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THE EFFECT OF NEURONAL REST ON THE OUTPUT OF SYMPATHETIC TRANSMITTER FROM THE SPLEEN

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Brown & Gillespie (1957) studied the output of sympathetic transmitter in the venous blood from the spleen of the cat resulting from stimulation of the splenic nerves. They showed that the output depended upon the frequency of stimulation, being maximal at 30/sec, and falling away at higher and lower frequencies. No noradrenaline could be detected in the venous blood at frequencies lower than 10/sec. The administration of the adrenergic blocking agents dibenamine and dibenyline increased the output at all frequencies below 30/sec. The explanation suggested for these results was that combination with tissue receptors precedes the metabolic removal of liberated noradrenaline; at frequencies below 10/sec the unpoisoned receptor mechanism can remove all the liberated noradrenaline, and so none can be detected in the venous effluent. At frequencies between 10 and 30/sec increasing amounts overflow into the circulation because the receptor mechanism is swamped. The administration of a blocking agent prevents the uptake of transmitter by the tissue, and the noradrenaline then appearing in the venous blood gives a measure of the amount liberated by the nerve endings. It is therefore possible by measuring the amount of transmitter in the venous blood, before and after a blocking agent is given, to determine, for any frequency of stimulation, the amount of transmitter liberated, the amount taken up by the tissue, and the amount normally overflowing.

Some experiments made for another purpose suggested that previous activity might modify the amount of the transmitter overflowing when the nerves were stimulated. It had been found, for instance, that a first group of stimuli at 30/sec given to the nerve after a rest of 1–1½ hr yielded 450 pg/stimulus, whereas a second, given 10 min later, produced an overflow of 1025 pg/stimulus (mean of 5 observations). These experiments led us to think that interruption of the normal constant centrifugal discharge of the sympathetic neurones might alter the peripheral processes of liberation

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and uptake of transmitter and yield information on the changes underlying the well-known sensitizing effect of decentralization. The innervation of the spleen by post-ganglionic fibres, with a cell station in the coeliac ganglia, provided an opportunity of studying the effect of resting the neurones by cutting the splanchnic nerves and so decentralizing them. Rest in this way has been found to modify the overflow of transmitter into the venous blood, but the picture is complicated by even greater changes in the amount of transmitter liberated by the nerve endings and in the amount of liberated transmitter taken up by the effector cells of the spleen. Preliminary accounts of some of this work have already been published (Brown, Davies & Ferry, 1959; Brown, 1960).

METHODS

Cats were used for all the experiments on the spleen. Before the operation to decentralize the spleen, the animal was given 1 mg atropine sulphate by subcutaneous injection and then anaesthetized with ethyl chloride and ether. The left splanchnic major nerve and usually one or more minor nerves were divided through an incision in the left flank, with strict aseptic precautions. In the subsequent experiment, between 19 hr and 6 days later, the cat was anaesthetized with ethyl chloride and ether and then chloralose 80 mg/kg was given intravenously. The abdomen was opened in the mid line and the splenic nerves were dissected off the splenic artery and tied. The arrangements for nerve stimulation, for collection of blood and for the assay of its noradrenaline content on the blood pressure of the completely pithed rat were identical with those described by Brown & Gillespie (1957).

Section of the left splanchnic major nerve almost completely decentralizes the post-ganglionic neurones to the spleen. In four experiments stimulation of the left splanchnic major nerve at 30/sec gave a mean output of noradrenaline of 1014 pg/stimulus. This figure is within the normal range of output when the splenic nerves are stimulated at this frequency. Stimulation of the right splanchnic major nerve in the same animals gave a mean output of 224 pg/stimulus. Hence the left splanchnic provides 80% of the pre-ganglionic fibres supplying the spleen.

In all experiments division of the splanchnic nerve was confirmed by post-mortem examination.

The adrenergic blocking agents used in the investigation were: dibenyline, dibenzyline (*N*-phenoxy*iso*-propyl-*N*-benzyl-β-chloroethylamine) 10 mg/kg by intravenous injection; hydergine (Sandoz; a preparation of dihydro-ergocornine, dihydro-ergocristine and dihydro-ergokryptine) 0·5 mg/kg by intravenous injection; phentolamine (Rogitine, Ciba; (2-*N*-p-tolyl-*N*-m-hydroxyphenyl-aminomethyl)-imidazoline methanesulphonate) was used in one experiment only.

RESULTS

Overflow of transmitter in normal animals. All the experiments described in this paper have been made with two frequencies of stimulation only, 30/sec which gives maximal overflow, and 10/sec which gives the least detectable overflow of noradrenaline in the venous blood. The figures we have obtained differ a little from those published by Brown & Gillespie, but have not substantially changed the characteristic output–frequency curve (Fig. 1 and Table 1).

The effect of adrenergic blocking agents in normal animals. The effect of the blocking agents dibenamine and dibenyline on the output of noradrenaline was investigated by Brown & Gillespie, who gave them 24 hr before an experiment and report only two experiments in which dibenyline was given in the course of an acute experiment. We have now accumulated a number of results in which dibenyline or other blocking agents were given either immediately after adrenalectomy or immediately after the first stimulation sample had been collected. In both instances half to one hour was allowed to elapse before sampling was continued.

