studied the effect of denervation of the nictitating membrane of the cat on the response of this smooth muscle to a series of sympathomimetic amines. They obtained good evidence for the view that three groups of amines can be distinguished: (a) those which are potentiated by denervation (the directly acting amines), (b) those which are not much affected (assumed to have both direct and indirect actions), and (c) those which are clearly less effective after denervation (the indirectly acting amines). The prototypes for these three groups are norepinephrine (directly acting), ephedrine (mixed actions), and tyramine (indirectly acting). The study of Fleckenstein and Burn suggested the following structure-action relationships: All catecholamines have direct actions; if a substance has only one or no phenolic hydroxyl group, then it has an indirect action. However, in addition to this indirect action, a direct action is conferred on the amine by the presence of an alcoholic hydroxyl group in the *beta*-position (ephedrine-like amines); the absence of the alcoholic hydroxyl group puts the amine into the group of purely indirectly acting (tyramine-like) amines.

Soon afterwards very similar results were reported by Fleckenstein and coworkers (55, 57) who carried out experiments of the same kind in spinal cats after the administration of cocaine. These extensive and well-documented studies established that there are three groups of sympathomimetic amines, and they confirmed the earlier finding that supersensitivity to epinephrine or norepinephrine seems to be accompanied by subsensitivity to tyramine. Moreover, they demonstrated that the effects of denervation and of the administration of cocaine are strikingly similar.

At about the same time Innes and Kosterlitz (77) drew attention to the fact that both denervation and the administration of cocaine seem to discriminate between amines which have a single phenolic hydroxyl group in the *meta*-position and those which have it in the *para*-position; only the former compounds are markedly potentiated by either denervation or cocaine. Holtz *et al.* (75) also reported such differences.

When reserpine became available as a pharmacological tool for the depletion of the norepinephrine stores of peripheral organs, the classification of sympathomimetic amines was studied again. Carlsson *et al.* (39) showed that the prototype of the purely indirectly acting amines (tyramine) lost its effectiveness after pretreatment of the cat with reserpine. Burn and Rand (28) confirmed and ex-

tended this study by testing the effect of a variety of sympathomimetic amines in normal and in reserpine-pretreated preparations. These authors found that their schedule of pretreatment with reserpine resulted in supersensitivity to some (*i.e.*, to the directly acting) amines and subsensitivity to others (*i.e.*, to the ephedrine- and tyramine-like amines). Since they used only single test doses of the amines rather than dose-response curves, differences between ephedrine-like and tyramine-like compounds were not reported. Bejrablaya *et al.* (15) obtained similar results in the heart-lung preparation of the dog; here again, pretreatment with reserpine abolished the rate-increasing effect of ephedrine-like and tyramine-like amines.

These findings clearly demonstrated that pretreatment with reserpine abolishes the indirect actions of sympathomimetic amines; the reports also suggested that pretreatment with reserpine causes supersensitivity to norepinephrine, again indicating that the phenomena of supersensitivity to norepinephrine and of subsensitivity to tyramine may be linked to each other; finally, the previous clear distinction between the groups of ephedrine-like and of tyramine-like amines was no longer observed. This latter finding does not agree with the observation by Innes and Krayer (78) that heavy pretreatment of dogs with reserpine reduces but never abolishes the response of the pacemaker of the heart-lung preparation to ephedrine. This difference finds its explanation in the fact that Bejrablaya *et al.* (15) used single test doses of ephedrine, while Innes and Krayer determined the whole dose-response curve. Liebman (93) studied this problem and demonstrated in the heart-lung preparation of the dog that pretreatment with graded doses of reserpine causes a depression of the maximum of the dose-response curve for indirectly acting amines (tyramine and amphetamine) without causing a horizontal shift of the maximum of the curve. Phenylpropanolamine differs from amphetamine by the presence of an alcoholic hydroxyl group in the *beta*-position and consequently has an ephedrine-like action; it is antagonized by pretreatment with reserpine, but with this compound a graded shift of the dose-response curve to the right is observed. These findings demonstrate that differences between ephedrine-like and tyramine-like amines can easily be detected when full dose-response curves are obtained. Recent observations on isolated guinea pig atria confirm this view (149). However, observations on the rate of beat of the isolated heart are complicated by the fact that the typical dose-response curve for sympathomimetic amines has a descending limb following the usual sigmoid ascending limb. Pretreatment with reserpine causes a shift of the ascending limb and of the maximum of the dose-response curve for ephedrine-like compounds towards or into the descending limb; this results in a reduction of the maximal response. Pretreatment with reserpine does not seem to influence the descending part of the curve.

When short-term pretreatment is used (a single intraperitoneal injection of reserpine 24 hours prior to the experiment), supersensitivity of the nictitating membrane of the spinal cat to norepinephrine does not develop, although the norepinephrine stores are severely depleted, as judged by the very small response of the nictitating membrane to nerve stimulation (60). The absence of super-

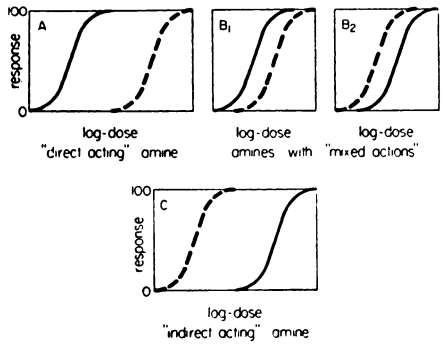
sensitivity to norepinephrine after such short-term pretreatment with reserpine has since been reported for the iris of the cat (97), the pacemaker of the dog heart-lung preparation (84), and the pacemaker of the isolated guinea pig atrium (44). The depleting effect of this short-term pretreatment with reserpine has been confirmed by direct measurements of the catecholamine content of the nictitating membrane (82, 152), the dog heart (112, 156), and the guinea pig atrium (44).

This observation permitted reassessment of the view that subsensitivity to tyramine is invariably linked to supersensitivity to norepinephrine. All previous studies of the effect of denervation, of the administration of cocaine, and of pretreatment with reserpine had suggested that this was so. However, when short-term pretreatment with reserpine was used, the response of the nictitating membrane, of the blood pressure, and of the cardiac pacemaker to tyramine was reduced (144), while the sensitivity to norepinephrine remained unchanged (60). Therefore it must be concluded that the two phenomena of supersensitivity to norepinephrine and of subsensitivity to tyramine can be separated experimentally; this conclusion is supported by similar findings on the cat iris (97), the heart-lung preparation of the dog (84, 93), and the isolated guinea pig atria (44). As in the isolated heart, pretreatment of the cat with reserpine depresses the effect of tyramine on the nictitating membrane and on the blood pressure mainly by a depression of the maximum of the dose-response curve of tyramine.

Ephedrine was found to differ clearly from both the purely directly acting norepinephrine (60) and the purely indirectly acting tyramine (144). Results obtained on the nictitating membrane indicated that short-term pretreatment with reserpine causes a pronounced shift of the dose-response curve of ephedrine to the right with no change in the magnitude of the maximal response to this substance (148). The investigation of a series of 16 sympathomimetic amines gave similar results (150); short-term pretreatment with reserpine either left the dose-response curves of the amines unaffected or it modified them as it modified those of ephedrine or tyramine. However, when the shifts of the dose-response curves are expressed quantitatively as the ratio "ED50 after pretreatment"/"ED50 without pretreatment," it is obvious that the various amines cannot be separated into three distinct groups. It is more useful to assign every sympathomimetic amine to a place between the two extremes of "purely direct" and "purely indirect" action, since there is a graded transition from one extreme to the other.

To overcome the rigidity which is inherent in any attempt to classify compounds into distinct groups, the following hypothesis is offered for consideration. Both directly and indirectly acting amines are known to be taken up into tissue stores (norepinephrine, 105, 159; epinephrine, 7; tyramine, 123), and there is growing evidence that nontransmitter amines may be able to displace norepinephrine from its storage sites (epinephrine, 130; tyramine, 123). Therefore it is possible that the relative importance of the direct and indirect effects of any given sympathomimetic amine is determined largely by the potency of its direct action. For instance, tyramine seems to be so weak in producing a direct action that for

all practical purposes it is an overwhelmingly indirectly acting amine. Epinephrine, on the other hand, seems to be a predominantly directly acting amine only because its high potency in producing a direct action normally masks its ability to displace norepinephrine from the tissue stores. On the basis of such a concept, seemingly qualitative differences between sympathomimetic amines can be resolved into quantitative differences of properties which are basically common to all sympathomimetic amines. The overall action of a sympathomimetic amine may thus be visualized as being determined by the relative position of two dose-response curves, one for the direct action and another for the indirect action (solid line and broken line, respectively, Fig. 3).



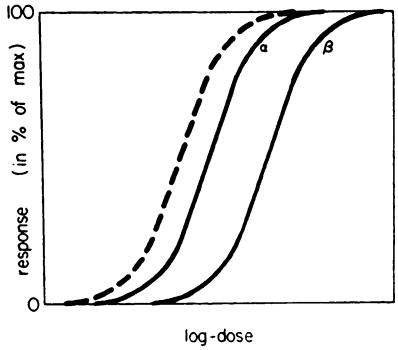


FIG. 3 (*left*). Hypothetical composition of the overall action of sympathomimetic amines. Represented are the three groups of epinephrine-like "directly acting" amines (A), of ephedrine-like amines with "mixed actions" (B_1 and B_2), and of tyramine-like "indirectly acting" amines (C). Direct actions—solid curves; indirect actions—broken curves. For details see text.

FIG. 4 (*right*). Hypothetical representation of the direct (solid curves) and indirect action (broken curve) of an ephedrine-like amine whose action on the nictitating membrane (alpha-receptors) is only slightly reduced by pretreatment with reserpine, whereas its action on the cardiac pacemaker (beta-receptors) is much more reduced by this pretreatment (for details see text).

This hypothesis can also account for the observation that the relative contribution of the indirect action to the overall (direct + indirect) action of a sympathomimetic amine with mixed actions sometimes varies from organ to organ or from receptor to receptor. Since it is well known that the potency of some purely directly acting amines on alpha-receptors is high in comparison to their potency on beta-receptors (*e.g.*, norepinephrine), whereas the opposite is true for other amines (*e.g.*, isoproterenol) (3), the situation illustrated in Figure 4 may exist. Phenylpropanolamine, for instance, has only a weak indirect action on the nictitating membrane (alpha-receptors) as shown by the very small shift of its dose-response curve to the right after short-term pretreatment with reserpine (150). When the response of the cardiac pacemaker (beta-receptors) to this amine is recorded, pretreatment with reserpine is found to cause a much more pronounced shift of the dose-response curve to the right; this indicates a relatively

greater contribution of the indirect to the overall action of phenylpropanolamine in the heart, a finding consistent with observations in the heart-lung preparation of the dog (93) and on isolated guinea pig atria (149). [illegible]rences may be resolved by the assumption that the indirect action of phenylpropanolamine is of equal intensity on both alpha- and beta-receptors but that the potency of its direct action is greater on alpha- than on beta-receptors (Fig. 4).

Holtz *et al.* (75) also reported that the classification of amines according to observations made on the nictitating membrane of the cat is sometimes very different from the classification according to observations made with isolated guinea pig atria; however, in these experiments it is not clear whether differences in species, organs, receptors, or experimental conditions (*in vivo* as against *in vitro*) are involved.

At the present time the hypothesis is put forward in a purely speculative way to stimulate interest in the possibilities mentioned. The problem requires much more extensive work before such a hypothesis is acceptable.

The structural requirements for direct and indirect actions of sympathomimetic amines were formulated in various of the previous studies (55, 56, 57, 99). According to Fleckenstein and Burn (56), the group of directly acting amines comprises all catecholamines (with or without an alcoholic hydroxyl group in the *beta*-position) and also phenylephrine, although this amine has a phenolic hydroxyl group only in the *meta*-position in addition to the alcoholic hydroxyl group in the *beta*-position. Phenylephrine was considered an exception in this series of amines, since all other amines with an alcoholic hydroxyl group in the *beta*-position but less than two phenolic hydroxyl groups seemed to belong to the group of ephedrine-like amines with mixed actions. Maxwell *et al.* (99), on the other hand, assigned not only phenylephrine but also its *p*-OH analogue, synephrine, to the group of directly acting amines. It has to be borne in mind that both these classifications were based on experiments in which there was not only a subsensitivity to tyramine but also a supersensitivity to norepinephrine. When synephrine was assigned to the group of amines with mixed actions (56), it was assumed that the potentiation of its direct effects cancelled out the subsensitivity to its indirect effects; *i.e.*, that the nictitating membrane was supersensitive to the direct actions of *all* sympathomimetic amines. However, there remains the alternative possibility that supersensitivity to norepinephrine is not necessarily accompanied by supersensitivity to the direct action of *all* sympathomimetic amines. It is quite possible that synephrine, though of predominantly direct action, is not potentiated by denervation or by the administration of cocaine. Recent experiments showed that this alternative possibility is true. Short-term pretreatment with reserpine, which does not cause supersensitivity to any sympathomimetic amine, does not lead to a shift of the dose-response curves of synephrine and its *m*-OH analogue phenylephrine to the right; both compounds must therefore be regarded as predominantly directly acting amines (150). In addition, norphenylephrine and *m*-OH-phenylpropanolamine also have predominantly direct actions, although they are not catecholamines. Since there are so many exceptions to the rule that only catecholamines have purely direct actions, the usefulness of the rule is questionable.

