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THE PRESSOR AMINES IN THE ADRENAL MEDULLA AFTER IRRADIATION

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There is evidence that the administration of pyridoxine hydrochloride successfully prevents the "radiation sickness" which may appear a few hours after a therapeutic dose of irradiation (Manfield, McIlwain, and Robertson, 1943; Shorvon, 1949). Pyridoxine has also been reported to reduce the mortality of mice given whole body *x*-irradiation (Goldfeder, Cohen, Miller, and Singer, 1948). In seeking for an explanation of this action we considered the relation of pyridoxine to the formation of the pressor amines, *nor*adrenaline and adrenaline, which occur in the adrenal medulla. One of us (Blaschko, 1939) has put forward the view that the enzyme dopa-decarboxylase (dopa is dihydroxy-phenylalanine) discovered by Holtz, Heise, and Lüdtke (1938) plays a part in the formation of *nor*adrenaline. Blaschko, Carter, O'Brien, and Sloane Stanley (1948) have demonstrated that the enzyme is absent in the livers of rats fed on a diet deficient in pyridoxine, and Blaschko, Carter, and Sloane Stanley (unpublished) have shown that the enzyme can be reconstituted in extracts of such deficient livers by adding pyridoxal-5-phosphate *in vitro*.

If it is true that dopa-decarboxylase is an important enzyme in the process of *nor*adrenaline formation, then rats fed on a pyridoxine-deficient diet should have difficulty in maintaining a normal content of pressor amines in the adrenal medulla. Blaschko, Burn, and Carter (unpublished) have found that, when the adrenal medulla is depleted as a result of the injection of insulin, rats fed on a pyridoxine-deficient diet cannot replenish the store of pressor amines so effectively as controls given a supplement of pyridoxine. The inability to replenish the store is, however, only evident in those rats in which the amount of dopa-decarboxylase in the liver is too small to be detected by manometric observations.

The experiments to be described have therefore been directed to examining changes in the amount of dopa-decarboxylase in the livers of irradiated rats, and also changes in the amount of pressor amines in the adrenal medulla both before and after the injection of insulin.

METHODS

Irradiation of rats.—Albino rats from an inbred laboratory stock were irradiated singly or in pairs in an aluminium box. Treatment factors were 240 kv. 15 mamp. H.V.L. 1.14 mm. Cu., tube distance 70 cm., dose rate 42 r./min. The field was uniform to $\pm 3\%$. The rats weighed from 250 to 350 g. and were usually litter mates.

Estimation of dopa-decarboxylase.—The livers were at once frozen in an ice-cooled mortar at almost $-10°$ C. and ground up with a little sand; 0.067 M-sodium phosphate

buffer solution of *p*H 7.4 was then added, 1 ml. for each g. of liver. The resultant suspension was then centrifuged for 5 minutes to remove the sand and connective tissue, and the supernatant was used in the manometric experiments. These were set up as described by Sloane Stanley (1949); in some of our experiments, however, the supernatant was diluted with an equal volume of water in order to reduce the rate of the enzymic reaction and thus to ensure linearity for a longer period.

Estimation of pressor amines.—In the adrenal gland of the normal rat, Burn, Hutcheon, and Parker (1950) found that the amount of pressor amines was 0.92 mg. per g., this being the mean figure for 78 animals, and that 90 per cent was adrenaline, the remaining 10 per cent being *nor*adrenaline. We have followed their procedure in estimating the pressor amines in the irradiated rats. Each rat was killed by a blow on the head; its throat was cut, and it was bled out; the adrenals were dissected quickly from fat and connective tissue and were weighed. They were ground in a mortar with a few grains of sand and 1.0 ml. of 0.02 N-HCl for each 10 mg. gland. This extract was transferred to a centrifuge tube, which was then immersed for 1.5 minutes in a boiling water bath. The tube was cooled, centrifuged, and the supernatant fluid transferred to a sample tube which was fitted with a stopper and kept frozen at −15° C. until the estimation was carried out. The estimation was performed either on the blood pressure of the spinal cat or on an isolated loop of the duodenum of the rabbit (Burn, Finney, and Goodwin, 1950).

