# THE AUTONOMIC NERVOUS SYSTEM[1]

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There has been, in recent years, a great upsurge of interest in the autonomic nervous system and particularly in adrenergic mechanisms. The reasons for this are many and varied: the application of biochemical techniques has led to a better understanding of the synthesis and elimination of catecholamines; the use of the electron microscope and the new histochemical method (see 33) for detecting catecholamines have given impetus to studies of the morphology of the components of the autonomic nervous system; electrophysiological techniques have yielded information on the pattern of autonomic innervation and on some of the details of autonomic neuromuscular transmission. Perhaps the most important stimulus has been the desire to investigate the mode of action of recently developed drugs such as guanethidine and bretylium and to re-evaluate others such as reserpine and the sympathomimetic amines. The investigation of drug actions by these new techniques and by the more conventional ones has led to a much better understanding of basic adrenergic mechanisms. Although the bulk of the research on the autonomic nervous system has been directed towards the clarification of adrenergic mechanisms in the postganglionic neurons, research on the properties of ganglia has continued and has been recently reviewed (83). The mechanism of release of adrenal medullary catecholamines has continued to attract attention (32), as has the pattern of innervation of this gland (57). A few reports of investigations on autonomic reflex mechanisms have also been published (24, 34).

In this review it was decided to concentrate on several aspects of one main topic; the liberation and fate of sympathin,[2] the sympathetic noradrenergic transmitter.

## THE RELEASE OF SYMPATHIN

This section of the review contains a discussion of the synthesis and storage of norepinephrine, and the mechanism of release of sympathin.

### The Structure of the Adrenergic Synapse

The structure of adrenergic synapses has been studied by the conventional light and electron microscopical techniques and the distribution of

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

[2] The term "sympathin" denotes the norepinephrine released from adrenergic nerve terminals by nerve impulses, and is used to distinguish this from norepinephrine infused into the circulation.

catecholamines investigated with the fluorescence technique (see 33). The subject has been reviewed by Grillo (42) and by Burnstock & Holman (21). The terminal ramifications of the silver-stained nerve fibres in adrenergically innervated organs have a beaded appearance and fluorescent specimens show a series of bright varicosities (33, 39). The electron microphotographs show that the nonmyelinated fibres have occasional expansions where the Schwann sheath is absent or incomplete and which contain mitochondria and vesicles. These expansions are considered to be the site of norepinephrine release and storage.

The vesicles contained within the terminal expansions are of several types; they may be broadly classified as granular vesicles and agranular vesicles, although there may be no sharp distinction between the groups as there are variations in the density of granulation as well as in the diameter of the vesicles. In many situations, terminals tentatively identified as adrenergic contain at least two types of vesicles—one agranular and one densely granular. The dense-cored vesicles may contain norepinephrine because tritiated norepinephrine injected and taken up by nerves was found in the granulated synaptic vesicles of a number of different organs (84, 85). Furthermore, the granulated vesicles disappear after reserpine (47) but not after reserpine and monoamine oxidase inhibitors, procedures which respectively deplete or leave unaltered the tissue norepinephrine [see Grillo (42)].

The hypothesis that norepinephrine is contained in the granulated vesicles is not supported by the finding that some adrenergically innervated tissues and the norepinephrine-containing fraction of homogenized heart contain only a few granulated vesicles. However, it seems that this lack of granulated vesicles may be an artefact, as Richardson (64) demonstrated granulated vesicles in all tissues containing norepinephrine detectable by the fluorescence technique.

The function of the agranular vesicles is unknown. Some of them might be granulated vesicles from which the core has been washed out, or missed in the section, but it seems likely that there is a second population of vesicles in "adrenergic" terminals (63). It is likely that the vesicles are concerned with transmission processes, and the demonstration (55) that there are twice as many vesicles on the naked side of the axons as on the side covered by the Schwann cell supports this idea.

The distance of the presynaptic terminals from the smooth muscle surface differs from organ to organ. There are "close junctions" in which the synaptic cleft is 200 Å across, but in general these junctions are rare, although some organs, such as the vas deferens, contain a high proportion of them. The more usual arrangement is that the nerves are 400–4000 Å from the effector cells. In blood vessels, the nerve terminals are mainly confined to the medio-adventitial border and are at a minimum of 830 Å from the smooth muscle; those muscle cells lying deep in the media are at a considerable dis-

tance from the nerve terminals (55, 67). In the cat spleen, an organ much used for studies of adrenergic mechanisms, the innervation is sparse and remote from the smooth muscle (36). Thus, the cells which are far from the nerve terminals must be controlled either directly by the diffusion of transmitter or indirectly by electrical activity in nearby muscle fibres.

To summarize, the distribution of the adrenergic nerves suggests that they release sympathin into the extracellular space some distance from the effector cells. These cells show no subsynaptic structural specialization and it is possible that the receptors and other binding sites are evenly distributed over the cell surface. This hypothesis was put forward by Gillespie and Hamilton who found that norepinephrine infused into cat spleen in high concentration was located by the fluorescence technique mainly in the nerve terminals; but there was also a uniform fluorescence of the smooth muscle which suggested an even distribution of binding sites (38, 39).



## The Synthesis and Storage of Norepinephrine

The experiments designed to elucidate the synthesis of norepinephrine have recently been reviewed (70, 80). It seems that there may be division of the synthetic mechanism; the first and rate-limiting step, the hydroxylation of tyrosine, occurs in the mitochondria, and the later stages from DOPA to norepinephrine take place in the vesicles.

