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THE RELATION OF CIRCULATING NORADRENALINE TO THE EFFECT OF SYMPATHETIC STIMULATION

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From a comparison of the action of sympathomimetic amines in normal animals and in animals treated with reserpine, we were led to the conclusion that some of the simpler amines, such as tyramine, act by liberating noradrenaline from a store in the neighbourhood of sympathetic nerve endings (Burn & Rand, 1958*b*). The presence of noradrenaline in the walls of arteries and veins was demonstrated by Schmiterlöw (1948) and by von Euler & Purkhold (1951) in the spleen, the liver, the kidneys and the salivary glands. Treatment of animals with reserpine has been shown to cause a disappearance of the extractable noradrenaline from the heart (Bertler, Carlsson & Rosengren, 1956) and from the blood vessels, spleen and iris (Burn & Rand, 1957, 1958*a*, 1959). Sympathomimetic amines such as tyramine lost their action in animals treated with reserpine, but this action was restored following an infusion of noradrenaline into a vein. We concluded that the infusion of noradrenaline had replaced some of the noradrenaline in these organs.

In order to obtain information on the role of the noradrenaline normally present in the vessel walls, we have studied the vasoconstriction caused by sympathetic stimulation before and after the infusion of noradrenaline.

METHODS

Experiments have been made on dogs anaesthetized with chloralose 80 mg/kg after preliminary ether anaesthesia. After evisceration the lumbar vessels and sympathetic chain were divided between ligatures at the level of L4. The peripheral end of the sympathetic chain was threaded into the electrodes shown in Fig. 1, which were designed and made by Mr O. B. Saxby. The part of the chain enclosed in the electrodes was irrigated throughout the experiment with oxygenated Krebs's solution flowing from a reservoir at 37° C through the polythene tube E at a rate controlled by a drip. As the irrigating solution left the electrodes it was removed by another tube connected to a suction pump. For stimulation, square wave pulses of 2 msec pulse width were applied at a rate of 25/sec for periods of 10 sec or less. Vasoconstriction was recorded by measuring either volume changes or the venous outflow during perfusion of the leg.

Plethysmographic recording. The right hind leg of the dog was enclosed in a metal plethysmograph with a thin latex lining, filled with water at 37° C and connected to a piston recorder sufficiently sensitive to record the change in volume of the leg with each heart beat.

In some experiments blood was collected from a second dog into a vessel containing heparin, and put into a Marriotte bottle of 1 l. capacity. A cannula of as wide diameter as possible was tied in the left external iliac artery of the dog used for the experiment and was connected to the bottom opening of the Marriotte bottle. The air space above the blood in the bottle was continuous with the air in a 20 l. stone bottle, in which an air pressure was maintained equal to the dog's blood pressure. This device prevented variations in the dog's blood pressure.

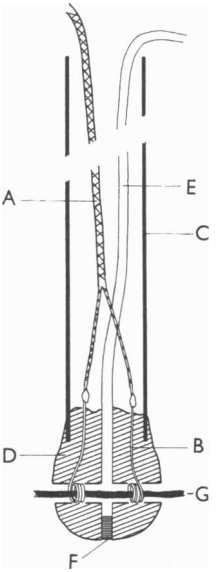

Fig. 1. Diagram of electrodes. A is twin 7/42 copper, flat laid, screened PVC sheathed cable. B is platinum wire, 0·35 mm. C, is a glass tube 20 cm long, outside diameter 6 mm. E is polythene tube 1 mm bore (Allen and Hanburys, No. 2). F is a cleaning hole, normally plugged. G is the nerve.

Perfusion of hind leg and recording of venous outflow. A dog under ether anaesthesia was bled into a vessel containing heparin and its lungs were then perfused with the blood, with a Dale–Schuster pump. This perfusion was continuous until the leg of a second dog was ready. The second dog was eviscerated under ether, and a cannula was tied in the left external iliac artery pointing to the bifurcation of the aorta. The aorta itself was tied below the origin of the two external iliac arteries and ligatures were also put in place around the aorta above the bifurcation. The right sympathetic chain was prepared for stimulation as already described, and mass ligatures were put in position so as to enclose the whole cross-section of the body wall about the level of the kidney. At a given moment the aorta was tied above the bifurcation, and a cannula was then tied in the inferior vena cava. The perfusion of the right hind leg was begun through the left external iliac artery by means of a second Dale–Schuster pump. The delay between arrest of the natural circulation and the start of the perfusion was usually 3 min. The mass ligatures were tied. The venous outflow was recorded with a Stephenson (1949) recorder. When the sympathetic chain was stimulated, constriction was recorded by the rise of pressure in the arterial cannula and by the fall in venous outflow. No anaesthetic was present during the observations; they were made 1–2 hr after the ether used during the preparation had been discontinued.

