CCXXIX. ELIMINATION OF AMINES IN MAN

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THE amine oxidase of mammalian tissues has been shown to oxidize adrenaline and a number of other amines to the corresponding aldehydes [Richter, 1937; Blaschko *et al.* 1937]. Of the 67 amines hitherto tested those of the general type $R \cdot CH_2NH_2$ were readily oxidized, but amines of the type $RR'CH \cdot NH_2$ showed no appreciable oxidation. The latter group includes the amines of the ephedrine series which are of particular interest owing to their selective action on certain parts of the nervous system and experiments were therefore carried out to determine what happens to them in the animal body and how they are eliminated.

Indications have been recorded in the literature that the elimination of amines may in some cases take a different course in laboratory animals from that followed in man. Slotta & Müller [1936] found for example that mescaline is oxidized in dogs and rabbits to the corresponding acid which they isolated from the urine, but they were unable to detect this acid in the urine in man.

In the present experiments a series of amines were given by mouth or intravenously to male adults of 60–75 kg. and believed to be normal. In agreement with the previous work with the isolated amine oxidase, amines of the type $R \cdot CH_2NH_2$ were rapidly destroyed *in vivo* while amines of the ephedrine series were found to be excreted in the urine unchanged.

With benzedrine and methylisomyn the non-toxic dose is of the order of 10 or 20 mg., and since the excretion extended over 2 days the resulting concentrations in the urine were too small to be estimated by the ordinary methods available; a sensitive micro-method for estimating amines was therefore worked out in order to follow the elimination quantitatively.

Micro-estimation of amines

The method depends on the fact that picric acid gives little colour while amine picrates are strongly coloured in 50% chloroform-toluene or chloroform-light petroleum solution: it is generally applicable to the higher amines and alkaloids which are extracted from aqueous solution by organic solvents [Richter, 1938]. 60 ml. of urine in a 250 ml. separating funnel were made alkaline with 4 ml. 2N NaOH and shaken with 6 ml. toluene, using a rotating motion to avoid froth formation. The toluene layer was run into a centrifuge cup, centrifuged for 5 min., cleared by stirring with a glass rod and centrifuged again. 0.1 ml. of 2% picric acid in chloroform was added to 3 ml. of the toluene solution and 3 ml. of chloroform in a dry test tube and the mixture was kept for 12 hr. The clear yellow solution of the amine picrate was then compared colorimetrically with solutions prepared in a similar manner from standard amine solutions.

By this method concentrations as low as $0.5 \mu\text{g./ml.}$ of β -phenylethylamine could easily be estimated. The urines were collected in bottles containing toluene to prevent amine formation by bacterial action. Normal urines were generally found to contain traces of amines coming apparently from the food (Lintzel [1934] observed an increased excretion of amines after feeding fish); but by giving a diet consisting chiefly of bread and butter the normal amine content of

the urine could be reduced to a value corresponding to 0.05 $\mu\text{g.}/\text{ml.}$ of phenylethylamine. Of the basic substances commonly present in urine which might be expected to interfere ammonia, methylamine, trimethylamine, trimethylamine oxide, indole and skatole at concentrations of 20 $\mu\text{g.}/\text{ml.}$ gave less colour than 0.5 $\mu\text{g.}/\text{ml.}$ of β -phenylethylamine and could be neglected. Bain [1914] claimed to have isolated *iso*amylamine from urine and that, if present, would interfere; but Guggenheim & Löffler [1915] were unable to confirm the presence of *iso*-amylamine in normal urine and concluded that it is formed only by subsequent bacterial action on standing. No interference by *iso*amylamine was observed under the conditions of the present experiments.

Ephedrine. $\text{C}_6\text{H}_5\text{CHOH}.\text{CH}(\text{CH}_3)\text{NH}(\text{CH}_3)$

(a) *Identification.* Urine was collected for 12 hr. after feeding ephedrine hydrochloride (104 mg. base). The urine was made alkaline with NaOH, extracted with ether and the ethereal extract shaken with a small volume of *N*/10 HCl. On evaporation and recrystallization of the residue a hydrochloride was obtained which crystallized in colourless prisms showing parallel extinction and strong interference in polarized light, crystal edge angle 130° and M.P. $215\text{--}217^\circ$. Ephedrine hydrochloride crystals showed similar optical properties, crystal edge angle 131° and M.P. $217\text{--}219^\circ$. The mixture melted at $216\text{--}218^\circ$.

(b) *Rate of excretion.* Urine was collected every 3 to 4 hr. before and after giving ephedrine hydrochloride by mouth and the ephedrine in the urine was estimated by the micro-method described. The rate of excretion was maximal

Fig. 1. Excretion of ephedrine in the urine. I, 26 mg. base; II, 52 mg. base; III, 104 mg. base. Given as the hydrochloride by mouth.

