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# THE ACTION OF EPHEDRINE

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THERE can now be little doubt that the active substance liberated by cholinergic nerves is acetylcholine, but the substance, or substances, liberated by adrenergic nerves have not yet been identified with certainty. The experiments described below represent part of an attempt to identify them. They originated in an unsuccessful attempt to demonstrate the liberation of substances by the vasodilator nerves in a rabbit's ear. Some of the results have been already mentioned in a general discussion of the action of ephedrine [Gaddum, 1938].

Experiments designed for the demonstration of the liberation of acetylcholine have been greatly facilitated by the use of eserine, which inhibits the hydrolysis of acetylcholine by choline esterase. If a substance could be discovered which would protect adrenaline as eserine protects acetylcholine, experiments with adrenergic nerves might be made much easier. According to Easson & Stedman [1936] the action of eserine on choline esterase is due to substrate competition. Eserine and acetylcholine are both esters which both combine with the enzyme in the same way, thus entering into competition with one another. The destruction of eserine by the enzyme is a comparatively slow process, which occupies the active patches of the enzyme for a long time leaving no room for acetylcholine. It therefore seemed likely that some substance allied to adrenaline in chemical structure might fill the place of eserine in studies on adrenergic nerves. Ephedrine appears to have an action of this kind, though it does not produce such striking effects as eserine.

The suggestion that ephedrine might act in this way came from the work of Blaschko *et al.* [1937*a*], who studied the destruction of adrenaline by tissues in the presence of cyanide. This destruction is probably due to amine oxidase [Kohn, 1937; Richter, 1937]. Ephedrine is not

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oxidized by the enzyme [Blaschko *et al.* 1937*b*], but inhibits the destruction of adrenaline, presumably by substrate competition.

Various writers have described results which might be due to the inhibition of amine oxidase by ephedrine. Schaumann [1928] was struck by the fact that ephedrine had much less action on isolated tissues than might have been expected from its activity in the body. He attributed this lack of activity to the absence of adrenaline, and found that when adrenaline ($10^{-7}$) was added to the fluid perfusing a frog's hind legs the preparation became very sensitive to ephedrine. Burn [1932] obtained similar results when a dog's legs were perfused with blood.

In other experiments ephedrine has been found to increase the effect of a subsequent dose of adrenaline. A potentiation of this kind has been seen in experiments on isolated uterine muscle from rabbits and on the blood pressure of dogs and rabbits [Schaumann, 1928; Launoy & Nicolle, 1928; Reinitz, 1929; Munch & Hartung, 1929; Koppanyi & Luckhardt, 1931] and man [Csépai & Doleschall, 1928]. Pak & Tang [1933] found that the application of ephedrine to a rabbit's conjunctiva sensitized the pupil to the subsequent local application, or injection, of adrenaline. None of these writers suggested that the potentiation was due to inhibition of an enzyme.

On the other hand, ephedrine has been found to antagonize the action of adrenaline on the perfused hindlegs of a frog [Schaumann, 1928], dog's blood pressure [Curtis, 1929], rabbit's intestine [Finkleman, 1930; Thienes *et al.* 1934; Reinitz, 1929], toad's intestine [Epstein, 1931] and dog's heart-lung preparation [Burn & Tainter, 1931]. It may even reverse the inhibitory action of adrenaline on virgin cat's uterus [Curtis, 1929].

Schaumann, who observed both potentiation and antagonism on frog's vessels found that the direction of the effect depended on the concentration of the ephedrine. Low concentrations caused potentiation and high concentrations caused antagonism. Reinitz [1929] came independently to similar conclusions regarding the action of ephedrine on rabbit's uterus. We have made similar observations of the effects of low and high concentrations of ephedrine in experiments with frogs' hearts and rabbits' ears. The experiments described below provide new examples of the fact that ephedrine may sensitize tissues to adrenaline, and show that it may also sensitize them to the effect of stimulating adrenergic nerves. Direct evidence that ephedrine preserves the substance liberated by adrenergic nerves is also presented.

