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LIBERATION OF *NOR*ADRENALINE FROM THE SUPRARENAL GLAND

BY

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The denervated nictitating membrane of the cat has frequently been used to detect the liberation of the humoral transmitter during stimulation of sympathetic nerves, because it was found to be more sensitive than the normal membrane (Cannon and Rosenblueth, 1933; Greer, Pinkston, Baxter, and Brannon, 1938; Gaddum and Goodwin, 1947). In a previous paper (Bülbring and Burn, 1938) we have studied the difference in the response of the normal and the denervated membrane to various sympathomimetic substances. We found that there were many exceptions to the rule that the denervated membrane is always more sensitive, and indeed for one and the same substance the relative sensitivity varies according to the dose injected. In a film demonstrating the release of Cannon's "Sympathin" by splanchnic stimulation, which we prepared for teaching purposes and which was shown at the International Physiological Congress, 1947, the responses of both the normal and denervated membranes were recorded. Fig. 1 shows such an experiment. The effect of splanchnic stimulation (Fig. 1*b*) on the membranes was different from that of the adrenaline injected in Fig. 1*a*, though the effect on the blood pressure was the same. Stimulation caused a larger contraction of the denervated, and a smaller contraction of the normal membrane. After removal of the suprarenals (Fig. 1*c*), stimulation caused contraction of the denervated membrane, but failed to affect the normal membrane, although the rise of blood pressure was again similar.

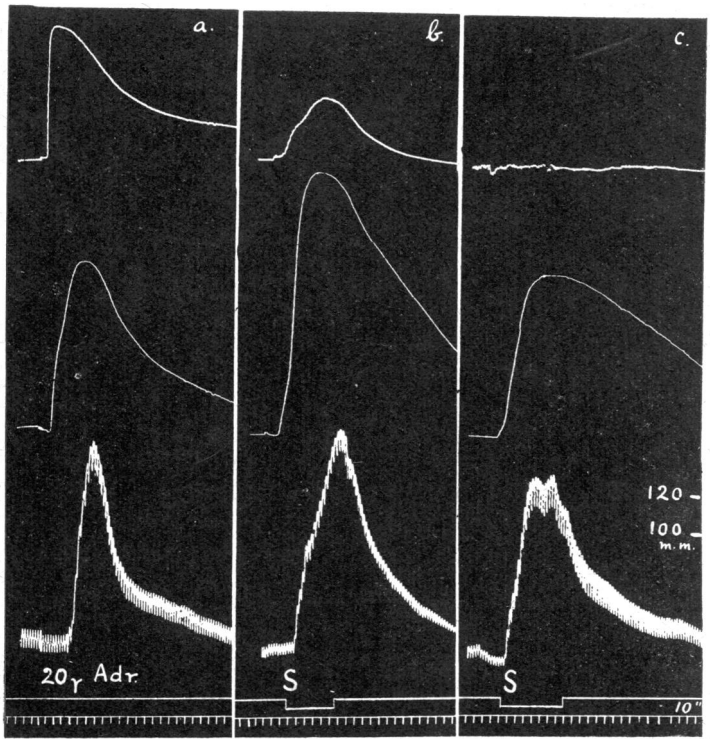

FIG. 1.—Spinal cat. Records from top downwards: normal nictitating membrane, denervated nictitating membrane, blood pressure. Effect of (*a*) 20 μg. adrenaline; (*b*) splanchnic stimulation before and (*c*) after adrenalectomy.

It was shown by Gaddum, Jang, and Kwiatkowski (1939) and also by Gaddum and Kwiatkowski (1939) that the substance liberated by stimulation of the sympathetic nerves to the blood vessels of the perfused rabbit's ear was adrenaline, since it gave the same match with adrenaline in pharmacological and colorimetric tests. We therefore supposed that the differences shown in Fig.1 between the action of adrenaline and that of stimulation were

most probably explained by the rate of entry of adrenaline into the circulation. It seemed possible that a sudden high concentration might stimulate the normal membrane as in (*a*), whereas a slowly rising concentration might stimulate less as in (*b*).