TABLE 1. Overflow of noradrenaline from the spleen on stimulation of the splenic nerves with 200 shocks

	Brown & Gillespie (1957)			Recent results		
Frequency	Mean	S.E. of mean	No. of expts.	Mean	S.E. of mean	No. of expts.
10/sec	166	20	14	169	28	25
30/sec	985	115	23	841	61	36

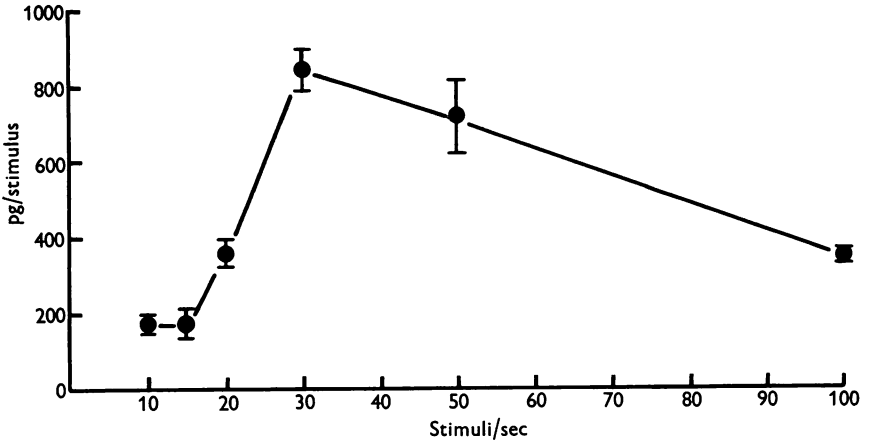

Fig. 1. Relation between overflow of noradrenaline and frequency of stimulation of the splenic nerves in normal animals. Each point shows the mean and S.E. of mean of several experiments. Points other than at 10 and 30/sec are from Brown & Gillespie (1957). For further details see Table 1.

At 30/sec there is a small, just significant increase, but at 10/sec the output in the venous blood is increased by a factor of nearly ten to give the high figure of 1710 pg/stimulus (Fig. 2, Table 2). This output after blocking agents we have taken to represent the liberation of noradrenaline by the nerve endings.

We have carried out experiments with two other adrenergic blocking agents, hydergine and phentolamine. The results of one experiment with

hydergine are shown in Fig. 3. In this experiment hydergine 0·5 mg/kg was given between groups of 200 stimuli at 10/sec and 30/sec. The output at 10/sec is raised to that of 30/sec. This pattern of output is what we expect when dibenyline is used. In our more recent experiments we have used hydergine regularly, as it has the advantage over dibenyline of quickly producing a nearly complete block to nerve stimulation. A sample

TABLE 2. The liberation of noradrenaline from the spleen on stimulation of the splenic nerves after dibenyline or hydergine

	Brown & Gillespie (1957)		Recent results		
Frequency	Mean	No. of expts.	Mean	S.E. of mean	No. of expts.
10/sec	1220	10	1710	280	8
30/sec	778	5	1060	132	14

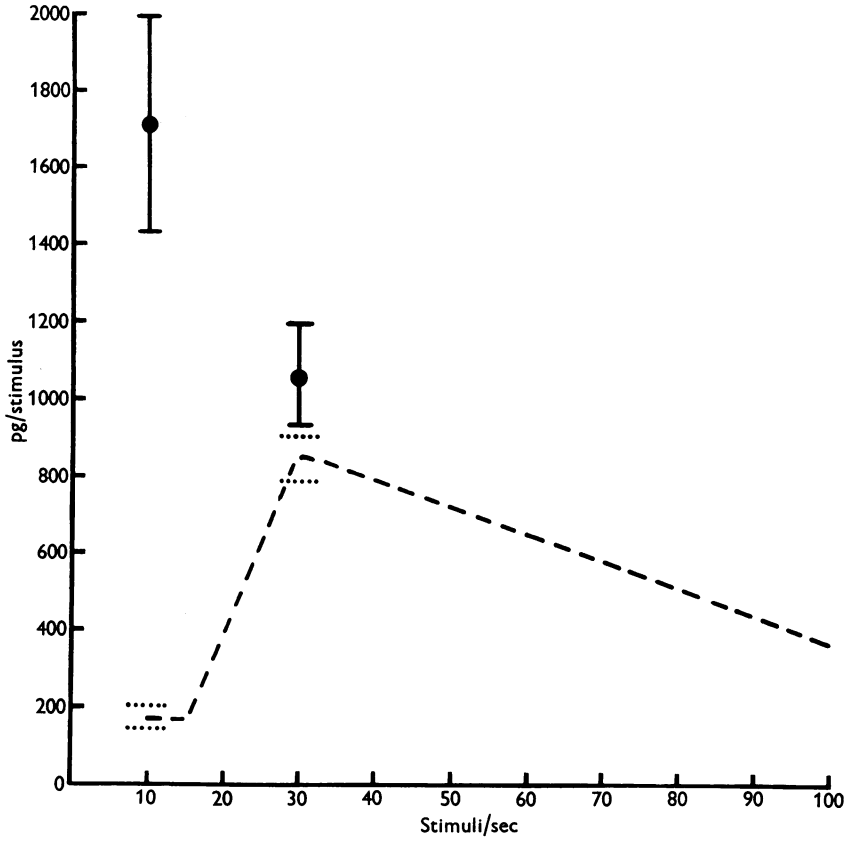

Fig. 2. Effect of adrenergic blocking agents on output of noradrenaline from the spleen with stimulation of the splenic nerves at 10 and 30/sec, indicated by the two single points. The interrupted line shows the normal overflow curve. For further details see Table 2.

can therefore be taken 10 min after administration of hydergine, whereas 30 min must be left between administering dibenyline and taking the next sample, during which time the preparation may deteriorate. Bilateral vagotomy is necessary before the administration of hydergine, to abolish the vagal inhibition of the heart otherwise produced by the drug.

