PHARMACOLOGY OF THE AUTONOMIC NERVOUS SYSTEM[1,2]

By Eleanor Zaimis

Department of Pharmacology, Royal Free Hospital School of Medicine, 8 Hunter Street, London

Introduction

This reviewer cannot hope to provide a complete coverage of all the very considerable literature in this field. My aim has therefore been to select certain aspects for closer scrutiny, and to examine their contribution to and influence upon the overall development of this special branch of pharmacology.

Autonomic Ganglia

The pharmacology of autonomic ganglia has during the past three years been the subject of several detailed reviews (117, 122, 135); however, the following reports of recent physiological investigations may be of interest to those working in this field.

Although the discovery of chemical transmission at both sites occurred almost simultaneously, the study of the ganglionic synapse lags behind that of the neuromuscular junction. Unfortunately, autonomic ganglia proved to be both anatomically and functionally very complex structures, and consequently the progress of electrophysiological studies was relatively slow and full of disappointments (135). In particular the lack of success in introducing microelectrodes into ganglion cells has been a great handicap, as no real analyses of synaptic events can be made without a follow-up of the activity of single neurones. A few years ago, however, Nishi & Koketsu (95) successfully studied in the frog the way in which action potentials are generated by sympathetic ganglion cells. More recently, Blackman, Ginsborg & Ray (11 to 14) applied further tests which resulted in a detailed analysis of synaptic transmission in the isolated sympathetic ganglion of the frog. Single cells were impaled with micro-electrodes, and action potentials elicited in response to antidromic and orthodromic stimulation were recorded. Their results suggest that the transmission is chemical, and although no direct evidence of the nature of the substance is available, the indirect evidence supports the authors' view that the transmitter is acetylcholine (ACh). Mecamylamine, hexamethonium, and tubocurarine blocked the responses to orthodromic nerve stimulation, while antidromic stimulation was not affected. Acetyl-

[1] The survey of literature pertaining to this review was completed in September, 1963.

[2] The following abbreviations are used in this chapter: ACh (acetylcholine); Nor (noradrelaline); [H^3]-Nor ([H^3]-noradrenaline).

choline applied iontophoretically depolarized the ganglion cells, and eserine or neostigmine sometimes increased the duration of synaptic activity. Miniature action potentials, like those seen at the neuromuscular junction, were recorded, and the results suggested that they were due to quanta of ACh released from the presynaptic terminals. Thus a very close similarity between transmission at the sympathetic synapse and that at the neuromuscular junction in the frog was clearly demonstrated. Eccles (44), reviewing mechanisms of synaptic transmission, comments that there are marked similarities in the essential features of synaptic actions of a wide variety of junctional regions in both invertebrates and vertebrates. Electron microscopy, moreover, has taught us that there is a remarkable uniformity in the minute structure of a great variety of synapses, although the same synapses may show important anatomical differences when examined by light microscopy. We may hope, therefore, that events taking place *in vitro* at the ganglionic synapse and the neuromuscular junction in frogs will, when direct evidence is finally forthcoming, prove not too dissimilar to synaptic events occurring in the intact mammal.

The transmission in the mammalian ciliary ganglion is chemical, and the transmitter substance is ACh. Martin & Pilar (86, 87), however, demonstrated a dual mode of synaptic transmission in ciliary ganglia isolated from chicks a few days old. Glass micropipettes were used to obtain intracellular records from both ganglion cells and presynaptic terminals. It was found that at many of the synapses there was, in addition to chemical transmission, direct electrical coupling between presynaptic and postsynaptic elements, and that this coupling was bidirectional. Very interesting was their finding that preparations from older chicks (4 weeks) showed an apparent increase in the relative number of electrically coupled synapses.

The Presence of Ganglion Cells in the Hypogastric Nerve

Many of the nerve trunks which are usually referred to as "postganglionic" have been demonstrated to contain aberrent ganglionic cells. For example, there is very good evidence that in the cat and rabbit the cervical sympathetic trunk contains a great number of ganglion cells [for references, see (41)]. The same conclusion was reached by Eccles (43). In 1895 Langley & Anderson (78) demonstrated that in the hypogastric nerves of the cat and the rabbit a proportion of nerve fibres are preganglionic and connect with ganglion cells normally situated near the innervated organ. More recently Vogt (121) studied the hypogastric nerve of the dog and in the core of the nerve found ganglion cells which were usually arranged like a string of beads. Moreover, large chromaffin bodies were found inside the inferior mesenteric ganglia, but these did not extend into the hypogastric nerve. Small elongated groups of 5 to 50 chromaffin cells, however, occurred either within the very core of the nerve or along its surface underneath the epineurium.

The presence of ganglion cells in the hypogastric nerve is also indicated by the following pharmacological observations. Sjöstrand (110) found that the

motor response of the isolated guinea-pig vas deferens to hypogastric nerve stimulation was blocked by azamethonium, hexamethonium, tetraethylammonium, lobeline and nicotine, and that chronic denervation (111) caused little or no decrease in the amount of noradrenaline (Nor) present in the vas deferens. This was considered a good indication of the presence of a peripheral cholinergic synaptic mechanism, because the Nor content of peripheral organs is known to be greatly reduced by postganglionic but not by preganglionic denervation. Moreover, Ohlin & Strömblad (96) showed that when electrodes are placed on the hypogastric nerve at a distance of one to five mm. from the vas deferens, contractions are produced which resist the action of hexamethonium. Finally Birmingham & Wilson (10) demonstrated that when the guinea-pig vas deferens is removed and stimulated transmurally, contractions are produced mainly by excitation of postganglionic adrenergic nerves.

Burnstock & Holman (25) were the first to study the isolated guinea-pig vas deferens electrophysiologically. Upon repetitive stimulation of the hypogastric nerve, junctional potentials were recorded in the smooth muscle fibres of the vas deferens. These junctional potentials facilitate and summate until, at a critical depolarization, a spike is initiated and contraction occurs. In the absence of nerve stimulation there is a spontaneous discharge of small potentials unaccompanied by changes in muscle tension. In a further study (26) preparations of vas deferens were taken from guinea-pigs 8 to 13 days after section of both hypogastric nerves. It was found that stimulation of the distal stump of the hypogastric nerve continued to give rise to contraction of the vas deferens. It thus appeared that only partial denervation was achieved, and the authors concluded that this was probably because of the presence of sympathetic ganglion cells in the pelvic plexus. They also concluded that the transmitter substance appeared to be located within the nerves themselves rather than stored and synthesized outside the nerves in structures such as chromaffin cells. This conclusion was reached because both the spontaneous potential discharge and the junctional potentials were markedly reduced in the partially denervated preparations. Burnstock & Holman argued that if the transmitter substance was stored in chromaffin cells outside the nerves, no reduction in the spontaneous discharge could be expected, even if the amplitude of the junctional potential was lowered. Kuriyama (77) studied the nervous pathway to the smooth muscle cells in the guinea-pig vas deferens and found both pre- and postganglionic fibres in the hypogastric nerve. The recording of neuromuscular junction potentials, action potentials and tension from single muscle cells showed that the hypogastric nerve contains two groups of nerve fibres. One group, presumably preganglionic, which was characterized by a low threshold and fast conduction velocity, triggered junction potentials of high amplitude which were blocked by ganglion-blocking drugs (0.1 mg./ml. hexamethonium bromide and 0.01 mg./ml. nicotine hydrochloride). The other group of nerve fibres, presumably postganglionic, had a high threshold and a slow conduction velocity; on stimula-

tion, junction potentials of low amplitude which were resistant to the action of ganglion-blocking drugs were elicited. When two pairs of electrodes were used for nerve stimulation, the delay in conduction between the nearest electrode and the muscle cell suggested the existence of ganglia somewhere close to the peripheral part of the hypogastric nerve. On the other hand, adrenergic blocking drugs (phentolamine 0.1 mg./ml. and bretylium tosylate 0.01 mg./ml.) prevented the generation of junction potentials for both types of stimulation.

Ferry (48) also found that the compound action potential of the hypogastric nerve showed two groups of fibres, one conducting impulses at 3–6 m./sec. which triggered the junction potentials, and the other conducting impulses at less than 1 m./sec. which did not trigger junction potentials. From his results, he concluded that the fast fibres were preganglionic B fibres supplying ganglion cells situated peripherally to the site of stimulation of the hypogastric nerve trunk. Ferry (49) later studied, in the guinea-pig anaesthetized with urethane, the electrical activity in the nerve bundles which run along the blood vessels in the connective tissue surrounding the vas deferens. Stimulation of the fast fibres in the hypogastric nerve was followed by a discharge in the vas deferens nerves. This discharge was increased by increasing the stimulus to the hypogastric nerve so that the C fibres in it were excited. He found that the response in the vas deferens nerves was abolished or greatly reduced by the intravenous administration of hexamethonium bromide (10 mg./kg.). In addition, he recorded the contraction of the longitudinal smooth muscle of the vas deferens in response to hypogastric nerve stimulation and found that it too was abolished by hexamethonium. In contrast, the contraction elicited by stimulation of the vas deferens nerve was not abolished.

