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THE HORMONES OF THE ADRENAL MEDULLA AND THEIR RELEASE*

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The evolution in recent years of sensitive tests for noradrenaline has led to a revival of interest in the adrenal medulla, especially because the medullary hormones were more likely to show differences in the extent of N-methylation in different animals and under different conditions than nerve sympathin, of which almost invariably very little is methylated. Among numerous recent investigations by several workers two in particular have a bearing on the present work. The liberation of adrenaline and noradrenaline from the adrenals of cats, when the splanchnic nerves were stimulated, was studied by Gaddum and Lembeck (1949), using the method of parallel quantitative assays; they pointed out, however, that the errors of the tests used were so large that convincing evidence was only obtained of the presence of a mixture of substances and not of their relative proportions. Adrenaline and noradrenaline in the adrenals of rats injected with insulin were estimated by Burn, Hutcheon, and Parker (1950). Their method involved the simultaneous recording of the blood pressure and of the contractions of the normal nictitating membrane of the cat. The latter test object gave a measure of the proportions of the two amines when various equipressor mixtures were injected.

In the present work studies were made of the changes in the adrenaline and noradrenaline content of the adrenals of rats under the influence of three drugs known to cause a release of the adrenal medullary hormones: insulin (Cannon, McIver, and Bliss, 1924), morphine (Elliott, 1912), and β-tetrahydronaphthylamine (Mutch and Pembrey, 1911). Experiments were also carried out on cats in which the adrenals were secreting spontaneously, or were being stimulated through the splanchnic nerves or by the injection of acetylcholine or potassium. The amines were estimated in the glands and in the plasma of the effluent blood.

METHODS

Rats of both sexes, weighing 150–200 g., were used. For the insulin experiments they were fasted overnight (17 hours) and during the course of the experiments. Groups of 4–8 rats were divided into two equal subgroups. One subgroup was injected subcutaneously with a drug and the other subgroup served as a control. The drugs and doses per 100 g. of rat were: soluble insulin (Burroughs Wellcome and Co. 20 units/ml.), 1 unit; morphine hydrochloride (Macfarlane), 2 mg. in a freshly made solution containing 2 g./100 ml.; tetrahydro-β-naphthylamine carbonate (B.D.H.), 7.5 mg. in a

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freshly made slightly acid solution containing 2 g./100 ml. Both injected and control animals were killed together by a blow on the head at various times after administration of the drugs. The adrenals were quickly removed and weighed. Both glands of each animal were placed in 0.5 ml. of 0.15 N-HCl in a "Pyrex" centrifuge tube. When all the glands from the particular experiment had been collected they were ground up in each tube with the assistance of a little acid-washed sand. The extracts were then prepared for application to a cylinder of filter paper for chromatography as described by Crawford and Outschoorn (1951).

Cats, weighing over 2 kg. if possible, were anaesthetized with ether and chloralose and eviscerated. Collections of blood were made in a similar manner to that described by Gaddum and Lembeck (1949) through a cannula in the inferior vena cava by transferring a clip on that vessel from below the site of entry of the renal veins to just below the liver. The renal vessels were always tied, but the kidneys were left *in situ*. In some experiments, in which the left adrenal vein joined the left renal vein, only the left gland was stimulated. The kidneys were still tied off, but the collecting cannula was tied into the left renal vein and the blood shunted through a rubber tubing by-pass into an external jugular vein. Adrenal venous blood was then collected through a T-piece in the rubber tubing by transferring a clip from a piece of rubber tubing on a stem of the T to the rubber tubing on the appropriate limb of the T. The splanchnic nerves were cut leaving a good peripheral length for electrical stimulation. Drugs were injected through a cannula in the stump of the coeliac artery, which was at other times occluded by a clip. The animals were heparinized (100 units/kg.) and the carotid blood pressure was recorded with a mercury manometer. The splanchnic nerves were stimulated with an alternating current from a Ritchie-Sneath stimulator, using 5–7.5 volts for platinum electrodes or 40–50 volts for a Collison's electrode (in which the nerve was surrounded by Locke's solution). Acetylcholine (100 mμg./ml. in saline) or potassium chloride (1 g./100 ml. water) was injected in volumes of 0.5–1 ml. either slowly over 5–10 min. or in a moment. Samples of adrenal venous blood were taken during control periods and when the glands were stimulated. Samples of arterial blood were sometimes taken as well. The blood was received into ice-cooled graduated centrifuge tubes, centrifuged (3,000 r.p.m. for 10 min.), and the plasma pipetted off and stored in ice until further treatment. Two types of experiment were performed. In the first series the periods of collection and stimulation were long (5–10 min.) and were accompanied by a progressive fall in the cat's blood pressure. The substances in the plasma were estimated by parallel assays on the rat's uterus, the rat's colon, and the rabbit's ear (Gaddum, Peart, and Vogt, 1949). In the second series the periods of collection were short (30–60 sec.) and the fall in blood pressure was much less; sometimes it was prevented by infusing blood from another cat between samples. Usually, however, it was sufficient to leave the animal for about 5 min. to let the blood pressure return to its original height after collection of a sample. The samples of plasma were subjected to preliminary tests on the rat's uterus and then prepared for paper chromatography.

