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ADRENALINE AND *NOR*ADRENALINE IN THE SUPRARENAL MEDULLA AFTER INSULIN

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When animals are injected with insulin in amounts sufficient to produce symptoms of hypoglycaemia, different mechanisms are set in motion to restore the normal blood sugar. One of these is discharge of adrenaline from the suprarenal medulla. Evidence supporting this was found by Burn (1923), who observed that ergotamine, which inhibits the hyperglycaemic action of adrenaline, greatly increased the hypoglycaemic action of insulin in the rabbit. Cannon, McIver, and Bliss (1924) injected insulin into cats from which the stellate ganglia had been previously removed to denervate the heart; they found that as the blood sugar fell the heart rate increased. The increase was not seen if the suprarenal glands were first removed. Burn and Marks (1925) showed that section of both splanchnic nerves in the cat increased the hypoglycaemic reaction to insulin. Poll (1925) found that the chromaffin reaction of adrenal tissues was much diminished by the injection of insulin in rats, mice, pigeons, and frogs. Gohar (1934) made similar observations using Folin's uric acid reagent. The doses of insulin were 10 units per rat given twice daily during 10–15 days; these were enormous doses. The adrenaline content was reduced in this way to 0.77 mg. per g. gland from 2.0 mg. per g. gland, which was the figure for control rats. Vogt (1947) also studied the effect of insulin injections on the suprarenal gland of the rat; she was mainly concerned with changes in the cortex. When insulin was injected, the dose being 0.12 unit/100 g., she found that "depletion of the adrenaline stores from the medulla was not yet visible, but it became obvious at 0.24 i.u. and was very conspicuous with all doses above this level." She observed the adrenaline by histological examination, fixing with Orth's fluid.

Evidence has already been obtained in this laboratory that *nor*adrenaline is a precursor of adrenaline in the suprarenal medulla. Bülbring (1949) has shown that minced suprarenal tissue from dogs and from cats, when incubated with adenosine triphosphate, converts *nor*adrenaline to adrenaline; she made the important observation that the conversion is most vigorous in tissue prepared from glands stimulated through the splanchnic nerve beforehand. Bülbring and Burn (1949b) have also obtained evidence of the conversion of *nor*adrenaline to adrenaline in the perfused suprarenal gland of the dog, by observing a disappearance of *nor*adrenaline and a corresponding increase in the amount of adrenaline.

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The present investigation was undertaken in the hope of observing that the proportion of adrenaline to *nor*adrenaline in the suprarenal medulla was less in the gland depleted by the injection of insulin. As the gland refilled its store, *nor*-adrenaline might accumulate at a rate greater than that at which it could be methylated. If this were so a lower proportion of adrenaline would be found than in glands from rats not injected with insulin.

METHODS

Wistar rats were used in groups of 5 to 11; they were mostly females from 100–150 g. In any experiment the different groups contained the same number of rats, the same proportion of males and females, and the same distribution of body weight. They were kept without food overnight and injections were given in the morning. The experiments were carried out between October and February. The dose of insulin injected (s.c.) was 0.2 unit per 100 g.; this was sufficient to produce symptoms of hypoglycaemia which in some experiments were severe. When symptoms were severe, injections of glucose were given not only to the affected rats but to all the rats.

At a given time after the injection of insulin the rats were killed by a blow on the head and bled out by cutting the throat. The suprarenals were removed, weighed, and the glands of one group were ground in a mortar in 6 ml. 0.1 N-HCl with a little sand. The contents of the mortar were then transferred to a centrifuge tube and N-NaOH was added to bring the reaction to *p*H 6. The tube was then immersed in a boiling water bath for 90 secs., taken out, cooled and centrifuged. The clear supernatant fluid was placed in another tube and adjusted to *p*H 4 with N-HCl. It was then kept in the cold until the test was carried out. Extracts of the glands from the non-injected rats were prepared at the same time.

The determination of the proportion of adrenaline and *nor*adrenaline in the extracts was made by the method we have recently described (Burn, Hutcheon, and Parker, 1950). This consists in determining in a spinal cat the effect of the extract in causing contraction of the nictitating membrane (not denervated) and a rise of blood pressure. The extract is compared with known mixtures of adrenaline and *nor*adrenaline, the ratio of the height of the contraction of the nictitating membrane to the rise of blood pressure being determined for the extract and for the different mixtures. In making these observations we have found it useful to ligate the suprarenal vessels of both glands, and also to cut the cervical sympathetic chain. Even in the spinal preparation there is sometimes central activity which affects the nictitating membrane.

