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THE OUTPUT OF SYMPATHETIC AMINES FROM THE CAT'S ADRENAL GLAND IN RESPONSE TO SPLANCHNIC NERVE ACTIVITY

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The release of sympathins from the adrenal gland as a consequence of electrical stimulation of the splanchnic nerve was first tested by Biedl (1897), Dreyer (1899) and Tscheboksaroff (1911). These were followed by the fuller investigations of Elliott (1912, 1913). Amongst other observations he found that, after prolonged excitation of the splanchnic nerve, the effectiveness of excitation was somewhat reduced, but he could not demonstrate any depletion of the adrenaline content of the adrenal gland. After these pioneer studies little attention seems to have been paid to the release of sympathetic amines by splanchnic nerve stimulation until recently. This cannot entirely be ascribed to lack of sensitive assay techniques, for Stewart & Rogoff (1917), investigating the resting output of the gland, had used the rabbit intestine and uterus as test objects for assay, and the great sensitivity to adrenaline of the fowl's isolated rectal caecum was exploited by Barsoum & Gaddum (1935). The realization that noradrenaline as well as adrenaline is released from the adrenal gland (Bülbring & Burn, 1949), together with the introduction of more sensitive assay techniques such as those using the rat uterus (de Jalon, Bayo & de Jalon, 1945) and the pithed rat blood pressure (Shipley & Tilden, 1947) have allowed a more precise assessment not only of the resting output of the gland (Vogt, 1952) but also of its output during electrical excitation (Outschoorn, 1952; Rapela, 1956; Eade & Wood, 1958). Investigation of the gland's output by pharmacological assay of the substances released into the adrenal blood can be made as rigorous as desired, but it is laborious and does not allow the time relationship of release to be easily determined. The method recently developed by Vane (1958), by which a strip of tissue sensitive to the amines can be included in a small extracorporeal circulation, offered an additional and more convenient method of estimating amine output. We have therefore used both methods in experiments to investigate the relation between output of adrenal medullary hormones

and various patterns of electrical stimulation, and also the susceptibility of the ganglionic synapse to fatigue and to specific blocking agents.

METHODS

Cats were anaesthetized with ether, after induction by ethyl chloride, and then given chloralose intravenously (80 mg/kg). A few animals were anaesthetized with intraperitoneal pentobarbitone sodium (30 mg/kg). For the collection of samples of blood from the adrenal vein, the abdomen was opened down the mid line, the stomach, intestines, spleen and pancreas were removed, and the blood supply through the hepatic artery was arrested. The vessels around the left adrenal gland were dissected out and branches joining the adrenolumbar vein lateral to the gland, either from the diaphragm, the abdominal wall or the renal vein, were tied off. Artificial ventilation was begun. By dissection under the diaphragm the left greater splanchnic nerve was freed and a loose ligature left round it as high as possible. The lesser splanchnic nerves and the sympathetic chain on the same side were also cut. The animal was then given heparin intravenously (10 mg/kg). A siliconed polythene cannula was inserted into the adrenolumbar vein just lateral to the adrenal gland; when the adrenal vein was then tied close to the inferior vena cava, blood from the gland flowed into the cannula. Because of the amount of residual blood in the cannula at the end of a control or stimulation period, which would otherwise be included with the next specimen, the cannula was provided with a side arm close to its insertion into the vein, whereby at the conclusion of the collection period the contained blood could be gently blown out. After the collection of (usually) two control specimens the splanchnic nerve was cut and the peripheral end laid across platinum electrodes (cathode distal) for stimulation with supramaximal rectangular (0·5 msec) pulses of 20 V, at varying frequencies. In experiments where a brief burst of rapid excitation was required two Bell square-wave stimulators were linked, the one providing the required stimulation frequency and the other a variable 'gate', up to 500 msec duration during which a relay was closed connecting the first stimulator to the electrodes. Stimuli were monitored with a Cossor oscilloscope to verify the absence of wave distortion.

Blood pressure was recorded throughout the experiment with a siliconed cannula in the right carotid artery as a check against any leak of pressor amines from the prepared gland into the general circulation.

Samples of venous effluent were collected in chilled siliconed graduated centrifuge tubes containing 1 mg heparin and standing in ice. They were immediately centrifuged at 3000 rev/min for 10 min in containers with ice (Gaddum, Peart & Vogt, 1949), to prevent the development of vasopressor activity unrelated to sympathin. After centrifugation plasma and cell volumes were noted and the plasma was pipetted off and kept in sealed bottles on ice until assay.

Circulating sympathins were assayed directly by the blood-bathed isolated organ technique of Vane (1958). The left greater splanchnic nerve was approached through a lumbar incision, after ligature and section of the posterior abdominal muscles. The adrenolumbar vein was divided between ligatures, and the greater splanchnic nerve identified and cut, together with the lesser splanchnic nerves and lumbar sympathetic trunk. The abdomen was then opened in the mid line and the right adrenal gland removed. After the injection of heparin (10 mg/kg) the left carotid artery was cannulated (the right carotid artery being employed for recording blood pressure) and connected with polythene tubing through a roller pump calibrated to deliver 10–15 ml. of blood/min to a siliconed organ bath, maintained at 37·5° C by an external water jacket and containing a rat-stomach strip preparation (Vane, 1957); this is approximately twice as sensitive to adrenaline as it is to noradrenaline. The blood overflowed from the organ bath through a side arm and dripped over the surface of another isolated organ, usually the chick rectum (West, 1950) which is 60–100 times more sensitive to adrenaline than it is to noradrenaline, before being returned by gravity feed to the right

external jugular vein. In the early experiments with blood-bathed tissues the extracorporeal circulation contained 10–15 ml. of blood; later this was modified, reducing the amount of blood circulating outside the body to 5–10 ml. The tone of the rat stomach strip and the chick rectum, which both contract when bathed with blood, was recorded by an auxotonic pendulum lever (Paton, 1957) of 16:1 magnification. A vibrator was mounted on the stand holding the two levers, to minimize friction between the writing points and the kymograph. For prolonged splanchnic stimulation the current to and from the electrodes was passed through 1 μF condensers, to prevent polarization of the electrodes (Garry & Gillespie, 1955). In all experiments the animals were artificially ventilated and their temperature was maintained at 38° C.

Noradrenaline was estimated by its pressor effect on pithed rats (Shipley & Tilden, 1947). Rats of 200–250 g were anaesthetized with ether and injected subcutaneously with 1 mg hyoscine. A tracheal cannula was inserted for artificial ventilation and the central nervous system was destroyed by introducing a wire rod into one orbit and through the foramen magnum into the spinal cord. Such rats have a blood pressure of 50–60 mm Hg. A venous cannula was placed in the left jugular or right femoral vein, and the arterial blood pressure was recorded from the right carotid artery. The venous cannula was connected through rubber tubing to a 5·0 ml. microburette filled with NaCl solution 0·9 g/100 ml. so that drugs or plasma samples injected through the rubber tubing could be washed into the animal. The total volume injected was always adjusted to 0·4 ml.

To assay adrenaline the rat uterus, in a 10 ml. bath, stimulated with acetylcholine or carbachol was originally employed (de Jalon *et al.* 1945); this is about 150 times more sensitive to adrenaline than to noradrenaline (Gaddum *et al.* 1949). It is now known (Vane, 1957) that there is in cat plasma about 2 μg/ml. of 5-hydroxytryptamine (5-HT); since this substance causes the rat uterus to contract, in later experiments bromlysergic acid 10^{-8} was left in the bath for 15–30 min, until (2·5 μg) 5-HT became ineffective.

The largest volume of plasma used was 0·3 ml. containing $< 0{\cdot}6$ μg 5-HT (Expts. 11, 13, 14). In other experiments (Nos. 15, 16) adrenaline was assayed by its reduction of maximum contractions of the rat uterus evoked by 5-HT, thus nullifying the effect of 5-HT in the plasma samples. The use of a pendulum auxotonic lever (Paton, 1957) assisted in regularizing contraction heights.

For both adrenaline and noradrenaline the assay was always done by bracketing the unknown solutions between doses of the standard differing as a rule by a factor of two, and assaying at two dose levels. Random specimens showing adrenaline-like activity were treated with Lugol's iodine and allowed to stand 5 min at room temperature. Excess iodine was destroyed with thiosulphate and the samples were retested to demonstrate the disappearance of adrenaline-like activity. The results are expressed as the amount of adrenaline and noradrenaline in the plasma that flowed from the suprarenal gland in 1 min.