## Methods

### (a) *Perfusion of rabbit's ear*

*Dissection.* Large rabbits (over 3 kg.) with large ears were anaesthetized with ether. The common carotid artery was dissected out. The external carotid was tied about 0·5 cm. above the carotid sinus, and all the branches central to this point were tied and divided, except the artery to the ear, which runs laterally from the carotid at about the level of the superior cervical ganglion (see Fig. 1). This artery was left undisturbed in

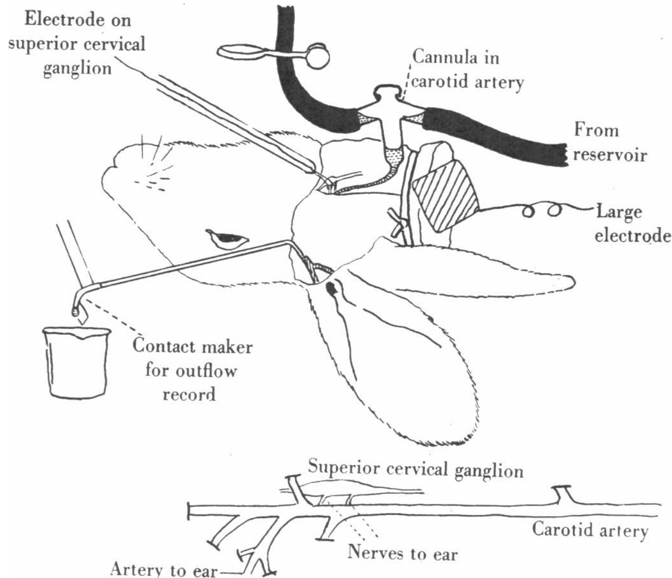


Fig. 1.

order to avoid damage to the post-ganglionic sympathetic nerves which run near it. The cervical sympathetic was then traced up to the superior cervical ganglion, and the post-ganglionic nerves which run laterally from the lower end of the ganglion were carefully dissected out for about 0·5 cm. The great auricular vein was then freed. After this preliminary dissection, during which the blood supply to the ear was undisturbed, the arterial cannula was tied in the carotid artery and perfusion with Locke's solution from a reservoir was started.

*Arterial cannula.* Fig. 1 shows the arterial cannula, which has two arms for perfusing different fluids. The cannula is largely filled with air, which separates the fluid coming from the reservoir from the fluid in the mouth of the cannula. Injections are made through the rubber cap. This

type of cannula has two advantages. First, injected fluid cannot mix with fluid coming from the reservoir, and secondly, it is easy to control the rate of injection so as to avoid pressure changes by watching the fluid in the mouth of the cannula. If the injection is made too rapidly the level of this fluid rises. Control injections with the perfusion fluid itself did not affect the outflow record in any way.

*Venous outflow.* As soon as perfusion started a piece of thin glass tubing was tied in the great auricular vein as a venous cannula. A strong ligature was then tied round the neck and the head was removed from the body. Blood and perfusion fluid were allowed to drain for some time from the region of the vertebrae. When the blood appeared to be all washed away the vertebral canal was blocked with plasticine. The outflowing fluid soon became almost or quite clear.

*Outflow record.* The rate of outflow was recorded by an apparatus working on the same principle as the Pulszeitschreiber [Fleisch, 1927]. The fluid from the venous cannula was led to a small silver tube at the end of which it formed drops. Each drop made contact between the silver tube and a piece of platinum wire. These were connected in the grid circuit of the radio valve in the apparatus described by Winton [1936] with the connexions so altered that the making of contact broke the anode circuit instead of making it. The anode current was led to an electromagnetic clutch which intervened between a small synchromotor and a writing lever. The apparatus was so arranged that the lever rose at a constant rate when there was no contact, and fell to the base-line when contact was made by the drop. By altering the position of the platinum wire relative to the silver tube it was possible to alter the size of the drops and the duration of the contact. In the tracings the height of the record measures the interval between successive drops. We are indebted to Messrs C. F. Palmer who made this apparatus at our request, and who are willing to make others. The apparatus has been used regularly for many months and has given no trouble.

*Stimulation of nerve.* A condenser ($2\mu$F.) was alternately charged, usually to 30 V., and discharged through a resistance of 2000 Ω. by means of a rotating commutator [Hill, 1934]. The electrodes and a resistance of 10,000 Ω. were connected in series to the two ends of the 2000 Ω. resistance. One electrode was a large wet pad, and the other a platinum wire which was hooked round the post-ganglionic fibres. In this way shocks of alternating sign were applied, usually at a rate of 36 per sec., and for periods of 3–10 sec. This voltage and this rate of stimulation were found to give maximal effects.