Table 1 summarizes the results of various attempts to classify the sympathomimetic amines. It is evident that it is difficult to assign many of these amines to one specific group; it is much more useful to emphasize the graded transition between the two extremes of "purely directly acting" and "purely indirectly acting." Both the table and recent quantitative determinations of shifts of dose-response curves (caused by short-term pretreatment with reserpine) (150) show that the position of the various hydroxyl groups is important. Of five pairs of *m*-OH and *p*-OH analogues, the *m*-OH derivative was always nearer to the extreme of "purely direct action" than the corresponding *p*-OH compound. Hence, it must be concluded that the presence of a phenolic hydroxyl group in the *meta*-position enhances the direct action of an amine, or decreases its indirect component of action, or does both.

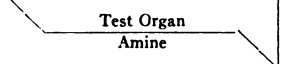
The preceding paragraph dealt with the lack of clear distinction between the group of directly acting amines and the group with mixed actions. A similar problem arises in separating the latter group from the indirectly acting amines. Fleckenstein and Burn (56) and Fleckenstein and Stöckle (57) found that, with amines which have either no or only one phenolic hydroxyl group, the presence of an alcoholic hydroxyl group in the *beta*-position is essential for the direct component of the overall action of the amine. Within this series all amines devoid of this alcoholic hydroxyl group had only indirect actions, *i.e.*, they were tyramine-like. Recent findings with *m*-tyramine indicate that this amine is an exception to the rule, since *m*-tyramine (no alcoholic hydroxyl group) has direct effects which are not abolished by short-term pretreatment with reserpine (150). The importance of the *m*-OH group in enhancing direct action is apparent from these observations. The importance of the alcoholic hydroxyl group in the *beta*-position is evident from the finding of Burn and Rand (28) that the levorotatory form of phenylethanolamine has predominantly direct actions, whereas the dextrorotatory form has predominantly indirect action. Recent observations by Blinks (18) suggest strongly that for certain receptors the dextrorotatory form of an amine possessing an alcoholic hydroxyl group in the *beta*-position acts as an amine with no alcoholic hydroxyl group; on isolated guinea pig atria, *l*-norepinephrine is 50 to 100 times more potent than *d*-norepinephrine, but the latter is equipotent with dopamine (the corresponding amine with no *beta*-OH group); *d*-epinephrine and epinine (the corresponding amine with no *beta*-OH group) are equipotent and have 1/50th to 1/100th the potency of *l*-epinephrine.

A quantitative determination of shifts of dose-response curves caused by short-term pretreatment with reserpine and a consideration of the structure-action relationship thus leads us to the conclusion that the concept of three distinct groups of sympathomimetic amines should be abandoned. It should be replaced by the concept that any given amine has a place between the two extremes of "purely direct" and "purely indirect" action. It should always be remembered that any classification is valid only for the organ or system under consideration.

These conclusions are in agreement with recent observations by Schmidt and Fleming (120). Twenty sympathomimetic amines were tested on the isolated rabbit gut, and full dose-response curves were obtained before and after ten days of pretreatment with reserpine. This long-term pretreatment with reserpine re-

TABLE 1

Classification of sympathomimetic amines

Substitution at	*p*	*m*	*β*	*α*	N	a	b	c	d	e	f	f	g	h	i	k	l	m	f	n
Test Organ / Amine						Nictitating membrane						B.P.	Heart rate							Ileum
Norepinephrine	OH	OH	OH	—	—	1	1	1		1	1	1	1	1		1		1	1	1
Epinephrine	OH	OH	OH	—	CH_3	1	1	1		1	1	1	1			1			1	1
Corbasil	OH	OH	OH	CH_3	—	1	1			1			1							
Dihydroxyephedrine	OH	OH	OH	CH_3	CH_3	1	1			1										
Dopamine	OH	OH	—	—	—	1	1	1	1	1										
Epinine	OH	OH	—	—	CH_3	1	1	1					1							
Keto-epinephrine (adrenalone)	OH	OH	O	—	CH_3				2											
Isoproterenol	OH	OH	OH	—	R															1
Phenylephrine	—	OH	OH	—	CH_3	1	1	1	1	1	1	1	1					1	1	
Synephrine	OH	—	OH	—	CH_3		2		1	1	1	1	1				1	1	1	
Norphenylephrine	—	OH	OH	—	—				1	1	1	1							1–2	
Norsynephrine	OH	—	OH	—	—	2			2	1	2	2							2	
Keto-Novadral	—	OH	O	—	—				3											
l-Phenylethanolamine	—	—	OH	—	—	2	2	1						3*						
d-Phenylethanolamine	—	—	OH	—	—			3*						3*						
Phenylpropanolamine	—	—	OH	CH_3	—						2	2	2				2	2	2	
m-OH-Phenylpropanolamine	—	OH	OH	CH_3	—				1	1	1							2	2	
p-OH-Phenylpropanolamine	OH	—	OH	CH_2	—				2		2	2						2	2	
Ephedrine	—	—	OH	CH_2	CH_3	2	2	3*		3*	2	2	2	3*			2	2–3	2	2
m-OH-Ephedrine	—	OH	OH	CH_2	CH_3						2	1						2	2	
p-OH-Ephedrine	OH	—	OH	CH_2	CH_3		2				2	2						2	2	
m-OH-Tyramine	—	OH	—	—	—	3					2	2						2–3	2–3	
Tyramine	OH	—	—	—	—	3	3	3	3	3	3	3	3	3		3	3	3	3	3
β-Phenylethylamine	—	—	—	—	—	3	3	3		3			3	3		3				
Amphetamine	—	—	—	CH_2	—	3	3	3	3		3	3	3	3			3	3	3	
p-OH-Amphetamine	OH	—	—	CH_2	—								3							
N-Methylamphetamine	—	—	—	CH_2	CH_3	3	3			3			3			3				
Pholedrine	OH	—	—	CH_2	CH_3	3	3			3			3			3				
Mephentermine	—	—	—	$2CH_2$	CH_3						3	3			3			3	3	

1 = amine with direct action; 2 = amine with mixed action; 3 = amine with indirect action; * = in this study no distinction was made between 2 and 3; R = isopropyl group.

a from (56), b from (55, 57), c from (28), d from (75), e from (122), f from (150), g from (99), h from (15), i from (131), l from (93), m from (149), n from (59).

sulted in supersensitivity of the gut to the inhibitory action of some amines and in subsensitivity to others. The shifts of the dose-response curves varied from a shift to the left by a factor of ten (supersensitivity) to a shift to the right by a factor of nearly two hundred (subsensitivity). There was supersensitivity to norepinephrine, no change in sensitivity to ephedrine, and marked subsensitivity to tyramine (59). The other seventeen amines did not fall into three distinct groups but were rather evenly distributed between the two extremes (120).

IV. TYPES OF SUPERSENSITIVITY AND OF SUBSENSITIVITY TO SYMPATHOMIMETIC AMINES

A. Cocaine

As mentioned in the preceding section, synephrine was classified by Fleckenstein and Stöckle (57) as an amine with mixed actions, because they assumed that cocaine causes supersensitivity of the nictitating membrane to the direct action of *all* amines. Since the action of synephrine is not affected very much by the administration of cocaine, it was reasonable to conclude (on the basis of this assumption) that this is due to a dual action of cocaine: (a) depression of the indirect and (b) potentiation of the direct component of the overall action of synephrine. However, synephrine has no pronounced indirect component of action, as indicated by recent experiments with short-term pretreatment with reserpine (nictitating membrane, blood pressure, and cardiac pacemaker of the spinal cat (150); heart-lung preparation of the dog (93)). If this is true, synephrine must be assumed to have a direct action which is not potentiated by cocaine. Or in other words, the problem arises whether Fleckenstein and Stöckle's assumption that cocaine causes supersensitivity to the direct action of *all* sympathomimetic amines is correct. Evidently, the selectivity of the sensitizing action of cocaine requires further analysis.

Before the sympathomimetic amines are considered, it is pertinent to inquire about the other substances capable of acting directly on the smooth muscle of the nictitating membrane (see Section II); all other substances (*e.g.*, histamine) can be left out of this consideration, since their effects are due to the release of epinephrine, or norepinephrine, or both from somewhere in the organism. Acetylcholine has been claimed to be potentiated by cocaine (83, 116, 135), but an analysis of this effect does not support the view that cocaine sensitizes the nictitating membrane to acetylcholine (145). Whereas the administration of cocaine shifts the whole dose-response curve of norepinephrine to the left, it affects only the lower third of the dose-response curve of acetylcholine. As pointed out in Section I, such a change can be explained by the assumption that the administration of cocaine enables the endogenous norepinephrine present in close proximity to the receptors to add its effects to those of the injected acetylcholine. This assumption is strengthened by two other observations: (a) Cocaine fails to potentiate acetylcholine after short-term pretreatment with reserpine (145); because of the depletion of the norepinephrine stores by this pretreatment, the endogenous norepinephrine is absent and can no longer exert its additive effect. However, cocaine

retains its full sensitizing action in regard to exogenous norepinephrine, when it is administered to reserpine-pretreated preparations (60). (b) Cocaine potentiates the response of the preparation of the nictitating membrane *in vitro* to acetylcholine only when the resting tone of this preparation is increased by cocaine (135). Hence, it must be concluded that the sensitizing action of cocaine is selective insofar as it causes supersensitivity to norepinephrine but not to acetylcholine.

The action of 5-hydroxytryptamine is also known to be potentiated by the administration of cocaine, but this effect has not been analyzed properly. It must be borne in mind that 5-hydroxytryptamine stimulates the adrenal medulla (90) and the superior cervical ganglion (139) in addition to its direct stimulant action on the smooth muscle of the nictitating membrane. Moreover, experiments with the isolated preparation of the nictitating membrane *in vitro* (135) have shown that cocaine influences the action of 5-hydroxytryptamine in much the same manner as it affects acetylcholine; *i.e.*, the action of 5-hydroxytryptamine is not enhanced when cocaine does not cause an increase in resting tension, but it is potentiated as soon as the resting tension rises. This observation is compatible with the view that the endogenous norepinephrine exerts an additive effect.

The true sensitizing effect of cocaine thus seems to be restricted to the sympathomimetic amines, and it remains only to consider whether cocaine potentiates the direct action of all amines or of only some of them. This problem is amenable to an experimental test, since it is known that short-term pretreatment with reserpine nearly completely eliminates the indirect action of sympathomimetic amines. Whatever action remains after this pretreatment must be assumed to be due mainly to the direct effect of these amines on the nictitating membrane. Sixteen amines were tested in such reserpine-pretreated preparations before and after the administration of cocaine. The ED50 was calculated from the dose-response curves of the amines, and measurements of the sensitizing action of cocaine were thus obtained (151). Two observations were made: (a) since the short-term pretreatment with reserpine virtually eliminates the indirect component of the action of these amines, cocaine never causes subsensitivity when studied in reserpine-pretreated preparations; (b) the sensitizing action of cocaine is not uniform for all sympathomimetic amines but is highly selective. Some amines (like norepinephrine) are strongly potentiated by cocaine; others (like synephrine) are not affected. Here again, the amines do not fall into two groups but are evenly distributed between the two extremes. One important factor influencing the location of an amine within this spectrum seems to be the position of its phenolic hydroxyl group. In each of the five pairs of *m*-OH and *p*-OH analogues under study, the *m*-OH derivative was always potentiated strongly by cocaine, whereas the *p*-OH derivative and the corresponding parent compound (no phenolic hydroxyl groups) were much less affected.

Similar findings were previously reported by Innes and Kosterlitz (77) and also by Holtz *et al.* (75), but these studies were made in non-pretreated spinal cats; *i.e.*, under experimental conditions which did not exclude the antagonistic effect of cocaine to the indirect actions (see below).

