XLI. OXIDATION OF ALIPHATIC AMINES BY BRAIN AND OTHER TISSUES

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In view of the finding of Quastel & Wheatley [1933, 1] that certain amines, such as *iso*amylamine, inhibit the respiration of brain tissue, an investigation has been made of the power of various organs to detoxicate such amines. It was found early in the work that *iso*amylamine is broken down by brain cortex, and this finding caused attention to be turned to the oxidation of the simpler aliphatic amines by brain and other tissues.

Technique

Thin slices of tissue were cut from organs freshly dissected from the animal, and were placed in manometric vessels containing phosphate-glucose-Locke solution,[1] with and without addition of amine. The vessels were filled with oxygen, and determinations of the oxygen uptakes were made using the Barcroft differential manometer. The experimental period with taps shut was 2 hours. The temperature was regulated at 37°. At the termination of the experimental period, the tissue slices were removed, washed, dried and weighed. Estimation of ammonia was carried out on the solutions in the manometric vessels, the washings of the slices being added. At the commencement of this investigation, the estimation of ammonia was only approximate, owing to the fact that a precise method of estimating small quantities of ammonia in presence of an excess of volatile basic amine had not yet been devised. This has since been done, and a description of the method has been published [Pugh & Quastel, 1937].

The thickness of the slices used in this work usually lay between 0·2 and 0·4 mm. Several slices were employed, the total dry weight in each vessel varying from about 15 to 30 mg. Since only small quantities of ammonia are formed from the amines, rather more tissue was employed than is usual in manometric experiments. With brain cortex, it was considered advisable to use the well mixed slices from two rat brains for a single experiment. With all other tissues, the results quoted represent the mean of duplicate experiments carried out with slices of the organ of one animal.

The solutions in which the tissue slices were immersed were made up to have an osmotic pressure approximately equal to 0·16 M NaCl. The hydrogen ion concentration was maintained at pH 7·4 in all experiments. All amines were used as their hydrochlorides.

Qualitative experiments on ammonia production with amines in presence of tissue slices

Qualitative tests were made in the first place for the ammonia produced by slices of liver, kidney and brain cortex, in presence of methylamine, ethylamine, propylamine and butylamine. The tests were carried out by adding Nessler

[1] Phosphate buffer = 0·022 M, pH = 7·4; glucose = 0·08%.

reagent to the solutions left in the manometric vessels after removal of the tissue slices. The colour was compared with that found in a control experiment where the amine was not added until the termination of the experimental period. The results are given in Table I, plus signs indicating the formation of ammonia from the amines.

Table I. *Ammonia production from aliphatic amines. Qualitative*

Guinea-pig:	Liver	Kidney	Brain
Methylamine	0	0	
Ethylamine	(+)	(+)	
Propylamine	+	±	(+)
Butylamine	+ +	+ +	+
Rat:			
Methylamine	0	0	
Ethylamine	0	0	
Propylamine	+	0	(+)
Butylamine	+ +	0	+

The results show that methylamine is either not attacked, or only feebly so, by rat liver and guinea-pig liver and kidney; rat kidney has little or no effect on the amines under investigation; butylamine is the most vigorously attacked by guinea-pig liver and kidney and by rat liver; while brain of both guinea-pig and rat has a definite deaminating action on butylamine and a feeble one on propylamine. Experiments with rabbit brain cortex slices showed a similar deaminating action on butylamine, with a feeble effect on propylamine.

Qualitative experiments with tissue extracts

Phosphate extracts of brain, liver and kidney were made on lines similar to those of Krebs [1933], and their deaminating effects on butylamine (0·014 *M*) were compared with those on alanine (0·014 *M*). Before applying the tests, protein was removed by addition of an equal volume of 10% CCl_3COOH, followed by neutralization with NaOH. The results are given in Table II, where

Table II. *Tissue extracts. Ammonia formation*

	Butylamine	Alanine
Rat brain extract	+	0
Guinea-pig brain extract	+	0
Ox brain extract	+	0
Rat liver extract	+ +	+ + +
Guinea-pig kidney extract	+	+ +
Rat brain slices	+	0

the plus sign denotes ammonia production as shown by the Nessler reagent. These results show that brain extracts, as well as those of liver and kidney, are able to deaminate butylamine.