A measured volume of plasma (up to 2 ml.) was added dropwise to 6 volumes of acid alcohol (0.1 per cent (v/v) conc. HCl in ethanol) and chilled in ice for 30 minutes. The tubes were centrifuged (3,000 r.p.m. for 5 min.) and the supernatant fluid was transferred to a 50 ml. round-bottomed Quickfit and Quartz flask; the precipitate was washed with 2 ml. acid alcohol which was chilled for 10 min., centrifuged (3,000 r.p.m. for 3 min.), and added to the first fraction. The cooled fluid was evaporated *in vacuo* at 55°–60° C. (external temperature). The residue was transferred with 5 washings of 1 ml. each of acid alcohol to a 10 × 1 cm. hard glass centrifuge tube which was chilled in ice for 15 min. and centrifuged (3,000 r.p.m. for 3 min.). The supernatant fluid was transferred to another 50 ml. round-bottomed flask, the residue being washed with another 1 ml. of acid alcohol which was chilled for 5 min., centrifuged (3,000 r.p.m. for

3 min.) and added to the fractions in the flask. The pooled fluid was evaporated *in vacuo* at 55°–60° C. (external temperature). The residue was leached with 0.7 ml. acid alcohol, which was applied to a paper cylinder for chromatography, and then with a further 0.2 ml. which was also applied.

In some experiments extracts of the adrenal glands were prepared for chromatography. In these experiments one gland was removed before cannulation of the inferior vena cava as a control while the other was left for electrical stimulation of the splanchnic nerve. This was carried out continuously for 1 hour during which blood samples were taken at half-hourly intervals. At the end of the period the remaining gland was removed. Each gland was weighed soon after removal, placed in 10 ml. of 0.15 N-HCl in a mortar, and ground. When well homogenized, 1 ml. of the liquid was pipetted off and added to 10 ml. acid alcohol. The preparation of the extract for chromatography was similar to that for the whole liquid containing a pair of rat's adrenals, save that the precipitation was aided by chilling in ice instead of leaving the sample at room temperature, and the centrifugings were for a shorter time (5 min.) at a higher speed (3,000 r.p.m.). The final leachings, with 0.7 and 0.2 ml. acid alcohol, were applied to a paper cylinder.

The application of the acid alcohol extracts to the paper, the development of the chromatogram with phenol as solvent, the elution of the appropriate strips of paper containing adrenaline and noradrenaline, and the assay of these amines in the eluates with the rat's blood pressure preparation were as described by Crawford and Outschoorn (1951), with the difference that the rats used for testing were treated with atropine and hexamethonium, which lowered their blood pressure and so decreased the effects of depressor substances in the samples. Atropine sulphate (100 μg./100 g.) was given intravenously slowly over about 2 min. and the animal left for about 10 min. to recover. Hexamethonium (C6) bromide (1 mg./100 g.) was then given intravenously, slowly over 2–3 min. The blood pressure usually fell to about 50 mm. mercury, and the rat was used immediately for assays. After about 0.75–1 hour the blood pressure had often risen to about 100 mm. mercury and this was accompanied by a reduction in the sensitivity and discrimination of the preparation. It was sometimes necessary to lower the rat's blood pressure again by repeating the injection of C6 in about double the dosage.