There was another possibility, however, that the differences between the effects of adrenaline and of splanchnic stimulation were to be explained by the release of another substance in addition to adrenaline. Bacq (1933) first suggested that liver sympathin might be *nor*adrenaline. Experimental evidence for this view was obtained by Greer, Pinkston, Baxter, and Brannon (1938), who compared the effect of hepatic nerve stimulation directly with that of *nor*adrenaline and adrenaline. In 1946 von Euler obtained evidence for the presence of *nor*adrenaline in sympathetic nerves, and in the following year Gaddum and Goodwin found that the substance released by hepatic nerve stimulation was probably *nor*adrenaline or possibly tyramine. In 1948 von Euler identified *l-nor*adrenaline in the splenic nerves of cattle, and Schmiterlöw similarly found it present in extracts from arteries. The foregoing observations suggested that the result shown in Fig. 1*c* might be due to the liberation of *nor*adrenaline, and the result in Fig. 1*b* to a mixture of adrenaline coming from the suprarenal glands, with *nor*adrenaline coming from sympathetic nerve endings.

The first indication that *nor*adrenaline might also be present in the suprarenal gland was given by Holton (1948), who found large amounts of it in suprarenal tumours of the type phaeochromocytoma. So far as the normal gland is concerned, observations by Meier and Bein (1948) led them to conclude that it released some other substances in addition to adrenaline. They found that a small dose of adrenaline, which caused an increased blood flow in the femoral artery, caused a decrease in flow after adrenalectomy; the normal response could be restored by slow infusion of *nor*adrenaline. Schümann (1948) has recently published a preliminary account of experiments in which he found that an extract of pig's suprarenal gland had a smaller hyperglycaemic action in the rabbit than an equipressor amount of adrenaline, but a similar hyperglycaemic action to a mixture of equal parts of adrenaline and *nor*adrenaline.

Our own experiments, to be described below, were designed to discover what was released by the normal gland during splanchnic stimulation. A brief note on this work has already appeared (Bülbring and Burn, 1949).

Methods

Cats were prepared by the removal of the right superior cervical ganglion in an aseptic operation carried out 1–2 weeks before. On the day of experiment anaesthesia was induced by ethyl chloride followed by ether, and a spinal preparation was made cutting the cord at the 2nd cervical vertebra and destroying the brain. The viscera were removed and the vessels to the left kidney were tied. In some experiments the vessels to both kidneys were tied. The left splanchnic nerve was prepared for stimulation with platinum electrodes embedded in perspex. Stimulation was applied for 30 sec. from an induction coil, the primary circuit being interrupted by Lewis's rotary contact breaker, at a rate of 20 per sec. The blood pressure was recorded from the femoral artery, and the contractions of both nictitating membranes were recorded by isotonic levers of the same weight and magnification. All substances were given by intravenous infusion into a femoral vein during a constant period of 30 sec., this being the same as the duration of stimulation. The sample of *nor*adrenaline was the racemic compound kindly given to us by Dr. M. L. Tainter of the Sterling-Winthrop Research Institute.

Results

Adrenaline, nor*adrenaline, and stimulation*

When the effects of adrenaline and *nor*adrenaline were compared with that of splanchnic stimulation the doses were chosen to give the same rise of blood pressure. It was found that in the eviscerated animal the pressor action of *nor*adrenaline was not stronger than that of adrenaline, as it is before evisceration, but was either equal to it or weaker. Thus in Fig. 2 the effect of 10 μg. *dl-nor*adrenaline is approximately the same as that of 5 μg. *l*-adrenaline, while in the experiment from which Fig. 3 was taken, 15 μg. *dl-nor*adrenaline was equal to 5 μg. *l*-adrenaline.