Respiration and ammonia production of tissue slices in presence of butylamine and propylamine

In Table III are shown typical results selected from a number of experiments, illustrating the increased respiration of liver slices of both guinea-pig and rat, and of guinea-pig kidney slices, in the presence of butylamine and propylamine. The increase in Q_{O_2} is greatest with guinea-pig liver or kidney and butylamine.

Table III. *Method, HgO-NaOH*

Organ	Amine *M*/60	Q_{O_2} (over first hour) Amine absent	Q_{O_2} (over first hour) Amine present	Q_{NH_3} Amine absent	Q_{NH_3} Amine present
Guinea-pig liver	Butyl	2·4	8·7	3·3	5·4
		3·5	8·3	3·5	5·0
	Propyl	2·5	5·9	4·2	5·8
		3·6	5·6	2·5	2·7
Guinea-pig kidney	Butyl	18·4	21·4	2·0	3·0
		20·5	25·0	2·5	3·8
	Propyl	19·3	22·6	4·3	4·5
		20·8	22·2	2·7	3·4
Rat liver	Butyl	9·8	11·7	6·7	8·8
		6·6	8·0	5·2	9·0
	Propyl	7·9	9·4	6·3	7·3
		8·6	10·2	4·8	4·6
Rat kidney	Butyl	28·5	27·4	7·8	6·4
		32·2	29·5	6·2	5·1

Butylamine does not appear to increase the Q_{O_2} of rat kidney. Propylamine does not produce as definite an increase in Q_{O_2} as butylamine with either guinea-pig liver or rat liver or guinea-pig kidney.

The ammonia estimations were carried out by adding mercuric oxide to the solutions from the manometric vessels, after removal and washing of the tissue slices and centrifuging to remove any tissue débris. The mercuric oxide-ammonia complex was decomposed with NaOH, according to Pugh & Quastel [1937], and the liberated ammonia was estimated by means of Nessler's reagent. The amounts of ammonia obtained were expressed as Q_{NH_3}, i.e. μl. NH_3 produced per hour per mg. dry weight of tissue. A correction was applied for the ammonia blank found when the experimental procedure was carried through without any tissue. This was found to be increased in the presence of amines owing to admixture of preformed ammonia with the amine. In all cases the results were calculated so as to minimize the apparent effect of tissue on amine; a negative result therefore is

Table IV. *Method, K_2CO_3-HgO-NaOH*

Organ	Amine *M*/60	Q_{O_2} (over first hour) Amine absent	Q_{O_2} (over first hour) Amine present	Q_{NH_3} Amine absent	Q_{NH_3} Amine present
Guinea-pig liver	Butyl	3·1	6·4	0·3	2·2
		3·9	7·4	0·2	1·2
	Propyl	3·0	4·1	1·2	1·8
		4·8	5·8	0·4	0·4
Guinea-pig kidney	Butyl	19·3	25·2	2·0	2·4
		18·7	22·9	1·3	1·7
	Propyl	23·2	23·1	1·6	1·4
		21·2	22·5	0·9	0·9
Rat liver	Butyl	9·1	14·4	2·9	4·5
		8·3	10·1	2·5	3·5
	Propyl	10·0	10·7	2·3	2·8
		8·2	8·9	1·1	1·2
	Ethyl	8·8	10·2	1·9	2·3
	Methyl	7·4	9·5	1·1	1·0
Rat kidney	Butyl	25·2	26·9	3·5	2·8

not necessarily absolute. The resulting Q_{NH_3} figures in the absence of added amines were found to be considerably greater than those which have been formerly recorded for the same tissues. In spite of this, however, it is clear from Table III that the presence of butylamine definitely increased Q_{NH_3} with guinea-pig liver and guinea-pig kidney and with rat liver. The increase is much less with propylamine. No increase is noted with rat kidney and either amine, and no definite increase with guinea-pig kidney and propylamine.