RESULTS

Rats killed after three days.—The irradiation given produced signs of its effect after 65–70 hours. The rats were first examined when these signs, disinclination of the rats to move, staring of the fur, were clearly seen. Usually two irradiated rats and two control rats were investigated in each experiment, and the content of pressor amines in the four extracts was determined side by side on the blood pressure of the same cat or on the same loop of rabbit intestine. The pressor amines were estimated in comparison with adrenaline and the amine content was expressed as adrenaline. Table I gives an example of the results of an experiment,

TABLE I

Exp. 4. Rats received 1,000 r. Killed 75 hr. after irradiation

Rat	Body wt. at death (g.)	Adrenal wt. (mg.)	Pressor amine μg. per 100 g. rat	Dopa-decarboxylase $\mu l.CO_2$/hr./mg. liver
Control	345	41.9	18.2	0.85
,, ...	275	39.0	18.4	0.86
Irradiated	260	58.5	8.3	0.26
,, ...	250	61.8	9.1	0.62

in which the rats were killed 75 hours after irradiation. Three points are evident; first, that the weight of the adrenal glands of the irradiated rats was nearly 50 per cent greater than that of the control rats; second, that the pressor amine in the adrenals, calculated, not per unit weight of the adrenals, but per 100 g. rat, was about one-half in the irradiated rats of what it was in the controls; third, that the mean figure for dopa-decarboxylase in the irradiated rats was also about half of what it was in the controls.

The other experiments in which these changes were studied are set out in Table II; all the irradiated rats received 1,000 r. and were killed at the times

TABLE II

Exp.	Control rats: Pressor amine μg./100 g. rat	Control rats: Dopa-decarboxylase μl.CO_2/hr./mg. liver	For hrs.	Irradiated rats: Pressor amine μg./100 g. rat	Irradiated rats: Dopa-decarboxylase μl.CO_2/hr./mg.
1	12.1 20.8		72 72	6.4 16.2	
2	15.3 13.4		73 73	14.5 9.8	
3	17.4 14.1	0.85 0.82	80 80	17.4 4.4	0.45 0.51
5	18.0 18.5	0.51 0.78	78 78	5 8 4.4	0.45 0.48
6	18.7 16 5	0.86 0.28	70 71 71.5	5.0 7.0 5.7	0 48 0.70 0.28
7	14.5 14.0	0 76 0 88	67.5 67.5	10.3 9.1	0.44 0.52
8	14.6	1.35	65.5 65.5	12 8 16.1	1.14 0.95
	18.4	0.65	71.6 71.6	7.8 14.2	0.49 0.50
9	— —	0.55 0.53	72 72	— —	0.48 0 33
Mean	16.4	0.75		9.7	0.53

stated, which varied from 65.5 to 80 hours. In each experiment the mean content of pressor amines in the adrenal medulla was less in the irradiated rats than in the control rats. In one experiment, not included in Tables I and II, when two recently dead rats and one moribund rat were examined, the pressor amine content was less than 5 per cent of the controls. These rats were irradiated 79 hours previously. The mean control figure for all experiments in Tables I and II was 16.4 μg. per 100 g. rat, and the mean figure for irradiated rats was 9.7, the standard errors of these means being ± 0.61 and ± 0.99 respectively. The difference between the means was therefore highly significant. The figures for dopa-decarboxylase in the liver were in every experiment higher in the control rats than in the irradiated rats except in one pair in Experiment 6 where they were the same. The mean figure for control rats was 0.75 and for irradiated rats 0.53, the respective standard errors being ± 0.076 and ± 0.054. The difference between the means was, therefore, highly significant.

Reactivation with pyridoxal-5-phosphate.—It was of interest to find whether the relatively low dopa-decarboxylase activity was due to a reduction in the amount of enzyme protein or of the prosthetic group. The dopa-decarboxylase activity of liver extract from an irradiated rat and its normal control was therefore determined

in the presence and absence of 10 μg. calcium pyridoxal-5-phosphate (synthetic codecarboxylase). This amount of pyridoxal phosphate, which we owe to the kindness of Dr. K. Folkers, restores the enzymic activity of moderately pyridoxal-deficient rats to a normal level (Blaschko, Carter, and Sloane Stanley, unpublished).

The result of one satisfactory experiment of this kind is set out in Table III, which shows that the activity of the extract from the control animal was slightly

TABLE III

Reactivation by added codecarboxylase; 10 μg. calcium-5-pyridoxal phosphate was added to each manometer flask

	Dopa-decarboxylase μl.CO_2/hr./mg. liver	
	Control	Irradiated
Without codecarboxylase	0.73	0.41
With codecarboxylase	0.80	0.78

raised by the addition of synthetic codecarboxylase. In the extract from the irradiated animal the activity in the absence of added codecarboxylase was low, but the addition of dopa decarboxylase raised the activity to a normal figure. We conclude that the low activity in the irradiated animal was not due to low content of enzyme protein, but to a lowering of the codecarboxylase content of the preparation.