Nervous Pathway to the Spleen

Brown & Gillespie (21) and Brown, Davies & Ferry (20) found that phentolamine and Hydergine greatly increase the output of Nor from the spleen when the splenic nerves are stimulated, and suggested that these blocking agents prevent the uptake by the splenic nerves of the liberated transmitter. Meanwhile, Boyd, Chang & Rand (17) reported that some adrenergic blocking compounds possess anticholinesterase activity and suggested that their effect in increasing Nor output was due to their action upon a "cholinergic link" in the sympathetic postganglionic pathway. Blakeley, Brown & Ferry (15), however, provided conclusive evidence that this is not so. They tested the effects of powerful anticholinesterase drugs on the output of Nor from the spleen and compared it with the effect of an adrenergic blocking compound. Their results showed that administration of eserine sulphate (0.3 mg./kg.) or neostigmine methylsulphate (0.1 mg./kg.), both of these doses being large enough to affect skeletal neuromuscular transmission profoundly, were without effect on the output of Nor. Subsequent administration of an adrenergic blocking agent increased the output by a factor of four. It is clear that adren-

ergic blocking agents do not owe their effects to any anticholinesterase activity they may happen to possess. The same workers showed that, if precautions are taken to maintain a constant blood flow through the spleen, hexamethonium, in doses large enough to block autonomic ganglia, did not reduce the sympathetic transmitter output from the spleen when the splenic nerves were stimulated at a frequency of 10 or 30/sec. Blakeley et al. (15) also investigated the effect of intravenously administered cocaine (5 mg./kg.) on the output of transmitter from the spleen and found that cocaine affects neither the uptake nor the liberation of transmitter. In these experiments a frequency of stimulation of 10/sec. was used because at this frequency the output is low and capable of being raised by adrenergic blocking agents, whereas the output produced by a frequency of 30/sec. cannot be substantially increased. These results are in agreement with those of Trendelenburg (116), who found that after cocaine the presence in the plasma of injected catecholamines was prolonged, but the output of transmitter from the spleen in response to nerve stimulation was unchanged. Thus Blakeley et al. concluded that the conditions affecting the disappearance of injected catecholamines from the systemic blood are quite different from those affecting the entry into the venous blood of the transmitter liberated within the tissues by stimulation of the splenic nerves.

The hypothesis put forward by Burn and his co-workers [for references, see (22)] of a "cholinergic link" in the sympathetic postganglionic adrenergic pathway has continued to stimulate a great deal of investigation. According to this hypothesis, some of the adrenergic nerve fibres may not in fact be truly adrenergic, but rather cholinergic, acting on some chromaffin tissue from which Nor is released on stimulation. For example, the sympathomimetic effects of injected ACh on the spleen have been used by Brandon & Rand (18) as evidence to support the existence of a cholinergic link in the sympathetic postganglionic pathway. Ferry (50), however, found that in the chloralosed cat the injection of 10–250 μg. of ACh into the blood supply of the spleen evoked a vigorous centripetal discharge in the C fibres of the splenic nerve. The centripetal discharge continued after atropine (1 mg. I.V.) or after Hydergine (0.5 mg./kg.). Because these substances would reduce or abolish any direct effect of ACh on smooth muscle, or the effect of any catecholamines released by ACh, and would therefore prevent the excitation of the mechanoreceptors by splenic contraction, Ferry concluded that it is unlikely that injected ACh acts on any structure other than the C fibres. Hexamethonium (10 mg./kg.), on the other hand, abolished the ACh-induced centripetal discharge. He considered that this could not be due to a conduction block in the C fibres since Daly & Scott (39) and Blakeley et al. (15) had already demonstrated that hexamethonium has no effect upon the motor response of the spleen to stimulation of the splenic nerves. Finally, Ferry showed that the discharge could still be elicited undiminished after the sensory innervation of the spleen had degenerated. As non-myelinated fibres from other sources are almost or completely absent, Ferry concluded that

injected ACh excites the sympathetic post-ganglionic C fibres and that it is this excitant action of ACh that hexamethonium blocks. A similar conclusion for other sensory nerves had previously been reached by Gray & Diamond (60) and Douglas & Ritchie (42). It would appear, therefore, that injected ACh owes its sympathomimetic effects on the spleen to an excitation of motor fibres, which in turn leads to liberation of Nor with a consequent contraction of the spleen. Obviously these effects can no longer be regarded as evidence for a cholinergic link in the sympathetic postganglionic pathway.

Localization, Uptake and Binding of Catecholamines

The treatment of freeze-dried tissues with formaldehyde gas produces the formation of compounds with intensive green to yellow fluorescence. This fluorescence reaction seems to have a high specificity for certain catecholamines and tryptamines. Using this method Falck (46) was able to show that the concentration of the adrenergic transmitter is much higher in the nerve endings than in the rest of the neurone.

Klouda (71) studied the distribution of catecholamines in five different regions of the dog heart and found distinct and significant differences. The following average values expressed as μg./gm. were reported: right atrium 1.46, left atrium 1.13, right ventricle 0.8, left ventricle 0.63, septum 0.67. A higher concentration of Nor in the atria of the dog heart was previously reported by Shore et al. (109). This pattern of distribution, however, is the reverse of that shown in the cat heart, in which the left ventricle was found to contain a larger amount of catecholamines than the atria (29). Yet no known difference exists between the anatomical distribution of sympathetic fibres in the ventricles and the atria of either species. On the other hand Gillis (57) found that after electrical stimulation of the cardio-accelerator nerves (20 stimuli/sec., 1 msec. duration, 30 v.) cat atria retained a larger amount of exogenous Nor. Labelled Nor in a dose of 2 μg. base/kg. was administered, either immediately after or one minute before a 30-second period of stimulation. The heart was removed 15 minutes after the administration of the labelled Nor, and the atria and ventricles were homogenized separately and assayed for their catecholamine content. The results showed that sympathetic stimulation given immediately before the administration of Nor significantly increased, in particular in the atria, the total amounts of labelled Nor. On the other hand, nerve stimulation one minute after the administration of [H^3]-noradrenaline ([H^3]-Nor) caused no significant change in either atria or ventricles. According to Gillis, the increase in catecholamine content was unrelated to heart rate, since in the control animals the administration of [H^3]-Nor was always accompanied by tachycardia, whereas the combined effect of nerve stimulation followed by the administration of [H^3]-Nor produced a change in the heart rate which ranged from varying degrees of tachycardia to bradycardia. He argued also that if altered functional activity of the heart had been responsible for the increased retention of exogenous

amine, this could have been expected to be reflected specifically in the ventricles because of their greater tissue mass.

Uptake and binding, as well as metabolism, form an important inactivation mechanism of circulating Nor. Strömblad & Nickerson (112) demonstrated in rats that an intramuscular injection of 1 mg. of adrenaline or Nor caused a marked increase in the content of the corresponding amine in the heart and salivary glands and a decrease in the concentration of the other amine. They concluded, therefore, that adrenaline and Nor can be stored in tissues other than those in which they are synthesized and that most of the accumulation is in the sympathetic nerve endings since chronic denervation prevented the increase.

That the sites of Nor storage are nerve endings of the postganglionic adrenergic fibres was also the conclusion of Marks, Samorajski & Webster (85), who studied in mice the localization of injected [H^3]-Nor by a radioautographic procedure. About 1 μg. of Nor was injected directly into the left ventricle of the heart, and isotope distribution was studied at 15 to 120 seconds. In another group of animals, the amine was injected intraperitoneally for studies at longer time intervals (5 min. to 4 hr.). In the spleen, short fibres in trabeculae and small "buttons" in splenic pulp appeared to be storage sites. In the small intestine, marked uptake of Nor was observed in polymorphic masses scattered throughout the region of Auerbach's plexus, including those areas which could be demarcated as ganglia in this layer. Finally, in the adrenal, the medulla became heavily labelled, while the cortex demonstrated no storage of Nor. Specific and intense localization of Nor occurred in the ventricular myocardium. After 15 seconds there was a wide distribution of Nor in all the myocardial cells. After 1 minute, long thin fibres, less than 2 μ wide and as much as 50 to 100 μ long, were densely labelled by Nor.

Potter, Axelrod & Kopin (100) found that in the rat exogenous Nor remaining in the heart 48 hours after its administration was not available for release by tyramine. The animals received intravenously about 8 μg. [H^3]-Nor/kg. Tyramine (10 mg./kg.) given 30 minutes later released labelled and unlabelled Nor to about the same extent. On the other hand, tyramine (10 mg./kg.) given 48 hours after [H^3]-Nor preferentially released unlabelled Nor. In contrast, Chidsey & Harrison (36), in an attempt to determine whether exogenous Nor would be distributed uniformly with the endogenous material, found that the exogenous Nor could be released into the blood by tyramine or nerve stimulation at a time as early as 20 minutes or as late as 48 hours after its administration. Labelled Nor was administered to dogs intravenously in doses of 0.82, 1.6 and 3.3 μg./kg. Release of Nor from the heart was produced either chemically by the administration of tyramine (0.04 to 1 mg./kg. I.V.) or electrically by stimulation of the cardio-accelerator nerve. Its specific activity (ratio of the labelled to total Nor) was determined (*a*) in the coronary venous blood and (*b*) in the heart itself. Following tyramine

administration, there was an increase in Nor concentration in blood and a decrease in specific activity. The change in specific activity, which occurred up to five hours after the administration of labelled Nor, was roughly proportional to the amount of tyramine administered and, therefore, to the amount of Nor released from the heart. Similarly sympathetic nerve stimulation resulted in a concomitant increase of Nor concentration and a reduction in specific activity. The specific activity of Nor in coronary sinus blood greatly exceeded that in myocardial tissue during the control measurements; during release of large quantities of Nor by tyramine or nerve stimulation, the specific activity of Nor in coronary sinus blood decreased towards the level of that in the myocardium. In every instance, however, the value in blood remained higher than that of the tissue. A similar result was observed in two isolated heart preparations. Sixty minutes after the injection of tritiated Nor, the specific activity in blood exceeded that in the heart. In contrast, in experiments in which the specific activity was measured 24 and 48 hours after the administration of labelled Nor, no change in the specific activity of the blood was observed with augmented release, and the values were similar to those determined in cardiac tissue. Thus, a difference in the amount of exogenous amine relative to the total amine released was observed at these two times. The authors concluded, therefore, that exogenous molecules of Nor do not mix homogeneously with the endogenous neurotransmitter pool during the first few hours after administration. There is, therefore, incomplete penetration of the labelled molecule into the pool. This is presented as further evidence for considering the Nor pool to be non-homogeneous. Chidsey & Harrison attribute the discrepancy between these results and those of Potter et al. (100) either to species variation or to the much larger doses used by the latter authors.