RESULTS

Experiments with atropine

Plethysmographic recording. Since the effects of sympathetic stimulation in the dog's hind leg include vasodilator as well as vasoconstrictor changes (Burn, 1932*a*), the experiments were first made in the presence of atropine so as to exclude the vasodilatation produced by the cholinergic sympathetic fibres. The results shown in Fig. 2 illustrate the findings. At (*a*) the effect of stimulating the lumbar sympathetic chain for 10 sec is shown. When the strength of current was 0·3 mA, there was no effect;

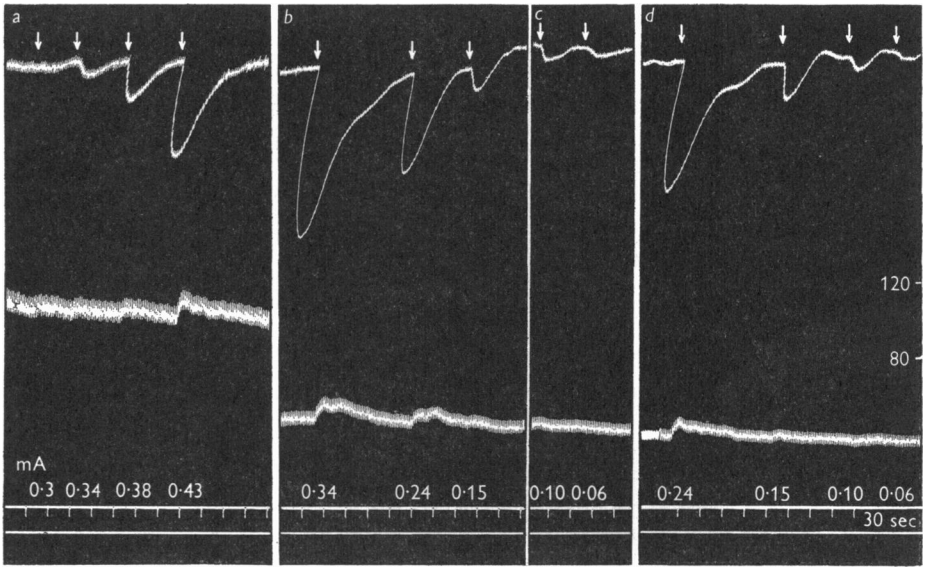

Fig. 2. Dog under chloralose. The upper record shows changes in volume of the right hind leg. At the arrows the right lumbar sympathetic chain was stimulated for 10 sec with square-wave stimuli of frequency 25/sec, duration 2 msec and of strength shown at the foot of the record. The lower record is the blood pressure. Observations were made in the presence of atropine. (*a*) shows that initially the threshold strength was 0·34 mA. Between (*a*) and (*b*) an infusion of 0·2 mg noradrenaline was given. (*b*) shows that the effect of 0·34 mA was greatly increased and (*c*) shows that the threshold was 0·06 mA. (*d*) shows that 30 min later the effects of 0·24 mA and of 0·15 mA were greater than in (*b*).

at 0·34 mA there was a trace of constriction; at 0·38 and 0·43 mA the constriction was progressively greater. The threshold strength therefore was 0·34 mA. Between (*a*) and (*b*) an intravenous infusion of 0·2 mg noradrenaline was given during 20 min. Sympathetic stimulation was then much more effective. The previous threshold strength, 0·34 mA, now

caused a large vasoconstriction, and the threshold strength (*c*) was reduced to 0·06 mA. After a further period of 30 min the effect of 0·24 mA, shown in (*d*), was greater than in (*b*), but the threshold remained at 0·06 mA.