The nictitating membranes reacted differently to equipressor doses. The sensitivity of the normal membrane was much greater to adrenaline than to *nor*adrenaline, while the denervated membrane was very sensitive to both. The effect of splanchnic stimulation on the normal membrane was intermediate between the two. In Fig. 2 stimulation produced similar effects on the blood pressure and denervated membrane to those produced by 10 μg. *dl-nor*adrenaline and 5 μg. adrenaline, while on the normal membrane its effect was very much greater than that of *nor*adrenaline but less than that of adrenaline. In Fig. 3, on the other hand, the effect of stimulation on the normal membrane was much closer to that of *nor*adrenaline; adrenaline produced a greater effect than either, in spite of the fact that the dose of adrenaline was too small to be equipressor and to produce the same contraction on the denervated membrane.

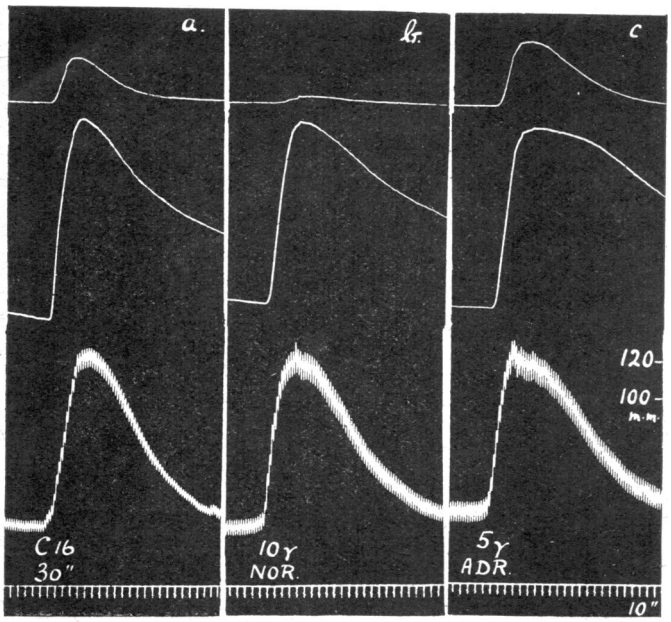

FIG. 2.—Spinal eviscerated cat. Records as in Fig. 1. Note that the response of the nictitating membranes to splanchnic stimulation (*a*) is intermediate between that to 10 μg. *dl-nor*adrenaline (*b*) and that to 5 μg. *l*-adrenaline (*c*).

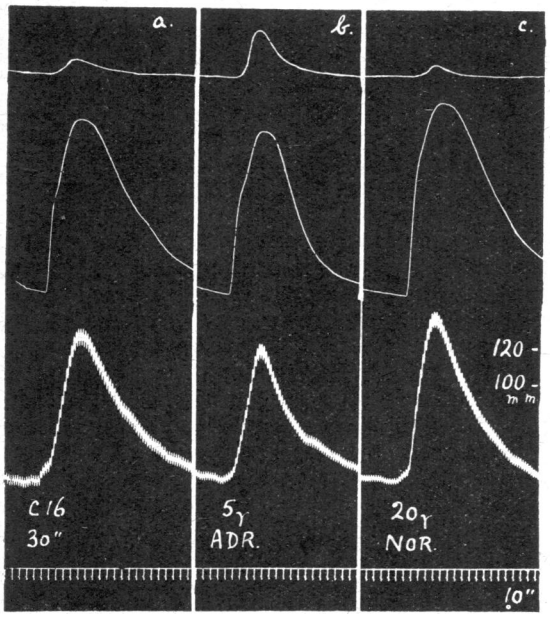

FIG. 3.—Spinal eviscerated cat. Records as in Fig. 1. Note that the response of the nictitating membranes to splanchnic stimulation (*a*) is very similar to that caused by 20 μg. *dl-nor*adrenaline (*c*) but not to that caused by 5 μg. *l*-adrenaline (*b*).

Ratio of the size of contractions

In twelve experiments we have compared the sensitiveness of the denervated with that of the normal membrane by calculating the ratio of the size of their contractions. The results are given in Table I. If the ratios for adrenaline in the different experiments are examined it will be seen that there are five exceptions to the rule that the denervated membrane is more sensitive. In Expts. 6, 7, and 10 the ratio is less than 1.0, and in Expts. 8 and 11 it is equal to 1.0; this may be due to the use of small doses only. The ratios for *nor*adrenaline are always greater than 1.0, as are those for stimulation.