Thus it can be concluded that, on the nictitating membrane, the sensitizing action of cocaine is highly selective and seems to be especially pronounced for all sympathomimetic amines possessing a phenolic hydroxyl group in the *meta*-position. Other structural requirements may also be of importance, since the sixteen amines under study did not fall into two distinct groups. Finally, it must be emphasized that these observations were made on the nictitating membrane of the spinal cat, and it remains to be seen how far they are applicable to other organs and to other species.

Cocaine is well known to cause subsensitivity to the effect of indirectly acting (tyramine-like) sympathomimetic amines (57, 133). A study of the effect of cocaine on the dose-response curve of tyramine showed that this antagonism is of the "surmountable" type; *i e.*, increasing doses of cocaine cause a graded shift of the dose-response curve of tyramine to the right, but nearly maximal responses of the nictitating membrane, the blood pressure, and the cardiac pacemaker of the spinal cat can be obtained by appropriate increases in the dose of tyramine (144).

It follows that the dose-response curve of any given sympathomimetic amine is subject to two opposing effects of cocaine: (a) a possible shift to the left due to the sensitizing action of cocaine, and (b) a possible shift to the right due to the antagonism by cocaine of indirect actions. Since short-term pretreatment with reserpine almost completely eliminates the indirect effects of amines, factor (b) above is virtually absent when cocaine is administered to pretreated preparations. However, in normal (non-pretreated) preparations (b) should reduce the effect of (a), and cocaine should have a less pronounced sensitizing effect in normal spinal cats than in those pretreated with reserpine. This has been verified by experimental observation (151). In fact, it is possible to account fully for the various positions of the dose-response curves observed in normal and in reserpine-pretreated spinal cats before and after the administration of cocaine. All thirteen sympathomimetic amines under study fell into one of the following four patterns: (a) predominantly directly acting and strongly potentiated by cocaine (Fig. 5a), (b) predominantly directly acting and hardly potentiated by cocaine (Fig. 5b), (c) with considerable indirect action but potentiated by cocaine (Fig. 5c), and (d) with considerable indirect action but hardly potentiated by cocaine (Fig. 5d). The two thin arrows in Figure 5c and d indicate how, in the non-pretreated preparation, the antagonistic action of cocaine reduces its sensitizing action.

As already mentioned, the antagonistic action of cocaine seems to be uniformly against the indirect action of *all* sympathomimetic amines. It is possible to test the validity of this hypothesis, for if this is true, the increase in sensitizing effectiveness of cocaine after pretreatment with reserpine should be directly related to the contribution of the indirect to the overall (direct + indirect) effect of the amine; *i.e.*, the increase in sensitizing action of cocaine after pretreatment with reserpine should be proportional to the reserpine-induced shift of the dose-response curve to the right. For the thirteen amines under study this correlation is significant ($r = 0.803$; $P < 0.001$) (151).

Thus, the experimental observations are consistent with the view that cocaine, in addition to its highly selective sensitizing action, rather uniformly antagonizes

indirect actions of sympathomimetic amines whether they be "pure" (as with tyramine) or "mixed" with direct effects (as with ephedrine). The importance of this antagonistic effect for the overall effect of cocaine is proportional to the importance of the indirect component in the overall action of the amine. Finally, the complex shifts of dose-response curves observed in normal preparations can be fully explained when these factors are taken into consideration.

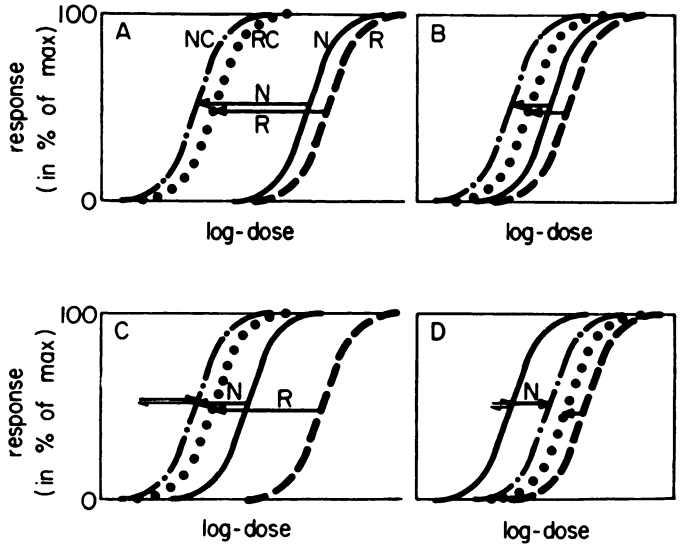


FIG. 5. Schematic representation of the position of dose-response curves of sympathomimetic amines in normal (N) and reserpine-pretreated preparations (R) before and after the administration of cocaine (NC and RC).

Shown are the four typical combinations: A, predominantly directly acting amine which is strongly potentiated by cocaine; B, predominantly directly acting amine which is hardly potentiated by cocaine; C, amine with mixed actions, the direct being strongly potentiated and the indirect being antagonized by cocaine; D, amine with mixed action, the direct being hardly potentiated and the indirect being antagonized by cocaine. The heavy arrows indicate the cocaine-induced shifts of dose-response curves as observed in normal and in pretreated preparations. The thin arrows in C and D indicate how the antagonistic action of cocaine (exerted against the indirect component of action) reduces the sensitizing effect (to the direct component of action) in normal non-pretreated preparations. N, ——; R, -----; NC, —·—·; RC, ·····. For details see text.

The fact that cocaine exerts two opposite effects must also be taken into account (a) when the effects of graded doses of cocaine are interpreted, and (b) when the effects of the administration of cocaine to two different preparations or systems are considered. Holtz *et al.* (75) have pointed out that the effect of small doses of cocaine may seem to be qualitatively different from that of large doses, the former resulting in supersensitivity, the latter in subsensitivity to a certain dose of tyramine. This has been confirmed for the nictitating membrane of the spinal cat (144), a structure known to be very strongly sensitized by cocaine;

therefore, the increased effectiveness of the released norepinephrine masks the fact that less norepinephrine is liberated by the tyramine after the administration of a small amount of cocaine. This explanation is supported by the observation that such a difference between the effect of a small dose and that of a large dose of cocaine is not found when the response of the cardiac pacemaker of the spinal cat to tyramine is studied (144). Because this structure is much less sensitized by cocaine than the nictitating membrane, even small doses of cocaine cause a distinct shift of the dose-response curve of tyramine to the right. These findings serve to illustrate how seemingly qualitative differences can be resolved into quantitative differences between different organs, between different doses, and between the two opposite effects which cocaine exerts.

B. Reserpine

As mentioned in the preceding section, short-term pretreatment of cats with a large dose of reserpine (3 mg/kg, 24 hours prior to the experiment) abolishes the indirect effects of sympathomimetic amines but does not cause supersensitivity of the nictitating membrane to norepinephrine (60). However, when a small amount of reserpine (0.1 mg/kg) is injected daily for 3, 7, and 14 days, supersensitivity of the nictitating membrane to norepinephrine is first observed after 7 days and becomes more pronounced after 14 days. Fleming (58) has recently extended this study to a pretreatment period of 28 days; he found no further increase in response to norepinephrine after 28 days. In Fleming and Trendelenburg's experiments the response of the nictitating membrane to nerve stimulation was used as an indicator of depletion of the norepinephrine stores by the pretreatment; the results suggested strongly that the depletion achieved by this schedule (low dose, long-term) was similar to that produced by the short-term pretreatment with a single large dose of reserpine (60); this was later confirmed by fluorimetric determination of the norepinephrine content of the smooth muscle of the nictitating membrane (152).

These observations indicate that pretreatment with reserpine is able to cause supersensitivity to norepinephrine, but that time is essential for the development of this supersensitivity. The importance of the time factor in the development of supersensitivity after the depletion of the norepinephrine stores by reserpine remained unrecognized for a long time, and as a consequence insufficient attention has been paid to the importance of the schedule of pretreatment. In all studies of this kind, a clear distinction should be made between short-term pretreatment, which causes depletion of the norepinephrine stores with no accompanying supersensitivity, and long-term pretreatment which results in both depletion and supersensitivity. Furthermore, it must be realized that the time factor may vary from organ to organ, since supersensitivity of the cardiovascular system develops within three days of long-term pretreatment but supersensitivity of the nictitating membrane only after 7 days (60). It is tempting to speculate that the time required for the development of supersensitivity to norepinephrine is inversely related to the number of tonic impulses sent from the central nervous system to the effector organ; it may be assumed that in the intact animal the

blood vessels and cardiac tissues receive a much larger number of impulses per unit of time than does the nictitating membrane. Whatever the reason for this difference in the magnitude of the time factor, the experimental evidence for the importance of the time factor is well documented.

It can be argued that the development of supersensitivity is delayed during long-term pretreatment with reserpine because depletion of the norepinephrine stores of the nictitating membrane is achieved only slowly with the small amount of reserpine (0.1 mg/kg per day). However, this cannot be the reason, since supersensitivity also developed within 14 days when an even smaller dose of reserpine was used (0.03 mg/kg per day); with this low dose the magnitude of the response of the nictitating membrane to nerve stimulation clearly indicated that the depletion of the norepinephrine stores was far from complete (60). This in turn indicates that even partial depletion of the norepinephrine stores may be associated with supersensitivity, provided there is enough time for its development.

These findings do not agree with certain previous reports. Schmitt and Schmitt (122), for example, reported supersensitivity of the nictitating membrane to norepinephrine 22 to 24 hours after pretreatment with a single dose of reserpine. However, these authors used anesthetized cats which were kept under anesthesia for the whole duration of the experiment (more than 24 hours); each cat then received sequential injections of a variety of sympathomimetic amines. The limitations of this experimental design have been pointed out in Section I. Burn and Rand (28) reported supersensitivity to norepinephrine after two days of pretreatment with 2.5 to 5 mg of reserpine per kg per day; however, their Figure 1 demonstrates only an increased response of the blood pressure to norepinephrine and not an increased response of the nictitating membrane of the spinal cat. In view of the variability of the responses from preparation to preparation, only a quantitative study of dose-response curves can decisively contribute to this problem. A quantitative study of this kind was reported by Burn *et al.* (26), who found that the isolated preparation of the nictitating membrane *in vitro* was not supersensitive to norepinephrine when the smooth muscle was taken from reserpine-pretreated animals; on the other hand, denervated preparations clearly demonstrated the phenomenon of supersensitivity. Thus there is good evidence for the view that depletion as such does not cause immediate supersensitivity to norepinephrine; as already mentioned, these observations on the nictitating membrane of the spinal cat are supported by studies performed on other preparations: cat iris (97); heart-lung preparation of the dog (84); isolated guinea pig atria (44).

It has been suggested that reserpine is able to block the norepinephrine receptors, since the exposure of isolated normal rabbit atria to a concentration of 4×10^{-6} reserpine causes a reduction of the rate-increasing effect of norepinephrine and nicotine (11). If such a blockade of receptors occurs also in other preparations, the presence of small amounts of reserpine (after short-term pretreatment with a large dose) could mask the supersensitivity to norepinephrine. However, this observation does not prove that reserpine is able to block norepinephrine receptors. Krayer and Fuentes (85) observed that a similar concentration of reserpine (5×10^{-6}) has a veratramine-like action in the heart-lung preparation of the

dog, and Hawkins (70) demonstrated that veratramine does not affect the norepinephrine receptors of the cardiac pacemaker. Hence, it seems likely that the antagonistic effect of reserpine observed in rabbit atria is a phenomenon restricted to the pacemaker of the heart and does not involve norepinephrine receptors; this conclusion is supported by the fact that such an antagonism of reserpine to norepinephrine has not been observed in any other organ.

Until now the discussion has dealt with the problem of supersensitivity after *pretreatment* with reserpine. Quite different results have been reported after acute injections of reserpine into spinal cats. A short time after such an injection, the response to norepinephrine is enhanced and remains so for a few hours (76, 109, 122). The time course of the development of this potentiation is similar to the time course of the elevation of the plasma level of catecholamines after intravenous injections of reserpine (86, 107). It seems likely that this phenomenon is again due to an addition of the effects of endogenous norepinephrine (increased in the proximity of the receptors because of the releasing action of reserpine) to the action of injected sympathomimetic amines. This view is supported by the finding that on the nictitating membrane during the first few hours after the intravenous injection of reserpine, tyramine is not only effective but actually more effective than normally. Hence, this type of supersensitivity is observed some time *before* the norepinephrine stores are depleted.