It appears that the norepinephrine in terminals is in several stores. The biochemical evidence is discussed by Kopin (52); the exchange of tritiated norepinephrine is multiphasic, and various depleting agents cause only partial depletion of total organ catecholamines. Evidence that only part of the norepinephrine in organs is available for release by nerve impulses has been afforded by a number of workers. Sedvall & Thorson (66) found that about 10 per cent of the original norepinephrine content of skeletal muscle resisted depletion by reserpine, but was released by nerve stimulation. Dearnaley & Geffen (30) stimulated cat splenic nerves, collected the sympathin overflowing in the venous effluent, and estimated the norepinephrine content of the spleen. At the end of a train of 9000 stimuli at 30/sec, the amount of norepinephrine in the venous effluent had fallen almost to zero, but the norepinephrine content of the spleen had fallen to about 70 per cent of the control. The fluorescence technique showed that in these stimulated spleens there was a reduction of the norepinephrine content of the terminals and a generalized fluorescence of the splenic tissue (28). It is likely that the splenic content of norepinephrine after stimulation included some of the transmitter released from the nerves as well as that in the nerves. Therefore, the amount of available transmitter in cat spleen may have been more than 30 per cent of the norepinephrine content. Boullin, Costa & Brodie (11) found that after about 9000 stimuli to the nerves to the cat colon, the overflow (see page 190) of previously labelled sympathin was almost nil, but the total norepinephrine content had fallen by only 10 per cent.

The subcellular distribution of norepinephrine has been investigated after prolonged nerve stimulation. There was 27 per cent decrease in the total amines of rat vas deferens and a corresponding fall in the amine content of the microsomal fraction (23); in rabbit heart there was a 10 per cent decrease in the supernatant fraction with less prolonged stimulation (69). Is sympathin released first from the supernatant which is then replenished from the microsomal norepinephrine store?

The possibility that the different stores of norepinephrine are in different intracellular structures or are situated in more accessible or less accessible parts of the cell has been discussed by Kopin (52) who concluded that there is no structural basis for the different stores of norepinephrine. Stjärne (70) concluded that it is unlikely that the vesicles represent the store of available transmitter as they are few and can be depleted of their norepinephrine content by reserpine. He suggested that these vesicles are a store from which the available transmitter is replenished. Further experimental investigation of the stores of transmitter is discussed on page 199. It appears that sympathin is not subject to the action of intraneuronal monoamine oxidases, for the amount "overflowing" (see page 190) is not increased by antiamine oxidase drugs (17, 68).



## The Mechanism of Release of Sympathin

It is inevitable that the mechanisms of the release of acetylcholine at the skeletal neuromuscular junction and of the release of catecholamines from the adrenal medullary cells should serve as models of the release of norepinephrine from adrenergic nerve. At these junctions, an important event in transmitter release is the entry of $Ca^{++}$ into the cell. At the adrenergic synapse there is evidence that $Ca^{++}$ is concerned in the release of sympathin, as the magnitude of the inhibition of intestinal smooth muscle after stimulation of the mesenteric nerves depended on the $Ca^{++}$ concentration of the bath fluid (19). Kuriyama (54) investigated adrenergic neuromuscular transmission in guinea pig vas deferens with intracellular micropipettes, and reported a number of effects of high concentrations of $Ca^{++}$; there was an increase in the junctional potential elicited by nerve stimulation. Furthermore, the technique of infusing tritiated norepinephrine in order to label the transmitter stores was used to show that when the nerves to cat colon were stimulated there was an efflux of tritiated norepinephrine only when $Ca^{++}$ was present in the perfusion fluid (10). Thus, at the adrenergic synapse, the mechanism of release of norepinephrine may involve $Ca^{++}$ movements in the nerve terminals.

*The link between the action potential and norepinephrine release.*—Burn & Rand (20) have suggested that adrenergic fibres release norepinephrine through the mediation of acetylcholine—the "cholinergic link" hypothesis. This hypothesis, originally proposed in 1959, and developed since then, can be summarized as follows. The action potential in the postganglionic fibre

causes the release of acetylcholine which increases the permeability of the membrane to $Ca^{++}$, which then enters from the extracellular space. The $Ca^{++}$ subsequently promotes the release of norepinephrine in some way as yet unknown. The earlier evidence for this hypothesis has already been discussed in a number of reviews by Burn and by Burn & Rand and more recently by Ferry (35), who concluded that the overall evidence for the existence of a cholinergic link was not strong.

## THE FATE OF NOREPINEPHRINE INFUSED INTO ADRENERGICALLY INNERVATED ORGANS

The distribution of infused norepinephrine has been extensively studied since 1959, and the experiments have recently been reviewed (1, 31, 39, 49, 52, 53, 59, 77). Part of the infused norepinephrine is O-methylated but most is taken up by the adrenergic nerve terminals. This theory of neutral uptake is supported by three kinds of evidence.

(*a*) Adrenergically innervated tissues take up norepinephrine, and this uptake is greatly reduced after degeneration of the nerves following surgery or the administration of the antinerve growth factor (50, 56).

(*b*) The norepinephrine taken up by innervated organs can be subsequently released by nerve stimulation (10, 41, 46).

(*c*) Norepinephrine infused into organs and taken up by them has been located in the granulated vesicles of the presynaptic terminals by autoradiography and electron microscopy or by autoradiography and fluorescence microscopy (39, 84, 85).

In addition to uptake by nerves, some of the infused norepinephrine is metabolized and some of it taken up by the effector cells. Small doses of norepinephrine infused into the cat spleen caused an increased fluorescence of the nerves, but with large doses there was also an overall fluorescence of the smooth muscle. It was suggested that postsynaptic uptake sites are distributed evenly over the smooth muscle. The application of the techniques of quantitative fluorescence microscopy and infusion of labelled or unlabelled norepinephrine have resulted in an estimate of the partitioning of infused norepinephrine between the various mechanisms of elimination in cat spleen. When norepinephrine was infused at 0.625 $\mu$g/min, 20 per cent flowed out of the spleen, 15 per cent was metabolized, 60 per cent was retained by the nerves, and about 5 per cent was bound to the muscle [Gillespie (39)].

Is it likely that infused norepinephrine and that released by nerve stimulation are similarly distributed in the tissues? There is little structural specialization of the adrenergic synapse, which seems to be adapted to a widespread diffusion of transmitter from the nerve terminals, and there seems no reason why infused norepinephrine and sympathin should not have access to the same structures. Thus, it is likely that norepinephrine released from nerve terminals should be reabsorbed by them, as was first suggested by

Paton in 1960 (62). One way of studying this hypothesis would be to compare the properties of the uptake mechanisms for infused norepinephrine and sympathin. This work is discussed in the next section of the review.

