FURTHER STUDIES ON THE FORMATION OF ADRENALINE AND NORADRENALINE IN THE BODY

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The exact method by which the body produces adrenaline is still not certain, although the last step from noradrenaline appears to be conclusive. It is now known, for example, that embryonic tissue in the suprarenal glands of man, cat, rabbit, guinea-pig and dog contains a high proportion of noradrenaline, whereas adult glands of these species possess relatively much more adrenaline[1]. In Blaschko's scheme[2] for the biosynthesis of adrenaline, dihydroxyphenylalanine and hydroxytyramine were considered to be the immediate precursors of noradrenaline. These two amines, however, were not detected in embryonic extracts[1] or in extracts of tumours of the adrenal medulla of man[3], both of which contain predominantly noradrenaline. They were also absent from extracts of organs of Zuckerkandl of children aged less than 8 days[4], and from extracts of the retroperitoneal tissue of many young mammals[5], both of which contain only noradrenaline. Fair quantities of hydroxytyramine have been found, however, in suprarenal medullary extracts of sheep and ox[3].

In 1939, Blaschko[6] put forward the view that *l*-dihydroxyphenylalanine decarboxylase might be responsible for the decarboxylation of dihydroxyphenylalanine as a step in the formation of adrenaline. When rats are fed on a diet deficient in pyridoxine, the amount of this enzyme in the liver is greatly diminished[7]; such rats are unable to replenish the store of pressor amines in the adrenal medulla when this is depleted by hypoglycæmia following the injection of insulin[8]. Thus dihydroxyphenylalanine decarboxylase may be a link in the normal mechanism of nonadrenaline formation, and Langemann[9] found this enzyme in the adrenal medulla of the ox. We have now given rats a diet deficient in pyridoxine (by removing pyridoxine or adding desoxypyridoxine or using both procedures) so that precursors of noradrenaline might be detected in tissues where the activity of dihydroxyphenylalanine decarboxylase is normally high (e.g. liver and kidney). We have also included the results of the administration of ethionine to animals, as this substance inhibits the action of methionine by substrate competition[10], with the result that methylation in the suprarenal gland might be reduced, and other precursors might be detected.

Methods

Daily intraperitoneal doses of desoxypyridoxine (1 to 4 per cent. aqueous solutions) were given for 3 weeks to a group of 6 albino mice (initial weights 20 to 25 g.) and to groups of 6 young and 6 adult albino male rats. They were then killed and their suprarenal glands, livers and kidneys removed and extracted with 0·01N hydrochloric acid. Another 12 young

albino male rats (initial weights 50 to 70 g.) received desoxypyridoxine (0·06 per cent.) in the drinking water for 5 weeks whilst on a normal diet of stock rat cubes. At the end of this period, 6 were killed and extracts made as above, whilst the other 6 received a subcutaneous injection of insulin (0·2 I.U./100 g.) 18 hours before being sacrified. 2 rabbits also received desoxypyridoxine in the drinking water for 3 weeks; 1 was then killed but the other received a subcutaneous injection of insulin (2 I.U./kg.) 6 hours before being killed.

3 further groups of 12 young albino male rats were fed for 6 weeks on a diet deficient in pyridoxine[7]. One of these groups also received daily doses of 0·1 mg. of pyridoxine per rat and served as controls. Another of these groups also received daily doses of 1 mg. of desoxypyridoxine per rat. 6 animals in each group were then killed; the other 6 received a subcutaneous injection of insulin (0·2 I.U./100 g.) 18 hours before being sacrificed.

Another 3 groups of 12 albino mice and 12 young and 12 adult albino male rats received daily intraperitoneal doses of a 1 per cent. solution of ethionine (50 mg./kg.) for 3 weeks before being killed. 6 rats in each group received a subcutaneous injection of insulin (0·2 I.U./100 g.) 18 hours before being killed. A further group of 6 young albino male rats received dibenamine (0·1 per cent.) in the drinking water for 5 weeks and were then killed.

All extracts of the suprarenal glands, kidneys or livers were made in 0·01N hydrochloric acid (0·5 g./ml.) and tested chromatographically and biologically (*see* Shepherd and West[1]). Concentration of extracts of kidneys and livers was effected by adding 4 volumes of ethanol, centrifugation, evaporation to dryness, and elution in a small volume of 0·001N hydrochloric acid.