The total activity in each extract was estimated by comparing its pressor action in the spinal cat with that of adrenaline. The pressor action of a given amount of adrenaline is usually slightly less than that of the same amount of *nor*adrenaline at the beginning of an experiment, but the difference disappears as the experiment continues, and the total activity can then be estimated in comparison with adrenaline without serious inaccuracy. In some experiments total activity was also determined in the isolated rabbit intestine.

RESULTS

Changes in total activity.—The changes in total activity in different experiments are shown in Table I. In Experiment 1, for example, there were three groups of rats, one not injected as the control group, a second group which was killed two hours after receiving insulin, and a third group which was killed four hours after receiving insulin. While the control figures in the different experiments were fairly close together, the mean figure being 0.92 mg. per g., the figures at a given interval

after insulin varied widely. Thus at six hours the lowest figure was 0.08 mg./g. and the highest was 0.84 mg./g. although the dose of insulin was the same in each experiment. The effect of insulin judged by symptoms of hypoglycaemia was, however, very different in different experiments although the conditions were similar.

TABLE I

CHANGE IN TOTAL PRESSOR ACTIVITY, ESTIMATED AS ADRENALINE (MG./G.), AFTER THE INJECTION OF 0.2 UNIT INSULIN/100 G.

Exp.	Control	Total activity (mg./g.) at various times after insulin					
		2 hr.	3 hr.	4 hr.	6 hr.	8 hr.	12 hr.
1	0.98	0.78		0.52			
2	1.01	0.69		0.57			
3	0.57		0.43				
4	1.07				0.84		
5	0.99				0.63		
6*	0.97				0.41		
7	0.60		0.25		0.08		
8*	1.0				0.48		
9	1.08					0.66	
10	0.79					0.38	
11	1.16					0.32	
12	0.74						0.55
13*	1.00						0.95
Mean:	0.92	0.73	0.34	0.55	0.49	0.44	0.75

*Sugar was injected in these experiments.

When the mean figures at the foot of the columns are examined, apart from the progressive fall in the first three hours, there is little sign of continuous change. If, however, each control figure is given the value 100, and the figures in the same experiment are expressed as a percentage, then, when the mean percentages are plotted, the curve (A) shown in Fig. 1 is obtained, which shows maximum depletion at 8 hours; at 12 hours recovery has already begun.

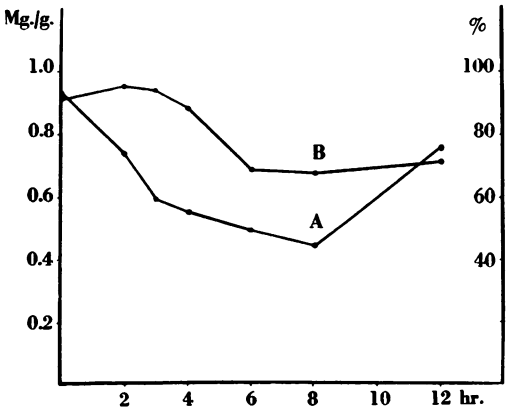

FIG. 1.—Curve A shows change in total activity (adrenaline + *nor*adrenaline) in rat suprarenals after insulin, 0.2 unit/100 g. Ordinate (on left) mg./g. Abscissa time in hours. Curve B shows change in adrenaline percentage (ordinate on right). Note that the adrenaline percentage does not begin to fall until 4 hr. whereas total activity falls at once.

Fall in adrenaline percentage.—In determining the proportion of adrenaline in the extract, the procedure followed is illustrated in Figs. 2 and 3. In Fig. 2, the first injection (*a*) was 5 μg. *l-nor*adrenaline ; the rise of blood pressure was accompanied by a small effect on the nictitating membrane. The last injection (*d*) was 5 μg. *l*-adrenaline ; the rise of blood pressure was the same, but the effect on the nictitating membrane was much greater. The second injection (*b*) was 0.4 ml. of extract prepared from animals injected with insulin six hours previously. The

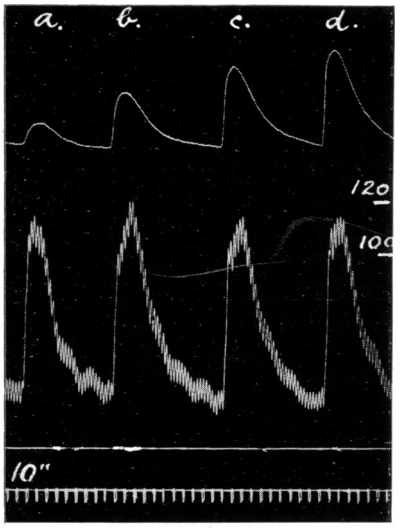

FIG. 2.—Spinal cat, normal nictitating membrane and blood pressure. (*a*) 5 μg. *l-nor*adrenaline; (*b*) 0.4 ml. extract of adrenal glands of rats given insulin 6 hr. previously; (*c*) 0.1 ml. extract of adrenal glands of control rats; (*d*) 5 μg. *l*-adrenaline. See text.