Effect of adding pyridoxine to the diet.—The results at this point suggested that the effect of irradiation in causing a fall in the amount of pressor amines in the adrenal medulla might indeed be explained as a consequence of a fall in the amount of dopa-decarboxylase. However, although the irradiated rats had less decarboxylase, the mean fall was to about 70 per cent of the figure for the control rats, that is to say, it was not a fall to zero. The unpublished work of Blaschko, Burn, and Carter, already mentioned, showed that in rats deficient in the decarboxylase as a result of excluding pyridoxine from the diet, the power to restore the pressor amine content of the adrenals after depletion by insulin was significantly reduced only in those rats in which the deficiency was so extreme that no detectable decarboxylase remained.

If the fall in pressor amines after irradiation was due to the lack of dopa-decarboxylase, the administration of pyridoxine should prevent the fall. Experiments were therefore carried out in which pyridoxine was added to the diet two days before irradiation and for the three days after it. Each rat received 1 mg. pyridoxine per day. It was found that this addition of pyridoxine raised the figures for liver decarboxylase to 80 per cent of the figures for the control animals, but that the fall in the pressor amines in the adrenal medulla occurred just as before. The results are shown in Table IV. It was thus clear that the fall in pressor amines in the adrenal glands was not a consequence of the fall in the amount of dopa-decarboxylase in the liver recorded in Table II. The fall in the amount of this enzyme is perhaps related to a diminished absorption of pyridoxine from the intestine, and if this is so the effect of the diminished absorption is evident in as short a time as three days.

TABLE IV

EFFECT OF ADDED PYRIDOXINE

Each rat received 1 mg. pyridoxine daily for 5 days

Exp.	Rat	Body wt. (g.)	Adrenal wt. (mg.)	Time since irradiation	Pressor amine μg./100 g. rat	Dopa decarboxylase μl.CO_2/hr./mg. liver
9	Control	270	48	—	23.3	1.05
	,,	245	47	—	17.4	1.01
	Irradiated	230	60	72 hr.	5.9	0.80
	,,	195	58	75.5	5.7	0.78
10	Control	280	55	—	13.4	0.65
	Irradiated	350	77	58 hr.	10.3	0.56
	,,	250	52	72	8.3	0.53

Pressor amines after insulin.—We have finally made experiments to determine the time at which the adrenal medulla first fails to maintain its store of pressor amines. There is no change to be observed at 48 hours after irradiation as shown in Table V, but a change of another kind can be observed at this time if the rats are injected with insulin. The hypoglycaemia which results causes a liberation of pressor amines from the adrenal medulla, the liberation depending on the degree of hypoglycaemia.

TABLE V

Exp.	Rat	Body wt. (g.)	Adrenal wt. (mg.)	Time since irradiation	Pressor amine μg./100 g. rat
11	Control	295	38	—	14.1
	,, ...	290	41	—	14.1
	Irradiated	270	35	48	13.5
	,, ...	285	43	48	16.6

A recent study of this effect of insulin has been made by Burn, Hutcheon, and Parker (1950), who found that, when rats were kept without food overnight and were then given 0.2 unit insulin per 100 g. body weight by subcutaneous injection, the total amount of pressor amine in the adrenal medulla fell to 60 per cent of the original amount in four hours after the injection. Rats vary greatly in the degree of hypoglycaemia caused by a given amount of insulin, the variation depending on the time of year, on their diet, and other factors. We found that 48 hours after irradiation the injection of a given amount of insulin produced fewer symptoms in the irradiated rats than in control rats, and that in consequence the adrenals of the irradiated rats were less depleted of their content of pressor amines than those of the controls when examined four hours after the injection. An example is given in Table VI. In this experiment two control rats and two irradiated rats were kept without food overnight, and insulin in a dose of 0.2 unit per 100 g. rat was injected 44 hours after irradiation into all four rats. Two hours later a second injection of half this amount was given to all the rats, which after a further two hours were then killed. The control rats were much more affected by the insulin than the irradiated rats. One control rat had convulsions, and the other was collapsed. Neither irradiated rat showed symptoms. The amounts of pressor amine in the

TABLE VI

RATS INJECTED WITH INSULIN. KILLED 4 HR. LATER

Exp.	Rat	Body wt. (g.)	Adrenal wt. (mg.)	Time since irradiation	Pressor amine μg./100 g. rat
12	Control	270	37	—	4.1
	,, ...	290	43	—	5.9
	Irradiated	280	37	48	15.8
	,, ...	300	43	48	11.5

adrenal medulla were in accordance; thus the amounts were very low in the control rats, and were almost normal in the irradiated rats. Thus at 48 hours the irradiated rats were much more resistant to insulin than the control rats, although at this point there was no adrenal hypertrophy.