The standards used were solutions of *l*-adrenaline, *l*-noradrenaline bitartrate, hydroxytyramine hydrochloride, *dl*-dihydroxyphenylalanine, tyramine hydrochloride, *p*-hydroxyphenylethanolamine, and *m*-hydroxyphenylethanolamine in 0·01N hydrochloric acid. All chromatograms were run in a solvent of butanol-acetic acid-water[1]; after drying, the first 4 substances were developed with a solution of 1 per cent. potassium iodate, whilst the other three substances were developed with the *p*-nitraniline reagent[11].

Results

Mice. Daily intraperitoneal injections of desoxypyridoxine (50 mg./kg.) did not produce any significant change in the concentrations of adrenaline and noradrenaline found in the suprarenal glands (Table I). Methylation was inhibited, however, in the experiments where daily injections of ethionine (50 mg./kg.) were continued for 3 weeks, the relative noradrenaline amount rising from 25 per cent. in the control mice (22 determinations) to 45 per cent. in the ethionine-treated animals. No hydroxytyramine or dihydroxyphenylalanine was detected in any of these extracts. Extracts of the livers and kidneys of these animals contained no detectable amounts of any of the seven substances listed above.

Rabbits. The oral administration of desoxypyridoxine (approximately 5 mg./kg.) slightly raised the adrenaline concentrations of the suprarenal gland. The treated gland gave a value of 610 μg./g., compared with a control value of 485 μg./g. (46 determinations). Following the insulin treatment, the treated gland gave a value of 425 μg./g., indicating that recovery of activity was not impeded. Noradrenaline and other precursors of adrenaline were not detected in any of these extracts.

TABLE I

THE EFFECTS OF DAILY INTRAPERITONEAL INJECTIONS OF DESOXYPYRIDOXINE (50 MG./KG.) AND OF ETHIONINE (50 MG./KG.) ON THE AMINE CONTENT OF THE SUPRARENAL GLANDS OF GROUPS OF 6 MICE

TIME OF TREATMENT—3 WEEKS

Treatment	Amine (μg./g.)		Percentage noradrenaline in the mixture
	Adrenaline	Noradrenaline	
Desoxypyridoxine ..	800	300	27
Ethionine	600	500	45
Saline	750	250	25

Rats. Daily intraperitoneal injection of desoxypyridoxine (50 mg./kg.) significantly increased the relative and absolute amounts of noradrenaline present in the suprarenal glands of young rats above that found in control animals receiving saline solution. The effect was less marked in adults, although the total amine content was increased (Table II). The oral

TABLE II

THE EFFECTS OF VARIOUS TREATMENTS ON THE AMINE CONTENT OF THE SUPRARENAL GLANDS OF GROUPS OF 6 RATS

Age of rat	Treatment			Amine (μg./g.)		Noradrenaline in Total per cent.
	Drug	Route	Time, weeks	Adrenaline	Noradrenaline	
Young	Saline	Intraperitoneal	3	900	100	10
Adult			3	1000	100	9
Young	Desoxypyridoxine	Intraperitoneal	3	1000	300	23
Adult			3	1600	300	16
Young	Desoxypyridoxine (insulin)	Oral	5	950	200	18
			5	1200	300	20
Young	Pyridoxine deficiency (insulin)	Oral	6	1050	150	12
			6	420	100	19
Young	Pyridoxine deficiency + Pyridoxine (insulin)	Oral	6	990	100	9
			6	250	20	7
Young	Full diet (insulin)	Oral	6	990	150	13
			6	810	100	11
Young	Ethionine (insulin)	Intraperitoneal	3	670	250	27
			3	570	190	25
Adult	Ethionine (insulin)	Intraperitoneal	3	1200	300	20
			3	1000	200	17
Young	Dibenamine	Oral	5	1200	300	20

administration of desoxypyridoxine (0·1 mg./kg.) also raised the relative noradrenaline value of the suprarenal glands of young animals. Subsequent treatment with insulin gave a similar result and indicated that the recovery process in the gland was not affected.

When young rats were fed on a diet deficient in pyridoxine (with or without desoxypyridoxine), no deficiency of the suprarenal gland was noted as compared with pair-fed rats which received pyridoxine. Following insulin treatment, however, an impairment in the power to restore the amine content of the exhausted gland was clearly demonstrated (Table II and Fig. 1). The combination of pyridoxine deficiency and desoxypyridoxine produced a greater effect (total amines—270 μg./g.) than pyridoxine deficiency alone (520 μg./g.) The relative noradrenaline amount in the latter group was raised above that found in the control group, but that in the former was not. Daily intraperitoneal injections of ethionine (50 mg./kg.) also significantly increased the relative and absolute amounts of noradrenaline in the suprarenal glands of both young and old animals, but subsequent treatment with insulin did not produce any further change. The oral administration of dibenamine for 5 weeks significantly increased the relative and absolute amounts of noradrenaline present in the suprarenal glands of young rats.