The view has been put forward that a continuous mechanism operates to pump the catecholamines across the membrane into the cell, and that the concentration of added or injected Nor which is taken up reaches a plateau only when levels of the amine within the cell are sufficiently high for passive outward diffusion just to balance the active inward pumping action [Shore 108)]. The amount of catecholamine administered in experiments in which effects on catecholamine stores are studied must be of the utmost importance. The need to use experimental doses of catecholamines comparable with those existing under physiological conditions was recently pointed out by Iversen & Whitby (69). These workers studied in the mouse the fate of a wide range of injected doses of catecholamines. Labelled adrenaline (0.1 μg. to 10 μg.) or Nor (0.1 μg. to 50 μg.) was administered intravenously. Thirty minutes after the injection, the animals were killed and their tissues analysed. The results showed that the uptake mechanism for each hormone was relatively more important at low dose levels than at high; a significant difference was found between the rate of disappearance of doses of 1 μg. of each hormone or less and all doses above this level. With the lower, more physiological, doses the rate of disappearance was slower than with the higher doses. The effects of

varying the dose of Nor were even more marked than in the case of adrenaline. This was in contrast to previously reported results in the same species (6), which indicated that there was no difference in the rate of metabolism of adrenaline for doses of from 1 to 10 μg. Iversen & Whitby concluded that the uptake of circulating catecholamines in the mouse is effected, at low injected levels, mainly by a tissue-concentrating mechanism but that this mechanism becomes saturated if the injected dose exceeds 1 μg. (30 μg./kg.). At doses greater than 1 μg. further uptake may be by passive diffusion, and the overall process of uptake becomes quantitatively less important as the proportion of the whole uptake due to the concentrating mechanism is progressively reduced. Eventually, for doses of 5 μg. or more, uptake is almost entirely by diffusion. The authors concluded, therefore, that the administration of massive doses of catecholamines appears to have little physiological relevance. In some of their experiments Iversen & Whitby administered combined doses of adrenaline and Nor, and their results suggested that circulating adrenaline and Nor appear to compete for entry into tissue storage sites and that a single concentrating mechanism may serve for the uptake of both hormones. They also found that with Nor the uptake was greater throughout the dose range tested than with adrenaline.

Metabolic Pathways of Bound and Drug-Released Adrenergic Transmitter

Trendelenburg (118) suggested that Nor is present in nerve endings in a labile "available" pool which slowly exchanges with a second stable pool of bound catecholamines. Later Potter, Axelrod & Kopin (101) and Kopin, Hertting & Gordon (74) provided more direct evidence for the presence of several bound forms of Nor. The last group of authors studied the fate of labelled Nor in the isolated perfused rat heart. A rapid infusion rate of 0.1 μg./min. of the amine was used, and the total radioactivity present in timed collections of perfusate and in the hearts at the end of the experiment was determined. Moreover, the various metabolites were separated. In both normal and reserpine-treated animals (2.5 mg./kg. I.P.), more tritium was found in the hearts than could be accounted for by the water content of the heart. This excess was considered to have been bound. Hearts from normal rats continued to concentrate the infused Nor at a constant rate during the first eight minutes, while those from reserpine-pretreated animals did so only for the first two minutes. After the infusion was stopped, there was a multiphasic exponential decrease in the rate of tritium loss from the heart. Four minutes after the infusion was stopped, nearly half of the tritium in the perfusate was present in the form of metabolic products of Nor. While normetanephrine was the major product in the normal hearts, reserpinized hearts mostly produced deaminated products. Twelve minutes after the infusion, the hearts of the untreated rats retained 9.8 per cent of the infused [H^3]-Nor, while those removed from reserpine-treated animals retained only 0.8 per cent. Because of this the authors suggested the presence of at least two pools

of bound Nor, one easily released, which is preferentially metabolized by O-methylation, and one more firmly bound (reserpine-depleted), which is deaminated without becoming active.

In order to find out whether there is a difference between the metabolism of circulating and bound labelled Nor, Kopin & Gordon (72, 73) studied *in vivo* the difference in the fate of Nor released by tyramine and by reserpine. Rats were injected intravenously with about 8 μg./kg. of [H^3]-Nor and their urine collected at various time intervals. Total radioactivity and the isotope, present as various metabolites in the urine samples, were assayed. At least two phases of isotope excretion were apparent. Following an initial rapid excretion of radioactivity, the rate of excretion became slower; and after about five hours this rate decreased exponentially, in a single phase, with a half life of about six hours. During the three hours following [H^3]-Nor administration, about two thirds of the amine was excreted. Unchanged Nor accounted for about 23 per cent and normetanephrine represented a total of about 29 per cent; about 37 per cent of the radioactivity was excreted as the deaminated O-methylated products. The metabolites present in the urine collected during the 10- to 13-hour interval after the injection of [H^3]-Nor were taken to represent the products of Nor which had been bound and were slowly being released and metabolized. During this period only 4 per cent of the administered radioactivity was excreted. Nor and normetanephrine accounted for a total of 18 per cent of the excreted radioactivity, but the deaminated O-methylated products represented over 60 per cent and the deaminated catechols about 7 per cent. The authors concluded that the metabolites excreted during the first phase indicate that O-methylation is the major route of metabolism of unbound or easily released Nor during the second phase, the [H^3]-Nor, which had been bound and slowly released, seemed to be metabolized primarily by monoamine oxidase. The animals excreted tritium more rapidly after tyramine (four doses of 10 mg./kg. I.V. at 15-minute intervals) and even more so following reserpine (2.5 mg./kg. I.P.). While more Nor and normetanephrine were found in the urine of tyramine-treated animals, the deaminated compounds showed the greatest increase following reserpine. The authors concluded that tyramine releases bound Nor directly into the circulation, mostly as free Nor, and that only a small part is metabolized in the tissue prior to the release by O-methylation. Reserpine, on the other hand, is much more effective in releasing catecholamines from the tissues, but metabolism proceeds mainly by deamination. Pretreatment of the rats, however, with a monoamine oxidase inhibitor (pheniprazine 10 mg./kg. I.P.) changed the pattern of metabolism, and almost all the reserpine-released radioactivity reached the systemic circulation as [H^3]-Nor or normetanephrine-H^3.

Drugs Affecting Adrenergic Mechanisms

Sympathomimetic amines.—It is clear that biochemical studies at the cellular level are crucial for the final analysis of drug action, and it is most

encouraging to see the progress already achieved in the area of biochemical pharmacology. In attempting to review the field synoptically, however, I feel that certain reservations must be placed on record.

The discovery that many drugs can influence the storage and uptake of the catecholamines has quite understandably aroused a great deal of interest. But there is now an almost compulsive desire to demonstrate, for all drugs which affect structures innervated by sympathetic nerve fibres, a drug effect in terms of either uptake or storage of adrenergic transmitter. However, only in a limited range of cases has there been any general agreement. One of the major causes of the varying results is that in this effort, the dose levels used have been very often well in the toxic range. Potter & Axelrod (99), for example, studied the effect in rats of large doses of various sympathomimetic amines on the storage of Nor. Thirty minutes after the animals had received approximately 1 μg. of [H^3]-Nor intravenously, the sympathomimetic amine under study (36 μM/kg.) was administered intramuscularly; two hours later the animals were killed, and their hearts assayed for both radioactive and endogenous Nor. The results, some of which are included in Table I, show that the degree of release obtained varied from considerable to negligible and that there was no clear relationship between releasing action and specific structure. Davey & Farmer (40) also attempted to correlate the sympathomimetic effects of tyramine, amphetamine, and ephedrine with changes in the Nor content of the isolated guinea-pig heart. A 100μg. dose of each amine was injected into the cannula close to the heart every five to ten minutes until no positive inotropic effect was observed. The absence of an inotropic response with tyramine was associated with a 50 per cent reduction in Nor content; in contrast to Potter & Axelrod's results, amphetamine produced no change, while after ephedrine there was a 50 per cent increase in the Nor concentration. In Table I the doses used by Potter & Axelrod for demonstrating effects on Nor stores are compared with LD_{50} doses as well as with doses producing pressor changes in the rat; furthermore the doses used by Davey & Farmer are compared with those which are known to stimulate the isolated heart of the guinea-pig. In both instances the doses claimed to alter Nor stores are well above those used in pharmacological analyses and are very often near or inside the toxic range. Such contradictory results are very confusing and make it clear that until serious attempts are made to correlate biochemical data with functional changes at dose levels within the pharmacological range, little is to be gained. When effects of various sympathomimetic amines on catecholamine stores are being discussed, it is useful to remember how small are the amounts which represent the upper limit of amine secretion in the intact animal. For example, in the cat strong reflex excitation of the vasomotor centre causes the release of 0.2–0.5 μg/kg./min. of catecholamines (70), and in prolonged asphyxia, which appears to be the most powerful physiological stress, only 1–2 μg./kg./min. are discharged (34, 123). Intense pain-fibre stimulation in lightly anaesthetized cats causes the release of 0.6–0.7 μg./kg./min. (123). From these results, Folkow (53) has reached the

TABLE I

A COMPARISON OF EFFECTIVE AND TOXIC DOSES OF SEVERAL SYMPATHOMIMETRIC AMINES WITH DOSES USED FOR DEMONSTRATING EFFECTS ON CATECHOLAMINE STORES

	RAT					GUINEA-PIG (*isolated perfused heart*)		
	Pressor dose μg./kg. *I.V.*	*Toxicity* μg./kg.	*Effect on Nor content (Heart)*			*Cardiostimulant dose (Total) in* μg.	*Effect on Nor content (Heart)*	
			Dose μg./kg. *I.M.*	*% change Nor* [H^3]*-Nor*			*Dose (Total) in* μg.	*% change*
l—adrenaline	0.05	40 I.V. 3500 I.M. *2500–4500 I.P.*				0.1–1		
d—adrenaline (about 4 times less active and less toxic)			6600	+64	−67			
tyramine	0.5		4900	−31	−37	10	3000	−50
dl—amphetamine } very *d*—amphetamine } similar	0.5–2.5	30,000 I.P.	4900	−20	−37	100–200	1200	no change
l—ephedrine } very *dl*—ephedrine } similar	0.5–5	70,000 I.V. 165,000 I.P.	5900	−15	−21	*50–100*	800	+50

conclusion that a rapid injection of 1 μg. of adrenaline or Nor administered intravenously to an average-sized cat is, from a physiological point of view, a very large dose, creating a peak concentration which in the intact animal might possibly be reached only in extreme stress.