(115 $\mu\text{g.}/\text{ml.}$) at about 4 hr. after administration, but with a large dose of 104 mg. a slow excretion at the rate of 0.25 mg./hr. could still be detected after 36 hr. The figures obtained for the fractions excreted at different times after administration were as follows:

Ephedrine (mg. base)	Time after administration		
	12 hr.	24 hr.	48 hr.
	%	%	%
26	77	97	97
52	59	92	99
104	59	77	82

These figures show that ephedrine is not appreciably oxidized in the body, but approximately 100 % may be excreted unchanged in the urine. The lower value of 82 % excretion obtained with 104 mg. ephedrine suggests that other methods of elimination, as in the sweat or faeces, may become significant when large amounts are given.

Benzedrine. $C_6H_5CH_2CH(CH_3)NH_2$

(a) *Identification.* Urine collected for 12 hr. after giving benzedrine sulphate (14.7 mg. base) by mouth was made alkaline with NaOH and extracted with toluene. The toluene solution was shaken with 10 ml. 1 % HCl and the aqueous solution evaporated to dryness. The residue was transferred to a Conway dish and the picrate, picrolonate and 2 : 4-dinitronaphthol derivative were prepared by allowing the base to volatilize in the presence of traces of the reagents on microscope coverslips [Richter, 1937]. The picrate crystallized in yellow prisms showing parallel extinction and strong interference colours in polarized light and crystal edge angles 107, 124 and 134°. Benzedrine picrate crystallized in similar habit and gave similar optical properties and crystal edge angles 106, 124 and 133°. The picrolonates and dinitronaphthol derivatives also agreed in crystal habit and optical properties: the pleochroism of the picrolonates was very characteristic.

(b) *Rate of excretion.* 20 mg. benzedrine sulphate (14.7 mg. base) were given by mouth and the urinary amines were estimated before and after administration, using light petroleum for the extraction. The rate of excretion was slower than for ephedrine and it was confirmed in a number of experiments that benzedrine could still be detected in the urine for 36 hr. after administration. With 14.7 mg. base the fraction excreted in the urine in 24 hr. was 40 % and in 48 hr. 70 %.

Fig. 2. Excretion of benzedrine (14.7 mg. base).

These figures make it unlikely that benzedrine is oxidized to any appreciable extent in the tissues. If the blood volume is taken as 7 l. the concentration in the blood for this dose would be at the most $2.1 \mu\text{g./ml.}$ and is probably much lower than this since the base is lipoid-soluble and is known to enter the nervous system. The concentration of benzedrine found in the urine was $10\text{--}13 \mu\text{g./ml.}$ a few hours after administration so that the kidneys must effect considerable concentration of the amine.

Methylisomyn. $C_6H_5CH_2CH(CH_3)NH(CH_3)$

Methylisomyn hydrochloride (8 mg. base) was given by mouth. As for benzedrine the rate of excretion was slow, being significant at 48 hr. after administration, and the recovery in the urine was 36 % in 24 hr. and 56 % in

48 hr. The maximal concentration found in the urine was $5\text{ }\mu\text{g./ml.}$ which again shows a considerable concentration by the kidneys such as was also found with ephedrine and benzedrine.

Fig. 3. Excretion of methylisomyn (8 mg. base).

Amines of the type $R\cdot CH_2NH_2$

The following amines were given by mouth in the form of the hydrochlorides: *isoamylamine* (100 mg. base), *benzylamine* (160 mg. base), β -hydroxy- β -phenylethylamine (50 mg. base) and β -phenylethylamine (300 mg. base). Although the experimental method was sufficiently sensitive to detect $1\text{ }\mu\text{g./ml.}$ of amine no increased amine excretion was observed after administration.

Attempts were then made to show the rate of elimination of β -phenylethylamine by looking for the corresponding phenylacetic acid in the urine: Guggenheim & Löffler [1915] showed that phenylacetic acid is formed from β -phenylethylamine in the rabbit.

Identification of phenylacetic acid. Urine voided after feeding β -phenylethylamine hydrochloride (300 mg. base) was heated for 3 hr. at 100° with 0.1 vol. of conc. H_2SO_4 to decompose phenylacetylglutamine [Thierfelder & Sherwin, 1914]. The acids obtained by extracting with toluene were separated by fractional crystallization and consisted mainly of benzoic acid and an acid which crystallized from water in triangular or hexagonal plates giving crystal edge angle 128° and having a characteristic smell. Phenylacetic acid crystallized in similar habit and gave the edge angle 128° and the same smell.

Fig. 4. Excretion of phenylacetic acid after taking β -phenylethylamine (300 mg. base) by mouth.