In the experiment described there was a rise of blood pressure during the infusion of noradrenaline; when the infusion was stopped the blood pressure fell to a lower level than before the infusion. However, the same result was obtained when these pressure changes were prevented by the use of a constant-pressure reservoir connected to the left external

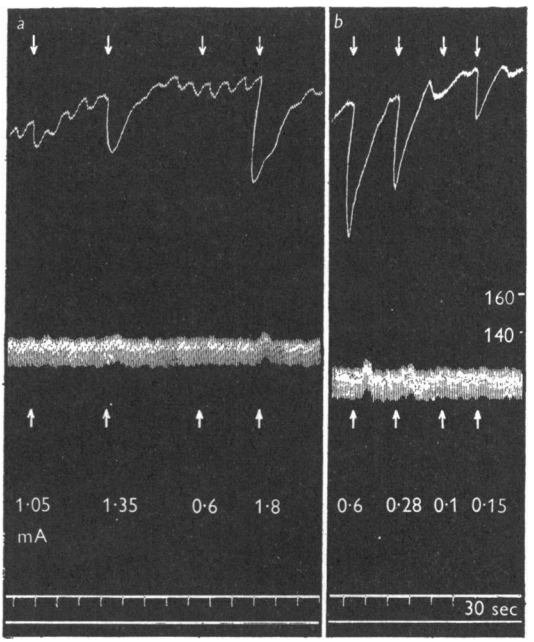

Fig. 3. Observations as in Fig. 2. In this experiment the blood pressure was kept approximately constant during and after the infusion of 1·0 mg noradrenaline by connecting the left external iliac artery to a reservoir of blood under pressure. (*a*) threshold strength was 1·05 mA before the infusion of noradrenaline. (*b*) threshold strength was 0·15 mA after the infusion.

iliac artery. Such an experiment is shown in Fig. 3. Before the infusion of noradrenaline (at *a*) the threshold strength was 1·05 mA and 0·6 mA was without effect. Greater effects were observed with 1·35 and 1·8 mA. After the infusion of 1·0 mg noradrenaline (at *b*) the threshold was reduced to 0·15 mA and the effect of 0·6 mA was greater than that of 1·8 mA before the infusion.

Table 1 shows the reduction of the threshold observed in seven experiments in which the plethysmograph was used, the threshold being reduced

to a percentage of the initial value varying from 14 to 75. In many experiments it was observed that the greatest fall in threshold did not occur immediately the infusion of noradrenaline was terminated, but after a delay of 30–60 min.

Perfusion of hind leg and recording of venous outflow. The effect of noradrenaline infusion is shown in Table 1 and Fig. 4. Table 1 shows the fall in thresholds in two experiments after noradrenaline infusion. Figure 4 shows an experiment in which the same strength of stimulation, 3·2 mA, was applied throughout. The effect of stimulation was small and declined during the course of the perfusion. The effects of the last two stimulations before the noradrenaline infusion are shown at (*a*) and (*b*). Between (*b*) and (*c*) 0·15 mg noradrenaline was infused. When the infusion was stopped

TABLE 1. Effect of noradrenaline infusion on threshold strength of sympathetic stimulation for vasoconstriction. Observations in presence of atropine

Type of experiment	Initial threshold strength (*A*) (mA)	Noradrenaline infusion (mg)	Threshold strength after infusion (*B*) (mA)	Threshold *B* as % of threshold *A*
Plethysmograph	1·05	1·0	0·15	14
	0·77	1·0	0·38	50
	1·16	0·025	0·87	75
	1·02	0·2	0·27	26
	0·42	0·175	0·27	64
	0·19	0·25	0·08	42
	0·34	0·2	0·06	29
Perfusion	1·28	3·2	0·92	72
	0·79	0·64	0·51	64

and the pressure in the arterial cannula had once more returned to its initial level, stimulation had an increased effect, but with repetition of stimulation the effect became progressively weaker (*c*). This decline of the effect was not observed in the plethysmograph experiments on dogs under chloralose and may be explained if the uptake of noradrenaline was relatively small for lack of some other substance in the perfusion circuit necessary to hold noradrenaline in the store. Thus, in the adrenal medulla noradrenaline and adrenaline are held in granules in which they are bound to adenosine triphosphate (Blaschko, Born, D'Iorio & Eade, 1956).

Effect of adrenaline. In three plethysmograph experiments 0·25 mg adrenaline was infused instead of noradrenaline. There was a fall of threshold to 80 % in one experiment, no change in a second, and a rise in a third experiment in which a fall of threshold was subsequently produced by an infusion of noradrenaline. Thus adrenaline was less effective than noradrenaline in lowering the threshold. A similar difference was obtained in experiments in which restoration of the pressor action of tyramine was investigated (to be published).