In dealing with amine mixtures the content of each amine is obtained from a knowledge of the noradrenaline and adrenaline equivalents on two discriminating test objects. From the equivalents two simultaneous equations may be set up and solved for the individual amine contents. A simpler graphical method is to make on linear graph paper a scale of noradrenaline equivalent per millilitre on one axis, and of adrenaline equivalent per millilitre on the other axis. For a given sample on a given test object a line can then be drawn joining the two equivalents; this line will pass through all the points corresponding to various noradrenaline–adrenaline mixtures which would have the same effect. A corresponding line is drawn for the same sample on the other test object and the point of intersection gives the independent amine contents. The method is quick and the slopes of the lines make obvious the different discriminating power of the two test objects. *Drugs* used were adrenaline bitartrate, noradrenaline bitartrate, acetylcholine chloride, bromlysergic acid (BOL 148), carbachol chloride, histamine acid phosphate, hyoscine hydrobromide, methonium salts (C_4, C_5, C_7, C_{10}) as iodides except for hexamethonium bromide, nicotine

hydrogen tartrate, probanthine bromide, serotonin creatinine sulphate, tetraethylammonium bromide (TEA), D-tubocurarine chloride. Doses are given as the amount of salt, except for adrenaline, noradrenaline, histamine and serotonin, which are measured as base.

RESULTS

Resting output from the adrenal gland before and after splanchnic nerve section

Before testing the responses of the glands to splanchnic nerve stimulation the opportunity was taken in 13 experiments of estimating the resting output of sympathins from the gland before nerve section. At least two samples were taken before the nerve was divided: Table 1 gives the figures obtained. There appeared to be a tendency for the output in the last control sample to be higher than the preceding ones; we believe, however, that the last control samples give the best values, since they were taken as long as possible (20–88 min) after completing the dissection, which might depress output for some time. In chloralosed cats output ranged from 3 to 198 ng noradrenaline/min and from < 1 to 209 ng adrenaline/min; with phenobarbitone sodium (cats 7, 8) the corresponding figures were noradrenaline 2–141 ng/min and adrenaline 1–99 ng/min. In the period 9–15 min after nerve section the output almost always fell unless it was initially barely detectable. On the other hand, output rarely became undetectable; and in eight experiments the output subsequently rose again in the absence of nerve excitation. For the whole group only the fall in noradrenaline output immediately following nerve section was significant ($t = 2{\cdot}26$, $P < 0{\cdot}05$).

The problem arose as to the reason for sustained output, sometimes considerable, in an acutely denervated gland. Asphyxia, circulating histamine or a rise in plasma potassium could all be excluded. Changes in blood flow through the gland may influence output. But we found no relation between changes in output and blood-flow rate: thus in one experiment the blood flow rose from 0·3 to 0·48 ml./min, and the total amine output rose from 45 to 101 ng/min; in another experiment blood flow fell from 0·21 to 0·12 ml./min, and the total amine output rose from 6 to 42 ng/min. All these figures refer to periods after dividing the nerve and before any stimulation. It may be that the rate of plasma flow rather than whole blood flow is more intimately linked with amine output. The mean plasma flow for the specimens before nerve section was 0·23 ml./min (25 samples) and after nerve section but preceding stimulation it was 0·215 ml./min (26 samples). Since there was no significant change in mean plasma flow, it is unlikely that the above changes in amine output depended on plasma-flow fluctuations.

Three possibilities remain. First, the denervation could have been

TABLE 1. Resting output of noradrenaline and adrenaline (ng/min) before and after splanchnic nerve section

	Expt. No. ...	1		2		4		5		6		7		8		10		11		13		14		15		16	
	Sample																										
Before nerve section	1	70	31	49	30	19	<1	8	3	57	84	39	40	4	4	11	3	17	20	3	<2	38	17	134	86	—	—
	2	198	160	50	29	23	<1	5	3	190	209	141	99	2	1	14	7	4	15	3	1	28	2	101	50	83	7
After nerve section	1	26	9	6	39	14	<1	3	2	32	115	29	32	28	1	1	1	4	2	3	1	5	<1	67	24	21	10
	2	14	7	52	39	20	12	4	3	83	98	131	498	4	4	1	4	9	7	4	4	44	17	11	14	7	11

	Sample	Mean values (ng/min) NAd	Ad
Before section	1	37·4	26·7
	2	64·8	44·9
After section	1	18·4	17·6
	2	29·5	56·2

incomplete; but all homolateral nerves known to influence secretion had been cut, and there is no evidence for contralateral innervation of the gland (Young, 1939). Secondly, since chloralose depresses the secretion by the gland (Malmejac & Neverre, 1950) it might be that its elimination in the body allowed a non-nervous element in the secretion to rise, but the action of chloralose is so prolonged that this seems unlikely. Thirdly, it is possible that the gland was in a state of mild 'exhaustion' before nerve section, sufficient to reduce *spontaneous* secretion, and that after a rest period following the section, spontaneous secretion at a higher rate could be resumed. This was borne out by the observation that the rise in resting secretion after nerve section tended to be high in those animals which had high outputs before section. This idea would imply that the non-nervous secretion represents a sort of overspill of amines from a gland, as soon as its stores are full.

The threshold frequency of excitation and number of shocks for the release of significant quantities of sympathetic amines

Although excitation of the splanchnic nerve at 10/sec produces a vigorous release of both adrenaline and noradrenaline, the output of these hormones with excitation at 1/sec usually does not increase output above the resting unstimulated level. An attempt was made, therefore, to determine the frequency of stimulation at which a consistently increased output could be obtained. Table 2 gives the results, which can be summarized as follows. Excitation at 1/sec for 5–15 min (totals of 300–900 shocks) produced a significantly increased output in three out of six experiments, whereas at 2/sec output was increased in all of three tests, and at 4/sec in all of eight tests. When the number of shocks was reduced to a total of 60 (excitation at 1/sec for 1 min), output did not increase in three tests. It appears, therefore, that only with prolonged excitation will a frequency of 1/sec occasionally prove effective, and that 2/sec is probably the lowest frequency which consistently increases the output from the medulla.

This conclusion was further tested in experiments with blood-bathed assay tissues (rat stomach strip, chick rectum) in an extra-corporeal circulation, and similar results were obtained. Excitation at 1/sec for 5 min was effective in one test; but in six tests with excitation for times varying from 30 sec to 3 min, no significant release of amines occurred. With a frequency of 2/sec, applying 60–180 shocks, release occurred in three out of four experiments; and at 3/sec 60 shocks were invariably effective (seven experiments).

To determine the minimum number of shocks which can cause a detetectable output, it is necessary to know the optimum frequency of stimulation. This was found (as described below) to be about 30–60/sec.

TABLE 2. Total amine output (ng/min) in adrenal venous blood in control and stimulation specimens after splanchnic nerve section. In parentheses, duration (min) of collection period*. Lower figures represent values in control period succeeding stimulation except in Expt. 4 where lower figure is for a later stimulation period at 1/sec

Expt. No.	Control total amine output	Total amine output on splanchnic nerve stimulation							Duration of stimulation or total stimuli
		1/sec	2/sec	4/sec	8/sec	10/sec	16/sec	32/sec	
1	28	29 (20)	—	—	—	717 (15)	—	—	15 min
2	95	120 (15)	—	—	—	1452 (20)	—	—	10 min
4	23	148 (22)	158 (15)	288 (14)	702 (12)	—	86 (13)	—	10 min at 2, 4, 8, 16/sec
		174 (12)							15 min at 1/sec
5	7	44 (31)	81 (21)	92	157 (12)	—	89	43 (12)	8 min
6	157	181 (11)	—	—	—	—	—	—	10 min
7	345	—	—	938 (19)	1804 (14)	3248	3139 (11)	—	10 min
				1614 (14)	1182 (11)	568 (12)			
8	6	98 (11)	70 (14)	—	—	—	—	—	5 min
		31 (14)	47 (12)						
10	4	6	—	20	—	—	6	9	60 stimuli
				8			5	12	
11	39	141	—	245	—	—	270	204	180 stimuli
				82			74	147	
13	6	6	—	20	—	—	40	100	180 stimuli
								13	
14	28	17	—	24	—	—	80	71	60 stimuli
							34	65	
15	51	40	—	67	—	—	48	215	60 stimuli
							147	116	

* Collection period was 10 min unless otherwise stated.

With this range of rates, 60 stimuli always produce a detectable output, sometimes large. With fewer stimuli sometimes no response occurs; but in one experiment 13 shocks at 128/sec caused a detectable response (Fig. 1).