The main point of Table I is that in each experiment the ratio for *nor*-adrenaline is greater than that for adrenaline, and that the ratio for stimulation is intermediate. Each ratio given in the Table is the mean of all the determinations made in that particular experiment. It is true that the ratio for any substance depends on the amount injected; thus in one experiment the ratio for 5 μg. adrenaline was 2.3, for 10 μg. it was 2.1, and for 20 μg. it was 1.85; but since the range of doses used to match splanchnic stimulation was narrow the effect of dose on ratio was negligible for our purposes.

TABLE I

RATIO OF CONTRACTIONS OF DENERVATED TO NORMAL MEMBRANE

Experiment	Adrenaline	Stimulation	*Nor*adrenaline
1	1.9	2.05	7.8
2	2.8	6.1	17.5
3	3.0	5.3	28.0
4	1.5	2.5	9.2
5	1.4	4.4	6.5
6	0.9	1.36	6.1
7	0.8	2.5	7.2
8	1.0	1.2	1.8
9	1.4	1.8	6.2
10	0.65	1.04	1.16
11	1.0	1.16	2.1
12	1.14	1.51	5.2

Matching the effect of stimulation

We were able to match the effect of stimulation on the nictitating membranes by infusing mixtures containing different proportions of adrenaline and

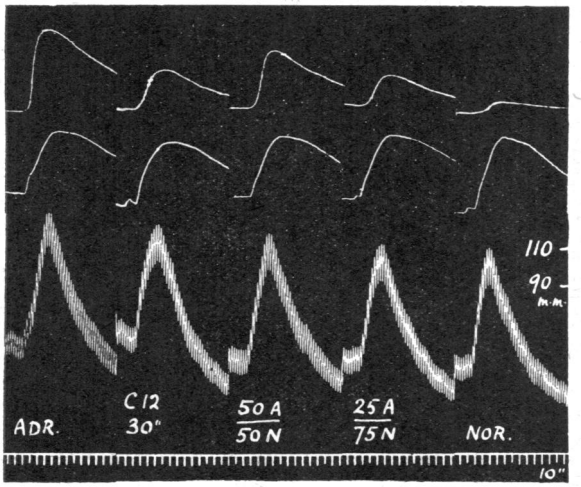

FIG. 4.—Spinal eviscerated cat. Records as in Fig. 1. Estimation of the proportion of adrenaline and *nor*adrenaline liberated by splanchnic stimulation (see text).

*nor*adrenaline. The solutions used were always 1.25 μg. adrenaline per c.c. and 2.5 μg. *dl-nor*adrenaline per c.c. A mixture described as 75 per cent adrenaline and 25 per cent *nor*adrenaline contained three parts by volume of the first and one part of the second, etc. The total amount of any mixture infused was adjusted to produce a rise of blood pressure equal to that of an adjacent stimulation. The mixture which also produced the same effect on the nictitating membranes both in size and in ratio was taken as a match. Fig. 4 shows the procedure. The effect of adrenaline was first recorded, followed by that of stimulation. From the response of the nictitating membranes it was obvious that stimulation did not release adrenaline only. A mixture was then infused of 50 per cent adrenaline and 50 per cent *nor*adrenaline. The effect on the membranes indicated that this mixture contained a larger proportion of adrenaline than was released by stimulation. Infusion of a mixture containing 25 per cent adrenaline and 75 per cent *nor*adrenaline produced a response which indicated that this mixture contained too small a proportion of adrenaline.

Estimation of proportion

The exact figure for the proportion of adrenaline and *nor*adrenaline was determined from a curve relating the ratios of the contractions to the mixtures infused in each experiment. Curves for three experiments are reproduced in Fig. 5. When the mean ratio for stimulation was found, reference to the curve gave the proportions of adrenaline and *nor*adrenaline released. In the three experiments represented in Fig. 5, the proportions were 55, 45, and 20 per cent adrenaline respectively.