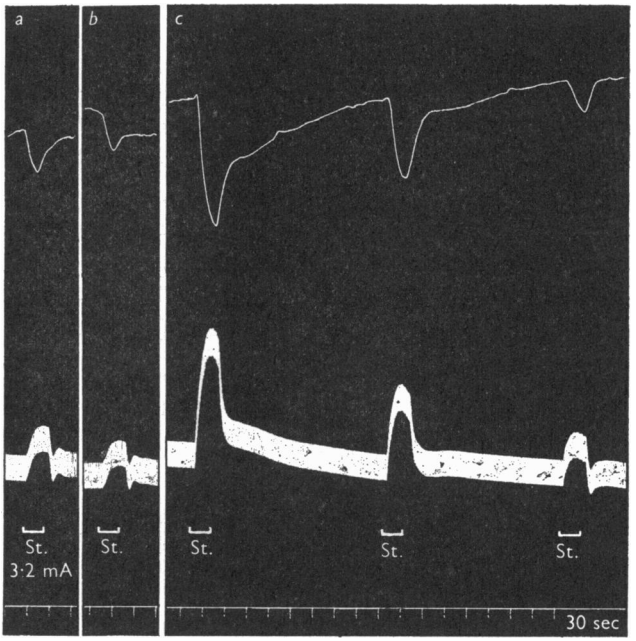

Fig. 4. Perfusion of dog's hind leg. (*a*) and (*b*) show effect of stimulating lumbar sympathetic chain for 0·5 min with a frequency of 25/sec, duration 2 msec and strength 3·2 mA. Between (*b*) and (*c*) an infusion of 0·15 mg noradrenaline was given. (*c*) shows the greater effect of the same stimulation, the increase diminishing with successive stimulations.

Experiments without atropine

Figure 5 illustrates a plethysmograph experiment in which the effect of an infusion of noradrenaline was examined without the use of atropine. The effect of stimuli of increasing strength before the noradrenaline infusion are shown at (*a*) and (*b*). A stimulus of 0·6 mA caused vasodilatation; one of 1·0 mA caused dilatation interrupted by constriction; and stimuli of 1·8 and 2·25 mA caused constriction only. After the infusion of 1 mg noradrenaline, during which the blood pressure was maintained constant by the use of the reservoir, the stimuli of 0·6 and 1·0 mA both caused dilatation followed by constriction. The stronger stimuli of 1·8 and 2·25 mA both caused constriction followed by dilatation, the constrictor phase being less than the constriction caused by the same stimuli before the noradrenaline infusion. Thus the infusion of noradrenaline appeared to increase the dilator effect of these stimuli to an extent which masked the increase in the constrictor effect.