Alternative possible explanations are 1) that the reserpine-induced release of norepinephrine has saturated nonspecific receptor sites (as defined by Koelle, 82a) and that consequently a larger proportion of the injected amines reaches their respective sites of action, and 2) that the increased response to tyramine is due to the summation of the releasing action of reserpine and that of tyramine; however, this second alternative does not account for the reported increase in response to norepinephrine.

The results obtained by Fleming and Trendelenburg (60) indicate that the supersensitivity to norepinephrine observed after long-term pretreatment with reserpine closely resembles the type of supersensitivity observed after chronic decentralization. This is discussed in the following section.

C. Decentralization

Chronic preganglionic denervation (= decentralization) is well known to cause supersensitivity of the nictitating membrane to various substances. The supersensitivity after decentralization is less pronounced than that observed after chronic postganglionic denervation (= denervation) in accordance with Cannon's law of denervation which states: "When in a series of efferent neurones a unit is destroyed, an increased irritability to chemical agents develops in the isolated structure or structures, the effect being maximal in the part denervated" (37). The question arises whether this difference between denervation and decentralization is of a qualitative or a quantitative nature.

Innes and Kosterlitz (77) postulated that it is a qualitative difference since decentralization, although it sensitizes the nictitating membrane to both epinephrine and norepinephrine, fails to affect the normal difference in potency between

these two amines; denervation (and the administration of cocaine), on the other hand, lead to a type of supersensitivity which is characterized by the finding that the normally less potent norepinephrine becomes as potent as epinephrine. However, one could argue that there may be a maximum of sensitivity; *i.e.*, a limit to which a dose-response curve can be shifted to the left. If this maximal sensitivity of the nictitating membrane is reached after denervation and after the administration of cocaine, then the normal difference in potency between epinephrine and norepinephrine is abolished and both substances are equipotent. This would then represent a quantitative rather than a qualitative difference between denervation and decentralization.

Supersensitivity after decentralization develops slowly and reaches its maximum about two weeks after the section of the preganglionic nerve (69); this characteristic time course is similar to that for the development of supersensitivity after long-term pretreatment with reserpine. Slow development of supersensitivity has also been observed by Emmelin and his group in their studies of the supersensitivity of the salivary glands of the cat (50). To obtain more information about this time factor, the following series of experiments was carried out (152). Dose-response curves were determined on the nictitating membrane of the spinal cat for three test substances (norepinephrine, tyramine, and acetylcholine) in normal preparations as well as after various pretreatment schedules designed to interrupt the normal pathway between the higher centers and the nictitating membrane. These pretreatments were: (a) 7 days of denervation; (b) 7 days of decentralization; (c) 7 days of ganglion block (produced by two daily injections of chlorisondamine); (d) 7 days of prevention of the release of norepinephrine from its peripheral stores (produced by daily injections of TM 10); (e) 7 days of depletion of the norepinephrine stores (produced by daily injections of a small amount, 0.1 mg/kg, of reserpine); (f) 7 days of blockade of the norepinephrine receptors (produced by daily injections of phenoxybenzamine). After some of these prolonged pretreatments the catecholamine content of the nictitating membrane was determined fluorimetrically. Furthermore, the effect of a single acute injection of chlorisondamine, TM 10 or phenoxybenzamine was also determined. The results of this study are summarized in Table 2. Short-term pretreatment with chlorisondamine, TM 10, or reserpine (agents c, d, and e, Table 2) had no effect on the sensitivity of the nictitating membrane to norepinephrine and acetylcholine, but long-term pretreatment with these drugs caused uniformly a moderate supersensitivity to both substances similar to that observed after decentralization. Denervation, on the other hand, caused the well-known pronounced supersensitivity to norepinephrine, but its sensitizing effect in regard to acetylcholine was not much stronger than that of decentralization. These differences are indicated in the table by the number of symbols. It is thus evident that there is a correlation between the development of supersensitivity and the time factor. No correlation was found between the development of supersensitivity to norepinephrine and the norepinephrine content of the nictitating membrane; however, the response to tyramine clearly depends on the presence of these stores. Comparison of the relevant columns illustrates that the response of the nictitating

TABLE 2

Changes in sensitivity of nictitating membrane after various agents and procedures

Agent or Procedure	Schedule	Sensitivity to			NE Content	Sensitivity to			
		NE	Tyr	ACh		*m*-Tyr	Eph	Syn	Phen.
a) Denervation	7 days	+++	−	+	−−−(1)	+(2)	−(2)	0(2)	+++(2)
b) Decentralization	7 days	+	+	+	0(1)	+(2)	+(2)	+(2)	+(2)
c_1 Chlorisondamine	30 min, ½ day	0	0	0					
c_2	7 days	+	+	+	0				
d_1 TM 10	30 min	0	0(3)	0(4)					
d_2	7 days	+	+	+	−				
e_1	3 mg/kg, 1 day	0(5)	−−−(3)	0(4)	−−−(6)	−(7)	−(7)	0(7)	0(7)
e_2 Reserpine	0.1 mg/kg, 7 days	+	−−	+	−−−				
e_3	0.03 mg/kg, 14 days	+(5)			−−(5)				
f_1 Phenoxybenzamine	30 min	−−−(4)	−−−(4)	−(4)					
f_2	7 days	−−−	−−−	0					
g Decentralization + 3 mg/kg Reserpine	7 days								
	24 hr				−−−(1)	+(2)	+(2)		
h 5 mg/kg Cocaine	30 min	++(5)	−(3)	0(4)	0(8)	0(2)	0(2)	0(2)	++(2)

If not otherwise stated, the results are taken from Trendelenburg and Weiner (152). (1) from (82), (2) from (151), (3) from (144), (4) from (145), (5) from (60), (6) from (60, 82, 152), (7) from (150), (8) from (15).

+ Increase in sensitivity; − decrease in sensitivity (or NE content of nict. membr.); 0 no change in sensitivity (or NE content of nict. membr.)

NE = norepinephrine; Tyr = tyramine; ACh = acetylcholine; Eph = ephedrine; Syn = synephrine; Phen. = phenylephrine.

membrane to tyramine is determined by at least two factors: (a) the norepinephrine stores and (b) the sensitivity of the smooth muscle to norepinephrine. This supports the view that tyramine acts through the liberation of norepinephrine from the stores.

The experiments with phenoxybenzamine were undertaken in the hope that prolonged block of the norepinephrine receptors would cause a supersensitivity similar to that observed after decentralization, *i.e.*, a supersensitivity to both sympathomimetic amines and acetylcholine. Unfortunately, phenoxybenzamine is an antagonist not only of norepinephrine but also of acetylcholine (though a weak one), so that after 7 days of pretreatment with phenoxybenzamine supersensitivity was not demonstrable with either norepinephrine or acetylcholine. However, Nickerson and House (111) have provided some evidence for the view that prolonged receptor block also causes supersensitivity of the nictitating membrane to norepinephrine.

It must be emphasized that all experiments presented in Table 2 (except some norepinephrine determinations) were performed under identical conditions although they have been taken from different publications. Furthermore, the sensitizing action of prolonged administration of chlorisondamine has been demonstrated previously by Emmelin (49), who observed the development of a moderate supersensitivity to norepinephrine and epinephrine which reached its maximum in about three weeks; this is in agreement with the reviewer's findings.

From the experiments presented in Table 2, it is concluded that decentralization causes a characteristic type of supersensitivity which is (a) of moderate degree, (b) not accompanied by a loss of the norepinephrine stores of the nictitating membrane, (c) nonspecific in that it is as prominent with acetylcholine as it is with norepinephrine, and (d) not associated with subsensitivity to any of the sympathomimetic amines. The most important factor responsible for the development of this type of supersensitivity seems to be a prolonged functional interruption of the pathway between the central nervous system and the nictitating membrane. Procedures or pharmacological agents which produce this effect also produce this type of supersensitivity. This is in agreement with the extensive studies of Emmelin and co-workers (50) who reached rather similar conclusions on the basis of experiments on the salivary glands of the cat. Emmelin proposed the term "pharmacological denervation" in connection with this type of supersensitivity, although Fleckenstein preempted the same term for the quite different type of supersensitivity observed after the administration of cocaine (55). Since the type of supersensitivity discussed in this section seems to be entirely different from that produced by the administration of cocaine the reviewer proposes in connection with it the term "pharmacological decentralization."

The nonspecificity of the decentralization-type of supersensitivity has been demonstrated by additional experiments with sympathomimetic amines other than epinephrine and norepinephrine. The nonspecificity is striking, since supersensitivity after decentralization is of similar magnitude for directly and for indirectly acting amines, and it is also of similar magnitude for *m*-OH compounds

and their *p*-OH analogues (*e.g.*, phenylephrine and synephrine; Table 2). In this respect the supersensitivity due to decentralization differs strikingly from that produced by the administration of cocaine (151).

The lack of importance of the norepinephrine stores in this type of supersensitivity is also illustrated by two series of experiments with ephedrine and *m*-tyramine. Both substances have pronounced indirect actions in addition to their direct effects, so that short-term pretreatment with reserpine markedly shifts their dose-response curves to the right. When this short-term pretreatment with a large dose of reserpine is carried out 6 days after decentralization of one nictitating membrane (the other serving as a control), then both nictitating membranes lose most of their normal norepinephrine content, since decentralization does not interfere with the depleting action of reserpine (82). On the other hand, since the horizontal distance between the dose-response curve of the unoperated and the decentralized side of such pretreated animals is of the same magnitude as in non-pretreated animals, depletion of the stores does not affect the sensitizing action of decentralization (Table 2) (151). Hence, the depletion of the norepinephrine stores does not reduce the supersensitivity which developed during 7 days following decentralization.

These additional observations all support the view that decentralization causes a moderate and nonspecific supersensitivity which is not related to the norepinephrine content of the sensitized tissue.

D. Denervation

The similarity of the sensitizing effect on the nictitating membrane of chronic postganglionic denervation (= denervation) and of the administration of cocaine is very pronounced and has been observed by numerous workers. However, this supersensitivity develops rather slowly after denervation and reaches its maximum after about two weeks (69), whereas the sensitizing effect of cocaine is observed within a few minutes after the intravenous injection of this substance (143a). The close similarity is convincingly demonstrated on the nictitating membrane by a quantitative study of the influence of denervation (56) and of cocaine (55, 57) on the dose-response curves of a large number of sympathomimetic amines. Fleckenstein and Bass (55) concluded from this study that the administration of cocaine causes a "pharmacological denervation." Moreover, the correlation between the effects of cocaine and of denervation is not restricted to sensitizing effects alone but extends also to their desensitizing effects towards amines with indirect actions (57). Therefore it is very tempting to speculate that the two procedures cause certain effects which result in the same type of super- and subsensitivity.

A recent study of dose-response curves of 16 sympathomimetic amines supports the view that there is a high correlation between the changes in sensitivity due to the administration of cocaine and those due to previous denervation of the nictitating membrane (151). All that has been said about the action of cocaine relative to the *m*-OH and *p*-OH group of an amine (see above) is also true for the sensitization of the nictitating membrane caused by denervation.

However, in addition to these similarities, there are certain differences between the effects of cocaine and those of denervation, and these differences appear to be of more importance than hitherto realized.

It has already been mentioned that the administration of cocaine fails to cause supersensitivity of the nictitating membrane to acetylcholine, whereas denervation does cause supersensitivity to this substance (145). Furthermore, the dose-response curves of all of thirteen sympathomimetic amines are shifted more to the left by denervation than by cocaine (151). It could be argued that this is simply a quantitative difference which would be reduced by the use of a larger dose of cocaine. However, this cannot be true, as can be shown in the case of ephedrine. Short-term pretreatment with reserpine shifts the dose-response curve of this substance to the right; this indicates that ephedrine has an indirect effect (which is nearly completely eliminated by pretreatment with reserpine) in addition to its direct action (which is unmasked by the pretreatment). Cocaine fails completely to cause any potentiation of this direct action (*i.e.*, when administered to a reserpine-pretreated preparation). When cocaine is administered to a non-pretreated preparation, the dose-response curve of ephedrine is shifted to the right since cocaine antagonizes the indirect actions of *all* amines (see above). In other words, the only demonstrable effect of cocaine on the action of ephedrine is antagonism. An increase in the dose of cocaine thus would not cause a shift of the dose-response curve of ephedrine to the left, but rather a further shift to the right. Hence, the difference between the effects of cocaine and of denervation must be more than a purely quantitative one.