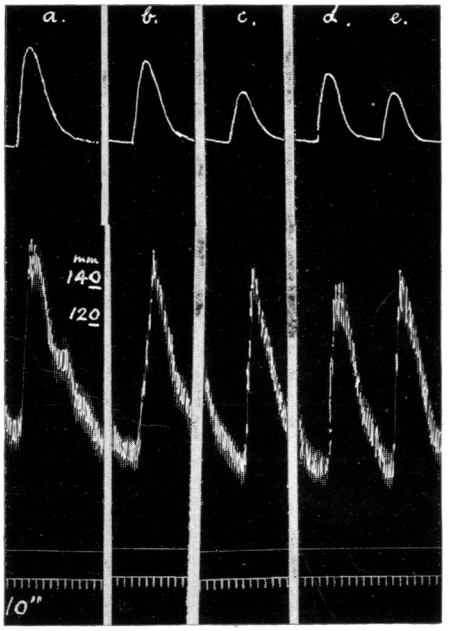

FIG. 3.—Records as in Fig. 2. (*a*) 10 μg. *l*-adrenaline; (*b*) 0.4 ml. extract (diluted 1 in 2) of glands of control rats; (*c*) 0.4 ml. extract of glands of rats given insulin 12 hr. previously; (*d*) a mixture containing 4 μg. *l*-adrenaline and 4 μg. *l-nor*-adrenaline; (*e*) a mixture containing 2 μg. *l*-adrenaline and 6 μg. *l-nor*-adrenaline.

third injection (*c*) was 0.1 ml. of control extract. Since the rise of blood pressure was similar in (*b*) and (*c*) the extract of glands depleted by insulin contained about one-quarter the total activity of the control extract. Since the contraction of the nictitating membrane was less for (*b*) than for (*c*) the proportion of adrenaline in the extract of depleted glands was less than the proportion in the control glands. Fig. 3, taken from an experiment in which insulin was injected 12 hours previously, shows at (*a*) the effect of 10 μg. adrenaline ; at (*b*) 0.2 ml. control extract, and at (*c*) 0.4 ml. of extract from depleted glands. Injection (*d*) was a mixture containing 4 μg. of adrenaline and 4 μg. of *nor*adrenaline, while (*e*) was a mixture containing 2 μg.

TABLE II

RATIOS OF MEMBRANE CONTRACTION TO RISE OF BLOOD PRESSURE × 1,000. EXP. OF DEC. 20, 1949

Adren.	75% adren. 25% *nor.*	50% adren. 50% *nor.*	Control extract	Extract of depleted glands
207	172	164	196	149
314		234	260	215
	284	219	328	273
334	250	197	303	212
			298	250
Mean 285	235	203	277	220

of adrenaline and 6 μg. of *nor*adrenaline. Inspection of Fig. 3 shows that the control extract contained between 50 and 100 per cent adrenaline, while the extract from depleted glands contained about 25 per cent adrenaline.

The procedure followed in arriving at the figures for the percentages of adrenaline in the extracts of control and depleted glands is illustrated by the following example. A series of 39 injections was given in which the rise of blood pressure and the contraction of the nictitating membrane were recorded, all volumes for injection being chosen so as to produce as nearly as possible the same rise of blood pressure. Each contraction of the nictitating membrane was measured and expressed as a ratio of the blood pressure rise. Thus 7.5 μg. adrenaline caused a 28 mm. contraction of the membrane and an 89 mm. rise of blood pressure. The ratio (× 1,000) was 314. In the course of the experiment the values for the ratio were those shown in Table II. The ratios in each horizontal line were obtained at the same stage of the experiment, and are therefore comparable. Each ratio for the control extract was appreciably greater than the corresponding ratio for the extract from the depleted glands. The actual percentages for the extracts were calculated by plotting the ratios for adrenaline and the known mixtures, and finding the values

TABLE III

PERCENTAGE OF ADRENALINE IN GLAND EXTRACTS AFTER INJECTION OF INSULIN 0.2 UNIT/100 G.