Various methods of perfusing the rabbit's ear were tried before the method described above was adopted. The cannula was tied in the carotid artery rather than in the auricular artery itself because it was found that the flow was less easily disturbed by accidental movements of the cannula. Perfusion at a temperature of 37° did not seem to have any special advantage. Perfusion with undiluted rabbit's blood has the advantage that it makes vasomotor effects visible, but it makes the pharmacological analysis of the effluent much more difficult. Stimulation of the peripheral mixed nerves, such as the great auricular, was abandoned because the peripheral course of the adrenergic supply to the ear was found to vary, and could not always be traced. A combined perfusion of the ear and the superior cervical ganglion was attempted, but the ganglion soon ceased to conduct impulses.

### (*b*) *Frog's heart*

The frog's heart (*Rana esculenta*) was suspended by Straub's method and filled with Locke's solution diluted 1·4 times with water, and containing atropine sulphate ($10^{-6}$). The vagus nerve, which contains adrenergic fibres was stimulated about 2–3 cm. from the heart.

### (*c*) *Cat's nictitating membrane*

Contractions of the nictitating membrane of etherized and spinal cats were recorded isotonically. In these particular experiments the suprarenal glands were not removed.

### (*d*) *Drugs*

Doses of adrenaline are weights of *l*-adrenaline, dissolved with HCl. Doses of ephedrine are weights of *l*-ephedrine hydrochloride.

## Results

When perfused by the method described above, the blood vessels of the rabbit's ear respond regularly with vasoconstriction to the injection of adrenaline or to the stimulation of the adrenergic nerves. Ephedrine, in concentrations up to $10^{-3}$, has no detectable effect by itself, but when ephedrine, in a concentration of $10^{-5}$, was added to the perfusion fluid, the effects of adrenaline and of stimulation of the nerves was increased.

Fig. 2 shows the increase of the response to adrenaline. Each portion of the tracing shows the effect of the injection of 0·4 $\mu$g. of adrenaline in 0·4 c.c. The third injection, which was made 6 min. after the perfusion

fluid had been changed for one containing ephedrine ($10^{-5}$), shows an increased effect, but 10 min. later the effect was increased still further.

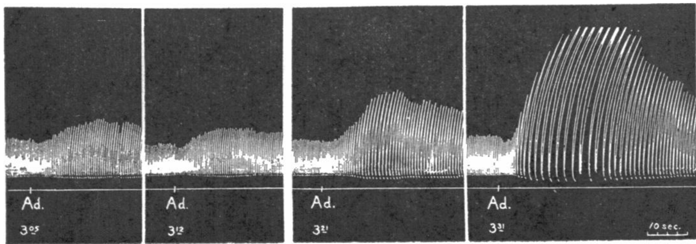


Fig. 2. Outflow from perfused rabbit's ear. The height of the record is proportional to the time interval between drops. *Ad.* injection of 0·4 c.c. adrenaline ($10^{-6}$). From 3.15 onwards the perfusion fluid contained ephedrine ($10^{-5}$).

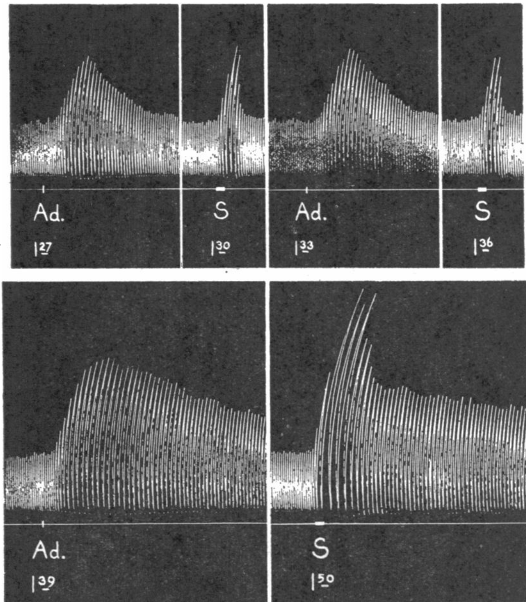


Fig. 3. Similar to Fig. 2. *Ad.* injection of 0·1 c.c. adrenaline ($10^{-7}$). *S*, stimulation of sympathetic, 6 sec. 25 per sec. From 1.37 onwards the perfusion fluid contained ephedrine ($10^{-5}$).