Considering that denervation (like decentralization) causes a prolonged interruption of the pathway from the central nervous system to the nictitating membrane, it is a reasonable concept that the decentralization-type of supersensitivity is an inherent part of the supersensitivity caused by denervation. Therefore, it was suggested that the sensitizing effect of denervation may be visualized as the sum of the nonspecific and moderate supersensitivity caused by the prolonged interruption of the central pathway and the highly selective supersensitivity which is qualitatively and quantitatively very similar to that observed after the administration of cocaine (151). The reasons for proposing this hypothesis are twofold. One is apparent from the graph presented in Figure 6. Plotted (on log-scales) are the shifts of the dose-response curves of thirteen sympathomimetic amines as observed after denervation of the nictitating membrane (ordinates) against the shifts due to the administration of cocaine (abscissae); the value 1 indicates "no change in sensitivity," values smaller than 1 indicate "increase in sensitivity," values greater than 1, "decreased sensitivity." The resulting regression line has two important features: (a) it has an angle of nearly 45° (regression coefficient b = 1.095), and (b) it does not pass through the crossing point of the two broken lines, the point of "no change" for both denervation and cocaine. The slope of the regression line is consistent with the view that the supersensitivity after denervation is qualitatively and quantitatively very similar to that observed after the administration of cocaine, as indicated by the regression coefficient of nearly unity. However, if there were

no additional difference between the two types of supersensitivity, the regression line should follow the course of the dotted diagonal line. The parallel displacement of the observed regression line is consistent with the view that the supersensitivity caused by denervation also comprises a moderate and nonspecific

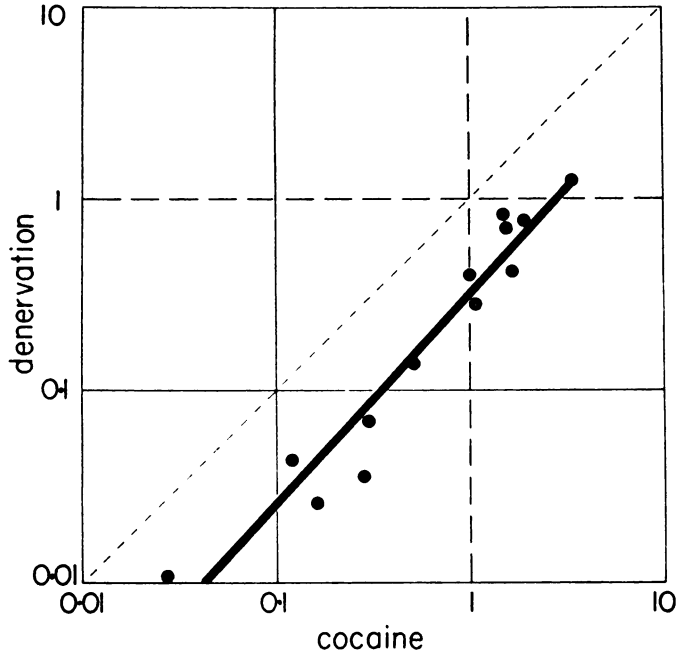


FIG. 6. Relation of the effects of cocaine to those of denervation.

Nictitating membrane, spinal cat. Abscissae: shift of dose-response curves of 13 sympathomimetic amines produced by the administration of 5 mg/kg cocaine. Ordinates: shift of dose-response curves produced by chronic postganglionic denervation (7 days). The shift of the curves is expressed as the ratio "ED50 after cocaine (or denervation)"/"ED50 in normal preparations"; the shifts are plotted on log-scales. Values above unity indicate shift to the right (= subsensitivity), values below unity indicate shift to the left (= supersensitivity). Broken lines indicate "no change in sensitivity." Solid line: regression line calculated from results. Dotted diagonal line: theoretical line on which the points should fall if the effects of cocaine and of denervation were quantitatively and qualitatively similar. For details see text.

type of supersensitivity similar to that produced by decentralization. The second reason for proposing this hypothesis is that both denervation and decentralization cause supersensitivity to acetylcholine, while cocaine does not (145). Here again, the earlier formulation "cocaine equals pharmacological denervation" does not agree with the experimental observations, whereas the new hypothesis does.

It has been mentioned that the resemblance of the effects of denervation to those of cocaine extends to the desensitizing action of denervation and cocaine. However, this resemblance is rather superficial, since cocaine reduces the effects

of tyramine by a "surmountable" antagonism, whereas the desensitizing effect of denervation on the action of tyramine is of the "unsurmountable" type; *i.e.*, the effect of denervation is especially pronounced for high doses of tyramine (144). This difference is explained by the fact that cocaine does not change the norepinephrine content of an organ (15) and probably antagonizes the effect of tyramine by some competitive mechanism (105), whereas denervation prevents tyramine from exerting its sympathomimetic effects by depleting the norepinephrine stores (29, 53, 67, 82).

Hence, the full descriptive equation should be stated as follows: The effect of chronic denervation is equal to the sum of the sensitization caused by decentralization, plus the highly selective supersensitivity similar to that produced by cocaine, plus the effect of depletion of the norepinephrine stores. The concept of Fleckenstein and Bass (55) that cocaine causes a "pharmacological denervation" should be considerably modified or abandoned. This consideration applies also to Emmelin's use of the term "pharmacological denervation" for something which seems to be more related to the decentralization-type of supersensitivity (50). It is the reviewer's contention that such simplified descriptions impede the attempts to unravel the problem of denervation supersensitivity; any theory which attempts to explain these phenomena must take into account the fact that the denervation-type of supersensitivity is a complex phenomenon.

E. Summary

In order to facilitate the understanding of the subsequent part of this review, which deals with mechanisms of action, a short summary of the most important findings is given here.

1) Sympathomimetic amines have direct or indirect effects, or both. These amines do not fall into three distinct groups but are distributed between the two extremes of "pure direct" and "pure indirect" action. It is suggested that these extremes exist only in theory and that every sympathomimetic amine has both direct and indirect actions.

2) The effects of denervation seem to be of composite nature. They combine the effects of decentralization, the effects of a supersensitivity very similar to that observed after the administration of cocaine, and the effects of depletion of the norepinephrine stores.

3) Supersensitivity after decentralization (a) is of moderate degree, (b) is nonspecific (*i.e.*, about equal for directly and indirectly acting amines, for *meta*- and *para*-OH derivatives, and for chemically unrelated compounds like acetylcholine), (c) is independent of the presence or absence of the norepinephrine stores, (d) is unaccompanied by subsensitivity to indirectly acting amines, and (e) requires time for its development. It can be produced by any procedure or agent which causes a prolonged interruption of the pathway from the central nervous system to the effector organ.

4) Supersensitivity after cocaine is characterized by its high selectivity for certain amines, especially those containing a *m*-OH group. In addition, cocaine antagonizes the indirect actions of *all* sympathomimetic amines; this antagonism seems to be of the "surmountable" type.

5) Depletion of the norepinephrine stores antagonizes the indirect actions of *all* sympathomimetic amines; this antagonism is of the "unsurmountable" type.

6) The effect of pretreatment with reserpine depends on the schedule employed for the pretreatment. Short-term pretreatment (24 hours) causes depletion of the norepinephrine stores with no concomitant supersensitivity to norepinephrine. Prolonged pretreatment (several days) results in depletion of the stores and in supersensitivity; the latter seems to be of the decentralization-type.

7) Supersensitivity to norepinephrine and subsensitivity to tyramine are *not* invariably linked. The former without the latter is observed after decentralization; the latter without the former is observed after short-term pretreatment with reserpine.

8) Short-term pretreatment with reserpine interferes neither with the sensitizing action of cocaine nor with the manifestation of supersensitivity after chronic decentralization.

V. MODE OF ACTION OF INDIRECTLY ACTING SYMPATHOMIMETIC AMINES

Current opinion holds that tyramine and similar amines exert their sympathomimetic effects through the release of norepinephrine from some storage site in the effector organ. Direct stimulation of the adrenal medulla by tyramine does not seem to be of great importance, since most workers have failed to find an increase in the plasma level of catecholamines in the adrenal vein blood after injections of tyramine (126, 129, 157). Yet, in the isolated perfused adrenal gland of cattle, the addition of tyramine or phenylethylamine to the perfusion fluid considerably increased the "spontaneous" release of catecholamines into the effluent (68).

The evidence suggests that in the nictitating membrane tyramine acts predominantly through the release of norepinephrine stored within the nerve terminals of postganglionic adrenergic fibers. Such a statement seems to be contradicted by the fact that the response to *small* doses of tyramine is increased after denervation and after the administration of cocaine and not decreased as one would expect. However, the response of an organ to tyramine is determined not only by the amount of norepinephrine available for release (or by the accessibility of the store) but also by the sensitivity of the organ to the liberated norepinephrine. The nictitating membrane differs from many other effectors in that denervation and the administration of cocaine increase its sensitivity to norepinephrine by factors of 100 and 50, respectively; in the spinal cat, cocaine causes much less potentiation of the pressor response and of the response of the cardiac pacemaker to norepinephrine (60). Hence, the response of the denervated nictitating membrane to tyramine would be about normal if tyramine released only 1% of the norepinephrine released normally; in other words, a 99% depletion of the norepinephrine stores by denervation would be masked by the very pronounced sensitization. There is no doubt that denervation of various organs causes a considerable loss of norepinephrine from the tissues (29, 38, 53, 67, 82, 128), but none of the published studies rules out the possibility that the

small amount of norepinephrine required for an increased response of the highly supersensitive smooth muscle to tyramine persists after denervation.

Histological evidence indicates that ganglionectomy fails to produce complete denervation of the nictitating membrane (89a; see also Section IIA). Moreover, Cooper *et al.* (42) had to perform a very elaborate operation to denervate fully the heart of the dog. After complete denervation no norepinephrine was de-

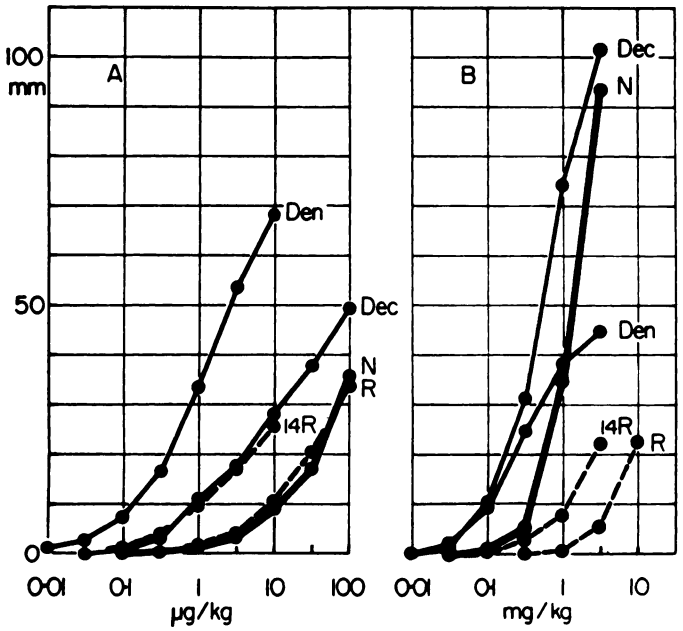


FIG. 7. Effect of various procedures on the dose-response curves of norepinephrine (A) and tyramine (B) on the nictitating membrane of the spinal cat.

Shown are the mean responses of the nictitating membrane (in mm on drum). N = normal preparations; R = 24 hours after pretreatment with 0.1 mg/kg reserpine i.p.; 14R = after 14 days of pretreatment with reserpine (0.1 mg/kg per day i.p.); DEN = 7 to 14 days after denervation; DEC = 7 to 14 days after decentralization. Values taken from (60, 144, 152). For explanation see text.

tectable and the heart failed to respond to tyramine, whereas the usual denervation by ganglionectomy only reduced the norepinephrine content of the heart. Such an elaborate operation is not possible with the nictitating membrane. The few nerve fibers which escape conventional denervation may sprout into the denervated parts of the organ; the process of sprouting has been shown to be very effective (104). Pharmacological evidence also indicates that conventional denervation of the nictitating membrane is not always complete, since some isolated preparations prepared 11 to 15 days after ganglionectomy still showed small responses to electrical stimulation and to nicotine (26).