A calculation was also made of the proportion of adrenaline liberated by successive stimulations in each experiment. The results are shown in Table II

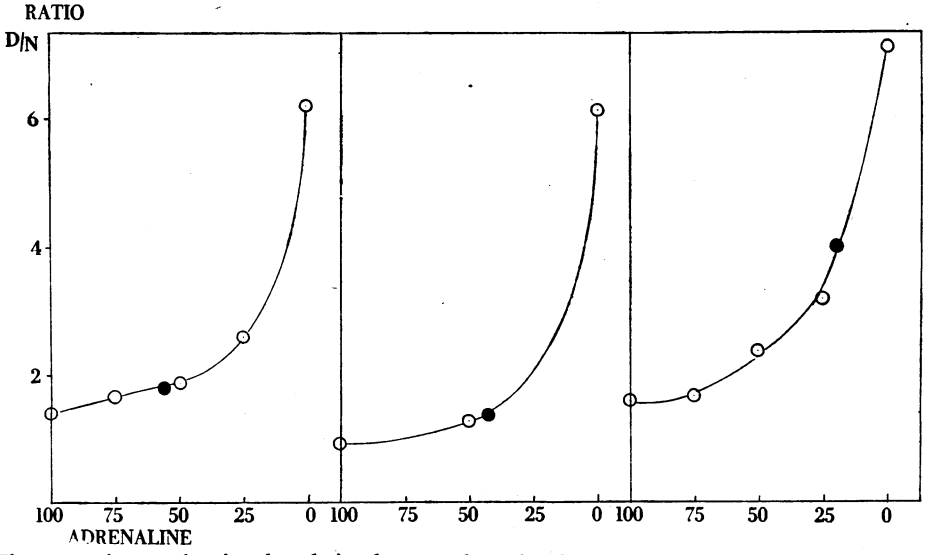

FIG. 5.—Three experiments showing the relation between the ratio of the size of contraction of the denervated to that of the normal nictitating membrane (ordinates) and the percentage of adrenaline present in the mixtures with *nor*adrenaline infused (abscissae). The black circles represent the mean ratio obtained by splanchnic stimulation in each experiment.

TABLE II

PERCENTAGE OF ADRENALINE RELEASED BY SUCCEEDING STIMULATIONS

Experiment	Stimulations 1st	2nd	3rd	4th	5th	Mean
4	98	95	86	82	42	81
5	65	75	50	10	0	40
6	80	55	40	32	37	49
7	15	12	32	34	25	24
8	32	28	32			31
9	40	67	85	47		59
10	35	42	35	27	22	30
11	70	42	37	38		47
12	95	95	35	45		67
Mean ..	59	57	48	39	25	47

in which it will be seen that in five experiments, in which initially the proportion was high, it fell steadily as the experiment proceeded. In the four experiments in which the initial proportion was lower, there was little change except in Expt. 9. The mean results for successive stimulations shown at the bottom of Table II clearly indicate a steady fall in the proportion of adrenaline released.

Experiments with other substances

While the experiments so far described gave results compatible with the assumption that splanchnic stimulation liberated a mixture of adrenaline and *nor*adrenaline, they did not exclude the possibility of other substances, such as *l*-dihydroxy*nor*ephedrine (corbasil), epinine, and hydroxytyramine, being present. We therefore investigated these substances. When their pressor effects were examined in the eviscerated spinal animal, it was found that the ratios of equipressor doses, taking *l*-adrenaline = 1, were *l*-dihydroxy*nor*ephedrine = 2, epinine = 17, and hydroxytyramine = 50. From these figures it follows that if epinine or hydroxytyramine is contributing a large part of the pressor effect of splanchnic stimulation, very large amounts must be released. Furthermore, when these two substances were injected they depressed the effect of a succeeding stimulation or injection of adrenaline. For this reason we did not consider them further. Dihydroxy-*nor*ephedrine remained. With this substance mixtures containing adrenaline could be prepared to give a curve similar to those in Fig. 5. The ratios obtained in three experiments are given in Table III.