Fig. 3 shows the result of another experiment. The top part of the figure shows effects obtained with the normal perfusion fluid, and the bottom part of the figure shows effects obtained when the perfusion fluid contained ephedrine. *Ad.* denotes the injection of adrenaline, and *S*

denotes stimulation of the sympathetic nerve. It will be seen that the vasoconstrictor response to adrenaline was increased by the ephedrine. In this particular experiment some sensitization of the response to adrenaline was seen when ephedrine had only been perfused for 2 min. In most experiments the sensitization developed more slowly than this. When the ephedrine solution was perfused for an hour or more the sensitization persisted, but it could be reversed by perfusion with ephedrine-free solution.

The same figure shows that the effect of stimulating the adrenergic nerves was also increased by the ephedrine. Stimulation of these nerves for 6 sec. at a rate of 25 maximal shocks per second caused a small and brief vasoconstriction. Fourteen minutes after the beginning of the perfusion with ephedrine this effect was increased in intensity and duration.

These effects of ephedrine appear to be a true sensitization because ephedrine itself had no vasoconstrictor action. The increase of the duration of the effects was not entirely due to the decrease in the rate of flow, which must increase the time during which adrenaline is in contact with the tissues, since in the presence of ephedrine the vasoconstriction persisted, not only for a longer time, but actually during the perfusion of a larger volume of fluid.

Fig. 4 shows the result of an experiment in which the colorimetric test for adrenaline with arsenomolybdic acid, devised in these laboratories by F. H. Shaw [1938], was applied to the fluid flowing from a perfused ear. In this experiment the rate of flow was made almost independent of vasomotor changes by the following device. The reservoir was raised to a height of 2–3 m. above the ear, and a piece of fine capillary tubing was interposed between reservoir and the ear. A simple arterial cannula was used, and no attempt was made to record the effect of injected fluids, but the effects of nervous stimulation were shown by a fine-bored water manometer connected to the cannula. With this arrangement the effect of nervous stimulation could usually still be detected on the outflow record, but the time taken to collect a sample of 10 c.c. was approximately constant at 15 min. Each sample of 10 c.c. was collected in a cylinder containing 10 c.c. of 10 % trichloracetic acid, and the apparent concentration of adrenaline was estimated and plotted as in Fig. 4. During the collection of the fourth sample the sympathetic nerves were stimulated for 5 sec. in each minute. This caused a small release of "adrenaline". After eight samples had been collected the perfusion fluid was changed for a similar fluid containing ephedrine. The ephedrine itself caused a

release of "adrenaline" in these and in each of a series of such experiments. Ephedrine itself has no direct effect on the colorimetric test.

After about 50 min. this immediate effect passed off. After 114 min. the sympathetic nerves were stimulated as before and a greatly increased concentration of "adrenaline" was detected in the effluent. This substance could still be detected for more than 15 min. after the second stimulation had ceased.

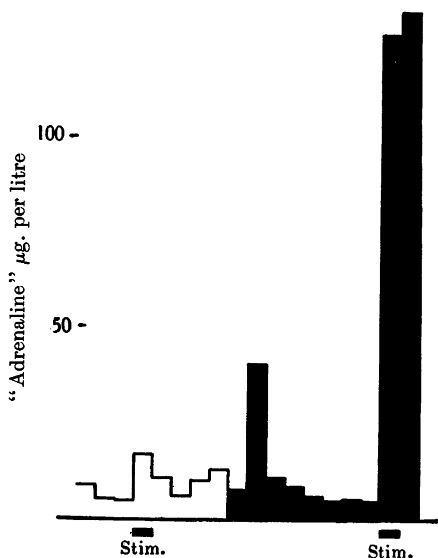


Fig. 4. Colorimetric estimates of adrenaline in successive samples of 10 c.c. of the effluent from a perfused rabbit's ear. *Stim.* stimulation of nerve for 5 sec. in each minute during collection of one sample. Black areas, perfusion with ephedrine ($10^{-5}$).