In one experiment we used phentolamine intravenously in a dose of 2·8 mg/kg. The results of this experiment are shown in Fig. 4, and it can be seen that the drug caused an elevation of the output at 10/sec in a way similar to the other adrenergic blocking agents.

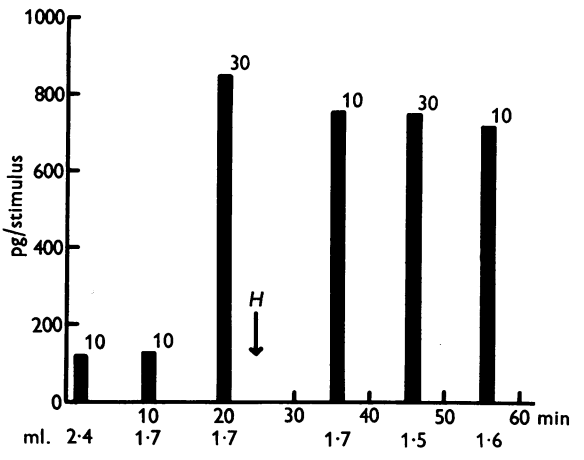

Fig. 3

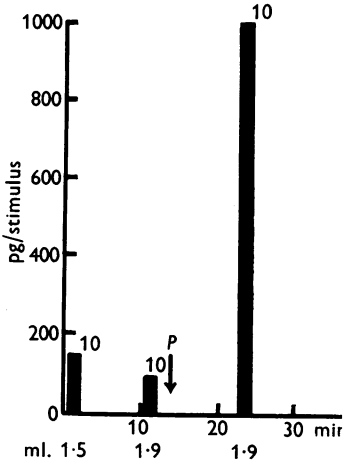

Fig. 4

Fig. 3. Cat 3·5 kg. Output of noradrenaline from the spleen in response to groups of 200 stimuli to the splenic nerve. The figures at the top of each block indicate the frequency of stimulation. The plasma volume of each sample is given below the abscissa. Between the third and fourth samples hydergine 0·5 mg/kg was given by intravenous injection.

Fig. 4. Drawn as Fig. 3. Cat 1·7 kg. Output of noradrenaline after 200 stimuli at 10/sec. Between the second and third samples phentolamine 2·8 mg/kg was given intravenously.

The effect of neuronal rest

Effect of rest on the overflow at 30/*sec.* When the abdomen was opened 19 hr to 6 days after cutting one or both major splanchnic nerves the spleen was seen to be large and engorged with blood. Stimulation of the post-ganglionic trunk caused a vigorous contraction and expulsion of blood with a high cell volume. When the overflow of noradrenaline with stimulation at 30/sec was determined, it was found to be significantly lower than the normal. In normal cats the overflow of noradrenaline from the spleen is 841 pg/stimulus (S.E. 61); in the 29 rested animals it was 394 pg/stimulus

(S.E. 30). This is illustrated in Fig. 5. It appears that section of the left splanchnic major nerve is sufficient to produce these effects. In 5 of the 29 experiments the splanchnic major nerves on both sides had been cut; in other experiments the left splanchnic minor and minimus had been cut as well as the left splanchnic major. There was no difference in the results attributable to section of nerves in addition to the left splanchnic major.

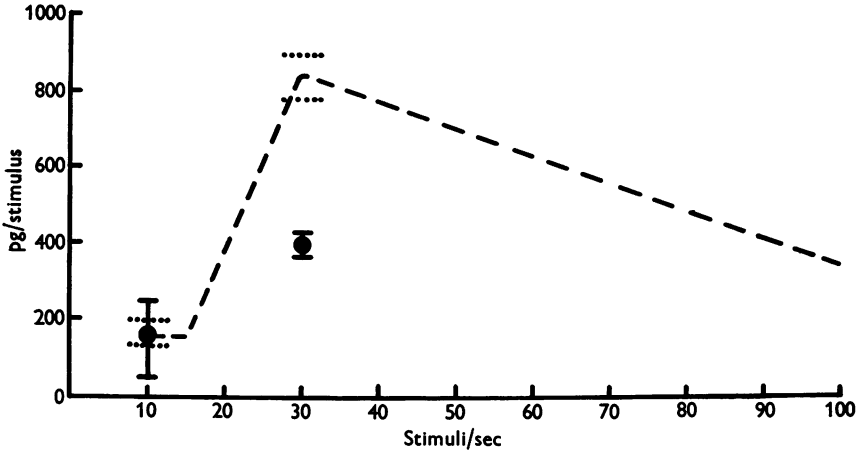

Fig. 5. Overflow of noradrenaline from the spleen when the splenic nerves were stimulated at 10/sec and 30/sec in the decentralized preparation. Interrupted line shows the normal overflow curve. For further details see text.