Inspection of Table III will show how small was the change in the ratio as increasing proportions of corbasil were introduced into the mixture. The difference in the curves for adrenaline *plus* noradrenaline and for adrenaline *plus* corbasil is

TABLE III

RATIO OF CONTRACTIONS OF DENERVATED TO NORMAL MEMBRANE WITH MIXTURES OF ADRENALINE (A) AND OF *l*-CORBASIL (C)

Experiment	Adren. 100%	A75% C25%	A50% C50%	A25% C75%	Corb. 100%	Stim.
13	0.65		0.74	0.76	1.25	1.04
14	1.0	1.02	0.98		1.86	1.16
15	1.14	1.25	1.25	1.4	2.4	1.51

illustrated in Fig. 6, which shows that the effect of stimulation in that experiment could be matched with either of these combinations, but the matching mixture with corbasil would contain only 10 per cent adrenaline. Corbasil is, moreover, excluded when the actual membrane contractions are considered as shown in Fig. 7. A mixture containing 25 per cent adrenaline and 75 per cent corbasil, which was equipressor to stimulation, produced contractions

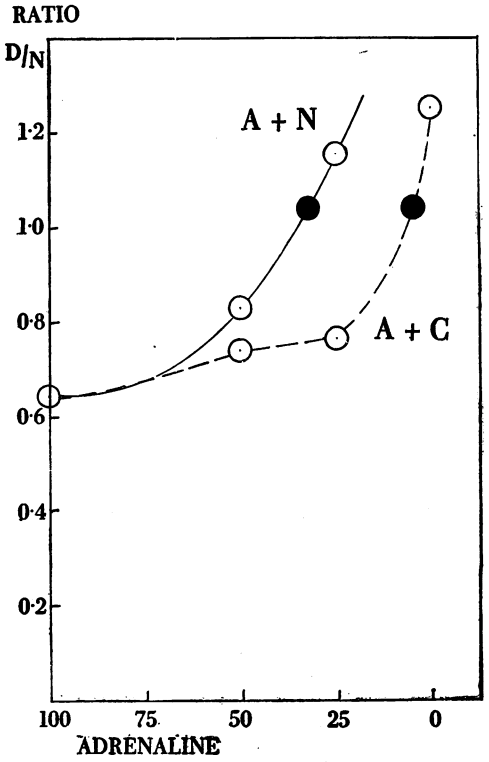

FIG. 6.—Curves relating the ratio of the contractions of the two nictitating membranes (ordinates) to the proportion of adrenaline (abscissae) present in mixtures containing *nor*adrenaline (continuous line, A + N) and corbasil (broken line, A + C). The ratio for splanchnic stimulation (black circles) may be found on both curves.

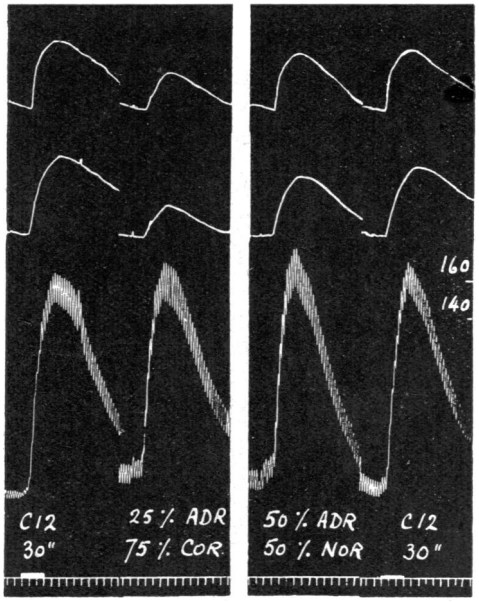

FIG. 7.—The ratio of the contractions produced by stimulation is very similar to that produced by a mixture of adrenaline and corbasil, but the actual size of the contractions is seen to be different. This is not so when stimulation is compared with the mixture of adrenaline and *nor*adrenaline.

much smaller than those caused by stimulation, and a higher proportion of corbasil, though matching the ratio, would have produced still smaller contractions. A larger amount of this mixture would have matched both the ratio and the actual size of contractions, but would have produced too great a rise of blood pressure. When stimulation was compared with adrenaline *plus nor*adrenaline, the blood pressure, the ratio, and the actual size of contractions could all be matched.