Rate of excretion of phenylacetic acid. The normal excretion of benzoic acid was reduced to a minimum by keeping on a diet free from fruit and vegetables.

Urine specimens collected before and after feeding β -phenylethylamine hydrochloride (300 mg. base) were heated for 3 hr. at 100° with 0.1 vol. conc. H_2SO_4 . 100 ml. samples of the urine were then shaken with 10 ml. of toluene and the toluene-soluble acids were titrated with $N/10$ NaOH using thymol blue. A partition curve was then prepared by treating standard solutions of phenylacetic acid in a similar manner and so the concentration of toluene-soluble acid in the urine, taken as phenylacetic acid, was estimated. No information is available as to the rate of conjugation of phenylacetic acid with glutamine to form the phenylacetylglutamine which is excreted, but in the present experiments the increased excretion of toluene-soluble acid corresponded to 62% of the theoretical for 300 mg. β -phenylethylamine in $4\frac{1}{2}$ hr.: the oxidation of the amine must therefore be comparatively rapid.

Mescaline. $(\text{OCH}_3)_3\text{C}_6\text{H}_2\text{CH}_2\text{CH}_2\text{NH}_2$

(a) *Identification.* Mescaline hydrochloride (191 mg. base) was given intravenously and the urine was made alkaline and extracted with toluene. The toluene solution was concentrated and treated with a solution of picric acid in chloroform. The picrate which crystallized out on standing formed diamond-shaped prisms giving an extinction angle of 10° in polarized light, crystal edge angle 141° and m.p. $221\text{--}223^\circ$. Mescaline picrate crystallized in similar habit and gave similar optical properties, extinction angle 10° , edge angle 142° and m.p. $221\text{--}223^\circ$. The mixture showed no depression of m.p. Mescaline was also identified in the same way in the urine after giving by mouth.

(b) *Rate of excretion.* When mescaline hydrochloride (191 mg. base) was given intravenously the fractional excretion in the urine was 52% in 24 hr. and with the same dose given by mouth 58% in 24 hr. The main bulk of the mescaline

Fig. 5. Excretion of mescaline after taking 191 mg. base as the hydrochloride by mouth.

is clearly excreted unchanged, but the amount found in the urine falls considerably short of 100% which suggests that the body may have a certain limited capacity for oxidizing mescaline or eliminating it in some other way.

DISCUSSION

The work of Ewins & Laidlaw [1910] and Guggenheim & Löffler [1915] led to the general conclusion that amines are rapidly eliminated by oxidation in the animal body. On the other hand there is pharmacological evidence that amines may in some cases remain active in the body for a considerable length of time. The action of ephedrine for example is known to continue for many hours and the effects of benzedrine have been observed to persist for more than a day [Guttmann & Sargant, 1937]. It has always remained doubtful, however, how far the various effects observed were due to the direct action of the amines and how far they were after-effects due to tissue damage caused by the amines: this could be decided only by accurate information as to the rate of elimination.

The present experiments *in vivo* confirm the previous observations on the significance of the amine oxidase since those amines which are oxidized by the enzyme (type $R\cdot CH_2NH_2$) were also destroyed in the body, while amines of the ephedrine series (type $RR'\cdot CH\cdot NH_2$) which are not oxidized by the enzyme were slowly excreted in the urine from which they could be recovered unchanged. The pharmacological data as to the persistent action of amines of the ephedrine series can therefore be explained in terms of the specificity of the amine oxidase.

With amines that are oxidized in the body the efficiency of the inactivation *in vivo* is shown in the case of β -phenylethylamine by the promptness with which phenylacetic acid appears in the urine and by the fact that even with doses of 300 mg. no amine excretion could be detected in the urine.

Mescaline, which is a phenylethylamine derivative, might be expected to be oxidized by the amine oxidase, but Blaschko *et al.* [1937] found little oxidation of mescaline by preparations of guinea-pig amine oxidase and it would appear that the bulky methoxyl groups modify the molecule so that it is able to resist oxidation. Slotta & Müller [1936] found that mescaline is completely oxidized *in vivo* in the rabbit and dog, but Bernheim & Bernheim [1938] concluded that some factor other than the amine oxidase is responsible for the oxidation. Whatever this factor may be it is clearly deficient in man and the case of mescaline illustrates the fact that with drugs of this type the behaviour in one kind of animal may be quite different from that in another.

SUMMARY

1. A sensitive micro-method for estimating amines has been used for studying the elimination of amines in man.
2. Amines of the ephedrine series and also mescaline are excreted unchanged and were isolated from the urine in the form of crystalline derivatives.
3. Amines of the general type $R\cdot CH_2NH_2$ are rapidly oxidized in man.
4. The rates of excretion of ephedrine, benzedrine, methylisomyn and mescaline have been measured.

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