Effect of rest on the overflow at 10/*sec.* Having found that rest caused a decrease in the overflow of noradrenaline when the nerves were stimulated at 30/sec, we expected that the overflow at 10/sec would be very small indeed. In six experiments, however, the initial overflow was 148 pg/stimulus (S.E. 100). This point is also shown in Fig. 5, superimposed on the normal overflow curve. It can be seen that there is no significant difference between the normal and the rested overflow at 10/sec.

The reduction in output of transmitter in the rested preparation is not due to any general effects of the previous operation nor to treatment of the animal with atropine or anaesthetics. In one animal, for instance, the left splanchnic nerve was not cut because of haemorrhage, and 24 hr later the output at 30/sec was 1000 pg/stimulus before, and 1100 pg/stimulus 1 hr after the administration of dibenyline, figures well within the normal range. In another animal the right splanchnic was divided and the left was sought, but not found. Four days later the output at 30/sec was 600 pg/stimulus. After 1000 stimuli at 30/sec the output remained at 600 pg/stimulus. This behaviour is characteristic of a normal unrested preparation with an output somewhat below the mean.

The diminution from the normal values of the overflow at 30/sec in the rested preparation could have been due either to diminished liberation of transmitter or to increased uptake by the tissue. The fact that there was no change in the overflow at 10/sec gave no clue to the cause of the reduction at 30/sec, and the only means of testing the point was to use blocking agents and thereby gain some information on the effect of rest on the amount of transmitter liberated.

Effect of adrenergic blocking agents on the output after rest. The administration of a blocking agent to a rested preparation showed that the liberation of noradrenaline after rest is greatly in excess of the normal. In 12 experiments the splenic nerve was tied as soon as the animal was anaesthetized with chloralose, and a blocking agent was given at a suitable time after adrenalectomy. In 7 cats the first stimulation was at 10/sec and the liberation was 3060 pg/stimulus (S.E. 465). In the other 5 animals the first stimulation was at 30/sec and the liberation was 1800 pg/stimulus (S.E. 206). The normal values for the liberation of noradrenaline are 1710 pg/stimulus at 10/sec and 1060 pg/stimulus at 30/sec. These points, together with the normal overflow curve, are shown in Fig. 6.

We conclude from these experiments that resting the post-ganglionic neurone leads to an increase in the amount of noradrenaline liberated per stimulus and also to an increase in the capacity of the tissue to take up the transmitter. At 30/sec the increase in liberation is less than the increase in uptake, and therefore the overflow falls. At 10/sec the increases in liberation and in uptake are about the same and the overflow, in consequence, is unchanged.

The background pressor activity of plasma. Furchgott (1959) has suggested that the elevation of the output from the spleen after dibenyline might be due to the release by the drug of noradrenaline from the nerve endings. During our experiments we have taken a number of samples of splenic venous blood in the absence of stimulation of the splenic nerves and assayed this background pressor activity in terms of noradrenaline. The initial venous control sample at the beginning of every experiment usually contains some substance with a pressor activity equivalent to about 10–20 ng noradrenaline/ml. The active substance in this sample is probably not noradrenaline, as it has a slow, long-lasting effect on the blood pressure of the pithed rat. During an experiment in the absence of blocking agents the background pressor activity gradually increases to about 30 ng/ml. and resembles that of catecholamines. We assume this additional activity to be produced by sympathetic activity in a deteriorating preparation which is being gradually exsanguinated during the experiment (cf. Brown, Davies & Gillespie, 1958).

If a blocking agent is administered, the background activity rises in a

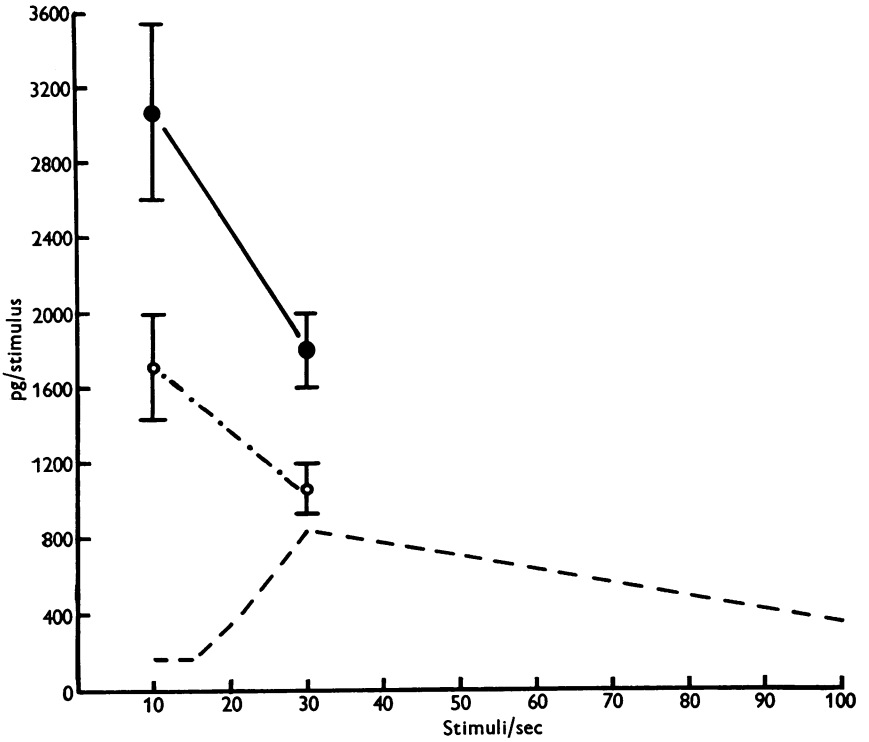

Fig. 6. Liberation of noradrenaline (mean and S.E. of mean) in the decentralized preparation (●), together with the liberation in the normal (○) and the normal overflow curve. For further details see text.