A special specific test was applied, in this experiment, to the third sample collected after the second stimulation. Shaw has shown that if adrenaline is subjected to suitable preliminary treatment with alkali the colour subsequently produced in the test is increased about five times. This increase appears to be a specific property of substances containing exactly the same side-chain as adrenaline, and can be made the basis of a specific test for this side-chain. When this specific test, as described by Shaw, was applied, an increase of colour similar to that due to adrenaline was observed.

The ordinary test makes it probable that the substances liberated by the nerve is a catechol derivative, since no other substances are known to give this test in low concentrations. The special specific test definitely excludes from consideration the substances whose molecules are identical

with that of adrenaline except for the absence of the alcoholic hydroxyl group (epinine), or the absence (noradrenaline), or transposition of the methyl group (corbasil). Adrenalone is also excluded. The results strongly suggest that the substance is adrenaline itself.

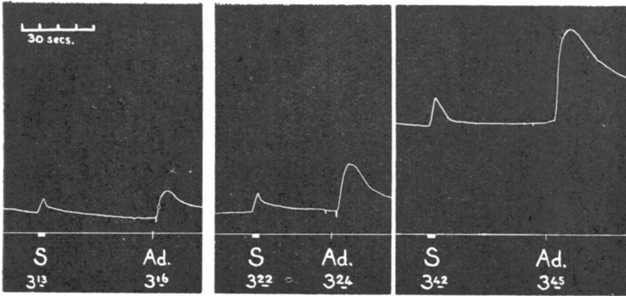


Fig. 5. Cat. Ether. Nictitating membrane. *S*, stimulation cervical sympathetic, 10 sec. 6·5 per sec. *Ad.* 7 $\mu$g. adrenaline, intravenous. At 3.21 0·1 mg. ephedrine, intravenous.

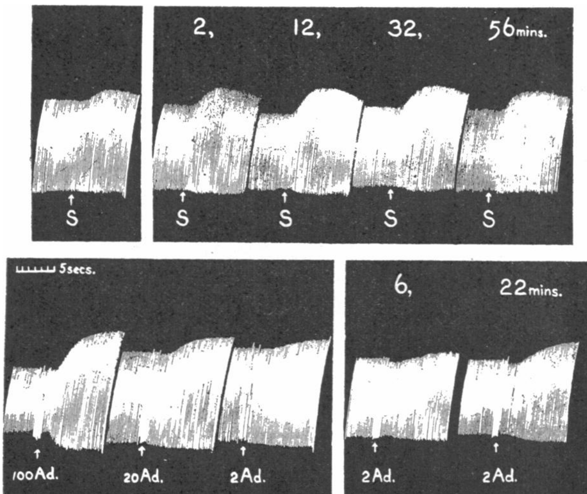


Fig. 6. Frog's heart. Straub's method. Atropine, $10^{-7}$. *S*, stimulation of vagosympathetic, 4 sec. *Ad.* adrenaline. Concentrations $\times 10^{11}$. Times after addition of ephedrine ($2 \times 10^{-9}$) to fluid.

The fact that the substance detected in these experiments is not noradrenaline is particularly interesting, because of Bacq's [1934] theory that noradrenaline is identical with sympathin E, the substance liberated by excitor adrenergic nerves [Cannon & Rosenblueth, 1937]. The nerves to a rabbit's ear are excitor and might be expected to liberate

sympathin E, but it would be unwise to reject Bacq's theory until we know more about the pharmacological properties of the substance liberated in the rabbit's ear.

*Cat's nictitating membrane.* Fig. 5 shows the effect of ephedrine on the responses of a cat's nictitating membrane. At *S* the cervical sympathetic was stimulated (10 sec.; 6·5 sec.). At *Ad.* 7 $\mu$g. of adrenaline was injected intravenously. At 3.21 0·1 mg. of ephedrine hydrochloride was injected intravenously. This caused a slow contraction of the nictitating membrane. The effects of adrenaline, and of nervous stimulation, were both increased in strength and duration. These increases developed slowly.