Exp.	Control	Percentage of adrenaline at various times after insulin					
		2 hr.	3 hr.	4 hr.	6 hr.	8 hr.	12 hr.
1	97	93		90			
2	93	95		85			
3	92		93				
4	99				88		
5	86				71		
6	88				67		
8	79				47		
9	96					62	
10	85					79	
11	84					60	
12	89						92
13	88						50
Mean:	90	94	93	88	68	67	71

for the extracts by interpolation. The control extract was thus found to contain 96 per cent adrenaline and 4 per cent *nor*adrenaline, while the extract of depleted glands contained 62 per cent adrenaline and 38 per cent *nor*adrenaline.

The results obtained in all the experiments are shown in Table III, where the figures indicate the percentage of adrenaline in the gland extracts. The fall in the adrenaline percentage begins to be evident after four hours, and is obvious in all experiments at six hours and eight hours. In one experiment at 12 hours there was no fall; in this the animals were very little affected by the injected insulin. In the other experiment at 12 hours the fall was still obvious; in this the animals showed severe symptoms and glucose was injected. Thus the results afford clear evidence that when the glands are depleted the proportion of activity due to *nor*-adrenaline is much greater than in control glands.

Discussion

The results show in the first place the change in the total amount of active material, adrenaline and *nor*adrenaline, present in the suprarenal medulla after the injection of insulin in sufficient amount to cause symptoms of hypoglycaemia. When insulin was injected in the dose of 0.2 unit/100 g. body weight of rat, the total fell, the mean fall reaching a minimum at eight hours, when the total activity was about 50 per cent of that originally present. After 12 hours the total activity was rising again. There is great variation in the response of rats to insulin on account of differences (*a*) in strain, (*b*) in diet, and (*c*) in season of the year. Consequently while the curve A in Fig. 1 represents the changes found between October and February in one laboratory, it may not have a general application to other rats injected with the same amount of insulin.

The results show in the second place the fall in the percentage of adrenaline present in the medulla. This fall is not parallel to the fall in total activity (see curve B, Fig. 1), and indeed it is only obvious six hours after giving insulin, although the fall in total activity is obvious two hours after insulin.

There are two explanations for the fall in the adrenaline percentage in the medulla. One is that adrenaline only is being discharged into the blood and not *nor*adrenaline. The other is that the work of replenishing the store of *nor*adrenaline in the medulla proceeds faster than the process of methylation. We think that the evidence is in favour of the second explanation, for if the first explanation were correct the curve showing the fall in adrenaline percentage ought to fall at least as quickly as the curve for the fall in total activity. There is, however, a period of more than three hours in which the progressive fall in total activity is not accompanied by a fall in adrenaline percentage.

We therefore consider that the present observations give further support to the evidence already referred to (Bülbring, 1949; Bülbring and Burn, 1949b) that *nor*-adrenaline is a precursor of adrenaline in the suprarenal medulla. Thus our experiments appear to us to demonstrate that when the suprarenal medulla is hard at work, pouring out its secretion for several hours because of hypoglycaemia, the process of methylation is rather slower than the processes by which fresh *nor*-adrenaline is accumulated.

The relative proportion of *nor*adrenaline and adrenaline which is released from the gland by nerve impulses (Bülbring and Burn, 1949a) probably depends on the

relative amounts present and there is no convincing evidence that one or other substance is released alone. Holtz and Schümann (1949) have claimed that when both carotid arteries are clamped in the cat, there is a reflex discharge of *nor*adrenaline only from the suprarenal glands. Their main evidence was the finding that after removing the suprarenals they no longer observed a contraction of the spleen on clamping the carotids. This evidence appears to us unsatisfactory. We believe that the contraction of the spleen observed when the carotids are clamped is due to impulses passing along the splenic nerves and not to a substance released from the suprarenal gland. The finding of Holtz and Schümann that the contraction did not occur after adrenalectomy can be explained by interference with the splenic nerves in carrying out adrenalectomy. Moreover, they give several illustrations that *nor*adrenaline caused inhibition of the intestine whereas clamping the carotid arteries did not.

Summary

1. When insulin is injected in sufficient amount, the activity (adrenaline and *nor*adrenaline) present in the suprarenal medulla declines. We have followed the course of the fall in rats, and have found that the fall is evident after two hours and progresses to a minimum at eight hours after which the amount of active material begins to increase.

2. After the injection of insulin there is also a fall in the percentage of adrenaline present in the gland. The fall is not parallel to the fall in total activity but is first seen after six hours and is still present at eight and twelve hours.

3. The evidence indicates that *nor*adrenaline is a precursor of adrenaline and that, after several hours' depletion of the suprarenal medulla, the supply of *nor*-adrenaline is restored more rapidly than it can be methylated to form adrenaline.

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