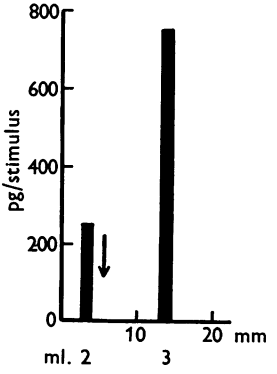

Fig. 7. Cat 2·7 kg. Left splanchnic nerves cut 24 hr previously. Output of noradrenaline after 200 stimuli at 30/sec to the splenic nerve. Between the two samples 1000 stimuli at 30/sec were given at the arrow.

similar way. After the administration of dibenyline an interval of at least 30 min was allowed to elapse before continuation of the experiment. During this time the background activity rose again to about 30 ng/ml. The use of hydergine allowed sampling to be resumed 10 min after administration of the drug, and the rise of background activity was usually absent or very small. Venous control samples taken some time after the administration of this drug showed a rise similar to that occurring after dibenyline. The rise in background pressor activity at various times after administering blocking agents is thus closely similar to the rise in their absence. We have therefore no reason to suppose that release of noradrenaline by the blocking agents is a significant factor in these experiments.

Effect of a conditioning train of stimuli on the overflow after rest. It seemed to us that, if the effects described above were due to the cessation of the normal discharge in the post-ganglionic pathways, a conditioning train of stimuli ought to return the transmission mechanism towards the normal. This proved to be the case, and we have found that the low initial overflow at 30/sec can be raised by further stimulation. The application of the standard sampling group of 200 stimuli alone has a definite effect, and the second sample always contains more noradrenaline than the initial sample, an effect referred to in the introduction to this paper. A more dramatic way of elevating the overflow is to give a conditioning train of 1000 stimuli at 30/sec. In five experiments the mean initial overflow at 30/sec was 381 pg/stimulus. After a train of 1000 stimuli at the same frequency the second mean overflow was 936/stimulus. One of these experiments is illustrated in Fig. 7.

It appears from these experiments that the effects of rest on the overflow at 30/sec can be removed by a period of stimulation of the nerves. The change could be due either to an increase in the liberation or to a reduction of uptake to within normal limits. This was tested by treating rested preparations with dibenyline *ab initio* and measuring the overflow with 200 stimuli, before and after 1000 stimuli at 30/sec. The results we obtained were not entirely conclusive, but in the few satisfactory experiments there was a small reduction in the liberation. It would appear therefore that the chief effect of 1000 stimuli is a reduction in uptake by the tissues.

Experiments with ganglionic blocking agents. We thought it might be possible to rest the post-ganglionic nerves to the spleen by the use of ganglionic blocking agents. Two drugs were tried, pentolinium tartrate (Ansolysen; May and Baker), and chlorisondamine (Ecolid; Ciba). These are reported to block ganglia maximally for 4 and 8 hr respectively. We injected them subcutaneously in doses of 3 mg/kg and used the nictitating membrane as an indicator of the state of block. With chlorisondamine the

nictitating membranes were relaxed across the eyes within 10 min of the injection and remained so for about 6 hr. After this time the block gradually wore off, and we could therefore not keep a cat blocked overnight. We found that the effect of a subcutaneous injection could be prolonged up to 16 hr by injecting chlorisondamine (4 mg/kg) in aluminium stearate in arachis oil.

In four experiments in which the block had been effective for 20–24 hr there was a clear reduction in the initial output of transmitter in response to 200 stimuli at 30/sec, the mean value being 270 pg/stimulus, and in all these experiments the outputs could be increased either by a conditioning train of stimuli at 30/sec or by dibenyline. In experiments in which the block was in operation for under 4 hr there was no evidence of any lowering of the initial output, and conditioning stimulation did not cause a significant increase in output. With periods of block between 4 and 20 hr the results were equivocal, the effect sometimes being evident and at other times absent, with no obvious relation to the duration of the block.

Effect of injecting noradrenaline into the spleen. Resting the postganglionic neurones to the spleen reduces the initial overflow of noradrenaline when the nerves are stimulated at 30/sec. We have interpreted this reduction as being mainly due to a temporary increase in the ability of the tissue to take up the transmitter. This increased avidity of the tissue can be returned towards normal by a conditioning train of stimuli. Theoretically a close-arterial injection of noradrenaline into the spleen should satisfy this avidity and increase the overflow of noradrenaline on subsequent stimulation of the splenic nerve.