The results in a series of such experiments carried out at varying times after irradiation are given in Table VII. In each experiment one or two control rats were

TABLE VII

THE ADRENAL GLANDS OF IRRADIATED RATS INJECTED WITH INSULIN

Note that the figures for pressor amine in the third column indicate in Exps. 12–16 greater depletion in the controls, and in Exps. 17–18 greater depletion in irradiated rats

Exp.	Time after irradiation (hr.)	Pressor amine as % of control	Adrenal wt. as % of control
12	48	273	100
13	48	116	90
14	56	169	110
15	60	134	108
16	64	242	112
17	68	37	164
18	72	42	132

compared with two irradiated rats, and the irradiated rats were killed at the time after irradiation stated in the second column. The mean amount of pressor amine in the irradiated rats is stated as a percentage of that in the control rats, both groups having been injected with a given dose of insulin per 100 g. four hours previously. (If the control rats showed no symptoms two hours after the injection, a further injection of insulin was then given to all rats.) In the last column the weights of the adrenals of the irradiated rats are expressed as a percentage of the weights of those of the control rats. The method of drawing up Table VII can be seen from the figures for Experiment 12, which are given in detail in Table VI and in summary in Table VII. The latter Table shows that, up to 64 hours after irradiation, no deficiency in pressor amines in the adrenal gland was revealed by the injection of insulin. The relatively greater amounts of amine in the irradiated rats, taken together with the fewer symptoms of hypoglycaemia produced in them, probably indicates that the irradiated rats were less affected by insulin.

At 68 hours and 72 hours, however, the pressor amines in the irradiated rats were less than in the controls. This might indicate either a disappearance of insulin resistance or a failure of the synthetic activity of the adrenal medulla.

Change in adrenal weight and pressor amines.—The results in the different experiments for adrenal weight and pressor amine content have been grouped according to the time after irradiation at which the irradiated rats were killed. They are shown in Table VIII. The adrenal weights were calculated per 100 g. body weight, and the ratio of irradiated to control figures was calculated for each separate experiment. The same was done for the amounts of pressor amine. Each figure in Table VIII is the mean of all the ratios for the time interval concerned. It is seen

TABLE VIII

ADRENAL WEIGHT AND PRESSOR AMINE CONTENT OF IRRADIATED RATS AS PERCENTAGES OF THE CONTROLS

Interval between irradiation and death (hr.)	48	66–67	70–73	78–80
No. of rats: $\frac{\text{irradiated}}{\text{control}}$	$\frac{2}{2}$	$\frac{4}{3}$	$\frac{9}{7}$	$\frac{4}{4}$
Adrenal wt./100 g. rat as % of control ..	104	133	146	193
Pressor amine/100 g. rat as % af control ..	107	84	62	49

that adrenal hypertrophy in the irradiated rats is not appreciable at 48 hours, but thereafter rises progressively up to 80 hours, when the glands are twice as big as the controls. The same trend is also visible in Table VII. The pressor amine content, expressed in relation to body weight and not in relation to adrenal weight, on the other hand steadily falls to about half the control value in the same time.

Adrenal hypertrophy caused by hexoestrol.—In order to discover whether agents other than irradiation which lead to adrenal hypertrophy also cause the content of pressor amines to diminish, we followed the procedure described by Vogt (1945), implanting tablets of hexoestrol into rats. We used tablets of 250 mg. each, and performed two experiments each on eight rats, four rats receiving a tablet and four being kept as controls. The weights (90–120 g.) and sexes of the rats were similar in the control group and in the group implanted with hexoestrol tablets at the beginning of the experiment. The implanted rats gained much less weight during the experiment; thus, while the mean control weight rose from 95 g. to 128 g. in one of the two experiments, the mean weight of implanted rats rose from 101 g. to 106 g. All rats were killed 12 days after implantation. The results are given in Table IX. They show that there was no appreciable difference in the mean total amount of pressor amine in the adrenals of the control and of the implanted rats, although the adrenal glands of the implanted rats were 143 per cent of the weight of the controls. This result for the increase of adrenal weight agrees very closely with that of Vogt (1945).