In perfusion experiments in which atropine was not given the results of

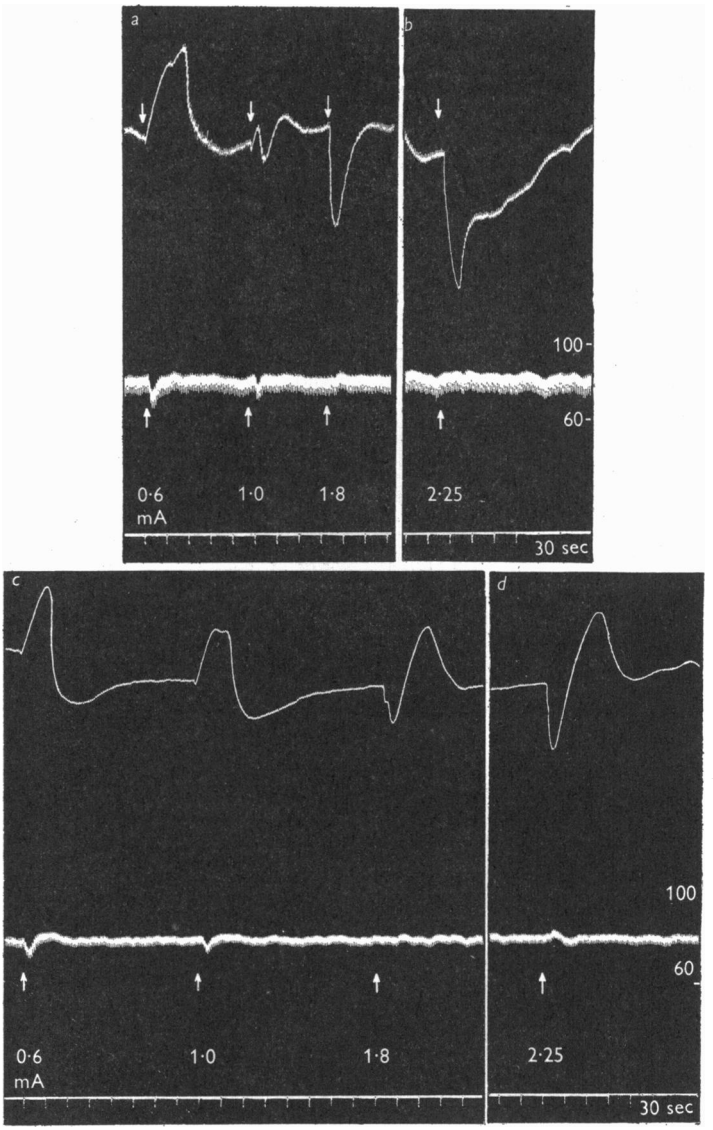

Fig. 5. Observations similar to those in Fig. 3 but made without atropine. (*a*) and (*b*) show the change in the response with increasing strength of stimulation from dilatation to constriction. Noradrenaline 1·0 mg was then infused. In (*c*) the effects of 0·6 mA and 1·0 mA were now dilatation followed y constriction, the effects of 1·8 and 2·25 mA were constriction followed by dilatation.

sympathetic stimulation were often a mixture of constriction and dilatation as is shown in Fig. 6. In (*a*) stimulation caused initial constriction, then dilatation and then further constriction. After the infusion of noradrenaline (*b*) the stimulation caused a more prolonged constriction without dilatation, which was probably masked. This result is consistent with the view that the noradrenaline infusion increases the constrictor response.

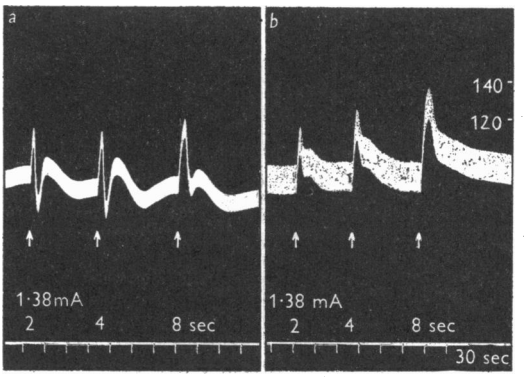

Fig. 6

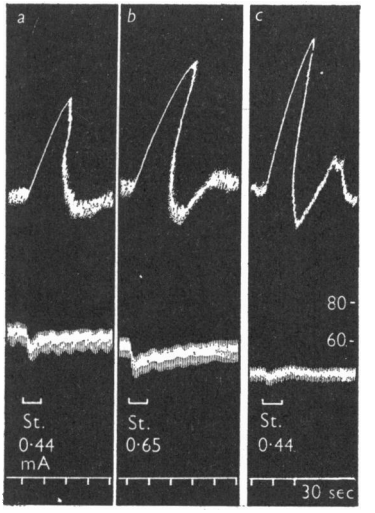

Fig. 7

Fig. 6. Perfusion of dog's hind leg. (*a*) initial responses were constriction with an intermediate phase of dilatation. (*b*) after the infusion of noradrenaline 0·25 mg, the constriction was now greater and masked the phase of dilatation.

Fig. 7. Plethysmograph observations on the dog's hind leg after removing the skin. (*a*) strength of stimulus 0·44 mA, causing dilatation only. (*b*) 0·65 mA dilatation followed by constriction. (*c*) 0·44 mA applied after a 0·25 mg noradrenaline infusion caused a greater dilatation than in (*a*), followed by constriction.

Since Bülbring & Burn (1935) showed that the dilator response to sympathetic stimulation was greatest in the skinned limb, we have also made observations in the hind leg of the dog after removing the skin. Figure 7 shows the plethysmograph record of a skinned leg, which responded to the stimulus of 0·44 mA strength with dilatation (*a*), and to the stimulus of 0·65 mA with a slightly greater dilatation followed by constriction (*b*). After an infusion of 0·25 mg noradrenaline, stimulation with 0·44 mA caused a still greater dilatation followed by constriction (*c*). Thus the noradrenaline infusion again increased both the dilator and the constrictor phase of the response.

DISCUSSION

The experiments described in this paper were prompted by observations (Burn, 1932*a*) that the vasoconstrictor effect of sympathetic stimulation in the perfused hind leg of the dog was increased when adrenaline was added to the perfusing blood. The effects observed were not great and were in the main found only when the adrenaline was actually present (Burn, 1932*a*). In more recent work we have found that when noradrenaline was used instead of adrenaline the constrictor action of tyramine was increased after the addition of noradrenaline had stopped and when its direct effect on the vessels had disappeared (Burn & Rand, 1958*b*). The present experiments have shown that, when vasodilator effects were excluded by atropine, the threshold strength of stimulus applied to the sympathetic chain for vasoconstrictor effects was reduced in some experiments to as little as one-seventh of the previous value by an infusion of noradrenaline.