Figure 7 shows dose-response curves for norepinephrine (7a) as an indicator of the sensitivity of the nictitating membrane, and for tyramine (7b). The position

of the various curves obtained with tyramine is consistent with the postulate that the response to this substance depends on the amount of norepinephrine available for release and the sensitivity to norepinephrine. Depletion of the stores without a change in sensitivity to norepinephrine (as produced by short-term pretreatment with reserpine = R) shifts the dose-response curve for tyramine far to the right. Depletion of the stores with moderate supersensitivity to norepinephrine (after prolonged pretreatment with reserpine = 14R) causes a smaller shift of the curve to the right. Depletion of the store together with pronounced supersensitivity to norepinephrine (as seen after denervation = DEN) brings part of the dose-response curve for tyramine to the left of the control curve, but the horizontal distance between the curves "DEN" and "R" (in their lower parts) is about equal for both tyramine (Fig. 7b) and norepinephrine (Fig. 7a). Finally, moderate supersensitivity to norepinephrine with no change in the norepinephrine stores (as seen after decentralization = DEC) causes a parallel shift of the tyramine curve to the left. This figure also demonstrates that depletion of the norepinephrine stores reduces the maximal response to tyramine, whereas the maximal response remains unimpaired after decentralization (which does not deplete the stores). Thus, a comparison of complete dose-response curves provides a full explanation for a problem which has aroused lively discussions (2).

Decisive evidence for the view that tyramine liberates norepinephrine from its peripheral stores—the demonstration of norepinephrine in the venous outflow of an organ *in vivo* or in the effluent of an isolated and perfused organ—has been achieved by various workers (41, 68, 94, 126, 131). Furthermore, Burn and Burn (23) have shown that the slow loss of radioactivity from isolated atria which had been exposed to labeled norepinephrine was increased when tyramine was added to the bath. It is clear that tyramine is able to release some norepinephrine, but the amounts detected are very small indeed. Two groups of workers measured the release of norepinephrine by doses of tyramine and of a nicotine-like agent which were equipressor (dog blood pressure, 157) or equiaccelerator (rabbit heart, 94); both found that the amount of norepinephrine escaping into the circulation is much greater after nicotine-like agents than after tyramine. The reason for this discrepancy is obscure. Brown and Gillespie (21) found that little norepinephrine escapes into the circulation when the rate of its liberation is low, whereas more norepinephrine escapes when the rate of liberation is high. Differences in the rate of release of the transmitter by tyramine and by nicotine-like agents may be the reason for the differences in the amounts of norepinephrine detected. Lindmar and Muscholl (94), on the other hand, postulated that tyramine not only is an indirectly acting amine, but also enhances the effect of norepinephrine at the receptor.[2] Weiner *et al.* (157) postulated that nicotine and

[2] Such a sensitizing action of tyramine has recently been demonstrated on isolated atria obtained from guinea pigs pretreated with reserpine. Although tyramine had no stimulant effect on their spontaneous rate, the sensitivity to norepinephrine increased 10-fold when this agent was tested in the presence of 10^{-5} g/ml of tyramine (Crout, personal communication).

tyramine liberate norepinephrine from different compartments of the norepinephrine store and that differences in the amount escaping into the circulation can be explained in this way. Clearly, a satisfactory answer to this problem must be found before the mode of action of tyramine can be regarded as being established.

Further evidence for the view that tyramine acts through the release of norepinephrine is provided by three reports that the injection or infusion of large amounts of tyramine is able to cause partial depletion of the norepinephrine stores (41, 128, 157). Finally, Schümann and Weigmann (124) showed that tyramine increases the spontaneous loss of norepinephrine from isolated heavy granules of adrenal medulla and of splenic nerve; this was confirmed by von Euler and Lishajko (52). Schümann and Philippu (123) then found that the tyramine-induced loss of norepinephrine was not accompanied by a loss of ATP and that tyramine was taken up into the heavy granules; in other words, tyramine seems to displace norepinephrine from its intragranular binding sites. Suggestive as these experiments are, it must be pointed out that isolated storage granules are only a "model," and that, as mentioned above, tyramine exerts actions on adrenal medullary granules which it normally fails to exert on the intact adrenal gland *in vivo*.

A completely different line of evidence for the indirect action of tyramine is that of the "refilling" of previously depleted norepinephrine stores. The first observation of this kind was made by Burn in 1932 (24). The preparation of an isolated perfused hind-limb of a dog made it necessary to interrupt its circulation for a period of 30 to 50 minutes. Although the perfused limb afterwards responded well to epinephrine, it failed to respond to tyramine. The response to tyramine was restored by an infusion of epinephrine. It was concluded that the interruption of the circulation depleted the stores of the sympathetic transmitter and thus prevented the effect of tyramine, and that the stores could be replenished by an infusion of epinephrine.

Similar observations have been reported by many workers after depletion of the peripheral norepinephrine stores by pretreatment with reserpine; the response to tyramine can then be restored by the administration of norepinephrine, epinephrine, or one of the norepinephrine precursors (15, 28, 30, 44, 102, 105). These observations present a puzzling problem: Although there is now general agreement that normal tissues are able to take up norepinephrine and to store it for a considerable time (23, 74, 105, 147, 159), it has been shown repeatedly that pretreatment with reserpine very greatly reduces the ability of the tissue to take up norepinephrine (6, 8, 45). Muscholl (105), for example, found that the norepinephrine content of the heart of a spinal rat was doubled by an infusion of norepinephrine; in identical experiments on reserpine-pretreated spinal rats, he observed restoration of the response to tyramine by an infusion of norepinephrine but no concomitant increase in cardiac content of norepinephrine. Pennefather and Rand (113), on the other hand, reported some increase in the norepinephrine content of organs previously depleted by reserpine when assays were performed after the infusion of norepinephrine, dopamine, or dopa.

Neither positive nor negative findings of this kind can settle the question, as long as we do not know how much norepinephrine has to be in the tissue to enable tyramine to exert its sympathomimetic effects. This information can be obtained only by a quantitative comparison of the norepinephrine content of an organ and its responsiveness to tyramine. In such a study, pretreatment of guinea pigs with increasing doses of reserpine (24 hours prior to the experiment) caused increasing depletion of the cardiac norepinephrine stores and also an increasing depression of the action of tyramine on rate of isolated atria (44). The relation was such that the response of the atria to tyramine was only slightly impaired when their norepinephrine content had fallen to about 50%, and tyramine had 50% of its normal effect when the norepinephrine content was only 10% of normal. This short-term pretreatment with reserpine did not change the sensitivity of the atria to norepinephrine. After heavy pretreatment with reserpine (5 mg/kg) the norepinephrine content was down to 1% of normal and the response to tyramine was abolished. However, when such atria were exposed for 10 minutes to 3×10^{-6} norepinephrine and then repeatedly washed for 45 minutes in order to eliminate the norepinephrine from the bath and from the extracellular space, they regained 70% of their normal responsiveness to tyramine. If the norepinephrine stores consisted of just one compartment, then—according to the previously determined relationship—the norepinephrine content after "refilling" should have been about 15% of normal. Although there was a significant increase in the tissue level of norepinephrine, the observed approximately 2% of normal is incompatible with the view that the whole store is "refilled" uniformly. The results are consistent with the view that the refilling is restricted to a small compartment of "available" norepinephrine (compartment A, (144)) and that it is this small fraction of the total store on which tyramine exerts its action. The results indicate also that reserpine causes depletion of the stores mainly by an action on the large compartment B ("bound" norepinephrine).

Since refilling of the depleted stores with norepinephrine has been reported to restore the response of various biological structures to nerve stimulation (28, 30, 66), and since the normal spleen takes up labeled norepinephrine, which is released again on stimulation of the splenic nerve (71), it seems possible that compartment A is also the compartment on which nerve impulses exert their action; however, more quantitative work must be done before this can be accepted as established. Recent experiments by Hertting *et al.* (74) and by Kopin and Gordon (82b) support the concept that the tissue store of norepinephrine consists of more than one functional compartment; after previous loading of the rat heart with labeled norepinephrine, the radioactive amine seemed to leak out from (a) extracellular space, (b) intracellular water, (c) a binding site of high turn-over rate (possibly = compartment A), and (d) a binding site of low turn-over rate (possibly = compartment B). An analysis of the labeled metabolites showed that the norepinephrine coming from (c) is metabolized mainly by catechol-O-methyl transferase, whereas that coming from (d) is metabolized mainly by monoamine oxidase. Furthermore, norepinephrine re-

leased by tyramine seems to be metabolized mainly by catechol-O-methyl transferase, whereas norepinephrine liberated by reserpine seems to be metabolized chiefly by monoamine oxidase.

The concept of different compartments of the norepinephrine stores is of importance, since it indicates that the determination of the changes in total norepinephrine content of an organ may be uninterpretable unless coupled with information as to which compartment is involved. Preliminary studies with guanethidine-pretreated guinea pig atria indicate that guanethidine depletes the norepinephrine stores mainly by an action on compartment A (and not, like reserpine, on compartment B); consequently the relation between the total norepinephrine content of the atria and their response to tyramine differs fundamentally from the relation established with reserpine-pretreated preparations (108).

Thus, experiments of different design and by different groups of workers provide evidence for the view that the "norepinephrine store" cannot be regarded as a simple entity. It must be pointed out that much more work is required before we have adequate knowledge of the physiological significance of the various compartments, their relative sizes, their location and their pharmacological importance. At the present time little can be said about the intracellular location of such compartments. The "available" and the "bound" norepinephrine may be in storage granules which contain different types of binding sites for the transmitter; or they may be in chemically similar granules, compartment A being equivalent to the granules close to the cell membrane and compartment B being equivalent to the granules close to the Golgi apparatus; or compartment A may be extragranular and B intragranular.

Differences in the size of the compartments may explain why Nasmyth (110) found that during the course of an experiment, perfused isolated hearts of guinea pigs gradually lose their ability to respond to tyramine without showing an accompanying fall in norepinephrine content; under such rather unphysiological conditions, it may well be that the small compartment A loses its norepinephrine whereas the large compartment B remains intact.

Although some of the experimental observations can be explained by the postulated differences in size of the compartments, others cannot. Kuschinsky *et al.* (88), for example, observed on isolated atria, papillary muscle, and ventricular strips of the rat heart that the response to tyramine can be restored by a concentration of norepinephrine which seems too low to refill compartment A. This phenomenon is distinctly different from the restoration of the response to tyramine after the exposure of isolated guinea pig atria to 3×10^{-6} norepinephrine with subsequent removal of the norepinephrine from the bath (44). In the experiments of Kuschinsky *et al.* (88) the concentration of norepinephrine in the bath was 10^{-9} (a subthreshold concentration); tyramine was effective in the presence but not in the absence of this small concentration of norepinephrine. The authors concluded that tyramine has a direct action which depends on the presence of small amounts of norepinephrine at the receptor. An alternative explanation could be made on the basis of Vane's hypothesis of the so-called

"silent receptors." Vane (153) obtained evidence suggesting that a large proportion of injected norepinephrine is temporarily bound to nonspecific binding sites which differ both from the true pharmacological receptors of the effector organ and from the storage sites of the nerve terminals. If this is true, tyramine might be able to displace norepinephrine from such nonspecific binding sites and thus increase the effective concentration of norepinephrine at the receptor. The possibility of the existence of such a "third compartment" has also been discussed by Weiner *et al.* (157). Furthermore, when Weiner and Trendelenburg (158) studied the uptake of labeled epinephrine and tyramine by the heart of the pithed rat, they found that neither the total radioactivity in the cardiac tissue nor the blood/tissue ratio was markedly affected by the administration of cocaine or by pretreatment with reserpine; the samples were obtained two minutes after the injection of the labeled amines. These observations can be interpreted as indicating that the uptake into specific compartments (A and perhaps also B) is very small and is masked by the ability of the tissues to take up temporarily large amounts of amines by a nonspecific binding process which is apparently not affected by the administration of cocaine or by pretreatment with reserpine.

Rather similar observations were made by Strömblad (127) who determined the radioactivity of normal and of denervated salivary glands after the injection of labeled epinephrine at the height of the secretory response. Although denervated structures have been found to be unable to "store" injected norepinephrine for any length of time (72), the amount of radioactivity found in the denervated gland very shortly after the intravenous injection of the labeled amine was quite normal.