The high figure for the Q_{NH_3} found without any amine led to a modification of the method of ammonia estimation [cf. Pugh & Quastel, 1937]. The results obtained with the modified technique, which is now adopted as the usual routine in this work, are recorded in Table IV. The conclusions to be drawn are much the same as those already given. Butylamine shows the greatest increase in Q_{O_2} and Q_{NH_3}. There is no apparent deamination of methylamine with rat liver, and the effects with ethylamine and propylamine are feeble. It would seem that with both guinea-pig and rat, liver has a greater deaminating action on aliphatic amines than kidney.

Deamination of aliphatic amines with rat liver slices

The effects of some of the higher aliphatic amines on the respiration and ammonia production of rat liver slices were next investigated. With such amines only low concentrations can be used, since their solubilities are low under the experimental conditions employed. In the work described in the earlier part of this paper, the concentration of the amines used was 0·016 *M*. For the higher amines a suitable concentration was found to be 0·0066 *M*. This was used in the experiments recorded in Table V.

Table V. *Method, K_2CO_3-HgO-NaOH. Rat liver*

	Q_{O_2} (over first hour)		Q_{NH_3}	
Amine *M*/150	Amine absent	Amine present	Amine absent	Amine present
Propyl	11·4	12·9	2·0	2·5
	8·5	11·7	1·1	3·7
Butyl	6·1	10·0	2·2	4·0
	8·5	10·0	1·1	4·7
Amyl	4·3	7·4	2·6	4·8
	9·5	11·0	1·8	5·6
*iso*Amyl	8·5	11·0	1·1	6·2
	8·6	9·4	2·0	7·2
Heptyl	6·1	9·1	2·2	4·7
	9·5	11·9	1·8	5·0

The most important feature of these results is the demonstration that deamination of higher amines such as heptylamine is accomplished by rat liver. The most marked deamination, however, appears to occur with *iso*amylamine. The oxygen uptake of rat liver is increased by these higher amines.

Deamination of aliphatic amines with rat brain cortex slices

Deamination of these aliphatic amines with rat brain cortex slices takes place as with liver, but the effect is much less marked. Typical results are given in Table VI.

Again it is clear that *iso*amylamine is the amine most vigorously attacked among the amines investigated. But whereas the lower amines slightly increase

Table VI. *Method, K_2CO_3-HgO-NaOH. Rat brain*

Amine	Q_{O_2} (over first hour) Amine absent	Q_{O_2} (over first hour) Amine present	Q_{NH_3} Amine absent	Q_{NH_3} Amine present
Methyl (*M*/60)	12·1	12·2	0·5	0·6
Ethyl (*M*/60)	13·6	15·0	0·6	0·9
Propyl (*M*/150)	10·7	11·0	0·7	0·9
Butyl (*M*/150)	10·6	11·0	0·5	1·3
Butyl (*M*/60)	10·9	11·2	0·8	1·4
Amyl (*M*/150)	10·2	11·4	0·3	0·9
*iso*Amyl (*M*/150)	10·2	9·4	1·0	2·2
Heptyl (*M*/150)	10·5	8·6	0·4	1·1

the respiration of brain, the higher amines have inhibiting effects; in spite of this, however, some deamination of the higher amines takes place. Brain cortex respiration is therefore inhibited by the higher amines, which at the same time undergo decomposition.