A comparison of the cardiac and vascular effects of the most commonly used pressor agents (Table II) shows that the various sympathomimetic amines may cause changes in blood pressure by altering either vascular tone or cardiac output or both. For example, the pressor responses elicited by methoxamine and phenylephrine are almost entirely due to vasoconstriction, those of ephedrine and hydroxyamphetamine mostly to their cardiac stimulant action, and those of methamphetamine and mephentermine almost entirely to cardiac stimulation. On the other hand, whereas Nor, metaraminol, ephedrine, and hydroxyamphetamine bring about cardiac stimulation and vasoconstriction, methamphetamine, and mephentermine are local vasodilators. That ephedrine acts more prominently on the heart than on the peripheral vessels and that its direct myocardial effects are the major factors in the cardiovascular responses the drug produces were well demonstrated by Chen & Schmidt (35). Amphetamine and ephedrine produce in man variable changes in peripheral resistance, and a fall in diastolic pressure is apparently not unusual (59). Moreover, methamphetamine has been shown to dilate blood vessels in both dog and man [for references, see (4)]. Nor, on the other hand, is a pure vasoconstrictor substance and therefore produces a rise in both systolic and diastolic blood pressure in man and animals. Ginsburg & Cobbold (58) demonstrated in man that Nor exerts purely constrictor effects; slow intravenous and intra-arterial infusions caused constriction by either route, and after Dibenyline this response was reduced or abolished but not reversed in any instance. Gaffney, Morrow & Chidsey (56) found that in dogs with chronically denervated hearts, the positive inotropic and chronotropic as well as the pressor responses to tyramine were markedly reduced. Their results showed, however, that in the normal dog a large component of the pressor effect of tyramine is produced by an increase in cardiac output, mediated by an increase in the force of heart contraction and the heart rate; an intravenous injection of 100 μg./kg. of tyramine produced in the control dogs an increase of 132 per cent in the contractile force, 25 per cent in heart rate and 42 per cent in mean arterial pressure.

The importance of venous tone in the regulation of the circulatory system is gaining increasing recognition. Sharpey-Schafer & Ginsburg (107) demonstrated that in man both intravenous and intra-arterial infusions of adrenaline caused marked constriction of the veins, and that the action of adrenaline on the arteries preceded that on the veins by about ten seconds. The authors concluded that the increased stroke output of the heart after 45 seconds following intravenous adrenaline depends more on peripheral venous constriction than on the direct effect on the heart. Nor also constricted veins, but its action was rather slower than that of adrenaline. Zimmerman, Abboud & Eckstein (137) compared the effects of several sympathomimetic

TABLE II[a]

Comparative Effects of Some Sympathomimetic Amines and Their Corresponding Effective Doses in Man

	Dose in mg.	Heart				Vascular Effects			Major Features in the Intact Animal and Man
		Rate		*Force of contraction*		*External iliac*	*Renal*	*Pulmonary*	
		Reflex activity		*Small doses*	*Large doses*				
		Normal	*Blocked*						
adrenaline (epinephrine)	0.5–1 S.C.	+ or −	+	+	+	c and d	c	c	Cardiac stimulation and ability to constrict *and* dilate blood vessels
noradrenaline (levarterenol)	2–8 μg./min., I.V. infusion	−	+	+	+	c	c	c	Pressor responses entirely due to vasoconstriction (unless reflex pathways blocked, when increased output may contribute)
metaraminol	5–10 I.V., I.M., S.C.	+ or −	+	+	depressed	c	c	c	As noradrenaline
phenylephrine	0.5–1 I.V., 5–10 S.C.	−	0	0	+	c	c	c	Pressor responses entirely due to vasoconstriction
methoxamine	5–10 I.V., 10–20 I.M.	−	0 or −	0	depressed	c	c	variable	As phenylephrine
ephedrine	15–50 S.C., I.M.	+ or −	+	+	depressed	c	c	c	Cardiac stimulant action more conspicuous than the vasoconstrictor action (in man in particular)
hydroxyamphetamine	5–10 I.V., 10–20 S.C.	+ or −	+	+	depressed	c	c	c	As ephedrine
methamphetamine	10–30 I.V., I.M.	+ or −	+	+	depressed	d	variable	d	Pressor responses chiefly by cardiac stimulation (in spite of initiating vasodilatation)
mephentermine	10–30 I.V., I.M.	+ or −	+	+	depressed	d	variable	d	As methamphetamine

0 = no effect; + = increased; − = decreased; c = constrict; d = dilate.

[a] This table (excluding doses) was compiled from a review article by D. M. Aviado (4).

amines upon the total vascular resistance and upon venous resistance in the perfused foreleg of the dog and found that the average percentage contribution of increments in venous resistance to increments in total resistance is as follows: noradrenaline 13.8; tyramine 8.0; metaraminol 7.2; ephedrine 3.3; mephentermine 1.9; phenylephrine 1.8; methoxamine 1.4. It appears, therefore, that for a given increase in total resistance, the venoconstrictor action of Nor was significantly greater than that of the other six amines. The most interesting finding was the differential ability of the sympathomimetic amines to constrict the veins of the foreleg; the increments in venous resistance were markedly different despite similar changes in total resistance. Zimmerman et al. concluded that sympathomimetic amines such as phenylephrine and methoxamine which were found to have a very weak venoconstrictor action and which are known to have relatively little positive inotropic effect would not be expected to improve cardiac performance.

Burn & Rand (23, 24), however, put forward the hypothesis that the cardiovascular effects of ephedrine, amphetamine, methamphetamine, and tyramine are indirect only and depend on the release of Nor from tissue stores. It is very difficult to see how the many pharmacological differences between the actions of these drugs can be adequately explained by such a single interpretation. Changes in systemic blood pressure have been extensively used by many workers, including Burn & Rand, in their attempts to find a correlation between the pressor effects of various sympathomimetic amines and their ability to alter catecholamine stores. Blood pressure changes, however, are as a rule the result of a combination of actions on several sites: actions on various vascular beds, direct effects on the heart, reflex actions from the vasosensory zones, central actions etc. Furthermore, slight constrictor action on the venous tree will mobilize a rather significant extravenous return to the heart. For all these reasons, blood pressure changes alone are a poor indication of what might be going on within the circulatory system.

Burn & Rand's conclusions on the "indirect" actions of ephedrine, amphetamine, methamphetamine and tyramine are mainly based on experiments on animals pretreated with very large doses of reserpine. Dogs were given 0.5 mg./kg., cats 2.5–5 mg./kg. and rabbits 3 mg./kg. of reserpine intraperitoneally on two successive days. Reserpine, however, is a very toxic drug, especially for the cardiovascular system. With doses of the order of 1 mg./kg. the heart goes into a most spectacular failure (134), and the sensitivity of the peripheral vessels to adrenaline and Nor is decreased (131). Moreover, histological examination of hearts removed from animals pretreated for one or several days with a total dose of reserpine of 1–1.25 mg./kg. revealed pronounced degenerative changes (134). The majority of muscle fibres were reduced in diameter and in their content of stainable sarcoplasm. Myofibrils well-preserved in some fibres were disorganized in others (106). Innes & Krayer (68) found that in dogs large doses of reserpine greatly impair the force of heart contraction; and Nayler (94) demonstrated that reserpine may exert a direct effect on the contractility of toad ventricular muscle,

apart from that which can be explained in terms of depleted catecholamine stores. All these results indicate that reserpine has a remarkable toxicity on the myocardium; and therefore, animals pretreated with large doses of this drug must be considered as most unreliable experimental tools. Large doses of all pressor amines except adrenaline and Nor are known to depress in both man and animals the force of heart contraction without, as a rule, inhibiting the activity of the sino-atrial node [for references, see (4)]. Large doses of ephedrine may even cause myocardial depression without evidence of preceding stimulation (35). It is quite possible, therefore, that in animals whose hearts are failing because of reserpine pretreatment, the same sympathomimetic amines produce a depression of heart contraction in doses which stimulate the normal heart. We have already demonstrated this for tyramine (131). In control animals, the administration of 1 mg. of tyramine was accompanied by a large rise in blood pressure and a marked increase in the force of cardiac contraction. In cats treated with 1 mg./kg. of reserpine, tyramine depressed cardiac contraction, and the rise in blood pressure was markedly reduced. After ouabain, however, the depression of cardiac contraction produced by tyramine was prevented and the rise in blood pressure increased. In general it was found that, in animals pretreated with large doses of reserpine, any improvement of the circulation was almost exclusively the result of an ameliora tion in the force of cardiac contraction.