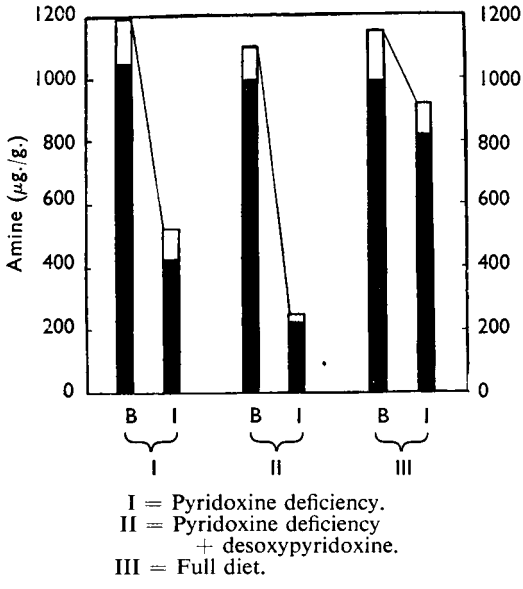

Fig. 1. The effects of pyridoxine deficiency (for 6 weeks) on the amine content of the suprarenal glands of groups of 6 young rats before (B) and after (I) insulin treatment. Shaded area, adrenaline; plain area, noradrenaline.

In all the above extracts of suprarenal glands and in all liver and kidney extracts, hydroxytyramine, dihydroxyphenylalanine, tyramine, and *p*- and *m*-hydroxyphenylethanolamines were absent. Adrenaline or noradrenaline were not detected in the liver and kidney extracts using the chromatographic and biological methods.

Discussion

The results show that treatment of rats with desoxypyridoxine to produce a deficiency of pyridoxine (with loss of hair) does not allow dihydroxyphenylalanine to accumulate in the suprarenal glands, kidneys or livers. There is no deficiency of the suprarenal gland in pressor amines as compared with pair-fed rats which received pyridoxine, but when such animals

are then given insulin, the normal production of both adrenaline and noradrenaline is reduced, and there is impairment of the power to restore the amine content in the exhausted gland. A similar result was also seen when rats were fed on a diet to which 1 per cent. of succinylsulphathiazole had been added to produce pyridoxine deficiency[8]. Further, if *l*-dihydroxyphenylalanine decarboxylase is part of the normal mechanism by which noradrenaline is formed and its activity is reduced, then dihydroxyphenylalanine might be expected to accumulate in tissues where the enzyme activity is normally high. Since it was not detected in these cases the question is again raised—"Is decarboxylation the reaction by which noradrenaline normally arises in the animal body?" Of the possible immediate precursors of noradrenaline, 3 may be noradrenaline-carboxylic acid, hydroxytyramine, and *p*-norsynephrine (*p*-hydroxyphenylethanolamine). The first substance is only slowly decarboxylated by enzymes and had not been found naturally[12]. The second has only been found in tissues of a few species[3]. The third has been found in the octopus[13], and can form noradrenaline under ultra-violet irradiation[14]; nevertheless, it has not been found in vertebrates. Further, adrenaline-carboxylic acid is not decarboxylated by enzymes[12], so that this method of formation of adrenaline is unlikely.

Evidence in favour of Blaschko's scheme was presented by Arman,[15] who found that if the suprarenal glands of rats were exhausted by insulin then only dihydroxyphenylalanine could replenish at once the catechol amines. It is possible that two processes may be taking place in the body concurrently—(1) Blaschko's scheme involving dihydroxyphenylalanine, hydroxytyramine, noradrenaline and adrenaline, and (2) that requiring tyrosine, tyramine, *p*-norsynephrine, noradrenaline, and adrenaline. Earlier experiments using thiouracil, alloxan and nicotine[16] failed to influence the normal production of these pressor amines and so far none of these precursors of noradrenaline have been detected: all possess feeble biological activity and are therefore only detected by the chromatographic method.

Ethionine reduces the amount of noradrenaline methylated in the suprarenal gland, but does not allow precursors to be detected. When ethionine-treated animals are exhausted by insulin, the normal mechanism by which noradrenaline is produced is unaffected.

Summary

1. Pyridoxine deficiency producing a deficiency of *l*-dihydroxyphenylalanine decarboxylase, or the administration of ethionine, do not result in precursors of noradrenaline and adrenaline being detected in the suprarenal glands, livers or kidneys of albino rats or mice.

2. It is possible that the steps leading to the production of noradrenaline in the body are complex, and even the decarboxylation of dihydroxyphenylalanine may be of little significance.

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