Relation of output to varying rates and durations of stimulation

Number of stimuli kept constant; experiments with adrenal vein cannulation. Five experiments were performed, three (Nos. 10, 14, 15) in which a total of 60 stimuli were given and two (Nos. 11, 13) with 180 stimuli, at stimulation rates of 1, 4, 16 and 32/sec, in random order to avoid systematic error,

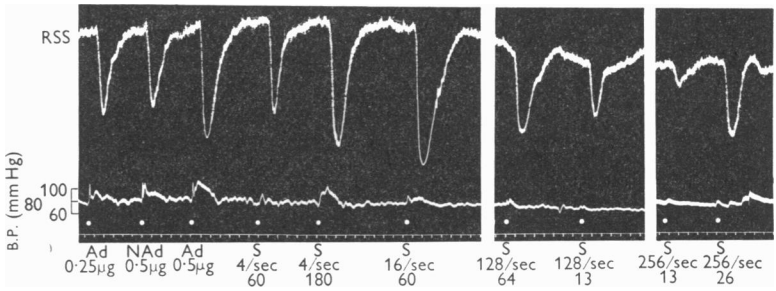

Fig. 1. Cat, 2·6 kg; chloralose. Response of blood pressure (B.P.) and of the blood-bathed isolated rat stomach strip (RSS) to intravenous injections of adrenaline or noradrenaline or to stimulations of the cut left splanchnic nerve at various frequencies. Total number of shocks 13, 26, 60, 64 or 180, as shown. Time marker, minutes.

due for instance, to the deterioration of the animal during the experiment. Both stimulation and control specimens were collected for 10 min periods. An arbitrary control value was arrived at by averaging the results for the two control specimens preceding the first stimulation and that for the control period succeeding excitation at 1/sec. Difficulty arises in a statistical analysis of the results because with the higher frequencies of stimulation considerably increased outputs of amines are to be found in the immediately succeeding control specimens. This could not be attributed to blood belonging to the stimulation specimen remaining in the dead space of the cannula in the adrenal vein, with subsequent carry-over into the next control sample. A correction was applied by adding to the stimulation output the excess found in the first control period after stimulation over the averaged control value. The mean values for the corrected total amine output (ng/min) in the control and stimulation specimens, and their range, are shown in Table 3. Analysis of variance of the data shows that the results for the control and stimulated specimens depart from homogeneity ($P < 0{\cdot}01$); and, as might be expected, there is a significant contribution to the total variance from the difference between the experimental animals ($P < 0{\cdot}001$). Comparison of the mean values (using a one-tailed t test)

shows that there is no significant difference between the total amine output in the control and stimulation at 1/sec specimens ($t = 0{\cdot}62$, $P = > 0{\cdot}5$), but that the differences between the total amine output in the control and stimulation specimens at 4/sec ($t = 1{\cdot}78$, $P < 0{\cdot}05$), 16/sec ($t = 2{\cdot}75$, $P < 0{\cdot}001$) and 32/sec ($t = 4{\cdot}19$, $P < 0{\cdot}001$) are significant, the significance mounting with frequency of stimulation. Analysis of variance of only the stimulation specimens showed them to depart significantly from homo-

TABLE 3. Combined adrenaline and noradrenaline outputs* (means and range, ng/min to the nearest whole figure) for the control and stimulation specimens after splanchnic nerve section. (Combined results of Expts. 10, 14, 15 (60 shocks) and Expts. 11, 13 (180 shocks).)

Indices	Control ($n = 15$)	1/sec ($n = 5$)	4/sec ($n = 5$)	16/sec ($n = 5$)	32/sec ($n = 5$)
Mean	26	46	85	117	165
Range	2–91	6–162	20–288	7–305	17–312

* Corrected as explained in text.

TABLE 4. Comparison of the mean combined adrenaline and noradrenaline outputs (ng/min; corrected) for the control and stimulation specimens with either 60 or 180 shocks after splanchnic nerve section

Total stimuli	Control	1/sec	4/sec	16/sec	32/sec
60 shocks (Expts. 10, 14, 15)	28	21	38	79	135
180 shocks (Expts. 11, 13)	23	84	154	174	210

geneity ($F = 7{\cdot}00$, $P < 0{\cdot}01$). Comparison of the mean values for the stimulation specimens (using a two-tailed t test) indicated that, whereas output of the 16/sec or 32/sec specimens differed significantly from that of the 1/sec specimen, and that of the 32/sec from the 4/sec specimen, the differences between the mean output at 1/sec and 4/sec, 4/sec and 16/sec and 16/sec and 32/sec were not significant. The data from these experiments were further subdivided to compare the mean total amine outputs for stimulation with 60 shocks with those occurring with 180 shocks to the cut splanchnic nerve (Table 4). As expected, outputs were found to be higher with the larger number of stimuli. Figure 2 shows the mean outputs at varying frequencies, plotted, not against frequency, but against the interval between successive shocks, since it will be discussed in these terms.

Proportionate outputs of adrenaline and noradrenaline. So far we have considered total amine output; the uncombined adrenaline and noradrenaline outputs (corrected) in the experiments just discussed were also calculated separately. The means, ranges and standard errors of the mean for these outputs, together with values for the ratio between the two amines, are shown in Table 5. Output of both amines rose with increasing frequency of stimulation, and there was again a considerable variation between different animals. Although a fall in the ratio of mean nor-

adrenaline to mean adrenaline output occurred at 32/sec stimulation, this was far from significant statistically, and our main conclusion is that the ratio does not change with brief periods of excitation at the serates. Since, however, Rapela (1956) has reported a specific increase in adrenaline at

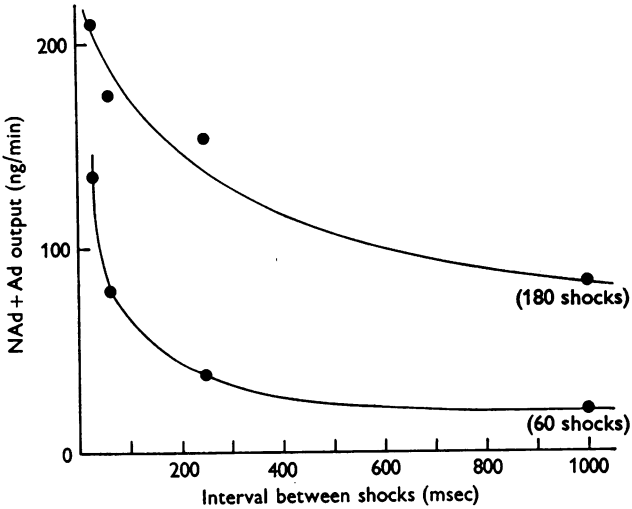

Fig. 2. The relation between the mean output of combined adrenaline and noradrenaline in the suprarenal venous blood (ng/min) to the interval (msec) between shocks administered to the cut left splanchnic nerve.

TABLE 5. The ratio of the means, the mean, the range and standard errors of the mean for the separate corrected noradrenaline and adrenaline outputs (ng/min) in the control and stimulation specimens after splanchnic nerve section (Expts. 10, 11, 13, 14, 15)

Indices	Control (n = 15)	1/sec (n = 5)	4/sec (n = 5)	16/sec (n = 5)	32/sec (n = 5)
Noradrenaline					
Mean	15·1	30·6	43·0	65·2	70·0
Range	1–67	3–117	4–152	< 1–211	1–180
S.E. of mean	4·87	19·49	24·75	33·89	30·98
Adrenaline					
Mean	10·5	16·6	42·4	52·0	96·4
Range	< 1–27	3–44	7–135	3–95	17–208
S.E. of mean	2·39	7·08	21·12	20·75	31·76
Ratio noradrenaline mean: adrenaline mean	1·4	1·8	1·0	1·2	0·7
Range of ratio, noradrenaline: adrenaline	0·05–5·0	0·75–2·7	0·19–2·0	0·14–13·3	0·06–3·5

high rates of excitation, this point was examined further; and in one experiment in which 51 shocks at a rate of 512/sec were given, an increase in output of adrenaline alone from 11 to 59 ng/min was obtained and in a second test from 17 to 44 ng/min; the noradrenaline output remained constant at about 18 ng/min. At 256/sec 26 shocks had a doubtful effect of the same

kind; 13 shocks at 128/sec were ineffective. It seems likely, therefore, that a selective increase in adrenaline secretion can be obtained, but not at frequencies of stimulation of physiological interest.