This change in threshold is an unusual phenomenon, which cannot be explained by any change at the site of application of the stimulus, since there was no circulation of blood through the chain at that point. The number of nerve fibres excited must therefore have been the same although the threshold fell after noradrenaline was infused. The following suggestion put forward earlier by one of us (Burn, 1932*b*) for adrenaline is likely to apply to the results now obtained if noradrenaline is substituted for adrenaline: 'If it is true that, when the sympathetic nerves are stimulated, adrenaline is liberated from the endings, then it is necessary to suppose that there is a store of adrenaline in the neighbourhood of the endings ready to be liberated when the sympathetic impulse arrives.... If now adrenaline is added to the circulation it may be supposed that the magazine or store at the end of each sympathetic nerve is replenished....'

In 1948 Schmiterlöw showed that noradrenaline could be extracted from blood vessels and in 1951 von Euler & Purkhold extracted it from the spleen, the kidney and other organs. This extractable noradrenaline is presumably the store. Von Euler (1956) has expressed the view that it is contained within the nerve terminations, since 'no evidence has been obtained for the assumption that noradrenaline may be located outside the nervous tissue itself.' This conclusion however led him to suppose that these nerve terminals might contain as much as 3–30 mg/g although the fibres of the splenic nerve outside the spleen contain only 15 μg/g. The view that the store of noradrenaline is within the nerves affords the simplest explanation for the disappearance of the store when the nerves degenerate (von Euler & Purkhold, 1951).

Another possibility has arisen from the finding of chromaffin cells in

human skin (Adams-Ray & Nordenstam, 1956; Burch & Phillips, 1958). Mr E. H. Leach has found similar cells in the skin of the rabbit ear (see Burn & Rand, 1958*a*), in the cat's nictitating membrane and in the arrectores pili muscles of the cat's tail. These cells disappear or lose their granules after the administration of reserpine or when the sympathetic nerves have degenerated, and in both circumstances the store of extractable noradrenaline disappears (Burn, Leach, Rand & Thompson, 1959). It is thus a possibility that the store of noradrenaline in the neighbourhood of sympathetic nerve endings is present in chromaffin cells.

Whatever be the precise nature and location of the store, the fact that it can be replenished from noradrenaline circulating in the blood means that the post-ganglionic sympathetic adrenergic nerve endings differ from the cholinergic nerve endings at the neuromuscular junction, where the acetylcholine seems to be produced by the nerve terminal exclusively. Thus at the post-ganglionic sympathetic nerve ending there is a mechanism for taking up circulating noradrenaline as well as for releasing it.

We see in these observations evidence of a possible function for the noradrenaline secreted into the blood by the adrenal gland. There has hitherto been no satisfactory explanation of this secretion and it now appears that it might be secreted in order to fill up the stores at the sympathetic nerve endings. Adrenaline is much less effective as regards this function. We can see further that, if the activity of the adrenal medulla in releasing noradrenaline is excessive, the tone maintained by sympathetic impulses may also become excessive.

The evidence further suggests that when noradrenaline disappears from the blood the disappearance may be partly due to its uptake and storage. Until now the natural assumption has been that when noradrenaline disappeared it was destroyed. This, however, is no longer the only possibility.

SUMMARY

1. Experiments have been carried out in which the threshold strength of stimulation of the lumbar sympathetic chain for producing vasoconstriction in the vessels of the dog's hind leg was determined. Vasoconstriction was measured by using a plethysmograph in the eviscerated and anaesthetized dog, and also by changes in arterial resistance and venous outflow in the perfused preparation.

2. After an infusion of noradrenaline had been given and after the direct effect of the infusion had disappeared, it was found that the threshold of sympathetic stimulation was reduced, often considerably, and that the effect of a given strength of stimulus was increased. An infusion of adrenaline had little or no effect.

3. It is suggested that the extractable noradrenaline present around sympathetic nerve endings forms a store from which noradrenaline is released by a sympathetic impulse. This store can also take up noradrenaline from the blood. The fall in the threshold following an infusion of noradrenaline suggests that before the infusion noradrenaline released by stimulation is at once returned to the store and is thus not available for an action on the effector organ, whereas after an infusion, when the store is full, the noradrenaline released is not taken up and therefore exerts an action.

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