The properties of the norepinephrine uptake mechanisms of rat heart have been studied by Iversen (49). There are two uptake mechanisms distinguishable by their different affinities for norepinephrine, epinephrine, and their optical isomers, and for various sympathomimetic agents and drugs. Uptake 1 preferentially accumulated l-norepinephrine and was saturated at a concentration of 5 μg/ml of norepinephrine. Uptake 2 preferentially accumulated epinephrine and exhibited no stereochemical specificity; it was saturated at about 85 μg/ml of norepinephrine. Both mechanisms were depressed after immunosympathectomy. In spleen and other tissues, the uptake mechanism was saturated at 100 ng/ml of norepinephrine.[3] In cat heart there is no equivalent of uptake 2 (77).

To summarize, the uptake mechanism for infused norepinephrine is saturable and can be blocked by cocaine, desmethylimipramine, dichloroisoprenaline and by the α-blocking agents phenoxybenzamine, phentolamine, and Hydergine as well as by many other substances (31, 40, 49). The effects of these drugs on the uptake of neurally released norepinephrine have been investigated in the heart, uterus, colon, and other organs, but mainly in the cat spleen.

## THE FATE OF SYMPATHIN

The hypothesis to be discussed is that sympathin shares the fate of infused norepinephrine, the bulk of it being normally taken up by the nerves and smaller amounts being taken up by receptors, acceptors, and enzymes or being lost into the circulation. This hypothesis of the fate of sympathin is represented diagrammatically in Figure 1. Sympathin released from the nerve terminals diffuses through the extracellular fluid and some reaches the receptors, enzymes, and acceptors of the postsynaptic cell some 200–4000 Å away. Some of the sympathin in the extracellular fluid overflows into the circulation, whence it can be collected and estimated. Two techniques have been used in studies of adrenergic transmission, the measurement of the response of the effector and the measurement of sympathin overflowing into the circulation. The amount of sympathin overflowing into the circulation is the difference between the amounts liberated and taken up (13, 17), and a change in the amount overflowing may be caused by a change in the amount taken up or in the amount liberated, or both. The response of the effector probably depends, amongst other factors, on the amount of sympathin liberated and its persistence. Thus, simultaneous measurements of the overflow of sympathin and the response of the organ may give information on the partitioning of sympathin between the various mechanisms of uptake and

[3] ng = nanogram = $10^{-9}$ gram

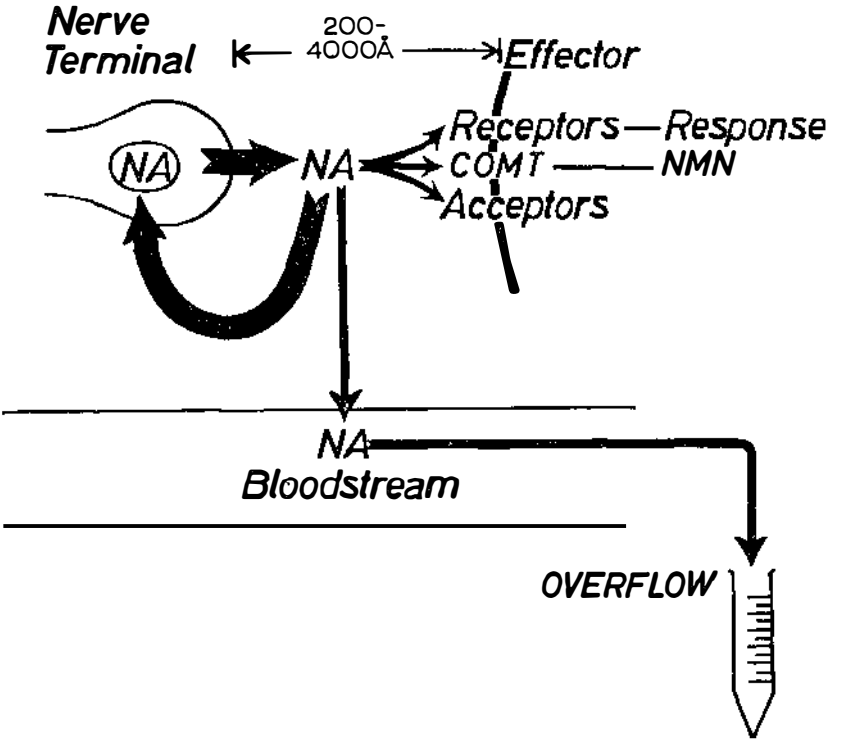


FIG. 1. A diagrammatic representation of the hypothesis that part of the norepinephrine released from the nerves is taken up by receptors, enzymes, and acceptors, that part is lost into the circulation and collected therefrom (the "overflow of sympathin") but that the bulk is reabsorbed by the nerve terminals.

COMT = catechol O-methyltransferase
NMN = normetanephrine

elimination. It has already been shown that about 60 per cent of infused norepinephrine is taken up by the nerves, 15 per cent by O-methyltransferase, 5 per cent by receptors and acceptors, and about 20 per cent overflows into the circulation. If sympathin were taken up by the same mechanisms, one would expect that drugs which block neural uptake would enhance the overflow of sympathin and the response of the effector, whereas drugs blocking only the uptake by receptors and enzymes would have a much smaller effect on the overflow. In the next section of the review, the evidence for this hypothesis will be presented.

## Estimation of the Uptake of Sympathin by Catechol O-Methyltransferase

It seems that little sympathin is destroyed enzymatically during the period of its release and the next 20 seconds or so. Several workers have infused tritiated norepinephrine into the cat spleen in order to label the transmitter store; on subsequent nerve stimulation, the venous outflow contained a mixture of one part normetanephrine and ten parts norepinephrine [Hertting & Axelrod (46)]. Gillespie & Kirpekar (41) found that tritiated normetanephrine appeared in the venous control samples, and during stimulation, the increase in radioactivity was due to tritiated norepinephrine. Similar results were obtained in the gracilis muscle (65) and cat colon (10). The inactivation of catechol O-methyltransferase had no noticeable effect on the overflow of sympathin from the spleen (4). From these experiments it appears that catechol O-methyltransferase takes up immediately only a small proportion of the released transmitter.