Effect on output of the plasma volume of the venous sample. Brown & Gillespie (1957) noted that even when both number of stimuli and frequency of excitation were kept constant, the noradrenaline output varied directly with the plasma volume of the sample but not with the whole-blood volume of the sample. This variation of output with sample volume might introduce serious errors in the interpretation of the results if, for instance, the sample volumes at the higher frequencies of stimulation were

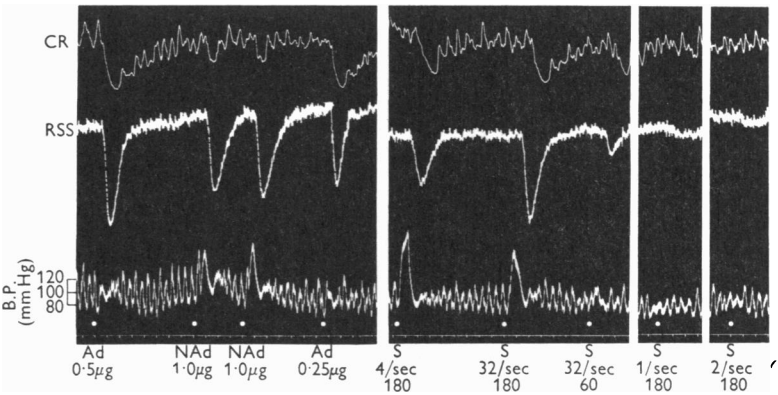

Fig. 3. Cat, 2·5 kg; chloralose. The response of the blood-bathed isolated chick rectum (CR), rat stomach strip (RSS) and blood pressure (B.P.) to intravenous injections of adrenaline or noradrenaline, or to stimulation of the cut left splanchnic nerve at various frequencies, for either 60 or 180 shocks. Time marker, minutes.

consistently greater than those of the lower. In fact, however, there was no consistent change of flow rate; the average plasma flow rate for the control period and stimulation at 1, 4, 16, and 32/sec respectively were 0·11, 0·11, 0·08, 0·08 ml./min in the experiments analysed above. There was a small reduction of flow with excitation at 16 or 32/sec, and so the values for output at these rates may be slightly underestimated.

Experiments with blood-bathed assay tissues

The observations just described, showing that excitation with a fixed number of shocks led to a higher output of amines if the rate of stimulation was increased, were extended by the less laborious assay technique of the blood-bathed organ. The comparative effect of stimulation at varying frequencies with either 60 or 180 shocks can be seen from Fig. 3, and Fig. 4 shows outputs determined by this method in three experiments. The same general result was obtained, that output is highest for frequencies of 30–60/sec (Fig. 5). A further experiment was made (Fig. 6) in which 50, 150, or

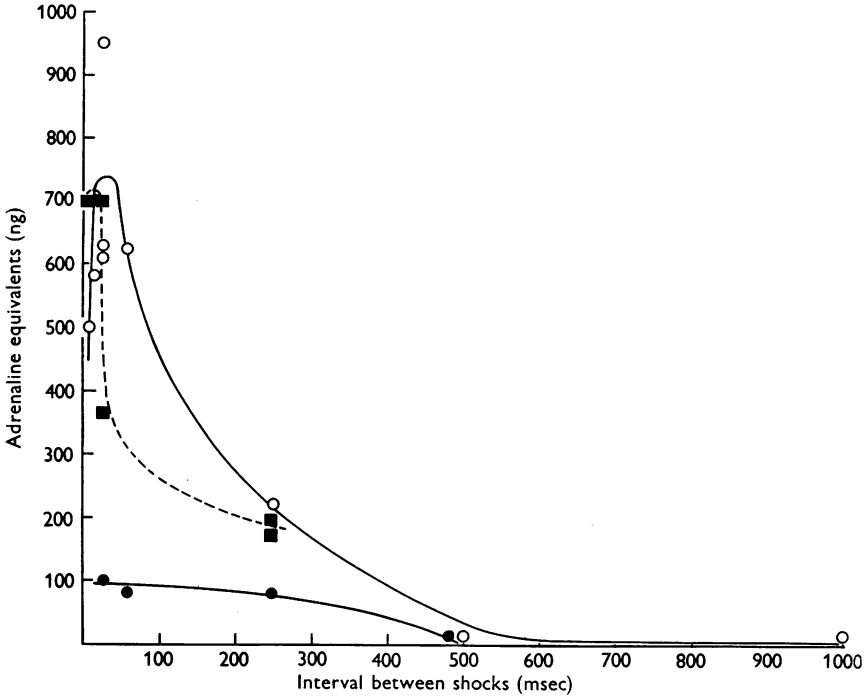

Fig. 4. The relation of the output of sympathetic amines (measured as adrenaline equivalents on the blood-bathed isolated rat stomach strip) to the interval between shocks given to the cut left splanchnic nerve. ●—● = 60 shocks; ■—■ and ○—○ = 180 shocks.

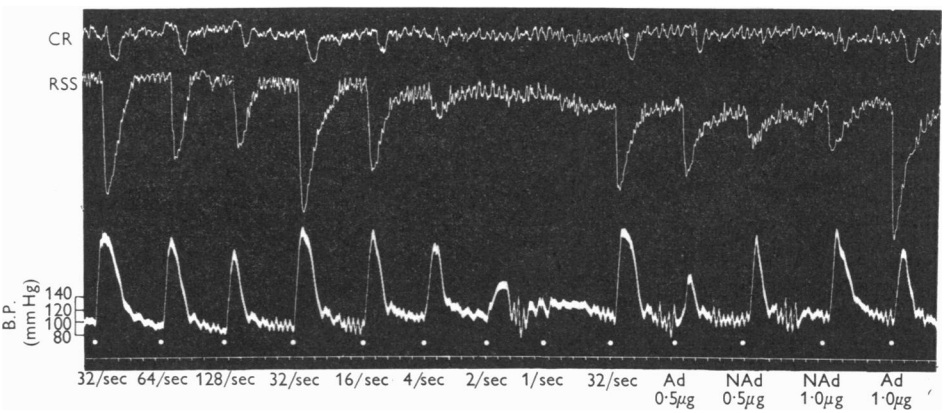

Fig. 5. Cat, 2·3 kg; chloralose. The response of the blood pressure (B.P.) and of the blood-bathed isolated chick rectum (CR) and rat stomach strip (RSS) to intravenous injections of adrenaline or noradrenaline or to stimulation of the cut left splanchnic nerve at various frequencies per sec for 180 shocks. Time marker, minutes.

450 shocks were delivered at frequencies ranging from 3·5 to 320/sec, to compare the response at very short stimulus intervals with longer intervals in one experiment: the various stimulations were delivered in a random order. This confirmed that at 30/sec stimulation output is maximal, and that it falls off sharply at faster rates. The interesting observation was made, however, that at 350/sec output recovered somewhat. It appeared, in short, that two separate processes in the response to successive shocks were occurring: the first on a short time scale, lasting only 10 msec or less; the second much slower, with a peak around 30 msec, and lasting up to 300

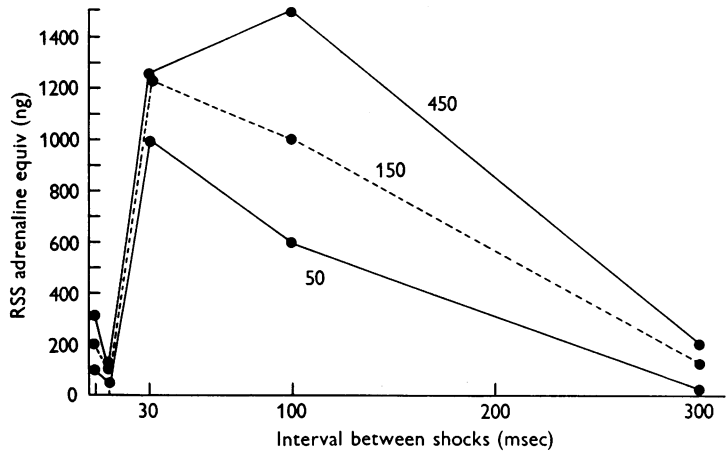

Fig. 6. The relation of the output of sympathetic amines (measured as adrenaline equivalents on the blood-bathed isolated rat stomach strip) to the interval between shocks given to the cut left splanchnic nerve. Upper line = 450 shocks; middle line = 150 shocks; lower line = 50 shocks.

or 1000 msec. The position of the peak is not constant, but varies with the number of shocks given, tending to a lower frequency as the volleys delivered increase.