Differentiation of amine oxidase from α-amino-acid oxidase

It has already been shown in this paper that brain extracts, as well as those of liver and kidney, deaminate butylamine, and it has been shown that brain cortex slices also deaminate amines. This is of interest in view of the fact that brain does not attack α-amino-acids, with the exception of glutamic acid [Weil-Malherbe, 1936; Quastel & Wheatley, 1932]. This fact has been confirmed, using rat brain cortex slices, with which even propylamine gave rise to an increase in ammonia production, whereas no increase was obtained with alanine. One experiment gave the following result.

	Q_{O_2}	Q_{NH_3}
Without added substrate	10·4	0·7
With propylamine	14·9	0·9
With alanine	10·5	0·6

It appears therefore that there exists in tissues an amine oxidase system distinct from the system which oxidizes α-amino-acids. In support of this conclusion may be cited the observed inactivity of rat kidney towards amines whereas this tissue actively deaminates α-amino-acids.

Products of metabolism of amines in presence of tissue slices

Butylamine. One product of metabolism of butylamine in presence of guinea-pig liver slices is acetoacetic acid. This may be demonstrated qualitatively by Rothera's reaction and it may be estimated manometrically by the aniline hydrochloride method [Quastel & Wheatley, 1933, 2]. One experiment showed $Q_{Ac} = 0{\cdot}77$ for guinea-pig liver in presence of Ringer solution containing $0{\cdot}016\,M$ butylamine.

iso*Amylamine.* When liver or brain slices are allowed to metabolize in presence of *iso*amylamine for 2–3 hours at 37°, the fluid in the manometric vessel develops a strong odour resembling that of *iso*amyl alcohol. On addition to the fluid, after removal of the slices, of a saturated solution of 2:4-dinitrophenylhydrazine in *N* HCl, an immediate yellow opalescence or a yellow precipitate occurs, resembling that due to the dinitrophenylhydrazone of an aldehyde or ketone. The yellow compound dissolves in alkali to form a fairly stable red or reddish brown solution. The formation of this reddish solution may be used as a sensitive

test for the breakdown of *iso*amylamine in presence of tissue slices. Experiments are now being carried out to isolate and analyse the substance responsible for the reaction with the dinitrophenylhydrazine reagent.

Importance of aerobic conditions

That oxygen is necessary for the breakdown of *iso*amylamine in presence of brain slices was shown by an experiment carried out under both aerobic and anaerobic conditions. In the presence of nitrogen the odour, reminiscent of *iso*amyl alcohol, which is always present when *iso*amylamine is attacked in presence of oxygen, is found to be absent. Moreover, there is no precipitation or opalescence on the addition of the dinitrophenylhydrazine reagent to the fluid in the manometric vessel, and no development of a stable red colour on the subsequent addition of alkali to the mixture. The experiment indicates the importance of aerobic conditions for the metabolism of the basic amines in presence of brain.

Summary

1. Deamination of butylamine, amylamine, *iso*amylamine and heptylamine takes place in presence of slices of brain cortex. With the lower amines, propylamine, ethylamine, methylamine, deamination is less marked or negligible.

2. Similar results are obtained with rat liver.

3. Guinea-pig liver and kidney deaminate butylamine, there being less or no action on propylamine. Kidney has a smaller effect than liver. Acetoacetic acid is formed by liver in presence of butylamine.

4. No measurable deaminating effect of rat kidney has been found with the amines investigated.

5. Brain extracts as well as those of liver and kidney possess the power of deamination.

6. The respiration of brain cortex (in presence of glucose) is lowered by the higher amines. The respiration of liver, on the other hand, is increased by the amines which undergo oxidation.

7. *iso*Amylamine is broken down by brain and liver to yield a substance giving a hydrazone with 2:4-dinitrophenylhydrazine. The possible formation of *iso*amyl alcohol is indicated by the development of the characteristic odour. The oxidation of the amine appears to occur only under aerobic conditions.

8. An amine-oxidizing system exists in brain and other tissues which is distinct from the α-amino-acid-oxidizing system.

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