There is, however, no need to use large doses of reserpine to reduce the Nor content of various peripheral tissues. In rabbits (65) and in guinea-pigs (38), 0.1 and 1 mg./kg. of reserpine produced, after 24 hours, almost complete depletion of the catecholamine stores; and in rats (27), 0.1 mg./kg. produced, after 6 hours, 96 per cent Nor depletion in the heart. My colleague, Michael Cymbalist, found that the Nor content of the atria, ventricles and spleen was almost nil in cats which received daily 20 μg./kg. of reserpine for two weeks. In the same animals the catecholamine content of the adrenals fell by about 60 per cent. Muscholl & Vogt (92) showed that daily doses of 0.1 mg./kg. were as effective as single large doses in depleting sympathetic ganglia of rabbits of their Nor, and in addition produced a highly significant effect on the adrenaline content of the ganglia. This was confirmed by Reinert (102), who found that in the superior cervical ganglia of rabbits there was little, if any, difference in the amine-depleting effect between 0.25 and 2.5 mg./kg. of reserpine. Weiner & Trendelenburg (127) found that the Nor content of the nictitating membrane fell to very low levels after one or two injections of 3 mg./kg. of reserpine (24 and 48 hr. prior to extraction) as well as after seven days of pretreatment with 0.1 mg./kg. per day. Similarly in Fleming's (51) experiments, the supersensitivity to Nor of the nictitating membrane of cats which received 0.1 mg./kg./day of reserpine for seven days was almost the same as that in a second group of animals which received 1.0 mg./kg. on the first day, 0.3 mg./kg. on the second and 0.1 mg./kg. for the next five days. It is quite obvious that doses of up to 0.1 mg. are sufficient to deplete peripheral tissues of their catecholamine content. The advantages of using minimal doses of reserpine are many: the general condition of the ani-

TABLE III

THE EFFECTIVENESS OF TYRAMINE ON PERIPHERAL CATECHOLAMINE STORES

REFERENCE	TYRAMINE		ASSAY		
	Species	*mg./kg.*	*in*	*of*	*% change*
(126)	dog	4–20 I.V.	Plasma adrenal venous blood	Nor Nor	Slight release 0
(61)	dog	200 μg./kg. /min for 1 hr.	atrial appendage	Nor	−30
(99)	rat	4.9 I.M.	heart	Nor [H³]-Nor	−31 −37
(100)	rat	10 I.V. (3 doses)	heart	CA [H³]-Nor	−58 −62
(126)	rat	100 S.C.	heart spleen	Nor Nor	−50 −50
(7)	rat	20 I.M.	ventricles	Nor	−36
(73)	rat	10 I.V. (4 doses)	urine	[H³]-Nor	+36
(40)	guinea-pig (isolated perfused heart)	3 mg. total	heart	Nor	−50
(42a)	fowl's adrenal tissue slices	1 mg./ml.			Slight release

mals is reasonably good; the adrenal medulla, in contrast to other peripheral tissues, is not markedly depleted; and the cardiovascular system, although affected, does not collapse.

Tyramine.—During the last few years, tyramine has been extensively used as a pharmacological tool for the study of processes taking place at the adrenergic synapse. Marked differences, however, exist between both the results obtained and the interpretations given to them. For example, it is still debatable whether tissue catecholamines are depleted by tyramine or whether the cardiovascular effects of tyramine are entirely indirect through the liberation of Nor from peripheral tissue stores. The overall results indicate (Table III) that even large doses of tyramine produce a limited decrease

in Nor stores. It is possible, therefore, that smaller, pharmacological doses do not produce a significant depletion at all.

Weiner, Draskóczy & Burack (126) found that in intact dogs tyramine, in doses producing marked increases in blood pressure (4–20 mg. I.V.), failed to release catecholamines from the adrenals; and they concluded that the observation (105) that tyramine accelerates the release of catecholamines from isolated adrenal chromaffin granules appears to be unrelated to the action of the drug *in vivo*. After phenoxybenzamine administration, the catecholamine levels in adrenalectomized dogs were moderately increased by tyramine. However, this appeared to be unrelated temporally to the pressor responses because the maximal plasma catecholamine increase occurred about 10 to 15 minutes after tyramine administration. Weiner and his colleagues had to admit, therefore, that their experiments failed to establish conclusively whether tyramine releases Nor directly from nerve endings. That the ability of guanethidine and tyramine to deplete Nor stores is very weak compared with that of reserpine was well demonstrated by Porter, Totaro & Stone (98). The concentration of Nor in the mouse heart was measured 16 hours after the administration of reserpine and guanethidine and one hour after tyramine. The following values represent effective concentrations of the three drugs in the tissue, in mg./kg. body weight dosage units, when the hearts were half depleted of Nor: reserpine 0.05; guanethidine 4.0; tyramine 28.5. Porter et al. showed also that after the administration of equipotent doses of the three compounds, heart Nor returned to normal at widely varying rates.

	mg./kg. (S.C.)	Time required for Nor concentration to return from 90 per cent to 10 per cent depletion.
Reserpine	0.17 and 0.5	20.6 days
Guanethidine	6.7 and 20	6.3
Tyramine	50 and 100	0.7 days

Potter & Axelrod (99) gave tyramine (10 mg./kg. I.M.) to rats at various time intervals (5 min. to 48 hr.) after the animals had received [H^3]-Nor (about 1 μg. per rat I.V.). This dose of tyramine, although large, reduced the average endogenous Nor level from 0.83 to only 0.61 μg./g. heart (about 27 per cent) and produced a quantitatively similar depletion of radioactive stores. In addition the specific activity of the depleted Nor and the specific activity of the tyramine-resistant store were determined. Reserpine (5 mg./kg. I.M.) was administered at several time intervals after the administration of [H^3]-Nor, and the animals were killed 45 minutes later. After reserpine, the specific activities of depleted and retained Nor were approximately equal. In contrast, after tyramine the specific activity of the released Nor was different from that of the remaining store. The authors concluded that there must be two bound forms of Nor. Tyramine releases Nor from a *small store* which initially contains a large fraction of the total bound [H^3]-Nor. This store has a

rapid turnover with a half life of several hours. Since the specific activity of the *larger* tyramine-resistant store increased during the first 30 minutes, the period when the smaller store had its higher specific activity, it was concluded that [H^3]-Nor was being transferred from the smaller to the larger store. After 30 minutes, the tyramine-resistant store showed a continuous decline in specific activity, approaching a constant half life of about one day. As the Nor liberated by reserpine had the same specific activity as that remaining in the heart after reserpine, the authors concluded that reserpine releases Nor from both the tyramine-releasable and tyramine-resistant stores.

Attempts have also been made to explain the phenomenon of tachyphylaxis in terms of Nor depletion. Potter, Axelrod & Kopin (100) measured blood pressure in rats during three successive injections of tyramine, each of 10 mg./kg. I.M. After the third injection, there was little or no blood pressure rise, yet considerable amounts of the catecholamine were present in the heart (about 42 per cent). It was concluded that the reduced response to tyramine is the result of the depletion of bound catecholamines easily available for release. Axelrod et al. (5) studied the action of tyramine in the isolated and perfused rat heart and reported that "with successive injections of tyramine the amount of labelled catecholamine released fell progressively, and there was a parallel decrease in the increment of amplitude and rate of contraction of the heart." However, the parallelism between release of [H^3]-Nor, heart rate, and heart contraction, is not well substantiated by the records which accompany the paper. Seventeen doses of 10 μg. of tyramine were administered at five-minute intervals. After the first five doses, there were marked increases in heart rate (from about 180 to over 250 beats/min.) which became progressively smaller with subsequent doses. The heart rate at the end of the experiment, however, was still at about the same level as before the administration of tyramine. In contrast, the amplitude of contraction, which increased moderately after the first two doses of tyramine, decreased markedly and progressively with subsequent doses. It is obvious that doses of tyramine which produce such a marked depression of the myocardium are not really suitable for a discriminating pharmacological analysis.

The complexity of the problem is very well portrayed in the following results: Harrison, Chidsey & Braunwald (61) found in dogs that the cardiovascular responses (blood pressure, force of heart contraction, heart rate) to tyramine (0.5–200 μg./kg.), amphetamine (100 μg./kg.), and tryptamine (100 μg./kg.) were potentiated for a period of three hours immediately after the administration of reserpine (3 mg./kg. I.V.). Reserpine, however, did not potentiate, during the same period, the responses to injected Nor (0.25 and 0.5 μg./kg.). Measurement of Nor released into the coronary sinus blood showed that tyramine consistently produced a greater release of Nor following the administration of reserpine, although reserpine alone did not induce significant changes. It was concluded, therefore, that reserpine potentiates the action of sympathomimetic amines by increasing their ability to release

Nor and not by releasing additional Nor. An infusion of tyramine (200 μg./kg./min. for one hour) following reserpine reduced the Nor concentration in the auricle by 35 per cent, an effect which did not differ significantly from that measured without reserpine. Because of this result, it was concluded that reserpine did not increase the size of the Nor store available for release by tyramine, and the suggestion was made that the augmented release of Nor by tyramine observed following reserpine may be due to the fact that reserpine interferes with the entry of Nor into the physiological compartments in the nerve endings. Bhagat & Shideman (8) found that in rats guanethidine (10 mg./kg.) injected intravenously inhibited the pressor action of tyramine (50 μg. I.V.) and amphetamine (60 μg. I.V.), but potentiated the action of Nor (0.2 μg. I.V.). The heart catecholamine concentrations, however, were not significantly altered. Similar results were obtained in the isolated atria. Bhagat (7) studied the influence of guanethidine on the catecholamine-depleting action of tyramine in the rat heart. The animals were given an intravenous injection of 5 mg./kg. of guanethidine 20 minutes before an intramuscular injection of tyramine (20 mg./kg.). Catecholamine determinations in the ventricular myocardium showed that guanethidine had no effect on the depleting action of tyramine. Bhagat concluded that the observation of Lindmar & Muscholl (83), that guanethidine significantly reduces the tyramine-induced release in the isolated perfused rabbit heart, appears to be unrelated to its pharmacological action in the intact animal. Campos & Shideman (29) separated by differential centrifugation homogenates of dog heart into three fractions, "coarse," "particulate" and "soluble," and showed that in the reserpinized animal, the uptake of infused Nor was greatest in the "soluble" and least in the "particulate" fraction. In a further investigation Campos et al. (30) attempted to discover whether tyramine exerts its stimulatory effect primarily through release of catecholamines from stores of Nor in the "soluble" fraction of the cell. In the isolated perfused cat heart, there was a continuous decline in the concentrations of the catecholamine which could be attributed to their continuous release from storage and removal by the perfusion fluid. The decline was more pronounced in the "soluble" than in the "particulate" fraction, which suggested to the authors the existence of at least two pools which differed in the ease with which their stores could be mobilized. The authors concluded, however, that the two fractions, the "particulate" and the "soluble," are not identical with the two cellular pools postulated by Potter et al. (100). While 1 μg./ml. of tyramine produced a significant depletion of catecholamine from both "soluble" and "particulate" fractions, the depletion of the "particulate" fraction increased with concentrations of 10 and 100 μg./ml. Cocaine had a biphasic effect on catecholamine levels. Low concentrations (10 μg./ml.) induced significant depletion of catecholamines; the depletion, however, was not accompanied by any cardiostimulant activity. Higher concentrations (20 and 40 μg./ml.) of cocaine prevented the spontaneous loss of amines which occurs during perfusion and antagonized the depleting action of tyramine. Thus a reversible, dose-