Output with constant duration of stimulation

In two experiments, continued stimulation of the splanchnic nerve at various rates with rather brief rest periods was associated with decline of output at excitation rates above 8/sec. But very large outputs might occur with stimulation at 8/sec or higher for a sustained period, if generous rest intervals were allowed. Thus in one experiment stimulation at 10/sec for 10 min produced a total of 36·5 μg combined amine, and a total of 38·0 μg was produced by excitation at 16/sec for 10 min. It was thus clear that with these longer periods of excitation output did not simply depend on rate of excitation, but that a phenomenon resembling 'fatigue' of the synapse

could be produced. Experiments were therefore made to produce it deliberately and to determine the factors governing its appearance.

Splanchnic nerve fatigue

Assays of collected adrenal venous blood. In two cats splanchnic nerve stimulation at progressively raised frequencies but with only brief rest intervals was found to cause a fall in output of noradrenaline and adrenaline into the adrenal venous blood when the rate of excitation, over a period of 8–10 min, exceeded 8–16/sec. In the first experiment the combined amine output at 16/sec was little more than a tenth of that at 8/sec. But this decline was not due to deterioration of the nerve, since subsequent excitation at 1/sec for 10 min (Fig. 7), after a rest period of 14 min, elicited almost the same output as an earlier stimulation at the same rate. In this animal the plasma flow through the gland during excitation at 16/sec was reduced by half. But this seems an unlikely reason for the decline in output, for in another experiment a similar decline was associated with a rise in plasma flow. In neither experiment was there any evidence that output of adrenaline might fail earlier than that of noradrenaline during splanchnic nerve excitation.

Assays with blood-bathed organs. Data from these investigations are shown in Table 6. It was possible to produce an apparent complete exhaustion of the response of the adrenal gland to splanchnic stimulation, this fatigue appearing sooner with high than with low rates of stimulation. Thus with a rate of 4/sec exhaustion was complete only after 193 min. In another animal no signs of fatigue were apparent after stimulation at 5/sec for 35 min, but exhaustion occurred after 50 min with stimulation at 8/sec, within 20 min at 16/sec, and 14 min at 32/sec. In another experiment only partial exhaustion was found after 60 min at 16/sec, while in yet another cat no exhaustion was seen when stimulating for 80 min at 10/sec. In two other animals stimulating at a rate of 32/sec led to complete exhaustion within 25 and 60 min respectively.

In all these experiments exhaustion was considered to have occurred when the blood-bathed assay tissue had regained its original tone. This return of tone during continuous splanchnic stimulation was not due to a developing insensitivity of the assay tissues to circulating sympathins as found in the experiments of Feldberg, Minz & Tsudzimura (1934); indeed, they usually became more sensitive to adrenaline, noradrenaline and isopropylnoradrenaline as the experiment progressed. Figure 8 illustrates how adrenaline and noradrenaline are still fully effective on the rat stomach strip at the time when tone had fully returned in the face of continuing splanchnic excitation.

That complete depletion of the sympathetic amines within the adrenal

gland was not the explanation of the unresponsiveness of the gland to nerve stimulation could be demonstrated by the intravenous injection of 0·25–0·5 mg nicotine. This was followed by prompt maximal relaxation of the assay tissues. Nicotine injected in 1/15th–1/12th of this dose into the polythene tubing leading to the blood-bathed assay tissues had no direct effect on the rat stomach strip; it relaxed the chick rectum slightly but

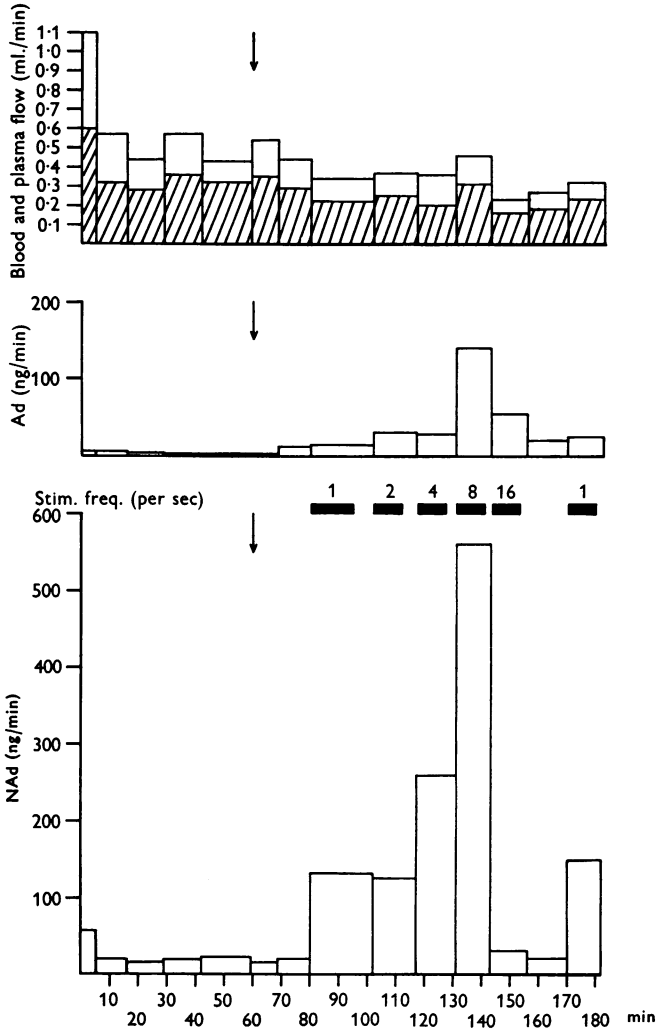

Fig. 7. The outputs of adrenaline and noradrenaline in the suprarenal venous blood before and after section of the left splanchnic nerve (downward pointing arrow) and in association with stimulation of the cut splanchnic nerve at varying frequencies. ■, duration of stimulation, 15 or 10 min. Frequencies of stimulation, reading left to right: 1, 2, 4, 8, 16 and 1 per sec. □, suprarenal venous blood flow, ▨, suprarenal plasma flow.

much less than did the injection of the larger dose of nicotine into the whole animal. The proportionate dose (1/15th–1/12th) was calculated on the basis of a blood volume in the cats of 120–150 ml. and a volume of extracorporeal blood of 10 ml. in the organ bath and connecting tubing.

The exhaustion of the response to nerve stimulation could not be due to electrode polarization (see Methods); nor was it due to the stimulus becoming submaximal by the short-circuiting effect of accumulating tissue fluid, since when fatigue was established doubling the voltage produced no significant alteration in response of the assay tissues. Moreover

TABLE 6. Exhaustion after prolonged splanchnic stimulation: blood-bathed organ experiments. (RSS = rat stomach strip; CR = chick rectum; RD = rat duodenum)

Expt. no.	Interval before stimulation (min)*	Stimulation: Rate (per sec)	Stimulation: Duration (min)	Assay tissues	Time (min) of complete return to resting level during continuous stimulation: Assay tissues	Blood pressure
18	310	16	60	RSS	60 (50 % return)	15
21	180	32	46	RSS	25	4
				CR	10	—
22	75	32	33	RSS	14	15
				CR	12	—
	230	16	37	RSS	20	11
				CR	9	—
	290	8	63	RSS	50	17
				CR	15	—
	370	5	35	RSS	No fatigue	9
				CR	—	—
23	96	4	201	RSS	193	35
				CR	35	—
24	332	10	80	RSS	No fatigue	27
				RD	—	—
31	40	32	60	RSS	60	26

* Interval between completion of the preparation and beginning stimulation.

20 V stimulation was used although it had been found earlier in the experiment that maximal relaxation of the stomach strip followed nerve stimulation with only 1–2 V. Neither could the length of the experiment nor the animals condition be incriminated. Thus fatigue appeared more rapidly at a stimulation rate of 32/sec, but more slowly at 8/sec some 215 min later (Expt. 22, Table 6). In another animal fatigue was incomplete after 60 min stimulation at 16/sec, even though 370 min had elapsed since recording was begun. In all the animals there had been at most an insignificant decline of blood pressure during the experiment, and splanchnic nerve stimulation invariably produced a substantial rise of blood pressure (50–140 mm Hg). The blood pressure immediately preceding stimulation was never lower than 60 mm Hg and in the majority of animals lay between 90 and 180 mm Hg.