*Frog's heart.* Fig. 6 shows the effect of ephedrine on the responses of a frog's heart. The upper part of the tracing shows that the presence of ephedrine increased the effect of stimulating the adrenergic nerves, and that this increase reached a maximum in about 12 min. The lower part of the tracing shows that the effect of adrenaline was simultaneously increased and that this increase also developed slowly.

## Discussion

*Sensitization.* The evidence that ephedrine potentiates the action of adrenaline by inhibiting amine oxidase is similar to the evidence that eserine potentiates the action of acetylcholine by inhibiting choline esterase. Both drugs have been shown to protect the chemical transmitter from the enzyme *in vitro* and to potentiate its action *in vivo*, whether it is injected or liberated locally by stimulation of the nerves. Both drugs may increase the yield of chemical transmitter liberated from the tissue when the nerve is stimulated.

Experiments on the effect of the degeneration of nerves confirm the theory that some of the actions of ephedrine resemble those of eserine. If the cholinergic nerves to the pupil are cut and allowed to degenerate the pupil becomes abnormally sensitive to acetylcholine [Shen & Cannon, 1936] and insensitive to eserine [Anderson, 1905]. Eserine's failure to act on the denervated pupil is easily understood if this effect of eserine is due to the inhibition of the destruction of the acetylcholine normally liberated, since the liberation of acetylcholine presumably ceases after degeneration of the nerves.

If adrenergic nerves are cut and allowed to degenerate, the pupil and various other tissues become abnormally sensitive to adrenaline [Meltzer & Auer, 1904] (for other references see Cannon & Rosenblueth, 1936). This effect is presumably analogous to the increased sensitivity to acetylcholine which may be produced by cutting cholinergic

nerves. Degeneration of adrenergic nerves affects different actions of ephedrine in different ways. It greatly diminishes its action on the blood vessels of a cat's leg [Burn, 1932] and eventually decreases its action on the pupil [for references see Pak & Tang, 1933], but it increases the action, of small doses at any rate, on the nictitating membrane [Bacq, 1936; Bülbring & Burn, 1937].

The first two of these actions of ephedrine resemble the action of eserine on the pupil in the fact that they are diminished by degeneration of the nerves and can be explained on the theory that they are due to inhibition of an enzyme which would otherwise have destroyed the chemical transmitter. The action of ephedrine on the nictitating membrane is clearly different since it is increased, like that of adrenaline, by nervous degeneration. Ephedrine probably causes the nictitating membrane to contract in exactly the same way that adrenaline does. This conclusion is not necessarily inconsistent with the theory that the potentiation by ephedrine of the action of adrenaline on the nictitating membrane is due to the inhibition of amine oxidase.

Further evidence comes from studies of the actions of substances allied chemically to ephedrine. Munch & Hartung [1929] studied the potentiation of the action of adrenaline on dog's blood pressure by compounds with the general formula $R_1.C_6H_4.CHOH.CHR_2.NH_2$. The substance in this series in which $R_1$ is H and $R_2$ is $CH_3$ differs from ephedrine only in the absence of a methyl group attached to the nitrogen. Potentiation occurred when $R_2$ was $CH_3$, but not when it was H, $C_2H_5$ or $C_3H_7$. $R_1$ was usually H, but might be OH or $CH_3$ without loss of the potentiation. Tainter [1931] observed potentiation with $(OH)_2.C_6H_3.CHOH.CHCH_3.NH_2$ (corbasil). These results are consistent with those of Blaschko *et al.* [1937*b*], who studied a more diverse series of substances and found that the presence of $CH_3$ in the position of $R_2$ conferred immunity to amine oxidase. The evidence is incomplete, but the fact that substances with $CH_3$ in the position of $R_2$ have been found to differ from allied substances both in their immunity to amine oxidase and in their potentiating action, supports the view that the potentiation is due to the enzyme becoming blocked up with substances which it cannot destroy.