dependent antagonism between the two drugs was observed. On the other hand, Weiner & Trendelenburg (128) found that neither cocaine nor pretreatment with reserpine blocks the immediate uptake of adrenaline and tyramine into the tissues. The total radioactivity of blood, heart, and spleen was measured in rats two minutes after the intravenous injection of either 20 μg./kg. of labelled adrenaline or 100 μg./kg. of labelled tyramine. After cocaine (20 mg./kg. I.V.) adrenaline produced a higher level of radioactivity in plasma than in control preparations, and this was accompanied by an increased pressor response. On the other hand, pretreatment with reserpine (3 mg./kg. I.P.) failed to cause supersensitivity to adrenaline and also failed to cause an increased level of radioactivity in the plasma. This is in contrast to results reported by Hertting et al. (64) who observed increased levels of [H^3]-Nor in the plasma of cats pretreated with reserpine. The pressor response to tyramine in Weiner & Trendelenburg's experiments was reduced by pretreatment with either cocaine or reserpine. Finally, after cocaine or reserpine, the tyramine-induced radioactivity of the heart did not differ from that of the control animals. These results contradict those of many other workers, a fact which Weiner & Trendelenburg explain by saying that in their experiments the uptake of catecholamines was determined two minutes after the injection of the labelled pressor amines, while in the other studies the catecholamine uptake was measured not earlier than 25 minutes and sometimes two hours later.

Several authors obtained results which are difficult to reconcile with the view that the action of tyramine is entirely the result of catecholamine liberation from tissue stores. In spinal cats during an infusion of Nor at a rate of 10 μg./min., Nasmyth (93) administered single injections of adrenaline, Nor, and tyramine. The results showed that, whereas the pressor response to tyramine was potentiated, those to adrenaline and Nor were slightly depressed. Doubling the infusion rate further increased the response to tyramine, whereas the responses to adrenaline and Nor were further depressed. When the infusion was stopped, the blood pressure immediately fell, and the response to tyramine quickly returned to its pre-infusion level, but the response to Nor and adrenaline remained depressed. In addition, Nasmyth tested in pithed rats pressor responses to intravenous injections of 20 ng. of Nor, 20 μg. of tyramine and 25 ng. of adrenaline before and during a continuous infusion of Nor (5 μg./kg./min.). Once again the response to tyramine was potentiated, but those to Nor and adrenaline were depressed. Similarly Farrant (47) found that in spinal cats Nor infusion at the rate of 2.75 μg./min. increased the pressor response to a single injection of Nor, but that higher rates depressed those to both adrenaline (5 μg.) and Nor (2 μg.). The responses to tyramine (200 μg.), however, increased up to an infusion rate of Nor of 11 μg./min. The effects produced by adrenaline infusions were very similar. Thus, an increase in the rate of infusion of either adrenaline or Nor progressively decreased the pressor responses to single injections of the two catecholamines but increased the responses to tyramine. Lindmar & Mus-

choll (83), using the isolated rabbit heart, found that tyramine (9–60 μg./ml.) increased rate and force of contraction and raised the Nor output. Doses of DMPP (1,1-dimethyl-4-phenyl-piperazinium iodide), however, which increased the rate by 67 per cent, liberated 6.7 times as much Nor as doses of tyramine, which increased the rate by 61 per cent. This was considered as an indication that tyramine "enhanced the action of Nor," apart from liberating it. Varma & Benfey (119) demonstrated that in spinal cats pretreatment with methyldopa (150 mg./kg. injected two days and 50 mg./kg. injected one day before the experiment) did not alter the cardiovascular responses to tyramine, or the effects of stellate ganglion stimulation. In contrast, reserpine (1 mg./kg.) injected intraperitoneally the day before the experiment reduced the pressor and inotropic effects of tyramine (1 mg./kg.) and the effects of the stimulation of the stellate ganglion on cardiac contraction. In animals treated first with methyldopa, followed by reserpine, the cardiovascular effects of tyramine were almost normal, although the response to stellate ganglion stimulation was still reduced. Similar effects were obtained with the isolated papillary muscle. The authors thought that the fact that methyldopa did not interfere with the effect of reserpine on the stimulation of the stellate ganglia was a good indication that reserpine had diminished the tissue catecholamine content; they concluded, therefore, that tyramine has a direct action. Angelakos & Torchiana (2) compared the inotropic effect which tyramine produces on isolated rabbit atria with its effect on catecholamine stores. During a continuous perfusion with tyramine (100 μg./ml.) there was a gradual decrease lasting several hours in the positive inotropic response. Changes in the inotropic response were compared with Nor concentrations at various time intervals. After 30 minutes the differences in Nor content between the controls and the atria perfused with tyramine were not statistically significant. Following longer exposure, the differences were quite definite. It was found, however, that the positive inotropic response was greatly reduced or completely abolished at a stage when the tissue content of Nor was still about 50 per cent of normal. In general, the results indicated that a progressive decrease in the tyramine response was associated with a reduction in Nor content; however, a strict correlation could not be found, and the authors concluded, therefore, that the results did not provide any direct proof that tyramine acts by releasing Nor from tissue stores.

Although it is generally said that, in animals pretreated with reserpine, Nor restores the responses to tyramine and to nerve stimulation, restoration of Nor stores has not been observed by all workers. Gaffney, Morrow & Chidsey (56) examined in dogs the contribution of cardiac catecholamines to the cardiovascular effects of tyramine and found that in both reserpinized and cardiac denervated dogs, the pressor responses to tyramine (3–100 μg./kg.), as well as the force of heart contraction and the heart rate, were reduced. A 30-minute infusion of 5 μg./kg./min. of Nor in the reserpine-treated dogs potentiated all three responses to tyramine and increased the myocardial Nor to approximately 30 per cent of normal. In contrast, in the cardiac

denervated dogs, only the pressor response was increased. It was concluded, therefore, that most of the inotropic and chronotropic effects of tyramine observed in the intact dog are the result of the release of only myocardial catecholamine and that the Nor content of the heart of animals pretreated with reserpine can be partially repleted by a prolonged infusion of high concentrations of Nor. On the other hand, Muscholl (90) suggested that the restoration of the tyramine effect in reserpine-treated animals following Nor infusion does not depend on the replenishment of the Nor stores. He found in his experiments that the concentration of Nor in the normal rat was considerably increased by an infusion of 1–20 μg. of Nor and that 40 minutes after the end of the infusion, the Nor concentration of the heart was still above its normal level. However, in reserpine-treated rats, a similar infusion of Nor did not produce any increase in the Nor concentration although the pressor action of tyramine (8–20 μg.) (which was markedly reduced after reserpine 5 mg./kg.) was restored by the infusion. Moreover, Luduena & Snyder (84) found that in spinal cats pretreated with 1–3 mg./kg. of reserpine, a slow intravenous infusion of *d*-Nor (2.5–5 mg./kg.) greatly increased the effect of tyramine (1 mg./kg.) on the nictitating membrane but left the blood pressure and heart rate responses almost unchanged. They concluded that their results cannot be explained by a replenishment of the Nor stores with *d*-Nor because the sympathomimetic activity of this amine is too low. Finally, Crout, Muskus & Trendelenburg (38) studied the effect of tyramine on isolated guinea-pig atria in relation to their Nor stores and showed that any given dose of reserpine produced a proportionately greater reduction of Nor stores than of the response to tyramine. Moreover, it was found that a substantial depletion of the Nor store had to be achieved before the response to tyramine began to decline. When the Nor stores were 50 per cent of normal, the response to tyramine was only slightly reduced, and 50 per cent reduction of the response to tyramine did not occur until the Nor content was approximately 10 per cent of normal. After exposure of the atria to 1 μg./ml. Nor, which produced nearly 80 per cent of the normal response, tyramine was still ineffective. A further increase in the Nor concentration (3 μg./ml.), however, partly restored the response to tyramine. The authors argued that if the restoration was due to a simple refilling of the Nor stores, then a Nor content of 10 to 15 per cent of normal should be expected. However the observed content of Nor following refilling was only 2.2 per cent of normal. In order not to abandon the concept of an indirect action of tyramine, it was suggested (*a*) that the Nor stores consist of two compartments, the smaller of which is important for the action of tyramine, and (*b*) that this smaller compartment can be at least refilled by exposure to a high concentration of Nor. Crout et al. admit, however, that their experiments do not give any indication of the functional importance of the store by which the Nor was taken up.