There are reports that catechol O-methyltransferase inhibitors slightly enhance the response to nerve stimulation (3). Axelrod (2) suggests that catechol O-methyltransferase is located close to the adrenergic receptors, as phenoxybenzamine and dichloroisoprenaline prevented O-methylation of norepinephrine in intact but not in homogenized heart. If the $\alpha$-receptors and catechol O-methyltransferase are linked in series or in parallel, block of catechol O-methyltransferase may increase the local concentration of sympathin and increase the proportion of transmitter taken up by the receptors, or prolong its duration of action. Thus, a greater response would be produced without altering the amount of sympathin liberated or overflowing.

To summarize, metabolism by catechol O-methyltransferase accounted for about 15 per cent of the norepinephrine infused into spleen and it seems that the enzyme takes up a similar proportion of sympathin. To this extent, infused norepinephrine and sympathin appear to suffer the same fate.

## The Effect of Block of Neural Uptake

According to the hypothesis of the neural uptake of sympathin, substances which block the uptake of infused norepinephrine ought also to block the uptake of sympathin and thereby increase its overflow. Some of the drugs which block the uptake of infused norepinephrine are the $\alpha$- and $\beta$-receptor blockers, and cocaine, desmethylimipramine, and similar compounds.

*$\alpha$-Adrenergic blocking agents.*—It has now been established that $\alpha$-adrenergic blocking agents prevent the uptake of infused norepinephrine (1, 31, 40, 49, 53).

Brown & Gillespie (17) demonstrated in 1957 that phenoxybenzamine increased the overflow of sympathin from the cat spleen, and they suggested that sympathin was destroyed after combination with the receptors. This observation has been confirmed many times (11, 13, 41, 51, 73, 76), see Table I, although its interpretation has been modified since the introduction

TABLE I

THE EFFECT OF DRUGS, THE FREQUENCY OF NERVE STIMULATION, AND NEURONAL REST ON THE OVERFLOW OF SYMPATHIN FROM THE CAT SPLEEN. THE OVERFLOW IS EXPRESSED AS PICOGRAMS ($10^{-12}$ GRAM) OF NOREPINEPHRINE/STIMULUS

| Stimuli/sec | | Overflow | Overflow after phenoxybenzamine | Overflow after cocaine | Overflow after named drug | References |
|---|---|---|---|---|---|---|
| Spleen | 10 | 169 | 1730 | 110 | | 8, 13, 17 |
| *in situ* | 30 | 841 | 1097 | | | |
| Spleen | 10 | 250 | 850 | 400 | | 51 |
| *in situ* | 30 | 670 | 1020 | 630 | | |
| Spleen | 10 | 275 | 2750 | | 272 desmethyl-imipramine | 37 |
| *in situ* | | | | | | |
| Saline-perfused | 6 | 640–1000 | 4300 | 1165 | 1240 imipramine | 71, 73–76 |
| spleen | 10 | 1070 | | | | |
| Saline-perfused | 0.5 | | 1500 | | | |
| spleen | 1 | | 4500 | | | 44 |
| | 2 | | 5500 | | | |
| | 4 | | 5500 | | | |
| Spleen | 1 | | 1414 | | | 27 |
| *in situ* | 3 | | 1671 | | | |
| | 7 | | 2689 | | | |
| | 10 | | 3174 | | | |
| | 30 | | 1418 | | | |
| Decentralized | 10 | 148 | 3060 | | | 13 |
| spleen *in situ* | 30 | 394 | 1800 | | | |

of the neural uptake hypothesis. The observation that sympathin overflow can be elevated by $\alpha$-adrenergic blocking agents has been extended to the intestine (11, 14), and to the drugs phentolamine and Hydergine (8). These other $\alpha$-adrenergic blocking agents are only half as effective as phenoxybenzamine in elevating the overflow of sympathin (12) and show a similar potency ratio with respect to the block of uptake of infused norepinephrine (40).

The elevation of the overflow of sympathin is usually attributed to block of uptake, but other explanations have been suggested to account for part or all of the enhancement. It has been suggested that the increased overflow is a consequence of a facilitation of liberation of norepinephrine due to the anticholinesterase properties of the $\alpha$-adrenergic blocking agents (18). This is unlikely, as eserine and neostigmine do not elevate the overflow (8, 76) and acetylcholinesterase is not found in cat spleen (36). Another suggestion is that changes in the overflow of sympathin are secondary to changes in the flow of perfusate, whether saline or blood, and the transcapillary transport of norepinephrine into the circulation (45, 65). There is no doubt that the flow through the organ affects both the uptake of infused norepinephrine (61) and the overflow of sympathin. Brown & Gillespie (17) showed that the overflow of sympathin depended on the plasma volume of the sample but considered it unlikely that variations in plasma volume accounted for the relation between the overflow of sympathin and the frequency of nerve stimulation. It is unlikely that the enhancement of overflow by the $\alpha$-blocking agents is due entirely to an effect on blood flow, as in many experiments there was little or no change in the plasma volume of samples after the drug (8, 13, 17), and in other experiments, spleens perfused at a constant rate with saline showed changes in the overflow of sympathin after drug administration (73, 74). It is not known with certainty how much of the change in overflow is caused by change in the microcirculation; Gillespie & Kirpekar (40) considered that changes in the distribution of blood in the spleen could not account for the changes in overflow of sympathin.

To summarize, $\alpha$-adrenergic blocking agents readily elevate the overflow of sympathin. This effect cannot be explained on the basis of blood flow changes; it is likely to be a result of the block of uptake of sympathin, whether by nerves or by receptors.

*$\beta$-Adrenergic blocking agents.*—The $\beta$-adrenergic blocking agents dichloroisoprenaline and pronethalol prevent the uptake of norepinephrine infused into organs (31, 49, 59). The effect of these drugs on the overflow of sympathin has been investigated in only a few organs. Dichloroisoprenaline increased the overflow of sympathin from rabbit heart to the same extent as cocaine; dibenamine was ineffective (59). The overflow of sympathin from cat uterus was doubled by dichloroisoprenaline but not by pronethalol (82).