One last point has to be discussed with regard to the mode of action of tyramine-like substances, *i.e.*, the antagonistic action of cocaine. It is very probable that cocaine antagonizes the entry of tyramine into the nerve ending. The site of action of cocaine seems to be on the cell membrane and not on the membrane of the storage granules, since cocaine fails to prevent the displacement of norepinephrine by tyramine from isolated heavy granules (124). In the preceding section it was postulated that cocaine uniformly antagonizes the indirect actions of *all* sympathomimetic amines. If this is true, cocaine should antagonize the uptake of all sympathomimetic amines into the relevant compartment(s). The evidence is far from complete, but what is available is consistent with this view. Muscholl (106) found that cocaine competitively antagonizes the uptake of norepinephrine into the heart and spleen of unpretreated pithed rats. Similar observations have been made by many workers (23, 45, 73, 160). In isolated atria of reserpine-pretreated guinea pigs, exposure to norepinephrine or epinephrine failed to restore the response to tyramine when the atria were exposed to these two catecholamines in the presence of cocaine; since both the catecholamines and the cocaine were carefully washed out before the response to tyramine was obtained, this effect of cocaine must have been due to a prevention of the "refilling" and not to a direct antagonism of the action of tyramine by cocaine. Furthermore, this effect of cocaine was clearly dependent on (a) the concentra-

tion of cocaine and (b) the concentration of the catecholamine used for refilling of the stores (146). Hence, it seems that cocaine by some competitive mechanism prevents the uptake of sympathomimetic amines into compartment A. On the other hand, the release of norepinephrine from nerve endings is not affected by cocaine, since this agent does not affect the appearance of the transmitter in the splenic vein blood of the cat after stimulation of the splenic nerve (81, 143a).

VI. MECHANISMS OF SUPERSENSITIVITY

During the last ten years a large number of hypotheses has been put forward to explain the phenomenon of supersensitivity of smooth muscle, cardiac tissue, and gland cells. The number of substances claimed to cause some kind of supersensitivity has grown to such formidable magnitude that no endeavor will be made to mention them all. Many workers apparently consider that the problem of the mechanism of supersensitivity is practically solved; others (including the reviewer) feel that in the last ten years the main contribution has been the realization that the whole problem is much more complex than was previously assumed. The reasons underlying such a view will be discussed in this section, which will be concerned with a critical review of current hypotheses.

A. Catabolic enzymes

Ten years ago monoamine oxidase was generally believed to be the most important enzyme involved in the catabolism of norepinephrine. Since high concentrations of cocaine inhibit this enzyme (61, 114) and since denervation reduces the monoamine oxidase activity of the organ concerned (32), inhibition of the activity or disappearance of the enzyme was proposed to be responsible for the supersensitivity to norepinephrine (25). However, when more potent inhibitors of monoamine oxidase became available, this hypothesis became untenable, since these compounds failed to cause a degree of supersensitivity to norepinephrine which could be quantitatively related to their enzyme-inhibiting effect (13, 61, 79, 121). On the other hand, their enzyme-inhibiting effect seems to be related more closely to their ability to potentiate responses to tyramine, which is metabolized by monoamine oxidase much more rapidly than is norepinephrine.

When it was found that catechol-O-methyl transferase also is involved in the catabolism of injected norepinephrine, it was tempting to postulate that this enzyme terminates the active life of norepinephrine and that blockade of this enzyme could explain the phenomenon of supersensitivity. This possibility can be ruled out because (a) cocaine fails to block catechol-O-methyl transferase (164), (b) cocaine produces supersensitivity to amines which are not substrates of this enzyme (*e.g.*, phenylephrine (5)), and (c) inhibitors of this enzyme (*e.g.*, catechol and pyrogallol) produce no pronounced supersensitivity to norepinephrine (43, 164) or epinephrine (91).

Two important conclusions must be drawn from these observations: (a) supersensitivity after denervation, decentralization, or the administration of cocaine cannot be explained solely in terms of enzyme inhibition, and (b) al-

though the enzymes implicated are important for the catabolism of epinephrine and norepinephrine, they do not appear to be required for the termination of the biological effects of norepinephrine and epinephrine, as appears to be the case for acetylcholinesterase and acetylcholine. The first step which ends the biologically active life of liberated (endogenous) or injected (exogenous) norepinephrine seems to be a yet unknown process prior to its enzymic breakdown. For detailed discussion of this point see the reviews by Blaschko (17a, 17b) and Koelle (82a).

B. Unifying theories

Since the supersensitivity to norepinephrine which is observed after denervation and after the administration of cocaine (and, under certain conditions, after pretreatment with reserpine) always seemed to be linked with a subsensitivity to tyramine, various authors have tried to explain these two phenomena as two aspects of one and the same phenomenon (55, 56, 96). Since it has been shown that the two phenomena can be separated experimentally (see Section V), such "unifying theories" cannot be valid. The subsensitivity to tyramine observed after denervation finds its explanation in the depletion of the norepinephrine stores (probably as a consequence of the degeneration of the postganglionic nerve fibers), and the subsensitivity to tyramine after cocaine is probably due to a surmountable antagonism by cocaine of the "uptake" of all sympathomimetic amines into the tissue stores (see Section V). Subsensitivity to tyramine after pretreatment with reserpine is clearly related to the depletion of the norepinephrine stores by this pretreatment.

Since we can account for the phenomenon of subsensitivity to tyramine, any "unifying theory" must at least be revised. There is no compelling reason for the view that cocaine must have only one site of action. Its sensitizing action may or may not be due to its preventing "uptake" of amines into certain tissue stores or compartments. This is discussed below.

C. Supersensitivity and norepinephrine content

Denervation is known to cause a considerable fall in the norepinephrine content of the denervated organ (29, 38, 42, 53, 67, 82, 128). Since cocaine causes a very similar type of supersensitivity, it was tempting to postulate that by virtue of its local anesthetic action cocaine can block the postganglionic fibers and thus cause "pharmacological denervation"; Fleckenstein and Bass (55) proposed this hypothesis on the basis of evidence which suggested that cocaine prevented the release of norepinephrine from nerve terminals following postganglionic stimulation. Macmillan (96) likewise postulated that cocaine interferes with the release of norepinephrine from the stores. This concept is unlikely since (a) there is evidence that cocaine does not impair the release of norepinephrine on nerve stimulation (81, 143a), since (b) compounds known to block the release of norepinephrine from nerve endings on nerve stimulation (such as TM 10 and bretylium) cause only a very slight supersensitivity to norepinephrine, which is quite different from that produced by either denerva-

tion or the administration of cocaine (19, 54), and since (c) other compounds which are at least as potent local anesthetics as cocaine fail to cause the typical cocaine-like supersensitivity.

When reserpine became available as a pharmacological tool for depleting the norepinephrine stores, experiments seemed to indicate that pretreatment with reserpine (like denervation and cocaine) *always* led to supersensitivity to norepinephrine, when depletion of the stores was achieved. Therefore, Burn and Rand (29) postulated that the sensitivity of an organ is inversely related to its norepinephrine content. However, the following experimental observations are not explained by this generalized form of the hypothesis: (a) decentralization is known not to deplete the stores (82, 115) but nevertheless it causes supersensitivity to norepinephrine; (b) short-term pretreatment with reserpine causes depletion of the norepinephrine stores without concomitant supersensitivity to norepinephrine (44, 60, 84, 152); (c) prolonged ganglion-block causes supersensitivity to norepinephrine (49, 152) without reducing the norepinephrine content of the tissues (152); (d) prolonged treatment with TM 10 or bretylium causes supersensitivity to norepinephrine (51, 152) with very little change in the norepinephrine content of the tissue (19, 152); (e) prolonged pretreatment with very small amounts of reserpine causes supersensitivity to norepinephrine although the stores of norepinephrine are not completely depleted, as judged by the reduced (but by no means abolished) response of the nictitating membrane to nerve stimulation (60).

Apparently in favor of the hypothesis of an inverse relationship between the sensitivity of an organ and its norepinephrine content is the observation that an infusion of norepinephrine into a reserpine-pretreated preparation not only increases its response to tyramine but also reduces its sensitivity to norepinephrine (15, 28). This phenomenon has been interpreted as a "normalization" insofar as the infusion of norepinephrine refills the originally depleted stores and reduces to normal the originally increased sensitivity of the organ. However, repetitive stimulation of the postganglionic fibers of a previously decentralized nictitating membrane also causes a reduction of the supersensitivity of this organ to injected epinephrine (163), although decentralization is known not to affect the norepinephrine content of this organ (82). Recent observations with isolated guinea pig atria likewise do not support the hypothesis. After short-term pretreatment with reserpine the norepinephrine content of the atria was only 1 % of normal, and they failed to respond to tyramine, but their sensitivity to norepinephrine was normal; although exposure to norepinephrine then restored their response to tyramine to 70 % of normal, their sensitivity to norepinephrine remained unchanged after the "refilling" of the stores (44). The difference between these and the previously mentioned experiments (with infusions of norepinephrine) can probably be explained by considering the time course of the desensitization observed by Burn and Rand (28). Their observation of a decreased sensitivity to norepinephrine was made immediately after the intravenous infusion of large amounts of norepinephrine. In skeletal muscle, the desensitizing action of a prolonged application of the transmitter is well known

(10, 80), but this is a phenomenon of very short duration and not necessarily the consequence of any "refilling"; it is probably a direct and short-lasting effect on the receptor. When, under the reviewer's experimental conditions, the norepinephrine (which "refilled" the stores) was carefully removed from the organ bath by a 45-minute period of repeated changes of the bath fluid, a desensitizing effect on the norepinephrine receptors of the atria was not detectable. Hence, it is likely that the well-documented reduction in sensitivity immediately after the exposure of a tissue to norepinephrine is the result of a transient "desensitization" of the norepinephrine receptor rather than of a "normalization."

In the preceding section it has been pointed out that the determination of the total norepinephrine content of an organ may be rather meaningless, since the distribution of this substance in different compartments of the entire storage site may be of greater importance than the total amount. Thus, although the generalized form of the theory that the sensitivity to norepinephrine is inversely related to the total norepinephrine content of an organ appears to be untenable, it may well apply to a particular compartment of the store. However, here again the available evidence does not support this modified theory. As mentioned in the previous section, reserpine seems to act predominantly on compartment B (44, 74, 82a) and there is no direct relation between supersensitivity and the depleting action of reserpine (objections (b) and (e); see above). Compartment A can also be ruled out, since its "refilling" failed to change the sensitivity to exogenous norepinephrine (44).

The various contradictory observations must find an explanation before the hypothesis under discussion is acceptable either in its generalized or in a modified form.

D. Deformation of receptors

Maxwell *et al.* (98) observed that cocaine reverses the blocking action of surmountable antagonists of epinephrine and norepinephrine (phentolamine) but does not affect the blocking action of an unsurmountable and irreversible antagonist (Dibenamine). From these experimental observations, which were made on the blood pressure of the dog, it was concluded that the site of action of cocaine was postsynaptic, probably at the norepinephrine receptor. It was thought that cocaine might produce its action by a change in the configuration of the receptor, *i.e.*, "deform" the receptor.

There is no evidence available to rule out the possibility that cocaine has a postsynaptic site of action, *i.e.*, an action on the smooth muscle cell or its norepinephrine receptor. But it must be emphasized that the above mentioned results do not prove such a postsynaptic site of action. If cocaine, by delaying the inactivation of injected norepinephrine in some as yet unknown way, would increase the local concentration of the injected drug at the receptor, then one would expect what actually has been observed: a reduction of the surmountable (competitive) type of antagonism (phentolamine), and no change of the unsurmountable (irreversible) type of receptor-block (Dibenamine). The actual site of action of cocaine could be presynaptic (block of uptake of norepinephrine into the

stores and therefore an increased concentration of norepinephrine at the receptor) or parasynaptic (block of enzymatic or binding processes which normally remove the injected norepinephrine quickly). Hence, it must be concluded that, although this effect of cocaine on the blockade of norepinephrine receptors is an interesting phenomenon, it does not establish the site or the mode of action of cocaine; "deformation" of the norepinephrine receptors is one of the theoretical possibilities which have to be considered in regard to the mechanism of supersensitivity, but at the present time there is no evidence that it exists.

E. Release of norepinephrine from nerve endings

Organs with an adrenergic nerve supply may be assumed to be under the influence of a resting secretion of norepinephrine from the postganglionic nerve ending comparable to the resting secretion of the adrenal medulla. This resting secretion may be visualized as consisting of two components: (a) the release of relatively large amounts of norepinephrine on the arrival of impulses from the central nervous system, and (b) the spontaneous release of relatively small "quanta" of transmitter from the nerve terminals. The importance of (a) is well documented for vascular smooth muscle by the role of the vasomotor center in the maintenance of the vasomotor tone. Until recently, (b) was only a matter of speculation; Burnstock and Holman (35, 36) have now established that in an isolated smooth muscle preparation with sympathetic innervation (vas deferens of the guinea pig), miniature junction potentials can be observed when intracellular records of membrane potentials are obtained. These miniature potentials resemble the well-known miniature endplate potentials of the neuromuscular junction. They seem to be due to the release of small quanta of norepinephrine from nerve endings, since their rate of appearance and their amplitude are reduced after denervation and after pretreatment with reserpine (36).