We therefore measured the initial overflow of transmitter at 30/sec and then injected noradrenaline into the spleen through a cannula in the hepatic artery. Ten minutes later the usual 200 stimuli at 30/sec were given and the overflow of noradrenaline determined. Ten micrograms of noradrenaline in 1 ml. of saline was used as the standard injection, since this amount caused approximately the same degree of contraction of the spleen as stimulation of the splenic nerves. These experiments proved rather unsatisfactory; the overflow in the second and third samples was greater than in the initial sample, but the results were almost identical with the controls in which the injection of noradrenaline was omitted. A possible cause of our failure to raise the reduced output is that the injection of such a large quantity of noradrenaline into the spleen caused a prolonged contraction, presumably through some of the noradrenaline being trapped in the closed vessels, and the blood flow, in consequence, was poor during the collection of the first, crucial sample after the injection. Smaller doses of noradrenaline had little effect on the spleen or on the overflow with a subsequent stimulation.

The nature of the transmitter liberated after rest. The transmitter liberated from normal splenic nerve endings is mainly noradrenaline (Peart, 1949; Mann & West, 1952; Brown & Gillespie, 1957), the adrenaline content being seldom more than 10% (Mirkin & Bonnycastle, 1954). It is conceivable that in the rested neurones a greater proportion of adrenaline might be present in sympathin released on excitation. As the pithed rat is less sensitive to adrenaline than to noradrenaline, a change in the ratio of the two substances in the venous effluent might explain some of our results. In one experiment in a cat 3 days after section of the left splanchnic nerves hydergine was administered and the splenic nerve was stimulated at a frequency of 30/sec. The plasma was treated so as to separate catecholamines (Vogt, 1952) and adrenaline was assayed on the carbachol-stimulated rat uterus (Gaddum & Lembeck, 1949), the noradrenaline being estimated in the usual way. The results showed that the sympathin liberated from rested neurones contained about 11% adrenaline. We conclude that resting the neurones does not alter the nature of the liberated transmitter.

The pattern of outflow of transmitter. The decentralized spleen contracts more vigorously in response to stimulation of the splenic nerves than does the control. It is possible that this might alter the time relations of outflow of transmitter, and the standard sampling time—the period of stimulation + 20 sec—might not allow collection from the rested preparation of the same proportion of total outflow as in the normal. We have found that the outflow pattern of the rested preparation does not differ significantly from the normal, and that 85–95% of the total outflow appears within the standard sampling time.

Effect of duration of rest. It has been shown by Govaerts (1935, 1939) and by McLennan & Pascoe (1954) that spontaneous activity of decentralized ganglion cells begins after 3 days and is fully developed 7 days after preganglionic section. The following figures show the number of experiments performed with different periods of rest:

Duration of rest in days	...	1	2	3	4	5	6
Number of experiments	...	15	3	6	2	1	2

The decrease in overflow at 30/sec was fully developed after 24 hr rest; no difference could be detected with durations of rest up to 6 days.

DISCUSSION

The normal preparation

The experiments that have been recorded in this paper have confirmed and extended those of Brown & Gillespie (1957) and have strengthened the evidence on which they based their conclusion that noradrenaline liberated from nerve endings is inactivated after combination with tissue receptors.

Inhibitors both of aminoxidase and of *o*-methyltransferase (Bacq, Brown & Ferry, 1960) are without effect on the overflow of the liberated transmitter, and a variety of substances with the common property of blocking adrenergic receptors have identical and dramatic effects on the overflow of noradrenaline. The hypothesis put forward by Furchgott (1959) that dibenyline might owe its effect not to its blocking action but to an inhibition of some unknown enzyme system would appear to be untenable, unless the enzyme is identical with the receptive substance for noradrenaline.

TABLE 3. Rates of liberation and uptake in normal and rested spleen; rates are expressed in ng/sec

	Normal			Rested		
Frequency	Liberation (*a*)	Uptake (*b*)	*b*/*a*	Liberation (*a*)	Uptake (*b*)	*b*/*a*
10/sec	17	15	0·88	31	29	0·94
30/sec	32	7	0·22	54	42	0·78

We have assumed that the output of noradrenaline in the absence of a blocking agent represents the overflow of transmitter, and that the output in the presence of a blocking agent represents the amount of transmitter liberated from the nerve endings. This cannot be strictly true because none of the blocking agents that we have used has produced a complete block to nerve stimulation, although, as is well known, the block to injected noradrenaline is total. We have no means of determining what proportion of the amount liberated is still taken up by the receptors, but it is likely to be small since Nickerson (1956) has shown that a maximal contraction of smooth muscle to histamine can be produced by a dose occupying not more than 1 % of the receptors.

From the figures of liberation and overflow the amount and rate of uptake of noradrenaline by the receptive substance can be calculated (Table 3). It is clear that at 30/sec the uptake is depressed, although the rate of liberation is twice what it is at 10/sec. This state of affairs is reminiscent of the desensitization of cholinergic receptors by the iontophoretic application of acetylcholine (Katz & Thesleff, 1957; Axelsson & Thesleff, 1958) or by repetitive stimulation of the motor nerve (Thesleff, 1959), and their suggestion that excess transmitter may inactivate the receptor fits well with our findings.