*Cocaine and desmethylimipramine.*—Cocaine and desmethylimipramine are as effective as phenoxybenzamine in blocking the uptake of infused nor-

epinephrine (40, 49, 59, 72) and enhance the response of the effector to norepinephrine (43, 78). The sensitization of smooth muscle to norepinephrine by drugs is considered to be due to the block of uptake of norepinephrine. The topic has been reviewed recently by Trendelenburg (79).

Cocaine has a variable effect on the overflow of sympathin. It doubles the overflow from the rabbit heart (48) and the isolated cat spleen (74), and has a range of effects on the spleen *in situ;* some workers report no change in overflow (8, 78) and some an increase to 180 per cent of the control (51, 60). However, it is now clear that in all experiments, phenoxybenzamine is much more effective than cocaine in elevating the overflow of sympathin (Table I). If it is assumed that the overflow in the presence of phenoxybenzamine approximates to the liberation of sympathin, it is possible to calculate the depression of the uptake mechanism. In the case of some of the experiments upon the spleen *in situ*, cocaine depressed the uptake of sympathin by 36 per cent (51), and in the isolated preparation by about 10 per cent (72).

The imipramine derivatives show a similar degree of activity to cocaine. Desmethylimipramine enhanced the contraction of cat spleen after nerve stimulation but did not elevate the overflow of sympathin from cat spleen, yet phenoxybenzamine increased it tenfold (37), Table I. Imipramine doubled the overflow from the saline-perfused spleen (75), but phenoxybenzamine has proved more effective. In cat uterus, neither cocaine nor desmethylimipramine increased the overflow of sympathin (82). Phenoxybenzamine increased the overflow of sympathin from cat colon, but cocaine or desmethylimipramine did not (25).

*Discussion.*—It is quite clear that cocaine, desmethylimipramine, and imipramine, which are potent inhibitors of the uptake of infused norepinephrine, do not block the uptake of sympathin to any great extent. The $\alpha$-adrenergic blocking agents, which also inhibit the uptake of infused norepinephrine, are effective blockers of the uptake of sympathin. This situation suggests either that the two groups of drugs act in a different way on the same uptake mechanism or that infused norepinephrine and sympathin are taken up by different mechanisms.

The possible uptake sites are the adrenergic nerve terminals, the receptors on the effector cell, enzymes (e.g., catechol O-methyltransferase, amine oxidase), and the acceptors. It seems that enzymes account for only a small proportion of the uptake of infused and released norepinephrine and can be excluded from further discussion. It is not known how much of the sympathin is attached to acceptors. The amount may not be large, for it has been shown that hexamethonium, which enhances the responses of smooth muscle to added norepinephrine (81), does not have much effect on the overflow of sympathin (8). The two remaining uptake sites are the nerve terminals and the receptors. All of the drugs mentioned above can block neural uptake; the adrenergic blocking agents also prevent uptake by the receptors. Thus, the fact that $\alpha$-adrenergic blocking agents are more effective in elevating the

overflow of sympathin may mean that uptake by the receptors is an appreciable proportion of the total uptake and that infused and released norepinephrine are handled differently. The original interpretation by Brown & Gillespie (17) of their results was that the $\beta$-haloalkylamines elevated the overflow of sympathin by preventing its uptake by the "receptive substance." This view is upheld by Costa et al. (25) on the grounds that phenoxybenzamine increased the overflow of sympathin from cat colon at a time when it had no effect on the uptake of infused norepinephrine. On the other hand, only a small proportion of infused norepinephrine is taken up by smooth muscle (39), and the distribution of sympathin studied histochemically suggests that only a small proportion of the transmitter is similarly bound (28). Furthermore, there is disassociation between block of receptors and block of uptake of sympathin, for in the saline-perfused cat spleen, phenoxybenzamine greatly reduced the contraction without elevating the overflow of sympathin, and after a larger dose of the drug the overflow was greatly increased without much further decrease in the contraction (73). Therefore, in the spleen it seems that the receptors may not be a quantitatively important uptake site. In other organs, the relevant evidence is sparse.

As there is no evidence of different uptake for infused norepinephrine and sympathin, is it possible that the $\alpha$-adrenergic blockers act in a different way from cocaine and the other neural uptake blockers? If so, the observed lack of parallelism between block of uptake of sympathin and block of uptake of infused norepinephrine may not contradict the hypothesis of the neural uptake of sympathin.

Cocaine is a competitive blocker of the uptake of infused norepinephrine (49, 58). Iversen (49) showed that cocaine blocked uptake 1 and phenoxybenzamine blocked uptake 1 and uptake 2. Phenoxybenzamine $10^{-5}$ caused 91.5 per cent block of uptake of norepinephrine $10^{-8}$ and 55 per cent block of uptake of norepinephrine $5 \times 10^{-6}$; cocaine $10^{-5}$ caused 95 per cent block with the low concentration of norepinephrine but cocaine $2 \times 10^{-4}$ caused only 34 per cent block of uptake of norepinephrine at the high concentration. Thus, the block of uptake of infused norepinephrine by phenoxybenzamine is little affected by the concentration of norepinephrine in rat heart. Whether or not a similar situation exists in cat spleen has yet to be determined. These experiments show there may be a difference in the block of uptake by cocaine and by phenoxybenzamine.

Further support for the idea that phenoxybenzamine and cocaine differ in their blocking action on the uptake of norepinephrine comes from experiments in which it was shown that phenoxybenzamine prevented the uptake of infused norepinephrine whether given before or after the infusion, whereas cocaine was only effective if given first (45). It was suggested that phenoxybenzamine had an action on the binding mechanism. There is no comparable data for the other $\alpha$-adrenergic blocking agents.