Evidence was obtained that output of adrenaline failed sooner than that

of noradrenaline during nerve stimulation. The chick rectum regained normal tone before the blood-bathed rat stomach strip during prolonged splanchnic stimulation (Figs. 8, 9) although it is much more sensitive to adrenaline than to noradrenaline, whereas the rat stomach strip is only about twice as sensitive to adrenaline as to noradrenaline. This pheno-

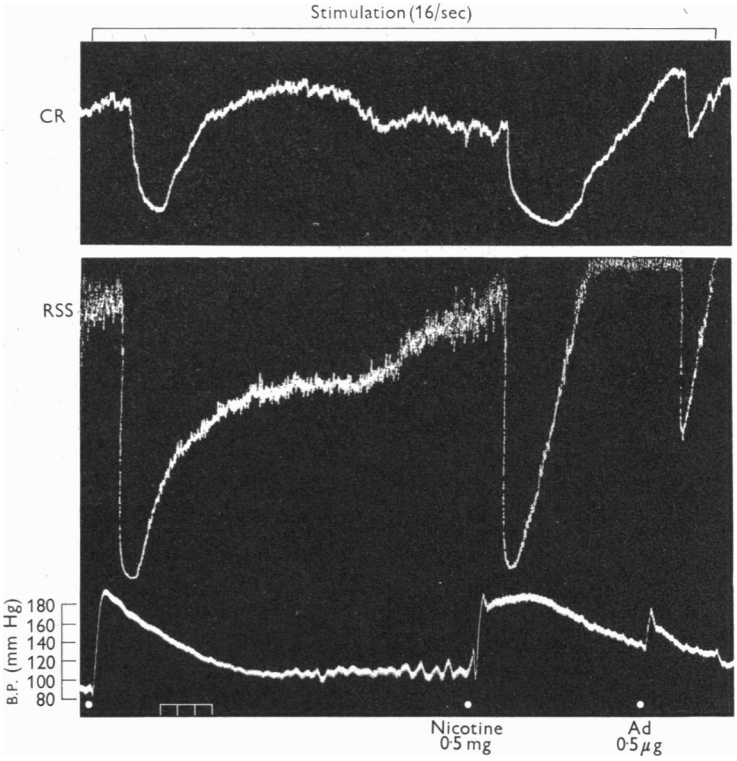

Fig. 8. Cat, 2·2 kg; chloralose. The response of the blood-bathed isolated chick rectum (CR) and rat stomach strip (RSS) and the blood pressure (B.P.) to prolonged stimulation at a frequency of 16/sec of the cut left splanchnic nerve. Note the early adaptation of the blood pressure to circulating sympathetic amines coinciding with the return of tone to the chick rectum, and the eventual return of tone to rat stomach strip. Considerable relaxations of both blood-bathed tissues following the intravenous injection of either 0·5 mg nicotine or 0·5 μg adrenaline. Time marker, minutes.

menon was seen whether the rate of excitation was 16/sec (Fig. 8) or 4/sec (Fig. 9). The result might only mean that the chick rectum has a greater facility to regain tone than the other assay tissues. This is excluded, however, by the fact that the relaxation of the chick rectum to adrenaline may follow the same time course as the stomach strip (Fig. 8) or may be somewhat slower (Fig. 3).

Adaptation to sympathetic amines

In the experiments on suprarenal exhaustion it was noticed (Table 6) that the cat's blood pressure returned to control levels considerably earlier than the rat stomach strip, and at about the same time as the chick

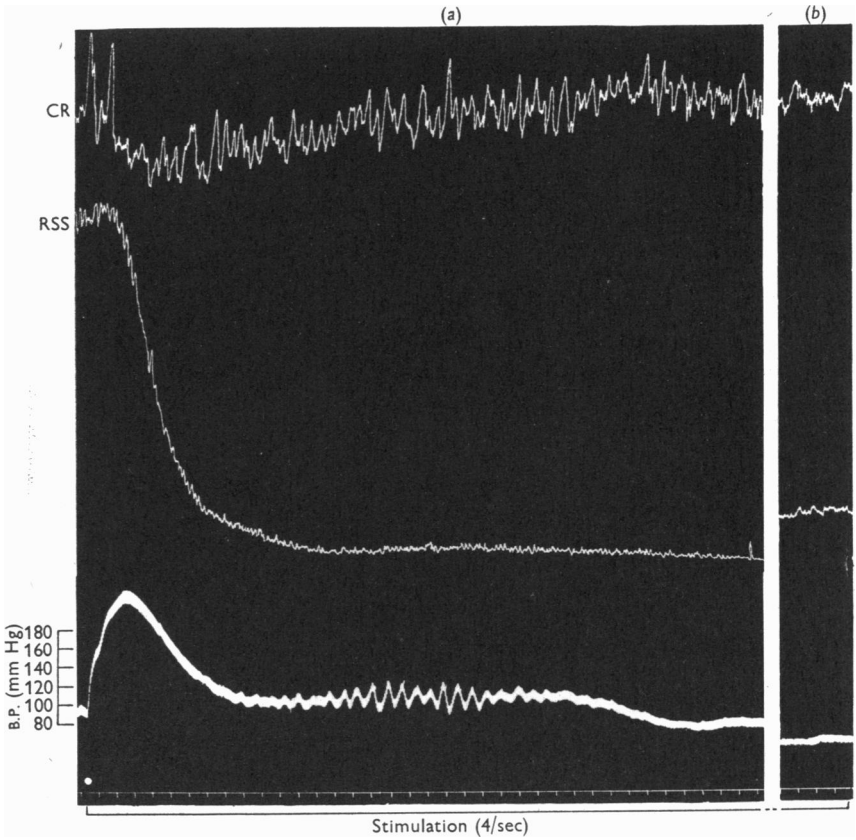

Fig. 9. Cat, 3·5 kg; chloralose. Effect of prolonged stimulation at a frequency of 4/sec of the cut left splanchnic nerve on the response of the blood-bathed isolated chick rectum (CR) and rat stomach strip (RSS) and of the blood pressure (B.P.). Note the early adaptation of the blood pressure and return of tone to the blood-bathed isolated chick rectum (*a*), while the rat stomach strip remains relaxed 125 min after commencing stimulation (*b*). Time marker, minutes.

rectum. Unlike the chick rectum, the blood pressure is more sensitive to noradrenaline than to adrenaline, so that a rising proportion of noradrenaline cannot account for the blood-pressure recovery. It suggests that there may be a difference in the manner in which various tissues accommodate to the effect of these amines. This was tested in four experi-

ments by infusing noradrenaline, adrenaline, or a mixture of these in equal parts, while recording the blood pressure and the responses of the blood-bathed stomach strip and chick rectum. In all cases the blood pressure returned to normal while the stomach strip was still fully relaxed; in only one experiment, with a mixture of noradrenaline and adrenaline, was there a partial recovery of tone with the chick rectum.

Effect of ganglion-blocking drugs on the splanchnic-suprarenal synapse

The experiments described above were undertaken to define the behaviour of the splanchnic-suprarenal synapse under various conditions of excitation. Once established, this knowledge could be applied to examining quantitatively the susceptibility of the synapse to blocking agents. Although a good deal of knowledge exists about the paralysis of homologous autonomic ganglia, our information about paralysis of the adrenal has advanced little since the study by Feldberg *et al.* (1934) of the effects of nicotine and atropine.

In early experiments ganglion-blocking drugs were tested during intermittent splanchnic stimulation at 10/sec for 30 sec; but the large relaxation produced by this excitation was little altered, for instance, by intravenous injections of hexamethonium up to 200 μg/kg. Larger doses (0·5 mg/kg) had a good blocking effect, but since other actions in the body became prominent, a more sensitive test seemed desirable. A better method was to test the action of the drugs by determining their effect on the tone of the blood-bathed assay tissues during sustained splanchnic stimulation at low frequencies (3–5/sec), which could be maintained for long periods without nerve exhaustion supervening; this stimulation rate produced a half maximal relaxation of the stomach strip. Provided the drug has no direct action on the strip, a paralysis of transmission shows itself by a recovery of tone in the strip and the relaxation re-establishes itself as the effect of the ganglion-blocking agents wears off.

Hexamethonium (C_6) was found to be the most potent of the ganglion-blocking members of the methonium series tested, with C_5, C_7 and C_4 in descending order of potency (50, 6·25 and 2·3 % respectively of the potency of C_6). C_6 was about five times as potent as tetraethyl ammonium bromide. The recovery of tone produced by, for example, 200 μg C_6 or 1 mg TEA/kg was incomplete and lasted about 7–10 min.