Brown & Gillespie (1957) thought that the liberation of noradrenaline per stimulus was probably constant from low frequencies up to 30/sec. Our more complete results with blocking agents show that the liberation per stimulus at 10/sec is considerably higher than at 30/sec. Unfortunately our experimental technique has not allowed accurate measurements at frequencies below 10/sec, but it would appear that from 10/sec to 30/sec

and beyond there is a steady decline of liberation. This is probably due to prejunctional failure, since the trunks we were stimulating were C fibres of small diameter, and even in the frog's skeletal muscle–nerve preparation prejunctional failure occurs when frequencies of stimulation much above 10/sec are used (Krnjević & Miledi, 1958, 1959).

The effect of rest

Decentralization for 24 hr of the post-ganglionic neurones supplying the spleen has been shown to bring about both an increased liberation of transmitter when the nerves are exposed to a short train of stimuli and an increased capacity of the receptive mechanism to take up the liberated transmitter. The peripheral sensitization both to nerve stimulation and to injected drugs does not reach its maximum until rather later (cf. Cannon & Rosenblueth, 1949), but we have avoided using longer periods than 6 days because of the possibility of the early re-innervation of the coeliac ganglion from adjacent cholinergic neurones.

The majority of our experiments have been done on animals 24 hr after splanchnic section, but we could find no evidence of a diminution of the effect of decentralization in animals examined up to 6 days after nerve section. It would appear, therefore, that the spontaneous discharge of the ganglion cells, to which we have already referred, has had no significant effect. We have shown that 1000 stimuli at 30/sec can abolish the effect of rest, but some experiments at present in progress suggest that stimulation at lower frequencies may be quite ineffective. It is possible, therefore, that removal of the effect of rest requires the presentation to the uptake mechanism of noradrenaline at a high rate. If this is so, it would explain the absence of effect of the irregular and infrequent spontaneous discharge and possibly also our failure to raise the rested overflow with injections of noradrenaline.

The doubling of the uptake of transmitter that we have observed suggests that the elimination of centrifugal impulses to the spleen brings about, in the smooth muscle cells, an increase in the area of receptive substance capable of taking up transmitter, just as denervation evokes a spreading of the area sensitive to acetylcholine in skeletal muscle (Axelsson & Thesleff, 1959; Miledi, 1960). A similar increase in receptor area can be produced in skeletal muscle by blocking conduction with botulinum toxin (Thesleff, 1960), and smooth muscle is sensitized to noradrenaline by reducing its noradrenaline content with reserpine (Burn & Rand, 1958), or by preventing the liberation of transmitter with bretylium (Green, 1960). There is clear evidence, however, that decentralization of the spleen for 3–14 days does not alter either its catecholamine content or the adrenaline: noradrenaline ratio (Rehn, 1958). The great increase in the amount of

transmitter liberated when the nerves of a rested preparation are eventually stimulated makes it probable that neuronal rest produces an accumulation of transmitter at the nerve endings, and it is possible to envisage a neuromuscular junctional apparatus, composed of nerve endings and receptive substance in close apposition, with a constant noradrenaline content, the partition between ending and receptor being controlled by the number and frequency of nerve impulses reaching the ending. Such a conception implies that when an amount of transmitter is liberated it combines with a given area of receptive substance or given number of receptors, activates the tissue and then remains for an appreciable time in combination with the receptors, which, as a result, are rendered inaccessible to further doses of transmitter. The easy reversibility of the effects of rest by 1000 stimuli at 30/sec is compatible with this view and particularly so since the effects of stimulation are of relatively long duration. A single train of 1000 stimuli abolishes the effect of rest, and if this is reinforced at 10 min intervals by groups of 200 stimuli for testing purposes, the preparation in an experiment lasting some hours never reverts to the rested state. We have some evidence that a rest of 1 hr can produce a reduction in transmitter overflow, but at least 12 hr must elapse before the reduction is beyond question. This implies that the processes responsible for removal of transmitter from the receptors take some hours to be effective and yet, during this period, the cells with some receptors occupied can still respond to further liberations by the nerve. These observations are compatible with Paton's (1961) suggestion that it is the 'rate of uptake of agonist' by receptors which determines activity of the effector cell and not the occupation *per se* of receptive sites.

SUMMARY

1. The output of noradrenaline from the spleen of the cat under chloralose in response to maximal excitation of the splenic nerves has been studied further.

2. The observation of Brown & Gillespie (1957) that adrenergic blocking agents (phenoxybenzamine) elevated the output of transmitter has been confirmed and extended to other blocking agents, hydergine and phentolamine. The output at a frequency of stimulation of 10/sec is increased by a factor of 10 and that at 30/sec is just significantly increased.

3. It is assumed that the output after blocking agents represents the amount of transmitter liberated and the output without a blocking agent represents the overflow. From these two values the tissue uptake can be determined.

4. At frequencies of stimulation of 10/sec 90% of the liberated transmitter is taken up by the tissue. At 30/sec the rate of liberation is twice that at 10/sec but only 28% of the liberated transmitter is removed by the tissue.

5. Degenerative section of the left splanchnic nerve decentralizes 80% of the post-ganglionic neurones supplying the spleen.

6. When the splenic nerve is stimulated at 30/sec 19 hr to 6 days after splanchnic section, the overflow of noradrenaline is reduced by half. At 10/sec the overflow is unchanged.

7. The liberation of transmitter (measured after blocking agents) is doubled at 10/sec and nearly doubled at 30/sec. The depression of overflow therefore is due to a great increase in the uptake of transmitter by the tissue.