The experimental observation that the neural uptake blockers are less effective than $\alpha$-adrenergic blockers in blocking uptake of sympathin can be explained on the basis of the competitive nature of the block by cocaine and the relatively noncompetitive block of uptake by phenoxybenzamine. It has been suggested that the high local concentration of sympathin overcomes the competitive block exerted by cocaine (40), but not that caused by phenoxybenzamine.

To summarize, pharmacological experiments to test the validity of the hypothesis that sympathin is distributed in the tissues in a similar way to infused norepinephrine have yielded results which neither confirm nor refute the hypothesis. The rationale of these experiments is to attempt to draw a parallel between the properties of the uptake mechanisms for infused and released norepinephrine. This has not been achieved, but the failure may be due to differences in the presentation of norepinephrine to the uptake mechanisms rather than to differences in the uptake mechanisms themselves. The techniques used to investigate the uptake of infused transmitter rely on the establishment of a steady-state of uptake, after several minutes of infusion. On the other hand, the techniques used to investigate the uptake of sympathin are employed during the rapid onset and decay of transmitter release and uptake. The concentration gradients of norepinephrine differ in the two situations. Hence it is not surprising that there is a certain lack of parallelism in the pharmacological properties of the uptake mechanisms for infused and released transmitter. The hypothesis of neural uptake of sympathin has been approached from other directions, and these experiments are described below.



### Saturation of the Uptake Mechanism

Biochemical studies (31, 49) indicate that the mechanism of uptake of infused norepinephrine is saturable. It has been suggested that the mechanism taking up sympathin is saturable, because the overflow with stimulation at 30/sec is greater than that with stimulation at 10/sec (13, 17). More recently, it has been shown that the amount of norepinephrine overflowing from the spleen when the nerves are stimulated during an infusion of norepinephrine is greater than the sum of overflows elicited separately, which suggests that infused and released norepinephrine are taken up, in part, by the same mechanism (5). The nature of this mechanism has still to be determined, but as most of the infused norepinephrine is taken up by nerves, this is a more likely mechanism than uptake by enzymes, receptors, and acceptors.

### Experiments on the Fatigue of Output

It seems that infused transmitter first enters the store of norepinephrine releasable by nerve impulses. If this store is replenished by reincorporation of the sympathin it releases, prevention of uptake ought to lead eventually to a

reduction in the liberation of sympathin. The overflow of transmitter is the difference between liberation and uptake, and hence the overflow of sympathin reflects changes in both its uptake and its liberation. The procedures which give overflows of sympathin from blood-perfused cat spleen in ascending order of magnitude are stimulation at 10/sec, at 30/sec, at 30/sec with Hydergine, at 10/sec with Hydergine, at 30/sec with phenoxybenzamine, and at 10/sec with phenoxybenzamine (12). When standard trains of 200 stimuli, at 10 or 30/sec, were repeated at 10 minute intervals and the overflow of sympathin returned to the system, there was no fall in overflow throughout the experiment. But when the overflowing sympathin was not returned, it was found that successive overflows were smaller and that the rate of decline of the overflow of sympathin depended on the magnitude of the initial overflow, i.e., was greatest at 10/sec with phenoxybenzamine and negligible at 10/sec (9, 12). This decline could be due either to a diminishing liberation of sympathin or to an increasing uptake throughout the experiment. It is unlikely that variations in uptake were the cause of the decline of overflow, as this was seen in preparations where uptake had been blocked. Furthermore, the responses of the spleen to stimulation declined at the same time as the overflow [Blakeley & Brown (7)]. The decline in overflow is likely to be due to the progressively decreasing liberation of sympathin. Thus, when the uptake of sympathin is prevented by drugs or by the removal of the overflow of sympathin from the system, there is a subsequent reduction in the liberation of sympathin, and this strongly supports the hypothesis of the neural uptake of sympathin.



## The Hypothesis of the Neural Uptake of Sympathin

The evidence for the hypothesis that sympathin is taken up mainly by the adrenergic terminals can be divided into two parts. The evidence involving a comparison of the pharmacological properties of the mechanisms taking up sympathin and infused transmitter neither supports nor refutes the hypothesis. This pharmacological evidence is compatible with the hypothesis, but there are too many alternative explanations of the results. The physiological evidence that fatigue of release of sympathin occurs if uptake is prevented strongly supports the uptake hypothesis. At the present time one cannot say with certainty that sympathin re-enters the nerve terminals whence it came. There is no evidence against the idea but not a great deal in its favour. It is to be hoped that the application of other techniques to the problem will be fruitful. The hypothesis of the uptake mechanisms are based mainly on biochemical and pharmacological evidence. The new fluorescence method for locating catecholamines *in situ* is beginning to be applied to the problem with most interesting results (39). The pharmacological evidence needs clarification; for instance, it would also be helpful to know if cocaine and phenoxybenzamine are located at the presynaptic terminals where they are supposed to act.

### Factors Affecting the Release of Sympathin

It is now clear that the amount of sympathin released by a nerve impulse is not uniform but depends on the frequency of stimulation and on the degree of synaptic activity in the animal before the experiment. The overflow of sympathin in the presence of phenoxybenzamine is assumed to be a measure of the liberation of sympathin. Stimulation of the nerves at different frequencies in the presence of this drug has shown that the maximum release of sympathin occurred at frequencies 4–10/sec (27, 44).

The liberation of transmitter also depends on the degree of synaptic activity; neuronal rest caused an increased liberation of transmitter (13) and an increased norepinephrine content of the spleen (15). There is potentiation of the junctional potentials in smooth muscle (22) and of the liberation of norepinephrine (16) during repetitive nerve stimulation.

### The Formation of "Available" Norepinephrine

Only part of the norepinephrine in nerve terminals is available for release by nerve impulses. During prolonged activity, it is possible for the available norepinephrine store to become depleted and the liberation of transmitter to decline, unless the store is replenished (9, 11, 26). The rate of formation of available norepinephrine has been estimated by determining the maximum rate at which sympathin can be lost without affecting the liberation, and by prolonging nerve stimulation until a steady-state is reached where, presumably, liberation and formation of available transmitter are equal (6). Available norepinephrine is formed at about 0.3 $\mu$g/hr in the whole spleen, but it is not known whether this is by enzymatic synthesis or by mobilization of preformed norepinephrine. It appears that uptake of sympathin contributes greatly to the maintenance of the splenic norepinephrine stores during intense activity, for in the absence of phenoxybenzamine the splenic norepinephrine content was reduced by 7.7 per cent following 3000 stimuli at 30/sec, but in the presence of the drug the depletion was 28 per cent [Dearnaley & Geffen (29)].