Experiments on rats.—There were several possible ways of presenting the results of the rat experiments. The concentrations of the amines could be expressed in terms of gland weight (mμg./mg.) or of body weight of rat (μg./100 g.). In the insulin experiments the effects of starvation and of the drug had to be considered. Vogt (1947) reported the influence of these on the weights of the adrenals. Fig. 1 shows the changes undergone by the mean weights of the glands in terms of body weight of rat in the present insulin experiments. Both control and injected groups agreed fairly closely in showing a fall in weight up to about 6–8 hours and an increase in weight after 16 hours. Owing to these changes the concentrations of adrenaline and noradrenaline in all the rat experiments were expressed in terms of body weight of rat, which presumably varied much less.

Wide variations were noticeable in the concentration of the amines in the adrenals of the control animals. Analyses of variance were made of the estimates of adrenaline and noradrenaline in the control rats of (*a*) the insulin experiments and (*b*) the morphine and tetrahydronaphthylamine experiments taken together. This was because the rats of the insulin experiments were fasted overnight while those of the other experiments were fed. For adrenaline and for noradrenaline there was in both series a significant variation between the control values from day to day

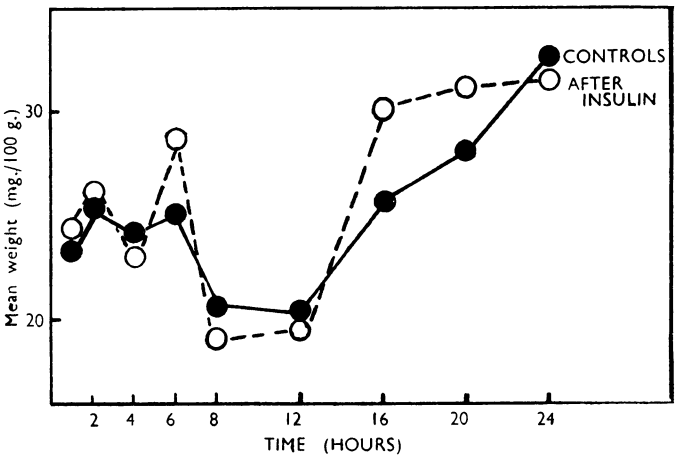

FIG. 1.—Weight of rats' adrenals (mg. per 100 g. body weight). Effect of fasting.

and at different times after the commencement of the experiments. On account of these variations among the controls it was decided that the concentration of the amines in each injected animal should be calculated as a percentage of the mean amount in the group of control rats treated in the same way and killed at the same time. A mean value for the amount of each amine found after a particular time of action of a drug was then calculated from the individual percentages. Table I

TABLE I

In this Table n = no. of injected rats; Adr. = adrenaline; Nor. = noradrenaline

Hours after injection	Calculated mean concentrations of pressor amines in rats' adrenals (as percentages of means of control animals) after											
	Soluble insulin			Morphine hydrochloride						Tetra-hydronaphthyl-amine		
				Single dose			Repeated doses					
	n	Adr.	Nor.	n	Adr.	Nor.	n	Adr.	Nor.	n	Adr.	Nor.
1	5	70	98	6	94	99						
2	21	99	147	3	137	158				3	68	81
4	5	54	112	3	88	64	6	43	67	2	66	131
5							3	59	131			
6	5	56	100									
8	5	55	87	3	79	105				3	25	81
12	5	22	52	3	111	188						
13										1	53	115
16	5	15	72	3	84	85				3	90	76
20	5	33	80	3	99	156						
24	4	28	112									
Range of mean conc. in controls (μg./100 g. Adr.		13.75—31.82			8.06—13.96			11.80—16.77			11.57—15.21	
Range of mean conc. in controls (μg./100 g. Nor.		0.95—3.48			0.94—2.86			2.17—3.33			1.82—3.22	

shows the results calculated in this way. However, when tests of significance were made, the absolute concentrations of each amine in the group of the injected rats was compared with those of the controls for the particular time of action of the drug even though that included experiments carried out on different days.

Adrenaline.—Insulin produced a significant depletion of this amine. In 12 hours the injected animals had about 20 per cent of the amount present in the controls and in 16 hours only 10–20 per cent. Thereafter and up to 24 hours a tendency towards recovery was noticeable. With tetrahydronaphthylamine the depletion was quicker. About 25 per cent of the adrenaline was left in 8 hours and recovery was almost complete by 16 hours. After only one injection of morphine there was no appreciable depletion of adrenaline over 20 hours' observation. A transient but significant depletion to 43 per cent of the original level was achieved with four injections of the drug at hourly intervals, the animals being killed 4 hours after the commencement. After 5 hours the depletion was less.