Apart from these observations nothing is yet known about the action of pharmacological agents on the two types of "resting" secretion from postganglionic nerve terminals. It is tempting to speculate that this resting secretion may be of importance for the development of supersensitivity. At the neuromuscular junction, for instance, it has been observed that supersensitivity begins to develop a few hours after cessation of the miniature endplate potentials (after either denervation or application of botulinum toxin); the development of full supersensitivity then takes several days. Since sympathetically innervated organs are innervated by a chain of two peripheral neurons (the pre- and the postganglionic neuron), one might postulate that preganglionic denervation (decentralization) removes the central contribution to the resting secretion from the postganglionic nerve terminals, whereas postganglionic denervation removes both components (tonic impulses and spontaneous random release of small quanta of the transmitter). This speculation may be extended to the concept that removal of the influence of "tonic" impulses leads to the decentralization-type of supersensitivity, whereas removal of the random release of norepinephrine quanta leads to the cocaine type of supersensitivity.

These theories are not new, and, in some form or other, have already been

discussed by Bacq (11a), Simeone (125a) and Cannon and Rosenblueth (38a). They are mentioned again in order to correlate them with recent electrophysiological advances. A careful and quantitative study of the time course of development of supersensitivity in relation to the disappearance of the miniature junction potentials (after denervation, cocaine, decentralization, or various schedules of pretreatment with reserpine) will greatly contribute to an understanding of the development of supersensitivity of smooth muscle. However, it should also be emphasized that although such studies have been carried out extensively on the neuromuscular junction, the fundamental problem has not been solved. It is now known that cessation of the miniature endplate potentials is soon followed by a spread of the acetylcholine-sensitive area and that this spread is probably responsible for the supersensitivity to acetylcholine and related substances; the actual mechanism responsible for this spread remains obscure (100, 101).

F. Deviation of norepinephrine from the "sites of loss" to the receptor

According to Veldstra (154) the "sites of loss" comprise all the mechanisms involved in the termination of the biologically active life of a pharmacological agent. In the case of norepinephrine these may include uptake of the transmitter into the norepinephrine stores, temporary binding to proteins ("silent receptors") diffusion from the receptor, enzymatic catabolism, or other as yet unknown mechanisms.

By far the most popular hypotheses concerned with the phenomenon of supersensitivity are those which consider supersensitivity to be the result of a deviation of liberated or injected norepinephrine from its usual "sites of loss" to the pharmacological receptor. This concept implies that the supersensitive effector organ is not changed at all but that the effective concentration of the injected agonist at the receptor is increased by the sensitizing agent or procedure. The enumeration of the various possible mechanisms which may be operating at the "sites of loss" immediately points out the weakness of this concept—the processes which normally terminate the biological action of norepinephrine are not known. If they were, we might be able to test these theories by specifically designed experiments. Since this is not yet possible, we can only postulate that the blocking or destruction of the "sites of loss" permits a larger number of norepinephrine molecules to reach the receptor and that this results in an increased response of the effector organ, *i.e.*, in supersensitivity.

A fairly good case can be made for this concept if only norepinephrine is considered; however, if the whole spectrum of sympathomimetic amines and the various types of supersensitivity are taken into account the available evidence is much less convincing. As an example, the following argument may be presented. It is quite possible that the uptake of norepinephrine into the stores is the most important of the "sites of loss." The administration of cocaine (73, 106, 160), pretreatment with reserpine (8, 45, 105, 118), and chronic denervation (30, 72) have all been reported to prevent this uptake. Consistent with this deviation concept is the observation that cocaine fails to cause any further

sensitization after denervation-supersensitivity has developed (87); entirely inconsistent is the observation that cocaine retains all its sensitizing effects after short-term pretreatment with reserpine (60, 151). Furthermore, although this hypothesis would account for the immediate sensitizing effect of cocaine, it completely fails to explain the phenomenon of slow development of supersensitivity after prolonged pretreatment with reserpine. Finally, the available evidence concerning the supersensitivity observed after decentralization does not fit into this picture. There is no known reason why chronic section of the preganglionic axon should impair the uptake mechanism in the postganglionic nerve terminal, since decentralization does not lead to any loss of norepinephrine from the tissue (and, therefore, presumably does not damage the stores). The alternative hypothesis, that decentralization and the consequent absence of tonic impulses from the central nervous system leave the relevant compartment of the nerve terminal so full of transmitter substance that it cannot take up any more, is not consistent with the finding that short-term pretreatment with reserpine [which depletes the stores of the decentralized nictitating membrane (82)] does not abolish the phenomenon of decentralization supersensitivity (151). Thus, the attractive original hypothesis that block of uptake into the tissues explains the phenomenon of supersensitivity cannot be maintained in such a generalized form; moreover, the argument becomes hopelessly entangled when other sympathomimetic amines are considered. What is the basis of the difference between the *m*-OH and the *p*-OH analogues? Neither denervation nor the administration of cocaine causes pronounced potentiation of the *p*-OH compounds. Two possibilities arise: (a) The *p*-OH analogues are not taken up into the stores; therefore, degeneration of the nerve terminals (after denervation) or block of the uptake mechanism (by cocaine) would not have any effect on the number of molecules of a *p*-OH compound reaching the receptor. Since it is well established that many of the *p*-OH analogues have a strong indirect action, the assumption that they are not taken up into the stores would be quite contrary to the accumulating evidence that indirectly acting substances *are* taken up into the stores (see preceding section). (b) The *p*-OH analogues are taken up into the stores but cocaine selectively blocks only the uptake of *m*-OH analogues and thereby potentiates the latter but not the former. This view is untenable, if it is accepted that degeneration of the nerve fibers (after denervation) leads to the disappearance of the stores; complete absence of the store should then cause an equal potentiation of the *p*-OH and *m*-OH compounds rather than the differential potentiation which is observed.

From these considerations it must be concluded that the hypothesis of deviation of norepinephrine from the "sites of loss" to the receptor cannot be regarded as established until much more is known about the "sites of loss."

G. Conclusions

The purpose of a critical appraisal of the various hypotheses concerned with the mechanism of supersensitivity to sympathomimetic amines is not so much to belabor the negative aspect (that none of them can, at the present time,

provide a full explanation) but rather to emphasize the pressing need for the recognition that this field is much more complicated than is generally realized. The main complications may be enumerated as follows:

1) The mechanism responsible for the termination of the biologically active life of injected or released norepinephrine is not known. Present evidence indicates that neither catechol-O-methyl transferase nor monoamine oxidase is involved; a binding process, a carrier mechanism, a storage process, or diffusion seems to be responsible.

2) The exact mechanism of action of indirectly acting (tyramine-like) substances remains a field of some justified disagreement. The evidence for actual displacement of the transmitter by the nontransmitter sympathomimetic amines with indirect actions has become increasingly, but not yet fully, convincing.

3) The concept that the norepinephrine stores of the tissue consist of only one uniform compartment must be abandoned in favor of the view that the stores consist of at least two (and possibly more) compartments of different size and different physiological and pharmacological importance. There is an urgent need for a proper definition of these compartments and for methods which will permit measurement of their norepinephrine contents individually. The usefulness of measurements of total norepinephrine content of tissues is questionable in the absence of detailed knowledge about the distribution of norepinephrine within the different compartments.

4) Supersensitivity should not be regarded as a simple phenomenon. As far as organs with adrenergic innervation are concerned, it should be recognized that decentralization supersensitivity is quite different from the type of supersensitivity observed after the administration of cocaine. Furthermore, it should be recognized that denervation supersensitivity is apparently a combination of both these types of supersensitivity. If, in fact, there are different types of supersensitivity, then entirely different mechanisms may be involved.

5) Any study concerned with supersensitivity should take into account the striking differences between sympathomimetic amines of closely related chemical structure. Any comprehensive theory of supersensitivity should also furnish an explanation for these differences. Comparative, quantitative studies of the pharmacology of such closely related compounds may be very helpful in elucidating the mechanisms involved in the development of supersensitivity.

6) It should be borne in mind that any well-established correlation does not necessarily indicate a causal relationship. For example, it has been found that the administration of cocaine delays the disappearance of injected norepinephrine from the circulation and that the resultant increase in the plasma level of norepinephrine has been correlated significantly with the increased response of the blood pressure to the injection of norepinephrine (143a). It is tempting to speculate that this supersensitivity is the consequence of the increase in the plasma concentration of norepinephrine. However, there are three serious objections to this view: (a) this observation fails to explain why the administration of cocaine increases the sensitivity of the cardiovascular system to norepinephrine by a factor of about 5 but increases that of the nictitating membrane by a factor of

about 50 (60); (b) prolonged pretreatment with reserpine also causes supersensitivity to norepinephrine, but fails to produce such an increase in norepinephrine plasma levels (152); (c) if the norepinephrine plasma level were of determining importance, then the administration of cocaine should cause a further increase in sensitivity of the already supersensitive denervated nictitating membrane; this is contrary to the experimental evidence which indicates that cocaine is unable to cause further sensitization beyond that already achieved by denervation (87). It is quite possible that the increased norepinephrine plasma level is the consequence of the impairment of the mechanism responsible for the uptake of norepinephrine into the tissue stores (or into one specific compartment of these stores), while a second and completely different action of cocaine is responsible for the phenomenon of supersensitivity. If it is assumed that cocaine prevents the uptake of sympathomimetic amines into the tissue (or a compartment of the store) by an action on the membrane of the postganglionic nerve terminal (*i.e.*, a presynaptic site of action), then it is not unreasonable to assume that cocaine in a similar concentration is also able to affect the postsynaptic membrane in such a manner that supersensitivity results.

7) There is growing evidence for the view that many of the compounds considered in this review have multiple sites of action. This is by no means astonishing, since the "master key," norepinephrine, must fit all of the following receptor-like sites: the postulated carrier mechanism responsible for uptake into the stores (or compartments of stores), the binding site inside the stores, the postulated less specific binding sites outside the actual stores, catechol-O-methyl transferase, monoamine oxidase, and the alpha- and beta-receptors. Any substance capable of fitting into one of these sites should be suspected of fitting into one, or some, or all of the others. This would account for the large variety of substances recently described to interfere with the storage, the release, or the action of norepinephrine.

8) Several years ago, presynaptic adrenergic nerve endings were generally believed to have only one function: to release norepinephrine in response to the arrival of a nerve impulse. When Burn (24) first advanced the idea that nerve terminals might also function as a site for uptake of sympathomimetic amines, the suggestion failed to find the proper receptor. During recent years this situation has changed markedly; in fact, it has changed to such a degree that it now seems necessary to point out that postsynaptic events are in danger of being neglected. Recent discussions about the mechanism of supersensitivity have dealt almost exclusively with hypotheses concerned with presynaptic events. In this connection, it may be worthwhile to point out that supersensitivity of skeletal muscle has clearly been demonstrated to be a postsynaptic event: after degeneration of the motor fiber the acetylcholine-sensitive area (which normally is restricted to the endplate region) extends until it covers the whole of the postsynaptic (muscle) membrane (9, 10, 48, 100, 101). Smooth muscle has no obvious equivalent of the endplate region, and norepinephrine receptors may be more or less uniformly distributed over the cell membrane. Nevertheless, supersensitivity of smooth muscle might be visualized as the consequence of an

increase in the number of receptors (in analogy to skeletal muscle). As a result of such an increase in the density of the receptor population, one would expect to obtain a uniform type of supersensitivity such as is actually observed after decentralization. Moreover, the time element involved in the development of decentralization supersensitivity in the nictitating membrane (69) resembles closely the time course of the development of denervation supersensitivity in skeletal muscle (48). Finally, just as a pharmacological interruption of the pathway to the endplate (by the administration of botulinum toxin) is enough to produce this type of supersensitivity in skeletal muscle (134), so also are various pharmacological procedures (all resulting in prolonged interruption of the pathway between the center and the end organ) capable of producing the decentralization-type of supersensitivity in the nictitating membrane. Hence, if supersensitivity in smooth muscle is due to a general increase in receptors, as appears to be the case in skeletal muscle, one could account for at least the decentralization-type of supersensitivity on a postsynaptic base. This is, of course, pure speculation; it has been claimed, to the contrary, that denervation of the iris results in a reduction of the number of receptors (155). But such considerations serve to point out that our intense interest in presynaptic events should not lead to the tacit assumption that postsynaptic events can safely be excluded. It is hoped that electrophysiological studies will help to classify the relative importance of these two possibilities.

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