It would appear that at physiological rates of stimulation, the bulk of the transmitter released is reabsorbed, and the small amount which overflows is replaced by synthesis. The amount of norepinephrine released after a maximal stimulus to the splenic nerves is about 1 ng. The total splenic norepinephrine content is about 9 $\mu$g, of which about 3 $\mu$g is available for release. Thus, a single impulse releases 1/3000 of the available transmitter. It has been suggested many times that the functions of the uptake mechanism may be to conserve transmitter and to terminate its actions. If only a small proportion of the sympathin combines with receptors, one wonders why so much is liberated. Perhaps a large amount of norepinephrine is released so that its concentration at the distant effector cells rises rapidly enough to avoid accommodation. Once the inhibitory or excitatory postsynaptic potential

has been generated, the "cloud" of sympathin about the nerve terminals might be reabsorbed to conserve transmitter; this would also withdraw the sympathin from the vicinity of the receptors and terminate its synaptic activity.

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## LITERATURE CITED


1. Axelrod, J., *Pharmacology of Cholinergic and Adrenergic Transmission*, 205–20 (Koelle, G. B., Douglas, W. W., and Carlsson, A., Eds., Pergamon Press, Oxford, 1965)
2. Axelrod, J., *Pharmacol. Rev.*, **18**, 95–113 (1966)
3. Bacq, Z. M., *Bull. Acad. Roy. Med. Belg.*, **15**, 697–710 (1935)
4. Bacq, Z. M., Brown, G. L., and Ferry, C. B., *Compt. Rend.*, **250**, 194–95 (1960)
5. Blakeley, A. G. H., and Brown, G. L., *J. Physiol.* (*London*), **172**, 19–20P (1964)
6. Blakeley, A. G. H., and Brown, G. L., *Mechanisms of Release of Biogenic Amines*, 185–88 (Pergamon Press, Oxford, 1966)
7. Blakeley, A. G. H., and Brown, G. L. (Unpublished results)
8. Blakeley, A. G. H., Brown, G. L., and Ferry, C. B., *J. Physiol.* (*London*), **167**, 505–14 (1963)
9. Blakeley, A. G. H., Brown, G. L., and Geffen, L. B., *J. Physiol.* (*London*), **173**, 22–23P (1964)
10. Boullin, D. J., *J. Physiol.* (*London*), **183**, 76–77P (1966)
11. Boullin, D. J., Costa, E., and Brodie, B. B., *Intern. J. Neuropharmacol.*, **5**, 293–98 (1966)
12. Brown, G. L., *Proc. Roy. Soc.* (*London*), *Ser. B*, **162**, 1–19 (1965)
13. Brown, G. L., Davies, B. N., and Ferry, C. B., *J. Physiol.* (*London*), **159**, 365–80 (1961)
14. Brown, G. L., Davies, B. N., and Gillespie, J. S., *J. Physiol.* (*London*), **143**, 41–54 (1958)
15. Brown, G. L., Dearnaley, D. P., and Geffen, L. B., *J. Physiol.* (*London*) (In press)
16. Brown, G. L., and Ferry, C. B., *J. Physiol.* (*London*), **167**, 48–49P (1963)
17. Brown, G. L., and Gillespie, J. S., *J. Physiol.* (*London*), **138**, 81–102 (1957)
18. Burn, J. H., and Gibbons, W. R., *Brit. J. Pharmacol.*, **22**, 527–39 (1964)
19. Burn, J. H., and Gibbons, W. R., *Brit. J. Pharmacol.*, **22**, 540–48 (1964)
20. Burn, J. H., and Rand, M. J., *Ann. Rev. Pharmacol.*, **5**, 163–82 (1965)
21. Burnstock, G., and Holman, M. E., *Ann. Rev. Pharmacol.*, **6**, 129–56 (1966)
22. Burnstock, G., Holman, M. E., and Kuriyama, H., *J. Physiol.* (*London*), **172**, 31–49 (1964)
23. Chang, C. C., and Chang, J. C., *Brit. J. Pharmacol.*, **25**, 758–62 (1965)
24. Coote, J. H., and Downman, C. B. B., *J. Physiol.* (*London*), **183**, 714–29 (1966)
25. Costa, E., Boullin, D. J., Hammer, W., Vogel, W., and Brodie, B. B., *Pharmacol. Rev.*, **18**, 577–98 (1966)
26. Davies, B. N., *J. Physiol.* (*London*), **167**, 52P (1963)
27. Davies, B. N., and Withrington, P. G., *J. Physiol.* (*London*) (In press)
28. Dearnaley, D. P., Fillenz, M., and Geffen, L. B., *J. Physiol.* (*London*) (In press)
29. Dearnaley, D. P., and Geffen, L. B., *J. Physiol.* (*London*), **184**, 75–76P (1966)
30. Dearnaley, D. P., and Geffen, L. B., *Proc. Roy. Soc.* (*London*), *Ser. B* (In press)
31. Dengler, H. J., *Pharmacology of Cholinergic and Adrenergic Transmission*, 261–76 (Koelle, G. B., Douglas, W. W., and Carlsson, A., Eds., Pergamon Press, Oxford, 1965)
32. Douglas, W. W., and Poisner, A. M., *J. Physiol.* (*London*), **183**, 236–48 (1966)
33. Falck, B., *Acta Physiol. Scand., Suppl. 197*, **56** (1962)
34. Fernandez de Molina, A., Kuno, M., and Perl, E. R., *J. Physiol.* (*London*), **180**, 321–35 (1965)
35. Ferry, C. B., *Physiol. Rev.*, **46**, 420–57 (1966)
36. Fillenz, M., *J. Physiol.* (*London*), **185**, 2–3P (1966)
37. Geffen, L. B., *J. Physiol.* (*London*), **181**, 69–70P (1965)
38. Gillespie, J. S., *Pharmacol. Rev.*, **18**, 537–40 (1966)
39. Gillespie, J. S., *Proc. Roy. Soc.* (*London*), *Ser. B* (In press)
40. Gillespie, J. S., and Kirpekar, S. M., *J. Physiol.* (*London*), **176**, 205–27 (1965)
41. Gillespie, J. S., and Kirpekar, S. M., *J. Physiol.* (*London*), **178**, 44–45P (1965)
42. Grillo, M. A., *Pharmacol. Rev.*, **18**, 387–400 (1966)
43. Haefely, W., Hürlimann, A., and Thoenen, H., *Brit. J. Pharmacol.*, **22**, 5–21 (1964)
44. Haefely, W., Hürlimann, A., and