Noradrenaline.—The amounts of noradrenaline did not follow the trend of the methylated amine. Neither morphine (single or repeated injections) nor tetrahydronaphthylamine produced any lowering of the content of noradrenaline below the range of the control animals. Of the three agents (in the particular dosages employed) the most powerful and longest lasting effect was produced by insulin. Even this produced only a transient, non-significant depletion of less than 50 per cent of the noradrenaline at 12 hours, while recovery was complete in 16–20 hours. On the other hand, 2–4 hours after the injection of the drugs there was an indication that the noradrenaline content was increased 30–50 per cent above that of the controls. Although this increase was small, transient, and only just significant ($P = 0.05$) in the insulin injected rats, a similar trend was observed in the experiments with the other two agents. Fig. 2 shows the changes undergone by the adrenaline and nor-

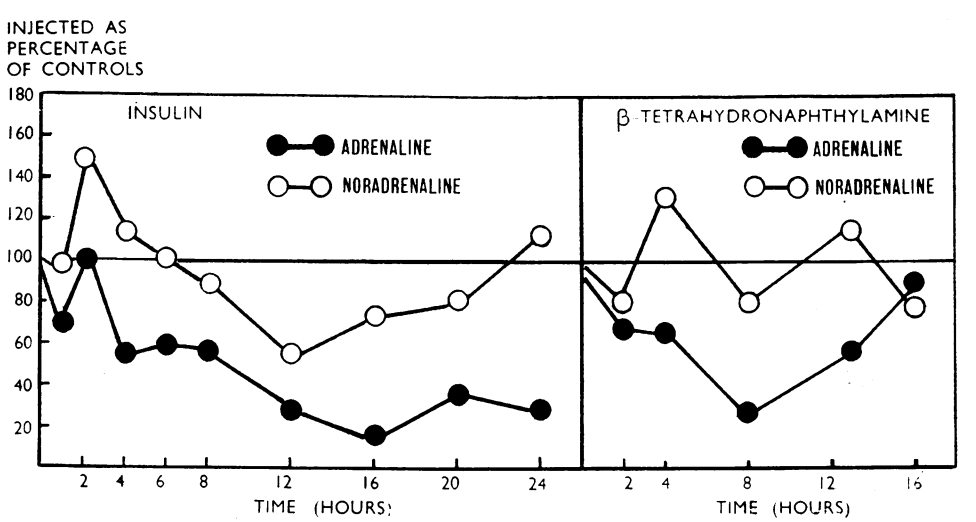

FIG. 2.—Amounts of adrenaline and noradrenaline in rats' adrenals (mg. per 100 g. body weight) expressed as percentages of figures for control rats. Effects of insulin and β-tetrahydronaphthylamine.

adrenaline in the adrenals of the injected animals. A figure for the morphine experiments was not constructed owing to the relatively small effects of that drug.

Experiments on cats.—The results of parallel assays on samples of adrenal venous plasma from the first series of experiments on cats are shown in Table II. In some

TABLE II

Cat: Estimates of adrenaline and noradrenaline in adrenal venous plasma by parallel assays R_u = dose ratio of noradrenaline to adrenaline required for equal responses on uterus. R_c = dose ratio of noradrenaline to adrenaline for equal responses on colon