Cats fed with methionine

With the possibility in mind that the release of *nor*adrenaline from the suprarenal gland might be due to deficient methylation of this substance, we fed a series of cats on a diet containing 0.2 g. *dl*-methionine daily for 6–10 days. Results which may be compared with those in Table II are shown in Table IV. The cats not only received methionine but were liberally supplied with kipper and other fish in order to encourage them to eat well. The figures in Table IV show that this treatment did not suffice to change the substance released by splanchnic stimulation to adrenaline only. The mean figures are slightly higher than those in Table II and the decline in the proportion of adrenaline released

TABLE IV

METHIONINE-FED CATS. PERCENTAGE OF ADRENALINE RELEASED BY SUCCEEDING STIMULATIONS

Experiment	No. of days fed	Stimulations 1st	2nd	3rd	4th	5th	Mean
16	7	92	95	96	97		95
17	7	57	57	45			53
18	10	70	67	100	65	53	70
19	9	33	30	45			36
20	10	75	78	78	95	100	85
21	6	42	44	43	43	35	41
22	7	77	66	43			62
	Mean	64	62	64	75	62	63

with succeeding stimulation is absent. In two of the experiments (Nos. 16 and 21) the condition of the cats was particularly good; in the first the proportion of adrenaline was almost 100 per cent; in the second, however, it was consistently below 50 per cent. We intend to investigate the effect of diet in a further series of cats.

DISCUSSION

During the last 12 years there has been a growing body of evidence that *nor*adrenaline is liberated as a sympathetic transmitter. Recent work of von Euler (1948) has established its presence in nerve extracts, and Peart (1949) has demonstrated its liberation. The evidence does not necessarily exclude adrenaline as another sympathetic transmitter and is, therefore, not in conflict with the findings of Gaddum and Kwiatkowski (1939). Till the last few months there has been no suggestion that the suprarenal medulla liberated anything but adrenaline. The chemical isolation of this substance nearly 50 years ago appears to have inhibited inquiry into the question. It is, however, a more important point than the identity of the transmitter, since while the latter has in the main only a local effect, substances liberated from the suprarenal medulla have a general action throughout the body. One difficulty in the way of this investigation has been the lack of suitable methods of distinguishing between adrenaline and *nor*adrenaline. The finding by West (1947) of the specificity of the rat uterus method for adrenaline has, however, proved a very useful advance. We have observed that the difference in the sensitiveness of the normal and denervated nictitating membranes provides a convenient test to distinguish the two substances in the blood, and have been able to show that splanchnic stimulation in the cat liberates both substances from the suprarenal gland. In the spinal cat the proportion of

adrenaline liberated is about 60 per cent, and the evidence is strongly in favour of the remaining 40 per cent being *nor*adrenaline. When stimulation is repeated the proportion of adrenaline steadily declines, and this observation suggests that *nor*-adrenaline is not only liberated but is a precursor of adrenaline in the gland. Holtz and Kroneberg (1948) have proposed epinine rather than *nor*-adrenaline as the precursor, on the ground that the latter substance, occurring naturally in the body—in the urine (Holtz, Credner, and Kroneberg, 1944–7), in sympathetic nerves (v. Euler, 1948), and as a constituent of the suprarenal medulla (Schümann, 1948)—is likely to be an end product and not a precursor.

A different view has been taken by Goldenberg *et al.* (1948). They compared the effect of intravenous infusion of adrenaline and *nor*adrenaline in a series of patients and found that the former produced a decrease of peripheral resistance and the latter an increase. They suggest that *nor*adrenaline is a precursor of adrenaline, and that essential hypertension results from a failure to effect methylation. We have tested the hypothesis of deficient methylation by feeding a series of cats on methionine, and have not obtained more than a slight and not significant increase in the proportion of adrenaline liberated from the gland. This proportion, however, was maintained with successive stimulations and did not decline as in other cats.