Thoenen, H., *J. Physiol. (London)*, **181**, 48–58 (1965)
45. Hertting, G., *Pharmacology of Cholinergic and Adrenergic Transmission*, 277–90 (Koelle, G. B., Douglas, W. W., and Carlsson, A., Eds., Pergamon Press, Oxford, 1965)
46. Hertting, G., and Axelrod, J., *Nature*, **192**, 172–73 (1961)
47. Hökfelt, T., *Experientia*, **22**, 56–57 (1966)
48. Huković, S., and Muscholl, E., *Arch. Exptl. Pathol. Pharmakol.*, **244**, 81 (1962)
49. Iversen, L. L., *Advan. Drug Res.*, **2**, 1–46 (1965)
50. Iversen, L. L., Glowinski, J., and Axelrod, J., *J. Pharmacol. Exptl. Therap.*, **151**, 273–84 (1966)
51. Kirpekar, S. M., and Cervoni, P., *J. Pharmacol. Exptl. Therap.*, **142**, 59–70 (1963)
52. Kopin, I. J., *Pharmacol. Rev.*, **18**, 513–24 (1966)
53. Kopin, I. J., *Pharmacol. Rev.*, **16**, 179–92 (1964)
54. Kuriyama, H., *J. Physiol. (London)*, **173**, 211–30 (1964)
55. Lever, J. D., Graham, J. D. P., Irvine, G., and Chick, W. J., *J. Anat.*, **99**, 299–313 (1965)
56. Levi-Montalcini, R., and Angeletti, P. U., *Pharmacol. Rev.*, **18**, 619–28 (1966)
57. Marley, E., and Prout, G. I., *J. Physiol. (London)*, **180**, 483–513 (1965)
58. Muscholl, E., *Brit. J. Pharmacol.*, **16**, 352–59 (1961)
59. Muscholl, E., *Pharmacology of Cholinergic and Adrenergic Transmission*, 291–302 (Koelle, G. B., Douglas, W. W., and Carlsson, A., Eds., Pergamon Press, Oxford, 1965)
60. Nasmyth, P. A., and Andrews, W. H. H., *Brit. J. Pharmacol.*, **14**, 477–83 (1959)
61. Paton, D. M., and Gillis, C. N., *Nature*, **208**, 391–92 (1965)
62. Paton, W. D., *Ciba Found. Symp. Adrenergic Mechanisms*, 124–27 (1961)
63. Richardson, K. C., *J. Anat.*, **96**, 427–42 (1962)
64. Richardson, K. C., *Nature*, **210**, 756 (1966)
65. Rosell, S., Kopin, I. J., and Axelrod, J., *Am. J. Physiol.*, **205**, 317–21 (1963)
66. Sedvall, G., and Thorson, J., *Acta Physiol. Scand.*, **64**, 251–58 (1965)
67. Simpson, F. O., and Devine, C. E., *J. Anat.*, **100**, 127–37 (1966)
68. Smith, C. B., *J. Pharmacol. Exptl. Therap.*, **151**, 207–20 (1966)
69. Stitzel, R. E., Campos, H. A., and Shideman, F. E., *Intern. J. Neuropharmacol.*, **4**, 327–36 (1965)
70. Stjärne, L., *Pharmacol. Rev.*, **18**, 425–32 (1966)
71. Thoenen, H., Hürlimann, A., and Haefely, W., *Helv. Physiol. Pharmacol. Acta*, **21**, 17–26 (1963)
72. Thoenen, H., Hürlimann, A., and Haefely, W., *Experientia*, **19**, 601–2 (1963)
73. Thoenen, H., Hürlimann, A., and Haefely, W., *Experientia*, **20**, 272–73 (1964)
74. Thoenen, H., Hürlimann, A., and Haefely, W., *J. Pharmacol. Exptl. Therap.*, **143**, 57–63 (1964)
75. Thoenen, H., Hürlimann, A., and Haefely, W., *J. Pharmacol. Exptl. Therap.*, **144**, 405–14 (1964)
76. Thoenen, H., Hürlimann, A., and Haefely, W., *J. Pharmacol. Exptl. Therap.*, **151**, 189–95 (1966)
77. Titus, E., and Dengler, H. J., *Pharmacol. Rev.*, **18**, 523–35 (1966)
78. Trendelenburg, U., *J. Pharmacol. Exptl. Therap.*, **125**, 55–65 (1959)
79. Trendelenburg, U., *Pharmacol Rev.*, **18**, 629–40 (1966)
80. Udenfriend, S., *Pharmacol. Rev.*, **18**, 43–52 (1966)
81. Vane, J. R., *Recent Advances in Pharmacology*, 95–121 (Robson, J. M., and Stacey, R. S., Eds., Churchill, London, 1962)
82. Vogt, M., *J. Physiol. (London)*, **179**, 163–71 (1965)
83. Volle, R. L., *Pharmacol. Rev.*, **18**, 839–70 (1966)
84. Wolfe, D. E., and Potter, L. T., *Anat. Record*, **145**, 301 (1963)
85. Wolfe, D. E., Potter, L. T., Richardson, K. C., and Axelrod, J., *Science*, **138**, 440–42 (1962)

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