A proportion of the total dose (1/15th) of these drugs, injected into the tubing in series between the cannulated carotid artery and the bath containing the blood-bathed stomach strip, did not produce any significant effect on the assay tissues. It was impossible, however, to determine the relative potencies of tubocurarine or probanthine, as these had a direct

atropine-like relaxing effect on the stomach strip, which masked the possible recovery in tone looked for.

The similarity to autonomic ganglia showed itself in two other aspects. First, the synapse was resistant to decamethonium, which in a dose of 10 mg/kg had no effect on the response of the stomach strip to splanchnic nerve excitation. Secondly, the synapse was first excited and later blocked by nicotine.

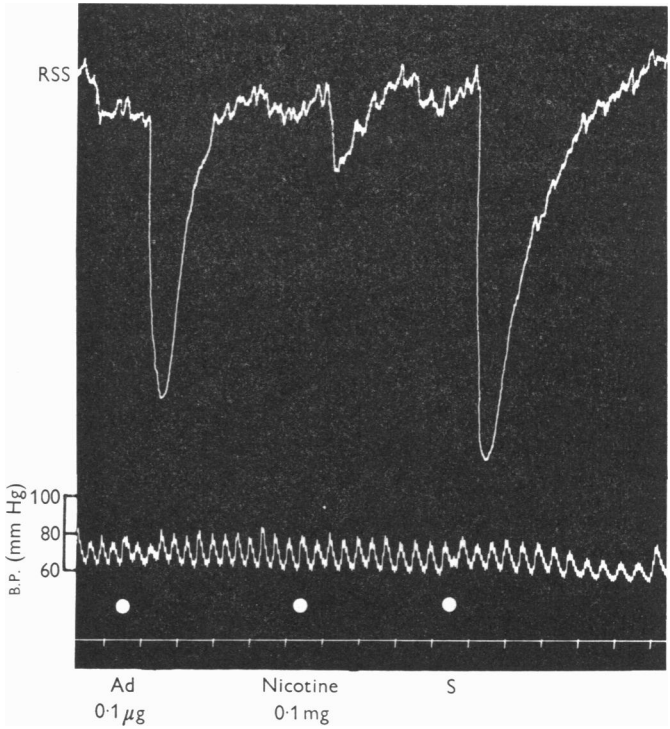

Fig. 10. Cat, 3·0 kg chloralose. The response of the blood-bathed isolated rat stomach strip (RSS) to intravenous injections of 0·1 μg adrenaline, 0·1 mg of nicotine or to stimulation of the cut left splanchnic nerve (S) at a frequency of 10/sec (100 shocks). Time marker, minutes.

Nicotine. The stimulant effect of nicotine on the adrenal medulla is well known, and nicotine in large doses has often been used to reduce or remove the response to splanchnic stimulation (Feldberg *et al.* 1934). Both these effects can be readily demonstrated with the blood-bathed stomach strip. It proved a very sensitive indicator of the stimulant action of nicotine on the medulla. Feldberg *et al.* required 0·1 mg given by arterial injection close to the gland to produce a good effect on the blood pressure. But with the stomach strip the same dose intravenously produced a good relaxation, the blood pressure being unaffected (Fig. 10). If the dose was

raised to 2 mg a slight reduction of the effect of splanchnic stimulation sometimes occurred. With repeated doses of 2 mg every 3 min up to a total of 12 mg a dwindling response was recorded (Fig. 11), and in the end splanchnic stimulation was greatly reduced.

The question arose, which appears not to have been previously considered, as to how far depletion of amine stores contributed to the fading response to stimulation. Two tests were applied. First, the release of amine to a fixed dose of histamine was tested before and after nicotine (the strip is exceedingly resistant to histamine). The release of amine by histamine was reduced by nicotine, but less so than the release to nerve stimulation

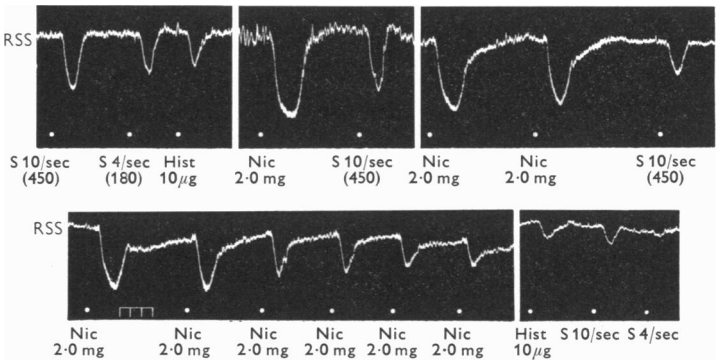

Fig. 11. Cat, 3·6 kg; chloralose. Effect of single or serial doses of nicotine (Nic) on the response of the blood-bathed isolated rat stomach strip (RSS) to splanchnic stimulation (S) at frequencies of 10/sec (total of 450 shocks) or of 4/sec (180 shocks) and to 10 μg histamine (Hist). Time marker, minutes.

(Fig. 11). If a massive amount of nicotine was given in divided doses in a relatively short time, complete abolition of nerve response occurred; the histamine response was reduced but still present. Secondly, it seemed improbable from our experiments on exhaustion that depletion could account by itself for the abolition of the response to nerve stimulation. This was tested directly in an experiment in which a series of brief nerve stimulations was substituted for the repeated doses of nicotine, producing relaxations of the strip comparable to those produced by the nicotine; no decline in response to splanchnic stimulation was found. It appears that the effect of nicotine on the adrenal synapse is a true synaptic block, although the reduced response to histamine may imply some contribution by amine depletion.

Since nicotine applied in sufficient doses directly to the strip may excite it, the relaxant effect could be somewhat underestimated. We believe, however, that the stimulant action of nicotine in the doses used, by the intravenous route, is virtually negligible. There is a circulatory latent

period of 1–2 min before the strip relaxes in response to a discharge of amines from the medulla; we have never seen any initial stimulant effect on the strip during this period, although it is then that the carotid blood contains nicotine at its highest concentration. As an additional control we have tested nicotine by injection into the extracorporeal circuit, in the same fraction of the intravenous dose as the ratio of the blood volumes of the circuit and the animal; such doses or slightly higher ones are ineffective.

DISCUSSION

Although early experiments on adrenal denervation (Stewart & Rogoff, 1916, 1917) indicated that amine output fell to undetectable levels, subsequent workers (Vogt, 1952; Duner, 1953; Rapela, 1956) agree that there is a constant small release of catechols from either the acutely or chronically denervated adrenal. Our results, restricted to the effects of acute nerve section, agree with those previously reported. In the normal cat under chloralose there is, before nerve section, a variable but sometimes quite large output of catecholamines from the adrenal (noradrenaline 3–198 ng/min, mean 54·5 ng/min; adrenaline < 1–209 ng/min, mean 36·2 ng/min), and under pentobarbitone similar figures were found. Nerve section reduced the output of both amines on an average by 60–70 %, sometimes to almost undetectable levels. An interesting finding was the subsequent rise in output after the nerve section, in the absence of nerve stimulation, or of any other obvious stimulus to secretion. We have concluded that before nerve section the stores of amine in the gland are slightly depleted by the normal activity of the splanchnic nerve; and that when this drain on the stores is prevented, amine accumulates until a spontaneous 'overspill' occurs.

When the splanchnic nerve is stimulated, there is a distinct 'threshold' frequency which the excitation must exceed for output to be raised. If duration of stimulation is unlimited then the rate, for a consistent response, must be 2–4 shocks/sec or more. If the optimal rate of excitation is used (about 30/sec) then something like 20 shocks are needed to increase output detectably. These properties are quite unlike those of, say, the nictitating membrane or neuromuscular junction, where single shocks produce distinct responses. It implies that, in the development of reflex activity by the adrenals, a more intense nervous discharge is called for from the splanchnic nerve than in the presynaptic fibres of other junctions. With effective nerve stimulation, output depended critically on the interval between successive shocks applied. As the interval was made shorter, with a constant number of shocks, output rose to a peak at about 30 msec intervals (stimulation rate 30/sec). Then output declined again, with more rapid shocks, to a minimum at about 10 msec interval. Finally, some degree of recovery occurred with stimulus interval made still shorter.

A number of mechanisms may be involved in this pattern of response. First must be considered a summation between the effects of acetylcholine released by successive shocks at the splanchnic–medullary-cell synapse. The wide distribution of cholinesterase (which also occurs in the medulla) and the invariable transience of action of acetylcholine exclude its direct participation in any prolonged response. But a process, capable of temporal summation, complete within 10 msec would be not unreasonable; so that the greater output at 3 msec intervals compared with 10 msec intervals could be attributed to persistence of the transmitter or of its immediate electrical effect for a few milliseconds.