While *l*-corbasil itself has a pressor action similar to that of *l*-adrenaline, *d*-corbasil has a much smaller pressor action, but when a large enough dose is injected the pressor effect is much more prolonged [Schaumann, 1931, 1937]. Since corbasil is immune to amine oxidase [Blaschko *et al.* 1937*b*] this enzyme is not the main cause of the short

duration of the pressor action of the more active of these isomers. The duration of the action seems to depend rather on the dose injected, so that amines with low activity appear to have a prolonged action because larger doses are used and these large doses remain in the circulation for longer than the comparatively small doses of more active amines, which may produce a larger immediate rise of blood pressure. These facts are consistent with the view that the disappearance of the pressor action is mostly due to the diffusion of the amines out of the blood into the tissues. On the other hand, the fact that certain inhibitors of amine oxidase may cause some increase in the pressor action of adrenaline suggests that amine oxidase does play some part in removing adrenaline from the circulation. If it was the only factor the potentiation might be expected to be much more marked than it actually is.

All these actions of ephedrine can be attributed to the inhibition of amine oxidase, but can also be explained in other ways. They might be attributed, like those of cocaine [Cannon & Rosenblueth, 1937], to an increase in the permeability of the tissues. On this theory the potentiation would be due to easier access, and the increased yield on stimulation of the nerves would be due to easier escape, of the chemical transmitter from the tissues. A similar theory was advanced by Burn [1932], who explained the fact that the presence of adrenaline was necessary for the action of ephedrine by suggesting that ephedrine acted by liberating adrenaline from a local store which became depleted unless refilled by circulating adrenaline.

The potentiation might be due to selective paralysis of the inhibitor actions of adrenaline leaving the motor actions comparatively unaffected. This theory would be the opposite of that advanced by Dale to explain the action of ergotoxine [1906].

The effects might be due to the inhibition of some other enzyme which shared with amine oxidase the property of being inhibited by ephedrine.

Similar theories could be advanced to account for the actions of eserine, but there is no need to adopt any of them at present because there is no evidence against the theories which attribute the effects to the inhibition of choline esterase and amine oxidase. It is simpler to explain the actions of eserine and ephedrine in terms of their known effect on enzymes, which can be demonstrated *in vitro*, than to postulate new properties for them.

If this theory is true, the observation that ephedrine potentiates the action of adrenergic nerves provides direct evidence that both the substance liberated into the circulation by these nerves and the enzyme

inhibited by ephedrine do really play an intimate part in the transmission of impulses.

*Antagonism.* The effect of high concentrations of ephedrine in diminishing the response to adrenaline can be explained on the theory that the ephedrine combines with the motor receptors and blocks them up [Curtis, 1929]. This theory is similar to the theory of substrate competition advanced above to account for the sensitization. Similar theories have been advanced to account for other specific antagonisms [Ringer & Morshead, 1880; Gaddum, 1936; Clark, 1937].

The fact that substances allied chemically to adrenaline may antagonize its action is well known [Loewe, 1927]. These other antagonisms may be accounted for by the same theory.

### *The immediate effects of ephedrine*

Some of the immediate effects of an injection of ephedrine by itself are probably due to the inhibition of amine oxidase, and some of them are not. The evidence of Schaumann [1928] and Burn [1932] that ephedrine has practically no vasoconstrictor action on perfused tissues in the absence of adrenaline suggests that the vasoconstrictor action of ephedrine is entirely due to the inhibition of amine oxidase. This conclusion is confirmed by the experiments, discussed above, on the effect of the degeneration of adrenergic nerves. The evidence suggests that the effect of ephedrine on the pupil is also due to the same mechanism, but that the effect on the nictitating membrane is not. The diminishing effect on the blood pressure of successive doses of ephedrine (*tachyphylaxie*) may perhaps be due to blockage of the motor receptors by ephedrine, which thus excludes the chemical transmitter through whose preservation from the enzyme the earlier doses of ephedrine produce their effect.

## Summary

1. An improved method of perfusing the rabbit's ear is described. Stimulation of the sympathetic nerves in this preparation causes vasoconstriction and the liberation of a substance which can be detected by a colorimetric test for adrenaline.

2. Low concentrations of ephedrine sensitize the rabbit's ear, the cat's nictitating membrane, and the frog's heart not only to adrenaline, but also to the stimulation of adrenergic nerves.

3. Ephedrine increases the yield of the substance liberated by the nerves so that its properties can be more easily studied. It is not noradrenaline, epinine, corbasil or adrenalone, but may be adrenaline.

4. These actions of ephedrine are attributed to the inhibition of amine oxidase. This effect is compared with the inhibition of choline esterase by eserine.

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