8. The depressed overflow at 30/sec can be restored by stimulation of the splenic nerve with a train of 1000 stimuli.

9. The effects of splanchnic section can be reproduced with ganglionic blocking agents.

10. Neuronal rest, therefore, appears to cause an accumulation of transmitter at the nerve endings and to give rise to a greatly increased capacity of the innervated tissues to take up liberated transmitter.

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REFERENCES

AXELSSON, J. & THESLEFF, S. (1958). The 'desensitizing' effect of acetylcholine on the mammalian motor end-plate. *Acta physiol. scand.* **43**, 15–26.

AXELSSON, J. & THESLEFF, S. (1959). A study of supersensitivity in denervated mammalian skeletal muscle. *J. Physiol.* **147**, 178–193.

BACQ, Z. M., BROWN, G. L. & FERRY, C. B. (1960). Action du pyrogallol sur la quantité de noradrenaline libérée par la stimulation des nerfs spleniques. *C.R. Acad. Sci., Paris*, **250**, 194–195.

BROWN, G. L. (1960). Release of sympathetic transmitter by nerve stimulation. *Ciba Foundation Symposium on Adrenergic Mechanisms*, pp. 116–124. London: Churchill.

BROWN, G. L., DAVIES, B. N. & FERRY, C. B. (1959). The effect of neuronal rest on the output of sympathetic transmitter from the spleen of the cat. *J. Physiol.* **147**, 13–14*P*.

BROWN, G. L., DAVIES, B. N. & GILLESPIE, J. S. (1958). The release of chemical transmitter from the sympathetic nerves of the intestine of the cat. *J. Physiol.* **148**, 41–54.

BROWN, G. L. & GILLESPIE, J. S. (1957). The output of sympathetic transmitter from the spleen of the cat. *J. Physiol.* **138**, 81–102.

BURN, J. H. & RAND, M. J. (1958). The action of sympathomimetic amines in animals treated with reserpine. *J. Physiol.* **144**, 314–336.

CANNON, W. B. & ROSENBLUETH, A. (1949). *The Supersensitivity of Denervated Structures*. New York: Macmillan Co.

FURCHGOTT, R. F. (1959). The receptors for epinephrine and norepinephrine (adrenergic receptors). *Pharmacol. Rev.* **11**, 429–439.

GADDUM, J. H. & LEMBECK, F. (1949). The assay of substances from the adrenal medulla. *Brit. J. Pharmacol.* **4**, 401–408.

GOVAERTS, J. (1935). Apparition d'une tonus cardio-accélérateur dans le ganglion stellaire déconnecté centralement. *C.R. Acad. Sci., Paris*, **119**, 1181–1184.

GOVAERTS, J. (1939). Nouvelles recherches sur l'activité spontanée des ganglions sympathiques déconnectés du nevraxe. *Arch. int. Physiol.* **49**, 426–448.

GREEN, A. F. (1960). Effects of bretylium and allied agents on adrenergic neurones. *Ciba Symposium on Adrenergic Mechanisms*, pp. 148–157. London: Churchill.

KATZ, B. & THESLEFF, S. (1957). A study of the 'desensitization' produced by acetylcholine at the motor end-plate. *J. Physiol.* **138**, 63–80.

KRNJEVIC, K. & MILEDI, R. (1958). Failure of neuromuscular propagation in rats. *J. Physiol.* **140**, 440–461.

KRNJEVIĆ, K. & MILEDI, R. (1959). Presynaptic failure of neuromuscular propagation in rats. *J. Physiol.* **149**, 1–22.

MANN, M. & WEST, G. B. (1952). The nature of hepatic and splenic sympathin. *Brit. J. Pharmacol.* **5**, 173–177.

MCLENNAN, H. & PASCOE, J. E. (1954). The origin of certain non-medullated nerve fibres which form synapses in the inferior mesenteric ganglion of the rabbit. *J. Physiol.* **124**, 145–156.

MILEDI, R. (1960). The acetylcholine sensitivity of frog muscle fibres after complete or partial denervation. *J. Physiol.* **151**, 1–23.

MIRKIN, B. L. & BONNYCASTLE, D. D. (1954). A pharmacological and chemical study of humoral mediators in the sympathetic nervous system. *Amer. J. Physiol.* **178**, 529–534.

NICKERSON, M. (1956). Receptor occupancy and tissue response. *Nature, Lond.*, **178**, 697–698.

PATON, W. D. M. (1961). A theory of drug action based on the rate of drug-receptor combination. *Proc. Roy. Soc.* B, **154**, 21–69.

PEART, W. S. (1949). The nature of splenic sympathin. *J. Physiol.* **108**, 491–501.

REHN, N. O. (1958). Effect of decentralisation on the content of catechol amines in the spleen and kidney of the cat. *Acta physiol. scand.* **42**, 309–312.

THESLEFF, S. (1959). Motor end-plate 'desensitization' by repetitive nerve stimuli. *J. Physiol.* **148**, 659–664.

THESLEFF, S. (1960). Supersensitivity of skeletal muscle produced by botulinum toxin. *J. Physiol.* **151**, 598–607.

VOGT, M. (1952). The secretion of the denervated adrenal medulla of the cat. *Brit. J. Pharmacol.* **7**, 325–330.