Discussion

Changes in weight and cholesterol content of the adrenal gland of the rat after irradiation have been determined by Patt, Swift, Tyree, and John (1947). The weight changes they recorded after 900 r. were similar to those recorded here after 1,000 r.; that is to say there was no change after 48 hours, and a substantial increase

TABLE IX

EFFECT OF HEXOESTROL ON ADRENAL WEIGHT AND ON PRESSOR AMINE CONTENT OF ADRENALS

Sex	Control		Implanted	
	Pressor amine per rat (μg.)	Adrenal wt. (mg.)	Pressor amine per rat (μg.)	Adrenal wt. (mg.)
Female	47.5	41.0	42.5	132.0
,, ...	50.0	48.0	62.5	50.0
,, ...	33.2	36.0	36.2	33.0
,, ...	37.8	35.0	34.9	45.0
,, ...	38.5	51.0	33.3	63.0
,, ...	36.0	48.0	30.5	47.0
Male	60.0	30.0	37.5	38.0
,, ...	47.5	27.0	57.5	46.0
Mean ..	43.8	39.5	41.9	57.0

at 72 hours after irradiation. The cholesterol content altered in the same way as the pressor amine content; at 48 hours both were normal, but by 72 hours both had decreased considerably. Whether there is any relation between cholesterol content and the pressor amine content it is from present knowledge impossible to say.

The increase in adrenal weight and depletion of cholesterol are signs of increased cortical activity. This should also lead to an increased deposition of liver glycogen, as was found by North and Nims (1949) in irradiated rats. Thus increased cortical activity provides one explanation of the increased insulin resistance of irradiated rats, since administration of cortisone or A.C.T.H. produces hyperglycaemia, and an apparent increased resistance to insulin (Conn, Louis, and Johnston, 1949). Liver glycogen is increased as early as 24 hours after irradiation (North and Nims, 1949), and the present work shows that insulin resistance, as judged by symptoms and the pressor amine content of the adrenals, is also present before hypertrophy of the adrenals has begun to occur. However, it is also possible that the early increase in liver glycogen is not a consequence of increased cortical activity, and that the increased insulin resistance is simply due to the increased glycogen store, whatever its cause may be. Although the increase in liver glycogen may not occur in adrenalectomized animals, the adrenal glands may not be directly responsible for it.

Patt, Swift, Tyree, and Straube (1948) found that hypophysectomy reduced the changes in adrenal weight and cholesterol content which followed *x*-irradiation of rats. The polyuria and polydipsia reported by Nims (1950) also suggest an alteration in pituitary function caused by whole-body irradiation.

This evidence is most easily interpreted by assuming that whole-body irradiation is merely one form of "stress" and that the changes in the adrenal are those characteristic of non-specific stress and mediated, at least in part, by the pituitary gland. There is as yet, however, no information on the effects of other forms of stress on the pressor amine content of the adrenals, and other evidence on the part played by the adrenals in the syndrome of whole-body irradiation is contradictory. Adrenalectomy has been stated (1) to have no effect (Straube, Patt, Tyree, and Smith, 1949) and (2) to make mice more sensitive (Chapman and Cronkite, 1950). Similarly, treatment by adrenal extracts has been said (1) to reduce mortality

(Ellinger, 1947; Graham, Graham, and Graffeo, 1950), and (2) to have no effect (Straube, Patt, Tyree, and Smith, 1949; Smith, Smith, and Thompson, 1950).

The cause of death after single doses of radiation is still obscure. Anaemia is usually insufficient in degree to be the immediate cause and infection is often not demonstrable. On the evidence given here it is not possible to say whether the deficiency in medullary pressor amines is a real contributory cause of death, or whether the deficiency is merely one consequence among many of a metabolic failure which is followed by death for some other reason.

SUMMARY

1. The content of pressor amines (*nor*adrenaline and adrenaline) of the adrenal gland of rats is progressively reduced after a lethal dose of *x*-irradiation of the whole rat. The reduction begins after 48 hours, and by 78–80 hours the content is about 50 per cent of normal.

2. The dopa-decarboxylase activity of liver extracts from irradiated rats is also reduced to some extent. Normal enzymic activity can, however, be restored *in vitro* by the addition of pyridoxal phosphate to the liver extract.

3. The fall in dopa-decarboxylase in the liver after irradiation is lessened by adding pyridoxine to the diet, but the fall in pressor amine is unchanged.

4. When the irradiated rats are injected with insulin from 48 hours onwards, they show fewer symptoms of hypoglycaemia than the controls, and their adrenal glands are less depleted of pressor amine as a result of the action of insulin than are those of the controls. This is true for the period 48 to 64 hours after irradiation. At 68 and 72 hours after irradiation the position is reversed, and the glands of the irradiated rats are more depleted by insulin than the glands of the controls.

5. After irradiation there is a progressive hypertrophy of adrenal glands beginning about 48 hours. This hypertrophy progresses as the pressor amine content declines.

6. When tablets of hexoestrol are implanted into rats, the adrenal glands hypertrophy as was shown by Vogt. This hypertrophy is not accompanied by any change in the pressor amine content.

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