We think that there is truth in both views. We believe that *nor*adrenaline is a precursor of adrenaline, as suggested by Blaschko (1942), and that in the healthy animal the process of methylation must be sufficiently vigorous to maintain a store of adrenaline which is not exhausted no matter how frequent the splanchnic impulses. If there is deficient methylation then, as Goldenberg *et al.* have suggested, hypertension may follow, and the recent work of Hartroft and Best (1949) showing that choline deficiency causes hypertension in rats may find an explanation along these lines. We believe in addition that *nor*adrenaline is released in the blood to exert a function of its own. Meier and Bein (1948) have shown that the effect of small doses of adrenaline in causing increased blood flow in the femoral artery is reversed after adrenalectomy, but is restored by the infusion of *nor*adrenaline. This suggests that the two substances are released together as is borne out by our own observations.

Summary

1. In the spinal cat the ratio of the contraction of the denervated nictitating membrane to that of the normal membrane is very much greater when *nor*adrenaline is injected than when adrenaline is injected.

2. If the cat is eviscerated and the splanchnic fibres to one suprarenal gland are stimulated the effect on the membranes is intermediate between that of adrenaline and that of *nor*adrenaline and can be matched by infusing a mixture of the two.

3. It is not possible to make such a match with a mixture of adrenaline and dihydroxy*nor*ephedrine, and other reasons exclude epinine or hydroxytyramine as substances which might be released from the suprarenal gland.

4. If the splanchnic is stimulated repeatedly, a gradual decline in the proportion of adrenaline secreted was observed in normal cats, but this decline seemed to be absent in cats fed on a diet rich in methionine.

5. The evidence indicates the release of *nor*-adrenaline as well as of adrenaline from the suprarenal gland, in amounts varying from 20–80 per cent of the total.

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References

Bacq, Z. M. (1933). *Arch. int. Physiol.*, **36**, 167.
Blaschko, H. (1942). *J. Physiol.*, **101**, 357.
Bülbring, E., and Burn, J. H. (1938). *J. Physiol.*, **91**, 459.
Bülbring, E., and Burn, J. H. (1949). *Nature*, **163**, 363.
Cannon, W. B., and Rosenblueth, A. (1933). *Amer. J. Physiol.*, **104**, 557.
Euler, U. S. von (1946). *Acta physiol. scand.*, **12**, 73.
Euler, U. S. von (1948). *Acta physiol. scand.*, **16**, 63.
Gaddum, J. H., and Goodwin, L. G. (1947). *J. Physiol.*, **105**, 357.
Gaddum, J. H., Jang, C. S., and Kwiatkowski, H. (1939). *J. Physiol.*, **96**, 104.
Gaddum, J. H., and Kwiatkowski, H. (1939). *J. Physiol.*, **96**, 385.
Goldenberg, M., Pines, K. L., Baldwin, E. de F., Greene, D. G., and Roh, C. E. (1948). *Amer. J. Med.*, **5**, 792.
Greer, C. M., Pinkston, J. O., Baxter, J. H., and Brannon, E. S., (1938). *J. Pharmacol.*, **62**, 189.
Hartroft, W. S., and Best, C. H. (1949). *Brit. med. J.*, **1**, 423.
Holton, P. (1948). Communication to Physiological Society, May 22. (Also *Nature*, 1949, **163**, 217.)
Holtz, P., Credner, K., and Kroneberg, G. (1944–7). *Arch. exp. Path. Pharmak.*, **204**, 228.
Holtz, P., and Kroneberg, G. (1948). *Klin. Wschr.*, **26**, 605.
Meier, R., and Bein, H. J. (1948). *Experientia, Basel*, **4**, 358.
Peart, W. S. (1949). *J. Physiol.* (in the press).
Schmiterlöw, C. G. (1948). *Acta physiol. scand.*, **16**, Supp. 56.
Schümann, H. J. (1948). *Klin. Wschr.*, **26**, 604.
West, G. B. (1947). *J. Physiol.*, **106** 418