Exp. No.	Adrenaline equivalents (mμg./ml.)					Calculated concn. (mμg./ml.)			
	Serial samples	Assay with uterus	R_u	Assay with colon	R_c	Adrenaline	Noradrenaline	% Adr.	
55	Control	<2.5	150	<50	0.5	<2.3	<24	—	
	Ach.	300	150	200	0.5	299	(−50)	100	
	Ach.	375	150	375	0.5	374	0	100	
	Control	75	75	87	0.5	75	6	93	
	Control	12	75	<50	0.5	12	<19	<39	
	KCl	3,000	75	3,000	0.5	2,979	0	100	
	Control	500	75	1,500	0.5	490	500	49	
57	Control	75	160	125	2.5	73	125	37	Assay with rabbit's ear / R_e
	Ach.	562	160	750	2.5	550	470	54	
	Ach.	1,200	160	1,500	2.5	1,177	750	61	
	Control	1,250	160	1,750	2.5	1,223	1,250	49	3,000 / 3
58	Control	30	175	<5,000	0.75	≥10	<3,727	—	
	Ach.	4,876* (s.e. 12.4%)	175	790* (s.e. 40.1%)	0.75	4,873	(−3,064)	100	*Statistical
59	Control	<5	150	Inter.	0.75	<5	—	—	
	Electr.	3,200	150	9,333	0.75	3,153	4,600	41	
	Control	1,500	150	2,000	0.75	1,490	375	80	
	Arterial	Inter.	150	Inter.	0.75	—	—	—	
60	Control	2.5	250	<250	3	(−0.5)	<742	—	Assay with rabbit's ear / R_e
	Electr.	2,500	250	2,000	3	2,476	(−1,500)	100	
	Control	562	150	1,500	0.5	558	469	54	
	Arterial	<2.5	250	<250	3	—	<742	—	10,000 / 1.87
61	Control	15	37	<500	0.75	≥5	<364	—	
	Electr.	6,000	37	6,000	0.75	5,880	0	100	
	Arterial	Inter.	37	Inter.	0.75	—	—	—	
62	Control	7.5	75	<250	0.37	≥6.2	<92	—	
	Electr.	1,500	75	3,000	0.37	1,485	555	73	
63	Control	7.5	100	<500	1	≥2.5	<492	—	
	Electr.	1,000	100	1,000	1	900	0	100	
	Ach.	12,360* (s.e. 16.8%)	100	17,866* (s.e. 25.7%)	1.5	12,092	8,259	59	*Statistical
64	Control	10	75	<500	0.75	≥5	368	—	
	Electr.	6,480* (s.e. 28.3%)	110*	1,693* (s.e. 51.2%)	0.7*	6,469	(−3,351)	100	*Statistical
	Ach.	19,540* (s.e. 27.9%)	110*	10,720* (s.e. 36.7%)	0.7*	19,472	(−6,174)	100	

assays the complete statistical procedure adopted by Gaddum and Lembeck (1949) was used, but significant amounts of noradrenaline were not found. The release of large amounts of active substances on stimulation of the adrenal medullae is evident. Generally stimulation samples taken after control samples showed an apparent increase in the percentage of adrenaline as well, whereas control samples taken after stimulation samples showed an apparent decrease both in total activity and in the percentage of adrenaline. Accordingly the hormones released must have consisted mainly of adrenaline. A better idea of the proportions of the two amines was afforded by the second series of experiments. Assays were carried out after chromatographic separation of adrenaline and noradrenaline, and the results are given in Table III. The percentage of adrenaline in the control samples could not be calculated from the rat's blood pressure assays only.. In one experiment (No. 68) the percentage was estimated as 5 per cent from

TABLE III

Cat: Estimates of adrenaline and noradrenaline in adrenal venous plasma separated by chromatography with the rat's blood pressure preparation

Comparison of different methods of stimulation: Control=unstimulated. Electr.=electrical stimulation (see text). Ach.=Acetylcholine injection. KCl=Potassium chloride injection.

Expt. No.	Sample: control or method of stim.	Adrenaline equivalent by direct assay with the rat's uterus mμg./min.	Estimates of separated amines with the rat's blood pressure mμg./min.		% Adr.
			Adrenaline	Noradrenaline	
65	Control	—	<551	412	<57
	Electr.	—	1,332	1,248	52
	Ach.	—	3,268	612	84
	KCl	—	4,960	1,550	76
	Ach.	—	4,799	2,800	63
	Electr.	—	2,400	600	80
66	Control	180	<300	<375	—
	Electr.	720	775	<186	>81
	Ach.	8,000	5,000	<625	>89
67	Control	<10	<1,000	<500	—
	Electr.	2,800	4,375	3,500	56
	Ach.	4,560	7,600	5,700	57
	KCl	5,000	10,000	5,000	67
	Ach.	1,440	3,000	1,500	67
	Electr.	—	3,256	4,888	40
68	Control	19	<950	357	<73
	Electr.	1,001	600	581	51
	Ach.	6,400	3,240	8,000	29
	KCl	2,855	2,800	2,500	53
	Ach.	4,800	3,528	1,500	70
	Electr.	925	1,388	462	75
69	Control	2.6	<525	<262	—
	Electr.	1,040	975	455	68
	Ach.	1,600	1,250	625	67
	KCl	4,400	3,300	2,475	57
	Ach.	2,666	2,500	1,500	62
	Electr.	3,466	3,250	1,950	62