Guanethidine.—A great number of workers have shown that guanethidine can reduce the Nor content of peripheral tissues. However, as Table IV shows, significant depletion occurs only with large doses of guanethidine, and

even then only after two or more hours. In contrast, similar doses of guanethidine abolish the effects elicited by stimulation of sympathetic nerves within a few minutes. It seems unlikely, therefore, that the two events are related. For example, Sanan & Vogt (104) found that in both rabbits and cats

TABLE IV

THE EFFECTIVENESS OF GUANETHIDINE ON DEPLETION OF PERIPHERAL CATECHOLAMINE STORES

REFERENCE	GUANETHIDINE		CATECHOLAMINE ASSAY		
	Species	*Dose mg./kg.*	*Tissue*	*Time after guanethidine hr.*	*% depletion*
(89)	guinea-pig (daily for 4 days)	0.5 I.P. 1.0 2.0	heart		9.5 32 62
(8)	rat	10 I.V. 25 I.M. (2 days)	heart		not significant almost complete
(33)	rat	15 S.C.	heart	1 2 6	20 30 80
(9)	rat	25 I.M.	heart		32
(76)	rat	25 I.V. 35 I.V. 150 I.P. 200 I.P. 400 I.P.	heart adrenals	0.5 1 2 4	11 22 57 90 0 10 53
(104)	cat rabbit	15 I.P. 15–20 I.V.	sympathetic ganglia 	2–4 2–4	40 65
(1)	cat	6–10 I.V.	postganglionic nerve fibres adrenals		no release no release
(3)	dog	1–10 I.V.	adrenals		no release

after guanethidine (15–20 mg./kg.), the loss of transmitter in sympathetic ganglia was too small and too slow to explain the failure of sympathetically innervated tissue to respond to electrical stimulation of its nerves; only about half of the ganglionic Nor was lost in the course of two to four hours, a loss which is not sufficient to cause functional failure (92). Cass & Spriggs (33) also attempted to correlate the rate of depletion produced by guanethidine with the onset of sympathetic blockade. Sympathetic function was assessed by measuring the pressor response to a standard intravenous dose of eserine, a method introduced by Lesić & Varagić (80). Guanethidine (15 mg./kg. S.C.) abolished this response immediately; the effect lasted up to six hours. The Nor levels in spleen, heart, and intestine were greatly reduced; but the effect, unlike that on sympathetic function, was apparent at one hour, and reached a maximum of 80 to 90 per cent depletion at 6 to 18 hours; only after 48 hours had some recovery occurred. The authors concluded, therefore, that as the rate of onset of sympathetic block did not parallel the rate of depletion of peripheral Nor, no obvious relationship can exist between Nor levels and the immediate blocking action of guanethidine. Falck (46) used a fluorescence reaction to examine in rats the effect of 1 to 30 mg./kg. of guanethidine administered subcutaneously. It was found that 24 hours after a dose of 1 mg./kg., the intensity of the fluorescence was normal and was only reduced at the higher dose levels. That adrenergic-blocking doses of guanethidine do not produce any significant depletion was also demonstrated by Niki Metaxas in our department. Three groups of guinea-pigs were injected for four days with 0.5, 1 and 2 mg./kg. of guanethidine daily. The catecholamine content of the adrenals remained unchanged; that of the heart was reduced by 9.5 per cent, 32 per cent, and 62 per cent respectively. The effects of guanethidine on the adrenal medulla were also studied by Athos, McHugh, Fineberg & Hilton (3). Denervated adrenals of dogs were perfused *in situ* with arterial blood obtained from donor dogs. Guanethidine in a dose of 10 μg./min., added for periods from 20 to 41 minutes neither had direct stimulatory action nor produced reflex stimulation. Furthermore, the intravenous administration of 1–10 mg./kg. to intact animals produced a distinct rise in blood pressure but was without effect on the Nor secretion from the adrenal glands. There was, however, a decrease of over 50 per cent in the secretion of adrenaline. It was concluded, therefore, that adrenal medullary catecholamines do not play a direct role in the acute pharmacological action of guanethidine. Similarly, Weil-Malherbe & Posner (125) found that guanethidine (60–120 μg./ml.) did not release adrenaline from rabbit adrenal granules. Herbeuval & Masse (62) compared the action of guanethidine in normal and hypertensive rats. The drug, dissolved in the animals' drinking water, was administered for four weeks. Five mg./kg. were given during the first two weeks and 10 mg./kg. during the last two. The blood pressure of the hypertensive rats fell from 165 mm. Hg to 110, but no change in pressure was seen in the control animals. In both groups, however, the levels of catecholamine excreted in the urine remained unchanged.

Hertting, Axelrod & Patrick (63) reported that in cats which had received [H^3]-Nor, the injection of guanethidine (1 mg.) or bretylium (1 mg.) into the splenic artery was followed, in the absence of stimulation, by a rise in [H^3]-Nor in the splenic outflow for three to five minutes. However, Abercrombie & Davies (1), who made a detailed study of the action of guanethidine in cats, were unable to demonstrate any Nor release from postganglionic nerve endings. An intravenous injection of 6–10 mg./kg. very rapidly abolished the rise in blood pressure produced by stimulation of the postganglionic fibres of the superior mesenteric or splenic nerves. Moreover, ten minutes after the intra-arterial administration into the spleen of 2 mg. of guanethidine, the release of Nor in response to splenic nerve stimulation was reduced by 70 per cent, and after a further ten minutes there was no detectable release of Nor. In the absence of nerve stimulation, however, samples of venous outflow, assayed for their Nor content before and after the administration of guanethidine, showed the same vasopressor activity. Furthermore, neither close-arterial injection of guanethidine into the adrenal gland nor its intravenous administration to the whole animal appeared to affect the release of catecholamines from the adrenal medulla in the absence of or following splanchnic nerve stimulation. The authors thus concluded that the initial vasopressor effect of guanethidine is the result of a direct sympathomimetic effect and is not due to the release of adrenaline or Nor from sympathetic nerve endings on the adrenal medulla.

In a number of papers, it is reported that in the presence of reserpine, guanethidine, and bretylium, the pressor responses of amphetamine, ephedrine and tyramine are depressed or abolished. This, however, is not a correct representation of the situation: the effects of the three sympathomimetic amines, when administered in their usual pressor doses and in the presence of fully effective doses of reserpine, guanethidine and bretylium, are either unaltered or potentiated in both animals and man. Depression sets in only when either large doses of the sympathomimetic amines are given or when the animals have been pretreated with unnecessarily large doses of the hypotensive drugs. Should hypertensive patients develop severe hypotension and an antidote be needed, it is a matter of great practical importance to know whether or not reserpine and guanethidine depress the action of sympathomimetic amines. The sweeping statement that reserpine and guanethidine depress the action of amphetamine and ephedrine may therefore prove dangerous, as it implies that the two sympathomimetic amines or other amines related to them will prove ineffective in antagonizing the action of these particular hypotensive drugs, which is certainly not the case: doses of bretylium, guanethidine, or reserpine used in the treatment of hypertension do not abolish the action of the sympathomimetic amines. Laurence & Rosenheim (79) made it quite clear that in patients treated with guanethidine, methylamphetamine effectively antagonized the postural fall in mean blood pressure. Wilson & Long (130) reported that amphetamine is fully active in the presence of bretylium. In six patients receiving daily doses of 600 mg. to 4

g. of bretylium, 25 mg. of amphetamine was administered orally. Immediately afterwards, blood pressure recordings, made in each case at 30 minute intervals for one and a half hours, showed a definite rise in blood pressure. After an interval of one and a half hours, five patients were given 25 mg. of amphetamine, and two patients, a placebo. The blood pressure of those given amphetamine showed a sustained rise, but there was a gradual fall in the two subjects given the placebo. Ephedrine also proved a very effective pressor substance in patients treated with reserpine (Krogsgaard, 75).

Boura & Green (16) made a detailed comparison of bretylium and guanethidine and showed in the cat that both the nictitating membrane and the pressor responses to tyramine, amphetamine, and ephedrine can be greater or less than in controls, depending on (*a*) the dose of the sympathomimetic amine and (*b*) the dose of the adrenergic blocking agent and the period over which it had been given. Effective doses of tyramine and amphetamine administered to animals pretreated for days or weeks with blocking doses of bretylium or guanethidine produced blood pressure and nictitating membrane responses which exceeded those of the control amines. Unfortunately, in most of the experiments unnecessarily large doses of bretylium and guanethidine were used, doses far above those needed to block sympathetic neurones. Moreover, the doses of guanethidine were similar to or very often larger than those of bretylium, although of the two, guanethidine is a much more potent drug. The use of large doses has been extended also to the sympathomimetic amines. Finally the various comparisons have been based on widely differing backgrounds of treatment. For example, the relaxation of the nictitating membrane of the unanaesthetized cat and the response of the nictitating membrane to the stimulation of the preganglionic sympathetic trunk were studied in animals pretreated with 3 mg./kg. of bretylium and 2.5–5 mg./kg. of guanethidine. In contrast, the pressor responses and the nictitating membrane responses to adrenaline and Nor and in particular to tyramine and amphetamine have been studied in animals pretreated with large doses ranging between 10 and 200 mg./kg. for bretylium and 10 and 50 mg./kg. for guanethidine. Obviously, under these conditions, any comparison becomes very difficult indeed.

In both humans (67, 115) and cats (136), the doses of guanethidine which produce hypotension, bradycardia, and an almost complete blockade of adrenergic neurones are very similar (Table V). In dogs reflex venoconstriction during carotid occlusion is reduced with an intravenous injection of 1 mg./kg. and abolished after 3 mg./kg. (55); moreover the effects of stimulation of the cardioaccelerator nerve are blocked with a dose of 3 mg./kg. I.V. Taylor et al. (115) studied in great detail the effects of guanethidine in both normal subjects and hypertensive patients whose blood pressure was continuously recorded and found that the intravenous administration of 1 to 2 mg./min. was never associated with subjective side effects or any hypertensive reactions. Moreover, in all cases the blood pressure started to fall within two to ten minutes of commencement of the intravenous injection; and 30 to

60 minutes later "the Valsalva manoeuvre showed unequivocal evidence of complete sympathetic blockade."