Secondly, one might postulate an excitatory state, set up in the gland cell by the transmitter, which, if sufficiently intense, leads to a discharge of amines. The fact that single shocks are ineffective, and that it needs, under the best conditions, about 20 shocks to produce a detectable response, indicates that the excitatory state evoked by a single volley is well below the threshold for amine discharge. If, then, shocks are repeated at sufficiently short intervals, this threshold will eventually be exceeded; and the magnitude of the total excitatory state achieved will be a measure (not necessarily linear) of the slowness of decline of excitatory state after each shock. Such a model will account for a rising output with shortening stimulus interval; but it does not account for the decline of output when the interval decreases from 30 msec, unless it is also supposed that during the rise of the excitatory state, or for some period after its start, the medullary cell is relatively refractory to additional excitation.

A third interpretation is as follows. First, the decline of output with shocks less frequent than 10–30/sec might be attributed to some process of local uptake by the gland, like that proposed for the spleen by Brown & Gillespie (1957), who found that the noradrenaline output from the splenic nerves wanes in a similar way. The situation here, however, is somewhat different. In the spleen the receptor tissue is in direct relation to the nerves, and uptake or destruction of the amines by splenic tissue is a plausible supposition. In the adrenal, however, the final receptor is the body as a whole; if, as in our experiments, adrenal venous blood is collected, the only tissue available to take up the amines released is the blood in the gland, the supporting tissues of the medulla, and the medullary cells themselves. Since the cells can regain their amine content after its exhaustion, a reabsorption by the medullary cells of amine secreted a moment earlier deserves consideration. Secondly, the decline of output with shocks more frequent than 30/sec could be attributed to a process of synaptic fatigue or, possibly, to a failure of nerve-terminal conduction at high rates.

The higher rates of output we have obtained, between 3·6 and 3·8 μg/min for a period of 10 min stimulation at 10 or 16/sec, are similar to those

found by earlier workers. The work by Eade & Wood (1958) is most similar in method to our own; they found that excitation at 20/sec for 4·75 min released 5·15 μg/min (average) of catechol amines into the adrenal venous blood. Put into relation with the figures provided by Butterworth & Mann (1957) for the amine content of cat's adrenals (range 178–992 μg), mean *ca.* 320 μg/gland), this means that between 1 and 2 % of the medullary contents can be released per minute. Such a figure implies, further, that exhaustion of the release for maximal rates of output should occur within 50–100 min at most, unless resynthesis or re-storage takes place.

Our experiments with continued excitation show that 'fatigue' in fact sets in considerably earlier than this, especially with the faster rates of stimulation. Thus, at 32/sec fatigue was sometimes quite rapid, being complete in two experiments in 14 and 25 min; at 4/sec, where the output is of the order of 0·2 % of gland content per minute, it required 193 min stimulation to exhaust the response. This in itself points to the existence of fatigue due to some cause other than depletion of the stores. This was confirmed by finding that after the response to continued nerve stimulation had failed nicotine could still elicit a vigorous discharge of amines. The fatigue, therefore, is presumably not post-synaptic in nature.

There is no information as to the physiological rate of nervous discharge in the splanchnic nerves. The properties of the synapse would allow it to work effectively up to rates of 30–100/sec, since increasing outputs are obtained with increase of rate up to this level. According to Folkow (1952), post-ganglionic sympathetic nerves to a limb do not fire faster than 6–8/sec; but these are performing a quite different function.

Previous workers have paid considerable attention to the proportion of noradrenaline to adrenaline released by nervous excitation. Thus Rapela (1956) reported that at a high stimulation rate a selective increase of adrenaline release occurred. In general, we have found that with brief periods of excitation the proportion of the amines did not vary with rate in any consistent way. But in one experiment excitation at 512/sec did produce an increase of adrenaline release only. More important is the question of a changing amine ratio during prolonged excitation. Bülbring & Burn (1949) found that in five out of nine experiments with repeated intermittent stimulation the adrenaline proportion fell. On the other hand, Lund (1951) and Outschoorn (1952) concluded that the amine ratio was unchanged. It is worth noting that, in any case, the amine ratio of cat's adrenal glands is extremely variable, ranging from 13·3 to 90·7 % noradrenaline (Butterworth & Mann, 1957). In our experiments with blood-bathed tissues there was a consistent tendency during prolonged excitation of the splanchnic nerve for the chick rectum (specifically sensitive to adrenaline) to regain its tone before the rat stomach strip (approxi-

mately equally sensitive to both amines), so that our evidence supports the conclusion reached by Bülbring & Burn.

During these experiments the observation was also made that the blood pressure returned to its control level much more quickly than did the rat stomach strip. Since noradrenaline is more effective than adrenaline on the blood pressure, a change in amine ratio does not account for this. The observation prompted experiments on the accommodation of blood pressure and of the blood-bathed tissues to infusions of amines. This showed that the blood pressure would always accommodate, completely if time were allowed, although the rat stomach strip would never do so. An interesting parallel finding is the record, in the experiments reported by Blacket, Pickering & Wilson (1950), that rabbits which had become tolerant to the pressor effect of noradrenaline or adrenaline infusions had grossly distended viscera. If accommodation to the excitatory but not to the relaxant effects of these amines is possible, an explanation of adrenaline or noradrenaline 'shock' immediately appears, that this is due to the unopposed vasodilator effect of these drugs after tolerance has developed to the vasoconstriction.

Finally, the possibility was explored in our experiments that the splanchnic-adrenal synapse might have a specific sensitivity to blocking agents, such that drugs might be found to paralyse it selectively, just as hexamethonium or decamethonium can be used at the ganglionic or neuromuscular synapses. By three tests, however, it resembled the autonomic ganglia with which it is homologous. First it was paralysed, better by hexamethonium than by its C_4, C_5, or C_7 homologues. As with autonomic ganglia, block was produced more readily during sustained excitation than with brief bursts of excitation. Secondly, decamethonium was inactive. Thirdly, nicotine initially excited and then paralysed the synapses. Such a response is typical of ganglia, but unlike that of cat's voluntary muscle, on which nicotine is almost devoid of competitive blocking action (W. D. M. Paton & E. C. Savini, unpublished). It is clear from the experiments by Feldberg *et al.* (1934) that the sensitivity of the synapse to block of an atropinic kind is rather small, and may, indeed, be not larger than that now known to exist at the autonomic ganglia. A selective blocking agent for the adrenal synapse, sparing the autonomic ganglia, would be of great interest and usefulness; but it seems that the faithfulness of the gland to its autonomic ancestry stands in the way of such a development.

SUMMARY

1. The output of sympathetic amines from the cat adrenal gland in response to nerve stimulation was studied both by assay of the amines in adrenal venous blood, and by their effect on isolated assay tissues

(rat stomach strip, chick rectum) included within an extracorporeal circulation.

2. The resting output of sympathetic amines (particularly noradrenaline) falls after acute splanchnic-nerve section, but rises again in the absence of nerve stimulation.

3. There is a threshold stimulation frequency for augmented amine release; thus excitation of the cut splanchnic nerve at 1/sec has virtually no effect unless prolonged for about 15 min; output is invariably raised with stimulation at a rate of 4/sec.

4. With adequate rest intervals output increases approximately linearly with increasing frequency of stimulation up to a rate of 30–60 sec, after which output declines. At such rates about 60 shocks are needed to increase output significantly; in one experiment 13 shocks were effective. Increased output above control values may be found with stimulation rates as high as 500/sec, given in a brief burst lasting 100 msec. It appears that two separate processes of interaction occur between successive shocks: the first on a short time scale lasting only 10 msec or less, the second much slower, with a peak at 30 msec, and lasting for 300–1000 msec.

5. Fatigue of the response occurs if stimulation is either protracted (even at excitation rates as low as 4/sec) or if inadequate rest periods are allowed between bursts of 10 min stimulation at rates of 4–32/sec; this appears to be a synaptic fatigue. During prolonged stimulation, the output of adrenaline declined before that of noradrenaline.

6. During a prolonged output of amines in response to splanchnic nerve stimulation, or during an infusion of noradrenaline or adrenaline, the pressor blood-pressure response accommodates, but the relaxant response of the stomach strip does not.

7. Ganglion-blocking drugs of the methonium series paralyse the adrenal synapse, especially during sustained stimulation. A range of drugs was tested to see whether a selective blocking agent was likely to exist; but none was found. The splanchnic-adrenal synapse appears to have the properties of autonomic ganglia with which it is homologous.

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