the result with rat's uterus. In two experiments (65 and 68) there was assayable noradrenaline in the control plasma. There was an increase in total activity on stimulation which was once (Exp. 68) accompanied by an increase in the percentage of adrenaline. The amines could both be estimated accurately in plasma collected during stimulation, and the percentage of adrenaline was practically the same after all three forms of stimulation. In a few experiments attempts were made to investigate the relation of the amounts of adrenaline and noradrenaline in the adrenal glands to those in the venous plasma. The results are shown in Table IV. In two experiments (70 and 71) the left adrenal was removed as a control and the right splanchnic nerve was stimulated. This proved unsatisfactory, probably owing to partial occlusion of the right adrenal vein when the inferior vena cava was clipped just below the liver. In the remaining experiments the right adrenal was removed as a control and the left was stimulated through the splanchnic nerve, with more satisfactory results. Tests on samples of plasma showed that after one hour's continuous stimulation the proportions of adrenaline and noradrenaline did not change.

TABLE IV

Cat: Estimations of adrenaline and noradrenaline in adrenal glands and adrenal venous plasma: separated by chromatography and assayed with the rat's blood pressure

Effect of continuous stimulation. Control gland removed before stimulation. Stimulated gland removed at end of period of stimulation.

Exp. No. (Wt. of cat)	Stim. for (hr.)	Adrenaline equiv.: Rat's uterus	Separated by chromatography, assayed with the rat's blood pressure						
			Venous plasma (mμg./min.)			Adrenal glands (μg./gland)			
			Adrenaline	Noradrenaline	% Adr.	Weight	Adrenaline	Noradrenaline	% Adr.
70 (2.85 kg.)	Control	<2	<48	<21	—	Control (142 mg.)	150	100	67
	0	27	<62	15	<80				
	½	30	<62	63	<50	Stim. (144 mg.)	75	50	67
	1	27	208	110	65				
71 (3.4 kg.)	Control	<2.8	<79	<25	—	Control (264 mg.)	75	25	75
	0	36	113	74	60				
	½	75	188	75	71	Stim. (246 mg.)	66.7	14.6	82
72 (4.2 kg.)	Control	<8.5	<36	70	<34	Control (238 mg.)	100	15	87
	0	360	240	450	35				
	½	315	275	275	50	Stim. (226 mg.)	75	10	87
	1	286	262	131	67				
73 (2 kg.)	Control	>>180	841	599	58	Control (120 mg.)	100	37.5	73
	0	800	333	500	40				
	½	75	125	62	67	Stim. (108 mg.)	50	25	67
	1	87	87	54	62				
74 (3.6 kg.)	Control	<9	<30	<15	—	Control (190 mg.)	100	75	57
	0	256	426	426	50				
	½	408	303	454	40				
	1	90	100	150	40	Stim. (218 mg.)	67	50	57
Mean depletion (per cent) after 1 hour							40	46	

In the same period there was an appreciable depletion of both amines to about the same extent (less than 50 per cent), so that the percentage of adrenaline in the stimulated gland was the same as in the control gland removed earlier. The percentage of adrenaline in the plasma was generally less than in the glands.

Discussion

The amounts of sympathomimetic amines in the adrenals of an animal are determined by a balance between utilization and synthesis. From the results for the rat adrenals as well as from those for the cats it would appear that with a sufficiently powerful and prolonged stimulus the utilization of adrenaline increasingly outpaces synthesis, with the result that the glands are depleted of that amine. In the rat adrenals the depletion of adrenaline was most marked in the insulin experiments, less pronounced in the tetrahydronaphthylamine experiments, and could only just be demonstrated in the experiments in which repeated doses of morphine were given. In the cats' adrenals even after a comparatively short stimulation period of 1 hour some depletion of adrenaline was evident. The amounts of noradrenaline showed different changes in the two species of animals. Under the conditions of the rat experiments the noradrenaline level kept within the normal range, although there was a tendency to depletion in the insulin-injected rats. This may be because there was either no output of noradrenaline or an output with which synthesis could keep pace. If the latter explanation is correct, the rat is perhaps better able to synthesize noradrenaline than adrenaline when its medullary resources are heavily taxed. On the other hand, in the experiments with cats the adrenals were depleted of noradrenaline to the same extent as of adrenaline. When the adrenals are stimulated there is an output of noradrenaline into the blood in the cat, but it is less than that of adrenaline. The parallel depletion of the two amines in spite of the smaller output of noradrenaline in relation to adrenaline shows that the cat has no special ability to synthesize noradrenaline quickly.