In many animal studies, however, unnecessarily large doses of this potent drug have been used. All that such large doses can do is to produce side effects of such magnitude as to obscure the basic pharmacological effect. For example, doses of guanethidine of 5–15 mg./kg. injected intravenously have been reported to produce in dogs a marked pressor response that lasts from 30 minutes to two hours (54, 88, 97). However, such doses are bordering the toxic range; Page & Dustan (97) reported that dogs during the intravenous injection of 10–15 mg./kg. "became quiet, lost interest in their environment, appeared apprehensive, and became tremulous"; and Maxwell et al. (88) reported that in the same species 10 to 30 minutes after an intravenous injection of 15 mg./kg., a long lasting period of "panting, quieting, piloerection, occasional emesis and diarrhoea" was observed. Also in rabbits Sanan & Vogt (104) found that after guanethidine (15–20 mg./kg. I.V.) all animals showed transient cardiac arrhythmias, gasping, and muscular weakness for a few minutes.

TABLE V

A Comparison of Selective and Toxic Doses of Guanethidine with Doses Used for Demonstrating Effects on Catecholamine Stores

	Guanethidine *mg./kg.*		
	I.V.	*Oral*	*I.P.*
MAN			
acute	0.5		
chronic		1	
CAT			
acute	0.5–1		
chronic		2	1–2
DOG			
acute	1–3		
doses used for pharmacological studies	5–15		
RAT			
LD_{50}	23.1[a] 48.0[b]		400[b]
doses used for depletion	10–35[c]		150–400[c]

[a] Maxwell et al. (88).
[b] Zaimis, E. Unpublished data.
[c] Kuntzman et al. (76).

If the pharmacological effects of a drug are already known to be produced in a particular species at a certain dose level, there is little to be gained by the administration of doses in excess of this when the mechanism of action of the drug is being analysed. The additional danger of using unnecessarily large doses in animal experiments is that a blurred picture is very often transferred from animal to man.

Conclusions

Studies of drug action are undertaken today by scientists from a wide variety of disciplines; physiologists, neurophysiologists, biochemists, clinicians, as well as pharmacologists. Each, naturally, brings to bear upon his problems an outlook which is the product of his own scientific training and experience, and the particular insights and perspectives which flow from them. This has resulted in important advances, since it has meant that pharmacological problems have been subjected to examination from many different angles. The inevitable corollary, however, has been that in certain cases generalizations have been based on studies which cannot by their very nature provide a safe estimate of drug action. For example, in homogenates of rabbit adrenal gland the same molecular concentrations of about fifty different substances were used in order to study their effect on adrenaline release (125); the list of drugs included phenothiazines, monoamine oxidase inhibitors, reserpine, insulin, morphine, ouabain, strychnine and thyroxine. Obviously, the conclusions reached from such an approach can have little relevance to the mechanism of action of substances producing a wide variety of physiological and pharmacological effects in the intact animal, and at dose levels which in terms of molecular concentration vary by several orders of magnitude.

Recently there has been a growing tendency to represent the pharmacological effects of drugs acting on structures innervated by adrenergic nerves as if they took place exclusively at the nerve endings, in other words, as if the effector cells played a minor role or none at all. For example, Potter & Axelrod (99) suggested a working model in which the effector cell does not appear at all. According to this hypothetical model, drugs such as the sympathomimetic amines, reserpine, bretylium, guanethidine, cocaine, and amine oxidase inhibitors produce all their pharmacological actions by affecting the storage, release and metabolism of Nor present in the adrenergic nerve ending. Another model, which they named "neurochemical transducer" system, was formulated for the nerve ending by Brodie & Beaven (19) and the action of a number of drugs (for example, mecamylamine, monoamine oxidase inhibitors, reserpine, guanethidine, the dopamine derivative of guanidine, metaraminol, bretylium, various guanidine derivatives, α-methyldopa, methamphetamine, amphetamine, ephedrine, etc.) was discussed in relation to this "neurochemical transducer," without reference to the effector cell. Such systems are unrealistic. Pharmacological studies at cholinergic synapses, our knowledge of which is much more complete than it is of adrenergic ones, have

taught us that drugs produce, as a rule, a wealth of effects by acting on the effector cells themselves and that only rarely do we come across drugs which act nowhere else than on the nerve ending. Moreover, effector cells innervated by adrenergic nerves are endowed with a variety of processes upon which the catecholamines normally act. Increasing knowledge about the cell and its functions has made it clear that the cell membrane and the membranes inside the cell, in the mitochondria, the microsomes, the cell nucleus, and in the cytoplasm, are not only diffusion barriers which separate water phases but that a large number of very important processes take place at these membranes. Sutherland and his associates [for references see (114)] discovered a few years ago that adrenaline has a catalytic effect on the cyclization of ATP to adenosine-3′,5′-phosphate and recently (113) reported that this precise site of action of catecholamines, which is of great biochemical significance and which may well account for many of the actions of catecholamines on a variety of specialized organs, is located in the cell membrane. It appears, therefore, that if a drug, by virtue of its chemical structure or other specific property, affects structures innervated by sympathetic nerves, it has as much chance of acting upon effector cells as upon the nerve ending, especially as most of these drugs penetrate cell membranes with great ease. A few examples of pharmacological effects produced directly on effector cells are given in the following papers.

Sutherland (113) found that dichloro-isoprenaline, but not dibenyline, blocks the stimulation of adenyl cyclase by catecholamines and that isoprenaline has of all sympathomimetic amines the most potent stimulatory effect. Weiss, Coalson & Hurwitz (129) demonstrated that cocaine possesses "a significant inhibitory action toward both the contractile response and the enhanced K^{42} efflux evoked by the application of a depolarizing solution to longitudinal smooth muscle," isolated from guinea-pig ileum. It was presumed that cocaine, acting at the membrane, impedes ion fluxes important for smooth muscle contraction. Moreover a partial or complete inhibition of smooth muscle contraction brought about by small-to-moderate concentrations of cocaine was easily reversed by calcium ions (66). This preparation, according to the authors, constitutes a tissue that is relatively free of cells other than longitudinal smooth muscle fibres. Gaffney, Braunwald & Cooper (54) found in cardiac-denervated dogs that bretylium has direct inotropic and chronotropic effects, while guanethidine has a direct negative inotropic effect. In doses of 0.3 mg./kg. I.V. guanethidine had no obvious effect on the ventricular contractile force, but doses of 1 to 10 mg./kg. produced up to 40 per cent depression. That guanethidine may affect myocardial contractility was also suggested by Richardson et al. (103) from observations in hypertensive patients. Skeletal muscle appears to be another candidate. Patients treated with bretylium complain occasionally of muscle fatigue. This muscle weakness was shown on electromyographic evidence to be due to the lack of function of a proportion of muscle fibres within each motor unit (28, 45). A similar direct action was demonstrated in cats. After bretylium and guanethi-

dine electromyograms recorded from the tibialis anterior muscle showed a myopathic pattern with shortening of the motor unit action potentials (120). Of the two, bretylium appeared to be more active. From these results it is obvious that the peripheral adrenergic neurone is not the only point of attack of guanethidine and bretylium.

When pharmacological effects of drugs affecting adrenergic mechanisms are being discussed, it is useful to remember that both cardiac and smooth muscle exhibit spontaneous rhythmic activity which is an intrinsic property of the muscle and is not due to external stimulation. Autonomic nerves, therefore, unlike motor nerves, are not essential for the contraction of either cardiac or smooth muscle. Peripheral or local mechanisms become prominent after the normal nervous control is interrupted; a few weeks after the surgical removal of the sympathetic nervous system, the peripheral vessels regain a measure of control which permits rapid adaptation and fairly normal activity. This does not mean that the sympathetic nervous system is not important, but indicates that additional mechanisms in the effector cells themselves can take over much of its function. One would, therefore, expect pharmacological actions, which are restricted to the nerve endings and result from a depletion of the Nor stores, to be more uniform and less severe than in fact they are.

Many years ago Cannon and his co-workers [for references, see (31)], showed that after the surgical removal of almost all the sympathetic ganglia, animals "within the confines of the laboratory" seemed not to differ markedly from the normals. More recently Levi-Montalcini and her co-workers [for references, see (81)] made the startling discovery that more than 90 per cent of the population of sympathetic nerve cells can be increased up to sixfold, or conversely wiped out, by supplying a protein or its antiserum to the new-born mammal. Animals (mice, rats, rabbits, kittens) injected at birth with this antiserum are permanently deprived of their sympathetic ganglia, but their adrenals are not destroyed. The destruction of the sympathetic ganglia, as judged by cell counts in the superior cervical ganglia, is from 90 to 95 per cent in mice and somewhat less extensive in rats, where a residual population of 15 to 25 per cent of neurones remain in the same ganglia. "Immunosympathectomy" (a term introduced by Levi-Montalcini and her coworkers) results also in a marked reduction of the Nor content (more than 75 per cent) in all peripheral organs which were investigated and especially in the heart. No changes, however, were observed in the Nor content of the brain. Large colonies of immunosympathectomized mice and rats have been raised already; these animals although deprived of their peripheral sympathetic nervous systems do not differ, on gross inspection, from controls. Levi-Montalcini & Angeletti (82) reported that "under the sheltered conditions of the laboratory, the injected and untreated animals did not differ from each other; immunosympathectomized mice became pregnant, nursed and took care of the litter as controls." Levi-Montalcini's immunosympathectomized animals provide ideal material on which to test the validity of present theo-

ries concerning the role of the sympathetic system and the way in which structures innervated by it can be affected by drugs.

In a field like pharmacology, in which one is attempting to assess the mechanism of action of drugs in relation to highly complex, and still inadequately understood, structures, the closest possible collaboration between disciplines must be sought. Collaboration, however, should not stop at the point of mere exchange of information; it should be pursued through combined experimentation and interdisciplinary team work. Such an approach is more likely to accelerate progress, reduce expenditure and spare unnecessary effort.

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