In the rat adrenals, owing to the much greater utilization of adrenaline than of noradrenaline which occurred after prolonged stimulation of the adrenal medulla, there tended to be a relative increase in the proportion of noradrenaline, which occurred 12 hours after insulin and 8 hours after tetrahydronaphthylamine. There was no evidence of an absolute increase of noradrenaline at these times. Burn, Hutcheon, and Parker (1950) in their experiments on rats showed that after 0.2 unit of insulin per 100 g. rat there was a 50 per cent depletion of the total amount of medullary hormones in the adrenals after 8 hours. At this time there was also a decrease in the percentage of adrenaline. They concluded that an increase in the relative amount of noradrenaline, as determined by their method of assay, was due to the process of methylation being slower than the processes by which fresh noradrenaline was accumulated, after the adrenals had been active for 4–6 hours. In their experiments the pooled adrenals of several rats were extracted and tested. In the present series the amines present in the pair of adrenals of each animal were estimated individually and separately. There was evidence of an absolute increase of noradrenaline in the rat adrenals 2–4 hours after injection of the drugs. During this increase of noradrenaline the amounts of adrenaline were hardly less than in the controls. The tendency for the earliest response of the adrenals of rats to be an increased production of noradrenaline, even while the

demand for adrenaline could still be met, is in conformity with the other evidence suggested above of the power possessed by the rat to synthesize noradrenaline. This early increase in the absolute amount of noradrenaline has also been reported by West (1951) in rabbits 2 hours after the injection of insulin. Assayable amounts of noradrenaline were found, which were not detectable at other times.

The percentage of adrenaline in the cat adrenals in these experiments showed no change during one hour's stimulation, whereas on repeated or prolonged stimulation Bülbring and Burn (1949a) and West (1949) noticed a fall in the percentage of adrenaline. The former workers had some evidence that this change was dependent on methionine deficiency in the animals' diets. The differences in the cat experiments reported here may be related to the intensity of the stimulus as well as to the fact that the depletion of total amines obtained was relatively small.

There was some difficulty in the experiments on rats in producing a depletion of adrenaline with morphine. This contrasts with Elliott's (1912) experiments on cats, where 20 mg. of morphine depleted the adrenals to 15 per cent of the control adrenaline content in 8 hours. With 2–3 c.c. of a 2 per cent (w/v) solution of tetrahydronaphthylamine only about 50 per cent of the adrenaline was depleted in the same time. Both drugs were used in the rats at about twice this dose per kg., but only tetrahydronaphthylamine produced an effect on the stores of hormone in the adrenal medulla of the rat. Evidently morphine seems more efficient in depleting the adrenals of cats than of rats, whereas the opposite is true for tetrahydronaphthylamine.

Since a certain degree of hypoglycaemia after insulin is essential for the initiation of the stimulus to the adrenals (Cannon, McIver, and Bliss, 1924), a check was made on the relationship between the changes in the amounts of the medullary amines and the blood sugar level. Blood sugar estimations (Hagedorn–Jensen method) gave mean values (of 3 rats in mg./100 ml. of blood) of 88 at the commencement, 54 in 2 hours, 51 in 4 hours, and 89 in 5 hours after insulin. The depletion of adrenaline appeared to have continued long after the blood sugar level had returned to normal.

The long duration (more than 24 hours) of the depletion of the adrenaline in the glands after insulin was like that observed in dogs injected with eserine by Edmunds and Smith (1932), who found only 10–20 per cent of the normal adrenaline after 24 hours and less than normal after 48 hours.

In the second series of experiments on cats, blood was injected in order to maintain the blood pressure at the same level at the commencement of each sampling so as to avoid the influence of haemorrhage on the adrenals as much as possible. In addition the types of stimulation were repeated thus: electric stimulation, acetylcholine, KCl, acetylcholine, electric stimulation, in each experiment. This was to prevent any change in the amounts of the amines or in the proportion of adrenaline due to a general shift caused by the condition of the animal passing unnoticed.

No differences were observed between the three methods of stimulation on the proportion of adrenaline to noradrenaline in the medullary hormones released in the cat. Either the method of investigation was not sensitive enough to detect such differences or the proportion of adrenaline depends on other factors, such as the supplies of noradrenaline available to the glands. Bülbring and Burn (1949b) found that noradrenaline added to the perfusing fluid of a dog's isolated adrenal resulted in an increase of adrenaline in the outflow.

The high output of active substances in the control plasma in one experiment (73) can be explained by an unintentional mechanical stimulation caused by respiratory movements displacing the electrode which was usually fixed in position before any samples (control or stimulation) were taken.

The suggestion of Meier and Bein (1948) that there was probably a constant discharge of noradrenaline from the resting adrenals is supported by the finding that this amine could be detected in the venous plasma of the control samples in some of the cat experiments. It is not likely that its presence was due to stimulation in operation, because it was not accompanied by a large amount of adrenaline. The instances in which it was possible to measure the percentage of adrenaline in control samples are too few to decide whether it is normally low in the conditions of these experiments. The experiments showed throughout that stimulation by any of the methods used caused a release of a mixture in which adrenaline predominated. It is, however, still doubtful whether a constant secretion of noradrenaline is part of the normal physiological function of the adrenals.

SUMMARY

1. Insulin, morphine, and tetrahydro-β-naphthylamine were injected into groups of rats. All three agents produced significant depletion of adrenaline in the adrenals after some hours. No significant depletion of noradrenaline occurred, although there was a tendency to depletion with insulin.

2. At the time of maximum depletion of adrenaline there was a relative increase of noradrenaline and thus a reduction in the percentage adrenaline content. An absolute increase of noradrenaline occurred within the first few hours of stimulation, at which time the amounts of adrenaline were still normal.

3. The adrenals of cats were stimulated through the splanchnic nerves electrically, and by acetylcholine and potassium. Small amounts of adrenaline and sometimes larger amounts of noradrenaline were found in the venous plasma from the resting gland. Stimulation caused the release of large amounts of adrenaline and smaller amounts of noradrenaline. The proportion of adrenaline to noradrenaline did not appear to depend on the method of stimulation, nor did it alter after continuous electrical stimulation for 1 hour.

4. In the same period the adrenal gland was depleted of less than 50 per cent of its pressor amines, but of both adrenaline and noradrenaline to the same extent. In this it differs from rat adrenals, where only adrenaline was depleted appreciably.

REFERENCES

Bülbring, E., and Burn, J. H. (1949a). *Brit. J. Pharmacol.*, **4**, 202.
Bülbring, E., and Burn, J. H. (1949b). *Brit. J. Pharmacol.*, **4**, 245.
Burn, J. H., Hutcheon, D. E., and Parker, R. H. O. (1950). *Brit. J. Pharmacol.*, **5**, 417.
Cannon, W. B., McIver, M. A., and Bliss, S. W. (1924). *Amer. J. Physiol.*, **69**, 46.
Crawford, T. B. B., and Outschoorn, A. S. (1951). *Brit. J. Pharmacol.*, **6**, 8.
Edmunds, C. W., and Smith, R. G. (1932). *J. Lab. clin. Med.*, **17**, 399.
Elliott, T. R. (1912). *J. Physiol.*, **44**, 374.
Gaddum, J. H., and Lembeck, F. (1949). *Brit. J. Pharmacol.*, **4**, 40.
Gaddum, J. H., Peart, W. S., and Vogt, M. (1949). *J. Physiol.*, **108**, 467.
Meier, R., and Bein, H. J. (1948). *Experientia*, **4**, 358.
Mutch, N., and Pembrey, M. S. (1911). *J. Physiol.*, **43**, 109.
Vogt, M. (1947). *J. Physiol.*, **106**, 394.
West, G. B. (1949). *J. Physiol.*, **110**, 9*P*.
West, G. B. (1951). *Brit. J. Pharmacol.*, **6**, 289.